











## PPO-Special Report no. 13

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### **Preface**

#### EuroBlight Workshop Hamar, Norway 28-31 October 2008

A European network of scientists and other specialists working on potato early and late blight meet every 18 months. The network combines two previous networks originating from European Concerted Actions and has 150 members.

- EU.NET.ICP: "European network for development of an integrated control strategy of potato late blight" (1996-2000). Coordinated by Huub Schepers
- EUCABLIGHT: "A potato late blight network for Europe" (2003-2006). Coordinated by Alison Lees.

The Eleventh Workshop was organised by Bioforsk the Norwegian Institute for Agricultural and Environmental Research in Hamar, Norway from 28-31 October 2008.

BASF, Bayer, Belchim, Bioforsk, Certis, Dow, DuPont, Germicopa, Nissan, Nordox and Syngenta sponsored the Workshop.

The Workshop was attended by 98 persons from 16 European countries, Russia, China and Japan. Representatives from all countries presented the late blight epidemic in 2007 and 2008 and recent research results regarding integrated control, decision support systems, resistance of varieties and population biology of the late blight pathogen in potatoes. Since early blight is an increasing problem in Europe, also reports on this disease are included.

The papers and posters presented at the Workshop and discussions in the subgroups are published in these Proceedings, PPO-Special Report no. 13. The Proceedings are also available on the internet www.euroblight.net.

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For further information please contact the network secretariat where also additional copies of this Proceedings can be ordered.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

# The development and control of Late Blight (*Phytophthora* infestans) in Europe in 2007 and 2008

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#### INTRODUCTION

The web application that generates the Euroblight late blight country profiles was launched in 2007 to keep track of the development of late blight and its control in Europe in individual countries and over years. The profile information for 2006 was presented at the workshop in Bologna, Italy in May 2007. Information for 2007 and 2008 was presented at the workshop in Hamar, Norway in November 2008 and is reported here.

#### **METHODS**

Country editors upload information to the Euroblight database via a dedicated web-page on the restricted side of the Euroblight website – http://www.euroblight.net. The country profiles have the following structure and content:

#### Summary

Write a short summary (max 200 words) about late blight development, fungicide use and
control of late blight in the country and year selected. This section will be used to generate a
summary report covering all countries. Additionally, this will be the starting point for the summary
report about late blight, fungicide use and effectiveness of control measures, published after each
Euroblight workshop.

#### Early outbreaks of potato late blight

- Select the date of first observation of late blight in covered or very early planted potatoes
- **Disease source for these attacks** (options: Seed, Cull pile, Volunteer plants, Covered crop, Waste pile, Oospores, Indications of Oospores, Other, Not known)
- Select the date when first infections were reported in more than 5 conventional, normally planted potato fields. This is the date when late blight is recorded in more than a few fields for the first time. After this event and if the weather is continuously blight favourable there will be a risk

- of epidemic developments in non-treated (and especially in susceptible) cultivars.
- Disease source for these attacks (options: Seed, Cull pile, Volunteer plants, Covered crop, Waste pile, Oospores, Indications of Oospores, Other, Not known).
- Write a short text (max 100 words) about early attacks. The report generator will include dates and disease sources in texts. Enter additional information in the text window.

#### Weather conditions and late blight development

- Weather based risk of late blight. Select whether the weather-based risk for late blight development
  was low, medium or high for the months May to September. Or, select 'Not known'.
- Write a short text (max 100 words) about the weather conditions related to late blight
  development. Mention if the information about weather conditions is general for the country,
  related to a specific region and if the risk is qualitative or based on calculations with a model or a
  DSS.

#### Use of fungicides and control strategies

- Enter the number of fungicide applications used in ware potatoes. What do the majority of
  conventional farmers do to control late blight in ware potatoes?
- Enter the number of fungicide applications used in all potatoes. Sometimes quantitative information is available as a mean of all types of potatoes e.g. in DK as calculated Treatment Frequency Index based on amounts of fungicide sold (normal dosage) and related to the total area of conventional grown potatoes
- Write a short text (max 100 words) about fungicide use and control of late blight.

#### Organic potatoes

- Select when outbreaks were recorded in fields with organic potatoes (Options: early, medium, late or not known compared to normal).
- Select the level of attack (Options: low, medium, high or not known compared to normal).
- Select the mean yield level in organic potato fields (Options: <20 t/ha, 20-30 t/ha, 30-40 t/ha, >40 t/ha or not known)
- Write a short text (max 100 words) about the situation in organic potatoes.

#### Tuber blight

- Select the level of tuber blight attacks (Options: low, medium, high or not known compared to normal).
- Write a short text (max 100 words) about tuber blight.

#### Alternaria spp

- Select when outbreaks were recorded (Options: early, medium, late or not known compared to normal).
- Select the level of attack (Options: low, medium, high or not known compared to normal).
- Write a short text (max 100 words) about Alternaria.

#### Characteristics of Phytophthora infestans

Write a short text (max 100 words) about pathogen characteristics. In the country reports graphs
for mating type distribution and virulence pathotypes are automatically included based on available
data from the Eucablight database.

#### Use of cultivars

Write a short text (max 100 words) about use of cultivars.

#### Use of DSS

• Write a short text (max 100 words) about use of DSS in the country.

One important motivation for partners to upload data into the Euroblight database is that the results are analysed and presented "in real-time" in a pan-European context, and the reports can be edited dynamically by a group of editors. When data are available over several years it will be possible to analyse the data over years and across countries.

The reports published below are the abstracts of the country reports taken directly from the database with only slight editing. The information and descriptions are the same as can be found on the Euroblight web site under the menu item 'Summary report'. More detailed information in national reports can be found under the menu item 'Country reports'.

## THE DEVELOPMENT AND CONTROL OF *PHYTOPHTHORA INFESTANS* IN EUROPE IN 2008

The abstracts of the country reports are provided by country alphabetic order. The first part covers 2008 and the second part 2007. General trends and observations on weather conditions, disease development etc. are discussed after each section (year). Information regarding "Date of first observation of late blight in covered or very early planted potatoes" and "Date when first infections were reported in more than five conventional, normally planted potato fields" for 2007 and 2008 is shown for all European countries on maps in Fig. 1-4. The same data are combined into marker plots per year in Fig. 5 and 6. The weather based risk of late blight development in Europe is shown in Fig. 7-8. Fungicide use is given in Tables 1-2. Finally, a list of DSS used in Europe is available in Table 3.

#### Relarus

In 2008, late blight development was moderate on conventional fields with fungicide treatments and epidemic in allotment gardens (we generally call them "personal subsidiary plots"), which constitute approximately 90% of the total potato plantings in Belarus. First disease symptoms were recorded on 18 June in the central part of the country (Minsk region) on early cultivars, susceptible to late blight. Between 23 June and 14 July, disease was found everywhere across the country and from 14 July late blight was abundant in potato fields. Nevertheless, disease pressure was high only in private gardens, where fungicides were not applied in a regular way. Moderate weather based risk of late blight during August and regular sprays conditioned moderate disease development in conventional fields at the end of the season. On average 2-5 sprays were used to control disease in conventional farms, whereas in private gardens fungicides commonly were not applied at all. The most popular fungicides used in Belarus are mancozeb and its prepack mixtures with systemic and translaminar ingredients (mefenoxam, metalaxyl, dimetomorph, fenamidone), cymoxanil, fluazinam and chlorothalonil. Due to increased temperature regime during August the level of tuber blight attacks was rather low.

#### Belgium

Around the time of planting for ware potatoes, first blight attacks were observed – not surprisingly on dump piles (April 22); the number of lesions on these piles increased strongly towards the end of April. First attacks in ware crops, with a foliage height of around 12 cm, were reported in the last week of May. Very favourable weather for late blight (with up to 15 consecutive infection days) coincided with the period of strong foliage growth. In the region of Flanders, more particularly the western part, an explosion of the disease was observed in the first half of June. By the end of this month, attacks of late blight were widespread and lesions could be found in a majority of the fields. During the summer months of July and August, the disease pressure remained very high – 40 infection days in two months – and a tight spray schedule with good performing fungicides had to be maintained. An average of 19 applications against late blight was necessary in a susceptible ware crop variety.

#### Czech Republic

Potato late blight had very favourable conditions for epidemic spread in decisive production regions of the Czech Republic in 2008. First secondary infections in the early potato-production region

were found in the end of May, in the potato-production region, despite of relative lack of infection sources in seed potatoes, they were found very early, in the first decade of June. Epidemic spread of the disease was recorded during July and intensive rainfalls supported simultaneous tuber infection. Extraordinary high infections were also determined in relatively resistant medium-early and medium-late potato varieties. Rainfall in the middle of August locally increased tuber infection. In medium-late and late varieties increased infection due to rainfall was also recorded in September. Most part of tubers infected in July was not harvested, these tubers decayed in the soil. Late blight in this year affected both yield amount and tuber quality. Intensive fungicide treatments increased yield by 82 %.

#### Denmark

Hot and dry weather in May and dry and relatively cold arctic air in June prevented early attacks from oospores and other inoculum sources. From first of May and until 18 June, not one single day was calculated as a "Dangerous day" with blight conditions. Blight was found first time on 3 July in the south of DK. The threshold for aphids was succeeded approximately 1 July and the first seed potatoes were desiccated from first week of July. Early appearance of aphids gave problems with virus. It was possible to keep the fungicide use at a very low level until 1 July due to our effective monitoring network for early attacks and a robust calculation of infection pressure. The risk of primary attacks from infected tubers calculated with NegFry was approximately 1 July. After a blight favourable spell in Mid July, Epidemic development was reported in several fields on 25 July. Weather in August was rainy and extremely blight favourable. Release of nitrogen left over from the previous dry period was released and attacks were found in cultivars with new growth, e.g. Kuras; but not in other cultivars without new expanding top leaves, e.g. Kardal

Copy this line to your browser and read more:

http://www.planteinfo.dk/BlightMgmt/BlightMgmt.asp?pi\_menu=1&item\_id=647&appl=647&language=UK. Select the menu "Fungicider og strategier" and see the Danish basic control strategies and fungicide information in English language.

#### England & Wales

Unfavourable conditions during April delayed planting in many regions of England and Wales in 2008. The majority of the crop was planted by the end of May/early June, several weeks later than in 2007. There were 255 incidents of late blight in GB in 2008, 132 of which were in England and Wales. The majority of reported outbreaks were from crops, with 7 originating from outgrade piles and 3 from volunteers. Only 9% of outbreaks were reported in May and September, with most reported in June (27%), July (31%) and August (37%). Agronomists and advisers recommended 7-day intervals for fungicide applications due to the high disease pressure in 2007 and the dominance of the 13\_A2 strain.

#### Estonia

The potatoes were planted in first half of May, which was dry and colder than usual. The potatoes emerged in normal time in the first decade of June. The dry period lasted until 17 June, when serious showers occurred in most parts of Estonia. The rainy period started at this time and lasted until the end of August. The first outbreaks of late blight were recorded in 8 July in home gardens where potatoes had been cultivated in the same soil for several years. These outbreaks had clear character of soil borne infection -lesions on lower leaves and many plants, evenly distributed in the plots were infected. Late blight progressed extremely fast in the first half of August. Most fields were waterlogged due to excessive precipitation in August what hampered fungicide applications and created suitable conditions for tuber infection. Tuber blight was a bigger problem than in the previous years. Tuber infection was favoured by inadequate control of late blight leading to

prolonged presence of infected foliage on the field and wet soil conditions. Fast developing late blight caused dramatic yield reduction in organic production. Several organic farmers lost the entire crop because of late blight damage.

#### Finland

First potato late blight outbreaks were reported during the first week of July. The date of outbreaks was rather normal, but the crop development was delayed due to dry and cool spring. First attacks were often present immediately after emergence. The weather was very favourable to blight from the second half of June until the harvest. First fungicide applications were generally started one week too late and severe blight outbreaks were reported at the end of July. Due to heavy blight pressure up to 9 fungicide applications were made and despite of that blight was present at most fields in the end of August, which is very exceptional in conventional production. Regardless of leaf blight situation very little tuber blight has been found in yield.

#### France

Surveys at the time of planting (around April 15, 2008) showed many refuse piles with abundant sprouting. The first late blight symptoms were observed about a month later (May 15, 2008) on refuse piles, and in early June in field crops. The disease severity increased throughout June, but the epidemic could be managed as sprays were performed in good conditions. Growers tended to use 'top end' active ingredients, in particular cymoxanil, fluazinam and cyazofamid, rather than dithiocarbamates. Despite these sprays, symptoms persisted on stems throughout the season. The crop was of good quality, with very little tuber blight recorded at harvest.

#### Germany

Planting of potatoes in Germany took place during beginning and end of April. It is a late planting date. Cold conditions after planting resulted a late emergence of potato plants (mid and end of May). The first outbreak of late blight in potatoes was in the mid of May in the early potato growing area. One week late we found a lot of late blight attacks in covered potato fields. In the first week of June late blight was observed in nearly all potato growing regions over Germany. The weather conditions for the development of late blight was low in May, low (northern part) to moderate (southern part) in June, moderate in July and moderate August. Only in the potato growing area around Munich the epidemic pressure was high in July. The number of fungicide treatments was normal in 2008. All kinds of products were used. The new product REVUS was registered and introduced this year. Very late in the season the Alternaria-fungicide SIGNUM was registered.

#### Latvia

Due to low air and soil temperatures in May, crop emergence was completed by the end of May – in some cases even the beginning of June. Cool and dry conditions delayed crop growth in spring. The first warning of the development of *Phytophthora infestans* and Alternaria solani was received on the 20th of June. Because of frequent rain and favourable temperatures for crop growth, development of early blight (eb) and late blight (lb) started on the 21st of June. The first protective application of fungicide (systemic + contact) was made before the infectious period. Temperature and humidity conditions were favourable for the development of both diseases. The first symptoms of A. solani were recorded on the 9th of July. In July 60 mm of precipitation was recorded - with frequent rainfall, an average temperature of 17°C and with high relative humidity. The second warning of the development of P. infestans was received on the 6th of July. The second protective application with fungicide (systemic or translaminar + contact, or translaminar + contact) was made. The first symptoms of P. infestans were recorded on the 16th of July. Applications with contact fungicides, mostly with mancozeb and fluazinam, were made in July and August. P. infestans and A. solani

progressed extremely fast in August when 95 mm of precipitation was recorded - with frequent rainfall and average temperature of 16,4°C. In the northern part of Latvia 155 mm of rainfall was recorded in August. In August and September the infection pressure on unprotected crops was very high due to frequent precipitation and optimal temperatures. Due to warm weather conditions in winter 2007/08, many volunteer potatoes were able to over winter, which is not common in Latvia's conditions. The first infection was recorded on fields where potatoes had been cultivated with 1 or 2 year intervals. The use of fungicides resulted in excellent control in all farms when the first application with systemic + contact fungicide was made at the end of June/ beginning of July. The control of lb was excellent to moderate in the 2008 season.

#### Lithuania

The weather conditions at the beginning of May were not favourable for the emergence of potato crop due to the low air and soil temperature. Also, potato emergence was delayed by lack of rainfall. Scarce amount of rainfall were recorded at the end of April and beginning of May. The summer period was exceptional compared with previous years. Average air temperature in June – August was recorded by few degrees higher than the long-term period. At the same time the amount of precipitation was lower by 20-40 percent compared with the long-term average. Such meteorological conditions were mainly favourable for early blight. Also, late blight was recorded in some regions and the fields but the disease didn't have a significant influence on potato crop yield. First symptoms of late blight were recorded on 27 June 2008. First infections in more than 5 conventional, normally planted potato fields were recorded on 10 July 2008. In some cases, first late blight symptoms were detected only at the beginning of September.

#### Netherlands

Planting of potatoes in the Netherlands took place during the first weeks of April. After a cold period in April the emergence in most fields of ware potatoes was about the third week of May. This is a normal situation for the Netherlands. April and the first weeks of May the weather was remarkably unfavourable for fungal diseases. During the last decade of May and the first week of June there was a big difference in the amount of precipitation over the country. In the southern part there was enough rainfall for a good start of the growing season and a prosperous crop development. In the central and northern provinces it was very dry and a large water deficit occurred lasting till the end of June. Due to this difference in conditions, the fungal development was diverse over the country. The first blight on a waste pile was found on the 26th of May in the South West. This was after the first few favourable days for blight. A few days later we got a report of blight in an early potato field, variety Ukema in Zeeuws Vlaanderen. In that region the conditions for blight and other Oomycete diseases were favourable during the first week of June. In the other regions hardly any blight was observed until the last decade of July. Overall there were few nights with the right infection conditions for Phytophthora during the period of canopy development. So the first weeks of the season, it was fairly easy to control blight. Blight favourable weather at the end of July initiated epidemic development in August. Despite the rainy conditions growers succeeded in controlling blight with fungicides.

#### Northern Ireland

Good weather during April-May and the first two weeks of June, with below average rainfall and above average temperatures, allowed planting on time and most crops were planted before the end of May. Rainfall during July was nearly twice the 30-y average and during August it was well over twice the average: on 16 August 68 mm were recorded in Belfast. Relatively few reports of blight were received during much of the season, probably because the dry start prevented development of primary infections. However, the exceptionally wet weather later resulted in 500 ha of potato crops being lost due to flooding. In addition to producing conditions very favourable to blight,

the wet weather made application of fungicides difficult. Growers made use of a wide range of different fungicides and generally maintained adequate control despite the adverse weather. Ground conditions for harvesting mostly remained poor during September; many crops were lifted very late (in November-December) and a few have still not been harvested. Although most crops had some foliage infection, few cases of tuber blight have been reported. This may be because of good tuber protection by some products and also because blighted tubers may have rotted before harvest. The very wet soil conditions have resulted in other fungal/oomycete rots, including pink rot (P. erythroseptica), being found in some crops.

#### Norway

Late blight appeared late in 2008, but from mid July onwards the disease appeared in several locations. The late blight weather was very favourable in August and many days had precipitation. The use of fungicide was a bit lower than normal and Shirlan was the most commonly used product. More tuber blight than normal is expected.

#### Poland

Beginning of the spring 2008 (April) was cool and very wet, what causes that the potatoes were planted relatively late in many region of Poland. The next two months (from the end of April to the beginning of the June), were the period of drought with minimal amount of rain. Therefore, in June the rainfall were as many as 90% lower, in comparison to long-term average. It was the main cause of late emergency of potato. Meteorological conditions in May and June (drought) didn't favour LB appearance. At the end of June more rainfalls were observed in southern regions of Poland. The first infections of the disease were noticed about 23rd June (BBCH 61). However in the North of the country, due to high air temperatures, only sporadic infection spots of LB appeared. More intensive rainfalls in the middle of July were conductive to appearance of late blight about 24th July (BBCH 69-73), but the disease developed slowly. Only heavy rain in August, especially in the middle of month, was the cause of violent development of LB. According to the weather conditions, the destruction of haulm on control plots increased from 30% to 94% in the time of ten days.

#### Russian Federation

Summer 2008 was very wet in most of the potato growing regions of European Russia. The severe development of late blight was registered in the northern, northwestern, western, central and some eastern parts of this territory. First attacks of *P. infestans* were reported at June 25 in the Bryansk region. Heavy summer rains resulted in extremely favorable blight conditions and complicated the spraying in some areas due to the heavy access to the fields. The moderate development of the disease was observed in the most of eastern and some southern regions. In other regions of European Russia, the development of the late blight epidemics began later, and the level of disease attacks was either moderate, or low. A high amount of rains before and during harvesting caused serious problems with the tuber blight (up to 16% of infected tubers) in the central and northwestern regions. The severe early blight attack was registered in some eastern regions on potato cultivars resistant to the late blight and untreated with fungicides. The most popular fungicides were Shirlan, Penncozeb, Tanos, Acrobat MZ and Ridomil Gold MZ. The average number of fungicide treatments in potato enterprises and private farms was 1-3; the maximum number was 10 (mainly for the protection of potato for processing). A small amount of farms used the DSS Plant Plus (Dacom) to control the late blight.

#### Scotland

2008 was less favourable for foliar blight development than the exceptional 2007. There were fewer Smith Periods early in the growing season than in 2007. The average across several sites in May,

June, July, August and September were 1, 0, 5, 4, 1. After the very high pressure and widespread foliar blight of 2007 growers were very well prepared for the blight threat. Most agronomists and advisors were recommending a maximum spray interval of 7 days unless there were extended periods of low risk. This was prompted by memories of blight in 2007 combined with concerns regarding the change in the UK blight population to predominantly the 13\_A2 genotype.

#### Slovakia

Very early potatoes were planted in Southern Slovakia on the end of March and the growing season was without late blight hazard. There were only 1-3 fungicide applications during vegetation period. Table and seed potatoes were planted on the end of April and beginning of May. There was noticed hot and dry May and June and no incidence of late blight infections was recorded on potato fields. Lot of rainfalls (141%) and higher temperature (+1,6°C) in July set up favourable conditions for late blight development that lasted in August as well. The first incidence of late blight in commercially grown potatoes was recorded July 8. Unprotected potato fields were completely destroyed by late blight infection in the beginning of August. Heavy rainfalls caused more tuber late blight infections than 2007. In general, late blight protection was moderately managed on majority of potato fields.

#### Sweden

Varying weather conditions resulted in a long planting period. Due to hot and dry weather in May the first attacks of late blight came later than normal. Only one report of late blight was recorded in the early potato districts. However, blight was recorded earlier than usual in the very North. In Midand South Sweden the infection pressure was low in June and July, but shifted in August. Rainy and cool conditions favoured blight in August and September and gave bad harvest conditions. There have been reports of tuber infection in both conventional and organic potato.

#### Switzerland

Up to the last third of June, the potato late blight situation in 2008 was similar to 2007. Until then the slope of the overall Swiss epidemic was even steeper as in 2007. However, due to a dry period of about 14 days, the 2008 epidemic was slowed down and overrun by the 2007 epidemic at the end of July. First late blight attack was announced in our decision support system PhytoPRE on 19.05.08 (cf. 14.05.07) in the western part of Switzerland and only one to four days later four new LB-attacks spread over the whole main growing potato area from east to west were announced by the cantonal plant protection services and other partners of our information network. As weather conditions were conducive during most of the potato growing season, late blight could develop easily and spread over the whole country. In 2008, infection pressure was extremely high in the western part and also high in the eastern part of Switzerland. As long rainy periods occurred it was difficult to control late blight and to apply fungicide treatments on time.

# THE DEVELOPMENT AND CONTROL OF *PHYTOPHTHORA INFESTANS* IN EUROPE IN 2007

#### Austria

In Austria there were some days with moderate rains followed by sunny and dry periods with high temperatures in most of the potato growing regions from the beginning of May to the end of July 2007, and late blight occurred only at low levels. First observation of blight in covered or very early-planted potatoes was at the end of May and on normally planted conventional fields from middle of June to middle of August. Rainy weather with moderate temperatures from the beginning of August to the first week of September caused an increase of infection pressure and late infections in some

fields. In Austria on average 6 fungicides applications were used (range 3 - 10) to control late blight.

#### Belarus

In 2007 first late blight was observed very early for local conditions – on 10 June, on unsprayed potato plants of cv. Satina in private garden (Minsk region). Next day (11 June) first LB attack was reported from southern Homel region and by 20 June first disease outbreaks were recorded in a lot of conventional fields. Conducive weather conditions of July (moderate temperature and increased RH regimes, 110 mm of precipitation per month with heavy rains (more than 10mm) favoured rapid disease development, but during dry and hot August (increased temperature, 25-30% of monthly rate of precipitation) it has been suppressed significantly. Finally late blight pressure was found to be moderate at the end of August and 2-5 sprays during the season were sufficient to control the disease effectively. The most popular fungicides used in Belarus are mancozeb and its prepack mixtures with systemic and translaminar ingredients (mefenoxam, metalaxyl, dimetomorph, fenamidone), cymoxanil, fluazinam and chlorothalonil. In commercial fields fungicides were applied regularly, whereas owners of allotment gardens usually don't use any fungicides.

#### Belgium

The potato season 2007 was characterised by massive, widespread, and often very serious attacks of late blight, forcing farmers to maintain a very tight spray schedule throughout the season. After a record dry spell in April – 36 consecutive days without rain and summer temperatures – the rains resumed at about the time of emergence for most of the ware potatoes. Two months of continuous rainfall (2 days out of 3) together with explosive leaf growth and numerous inoculum sources led to a very severe epidemic. Control was difficult and required a very high input of fungicides. The average spray interval for the season was only 5 days or less, often with a mixture of different commercial products. On average, 21 applications (18 tot 25) were made in ware potatoes; as a result, the level of tuber infection at harvest was relatively low.

#### Czech Republic

Conditions for planting and potato crop emergence were very favourable in 2007 and potato crops emerged 2 – 3 weeks earlier than usually. Late blight occurrence was considerably affected by extraordinary weather progress and limited infection sources. Meteorological conditions during growing season were not favourable for crop infection and epidemic late blight spread. Rainfalls in decisive months May to August were below long term normal and relatively high temperatures and unsuitable rainfall distribution did not allow longer leaf area wetness, which is a presupposition of the infection. Late blight spread through crops was very slow. Greater differences between untreated and treated crops in leaf area destruction were apparent in the second half of August in connection with longer leaf wetness due to night dew. Yield effect of a fungicidal treatment did not exceed 20 %. Weak occurrence of foliar late blight and lack of rainfall were also a cause of minimal tuber infection. Intensive rainfall was recorded in September; however, late blight did not affect yields and tuber infection any more. In the pathogen population 76 % isolates were found belonging to mating type A2. No resistance to fungicidal substances metalaxyl, dimethomorph and propamocarb hydrochloride was found.

#### Denmark

In 2007, crop emergence was 2 to 3 weeks earlier than normal. In early planted, non-covered potatoes potatoes, a blight favourable spell in late May caused primary attacks in the first week of June in South of Denmark. Then weather shifted into warm and dry and only one month later in early July attacks were recorded widespread in the country. The season is characterised by many days with rain and sometimes, heavy rainfall. It was difficult to spray in between the showers,

but generally the control was effective. The fungicide input probably will be the highest ever recorded in Denmark. The most used control strategy was 5-7 day routine schedule using Dithane (mancozeb). Ridomil and Typhon were sold out due to the need for extreme amounts this year. Even late blight was found (in low amounts) in nearly every field, problems with tuber blight were only limited due to low infection pressure in September. Yield in organic potatoes was low to medium due to epidemic development in many fields in early-mid July.

#### England & Wales

Most crops in England and Wales had been planted by early to mid-May, two weeks earlier than 2006, despite delays due to heavy rain in February and early March. There were 324 reported outbreaks of blight in GB in 2007, 233 of which were in England and Wales. Eighty-two percent of outbreaks in GB contained the A2 mating type and genotype 13\_A2 was widespread throughout England and Wales in the 2007 growing season. A combination of factors contributed to the severe foliar blight that occurred in 2007, including the occurrence of high-risk weather at the beginning of the growing season and stretched spray intervals due to wet soil conditions disrupting spray applications. The weather-based risk of late blight development across England and Wales was moderate in May, high from June to August and moderate in September, although there were differences in risk depending on the region. The majority of reported outbreaks were from crops, with 5 originating from outgrade piles and 13 from volunteers.

#### Estonia

The cold weather in May suppressed the emergence of potatoes. Crop emergence was in mid June. The first outbreak of *Phytophthora infestans* was on 13 July when the temperature and humidity conditions were favourable for the development of the disease. Low precipitation and high temperatures in second half of July slowed down the development of disease. The rainy and humid period in first decade of August was most favourable for late blight development. Further low night temperatures suppressed disease spread and development. Control of late blight was relatively easy and the majority of growers succeeded with good to excellent control in season 2007.

#### Finland

The blight season was relatively normal after two almost blightless seasons. The first outbreaks in unprotected crops were reported in the first half of July and blight was commonly present at the end of July. In unprotected crops the epidemic development was extremely fast due to rainy July. Conventional growers sprayed against blight normally and there were generally no severe failures in leaf blight control. Also tuber blight in general was in control. Most commonly used fungicides were mancozeb products and Shirlan. The normal number of applications was 4 to 6.

#### France

In 2007, surveys showed that potential sources of primary inoculum were very important from the beginning of May. The presence of such sources of infection is an important criterion, which is taken into account in the decision support system MilPV. In certain parts of Northern France, the first late blight symptoms were observed as early as May 10th on refuse piles, at a time when potato crops had not emerged yet. The first sprays on susceptible cultivars, advised when the Guntz-Divoux and Milsol models forecast sporulating spots from the third asexual cycle of *P. infestans*, were timed on May 10th in Northern France and Picardy, and around May 10th in Central France.

#### Germany

The weather condition and the fungal development was diverse over the country in 2007. The Northern and Western part of Germany had an early and severe late blight epidemic. In the Eastern

and Southern part we observed a normal late blight development. Planting of potatoes in Germany took place during end of March till mid of April. It is a normal planting date. Warm conditions after planting we found an early emergence of potato plants in the Southern part of Germany (end of April, beginning of May). The first outbreak of late blight in early potatoes was 21/05/2007 in the region Burgdorf (Niedersachsen). In the first week of June late blight was observed in nearly all potato-growing region over Germany. This time is very early for the Germany regions. The further development of late blight was completely different. In the Northern part of Germany there were very favourable weather conditions for the late blight development. The disease pressure was very high till end of August. In the Southern part only few infection periods were observed in July and August. The use of fungicides was very high in 2007. All kinds of products were used - mixtures were used in the Northern part of Germany.

#### Ireland

In the Republic of Ireland the weather conditions in 2007 were much wetter than 2006. June, July and August were very wet while September was relatively dry. There was an average rainfall of 3.69 mm per day in June (233% of 2006), 4.15 mm in July (411% of 2006), 3.43 mm in August (290% of 2006) and 1.27 mm in September (31% of 2006). There were only 8 days with no recorded rainfall in June 5 in July, 13 in August and 16 in September. There was a major wet period from June 12 to August 20. The average temperature for the 4-month period was 14.37°C and this was the coldest growing season in the last five years. During 2007, the relative humidity was higher than average for most of the season. As a result, the date of disease out break was much earlier than normal and progressed rapidly during July. Untreated areas reaching 100% defoliation by early August. The generally wet soil conditions resulted in relatively high levels of tuber infection. Despite the very severe blight epidemic in 2007, growers achieved good disease control. Most growers used a phenylamide-based product early in the season. During 2007, only a small number of isolates showed phenylamide resistance and no isolates were of the A2 mating type. All isolates were complex in terms of physiological race make up, with one isolate having all 11 resistance breaking genes.

#### Latvia

Crop emergence was completed by the end of May. The first warning of the development of *Phytophthora infestans* was received on the 20th of June, when the temperature and humidity conditions were favourable for the development of the disease. In the following 2 weeks 70 mm of precipitation were recorded - with frequent rainfall, average temperature 15-16°C and with high relative humidity. The first protective application was done with fungicide (systemic + contact) before this infectious period. The second protective fungicide application (systemic + contact or translaminar) was done at the beginning of July. The last week of July was extremely favourable for the development of late blight (lb). Applications were done with contact fungicides, mostly with mancozeb and fluazinam. Low precipitation and high temperatures in August slowed down the development of the disease. The lb development and infection pressure at the end of August was very high on unprotected crops due to increased precipitation and optimal temperatures. The control of lb was good to excellent in the 2007 season.

#### Lithuania

The weather conditions at the beginning of the growing season (in May) were favourable for the emergence of potato crop. The average air temperature in May was by few degrees lower during the first ten-day period but higher during the next ten-day periods. The amount of rainfall distributed very evenly during May and was close to the long-term average. The air temperature over the June – August period in most cases was by 0.1 - 3.3 °C higher compared with the long-term average. From the second ten-day period of June till the end of July rainy period started with rainfall occurring

almost every second day. The average amount of rainfall in July was by more than 50 percent higher than the long-term average. August was less rainy and average amount of rainfall accounted for only 31 % of the long-term average. Rainy weather favoured early late blight occurrence in the potato crop. The first disease symptoms were spotted on 23 June. Later, the amount of rainfall significantly declined which slowed down the disease development. The last ten-day period of July was rainy. In 7 days the total amount of rainfall amounted for 39.0 mm. Such weather conditions determined a fast increase in late blight severity.

#### Netherlands

After a wet and mild winter, there was an exceptional long dry period in spring, between 22 March and the 7th of May. All over the country there has been hardly any rainfall during this period. Most crops had been planted by mid-April, which is rather normal for the Dutch situation. After the dry spring it started raining and we got a very wet summer. This meant that the weather conditions for blight were very favourable. After crop emergence the disease pressure was in no time at a very high level. In the period of the canopy development it was hard for growers to keep their crop well protected (wet soil conditions disrupting spray applications). The use of fungicide was high in 2007 and all kind of products and mixtures were used to keep control of *Phytophthora*. The disease pressure over the whole season has never been that high over the last 10 years.

#### Northern Ireland

Good weather during March-May, with below average rainfall and above average temperatures, allowed planting on time and most crops were planted before the end of May. Rainfall during June-July was nearly twice the average and wet weather continued throughout August, which produced conditions very favourable to blight and made application of fungicides difficult. Growers made use of a wide range of different fungicides, including recently approved products, and generally maintained adequate control despite the weather and limited availability of fungicide products during July. A dry September and October allowed crops to be lifted in good conditions. Although most had some foliage infection, few cases of tuber blight have been reported. This may be because of good tuber protection by some products and also because blighted tubers rotted before harvest.

#### Norway

From the end of June to mid August the risk of late blight was very high. Use of fungicides was a bit higher than normal and Shirlan was the most commonly used product. A lot of fields were attacked during July. In the last part of the season the weather was less blight favourable and only slightly more tuber blight than normal was found.

#### Poland

Warm and wet spring (IV-V) provided optimum planting conditions and early emergence of potato plants. The end of May and beginning of June were warm and relatively dry and didn't favour LB infections. In the next period – the end of June the increase of rainfall were observed (160% more compare to long-term average) and continued through all July. Additionally, the weather conditions caused greater vulnerability of the potato plants to the pathogens. The most of LB infections started at the end of June and beginning of July (30.06 - 02.07, BBCH 38-69). Unusually strong development of the LB was indicated during that time. Despite low precipitation in August LB developed still strongly because of big differences of temperatures between days and nights and high humidity of soil, which caused premature haulm destruction. At unprotected plots the disease destroyed haulm completely at the beginning of August.

#### Russian Federation

The late blight infection was severe on untreated potato fields of the northern part of Russia (Arkhangelsk, Karelia, Vologda, and part of the Yaroslavl region), the territory of the Kaliningrad enclave, in some farms of the western (Bryansk region) and south-eastern (Mariy El) regions. The moderately severe development of the disease was registered in most parts of the northern, northwestern, western, and northeastern regions. In the other parts of the European part of Russia, the weak or depressed development of the disease was registered. An unusual early appearance of the primary late blight symptoms was reported from Kursk and Kaliningrad regions (shortly after the appearance of shoots). There were severe tuber attacks in Karelia and Kaliningrad: for other regions only minor infection of tubers were registered. The high amount of precipitation complicated the treatment with fungicides because of the inaccessibility of fields in the Kaliningrad enclave. The most popular fungicides were Shirlan, Penncozeb, Acrobat MC, Tanos, Sectin Fenomen, and Ridomil Gold MC. The average number of fungicidal treatments in agricultural enterprises and private farms was 2-3; the maximum number was 9. The owners of allotment gardens did not use any fungicides. Problems, connected with the phenylamide resistance of the late blight pathogen were registered in some regions of Russia; these problems were caused by the irregular use of Ridomil Gold MC and other phenylamide-containing fungicides. Last three years a small amount of farms, producing potato for the processing, used the DSS Plant Plus (Dacom) to control late blight. The severe early blight attack was registered only in the Kaluga region.

#### Scotland

2007 was the worst year for foliar blight in Scotland for decades. One explanation for the severe blight in 2007 is the concentration of Smith Periods early in the growing season. The average number of high-risk periods across several sites was 4.7, 2.6 and 1.4 in June, July and August respectively. In 2007 Smith periods were infrequent in May and September. In 2007 the combination of circumstances favored the pathogen, i.e. high-risk conditions when many crops were still at the rapid haulm growth stage, wet soils delaying spray applications or at least preventing optimum coverage of the crop. The very early high-risk conditions meant that most crops had received few fungicide applications prior to being challenged by blight. Also, the more aggressive and virulent *P. infestans* genotype 13\_A2 was widespread from the start of the growing season. In addition, there were delays in the supply of some fungicide products and other products ran out.

#### Slovakia

Very early potatoes were planted in the end of March, table and seed potatoes in the end of April. Rainy and warm weather in May and June accelerated very good crop development as well as late blight outbreaks. Spread of late blight infections was stopped during second half of July due to extreme high temperatures (over 35°C). Rainfalls in August helped to recover late blight infection on mid-season varieties but with lower intensity than in the beginning of growing season. Unprotected potato fields were completely destroyed by mixed infection of late blight and early blight in the end of August. In general, late blight protection was well managed on majority of potato fields.

#### Sweden

The early spring of 2007 resulted in 2-3 weeks earlier planting of the potato compared to normal. Despite of this the first reports of late blight did not come until in the first week of June from the South coast area in a covered early crop. In the second half of June blight were reported from occasional fields in the Southwest and east. In the south, infection pressure was very high in late June and early July due to heavy rain. Mid July was somewhat drier, but the rain came again in late July and the infection pressure during late July and August was very high. As in 2006 many growers had problems accessing their fields, which in many cases made it impossible to keep the

spraying interval short enough, resulting in very severe attacks of blight in some fields. Most fields had varying levels of late blight attacks at the end of the season. Mid Sweden did not experience late blight until mid July, and despite high amounts of rain with high infection pressure the problems were a lot smaller compared to South Sweden.

#### Switzerland

After two very dry spring months March and April, unsettled weather started in May '07 and during June, July and August many long periods with bad weather were registered. Therefore weather conditions were often favorable for the development of potato late blight. Under these circumstances it was hardly impossible to apply fungicide treatments on time and late blight could spread easily in all Swiss major potato growing regions. Only a few fields remained free of late blight. Late blight pressure was extremely high in the western part of Switzerland (canton Bern and Fribourg), in the eastern part late blight occurred later and the infection pressure was lower. Overall, 2007 was a year with high late blight pressure.

#### Turkey

The potatoes were planted in the middle and at the end of April. The plant emergence was completed on 20 May in 2007. The first outbreak of late blight was observed at the beginning of June (06.06.2007). The first application was recommended and made with the Ridomil MZ 68 WP. The first symptoms of late blight were observed at 10.06.2007 in the field. After that period 2 fungicide applications were made with Trooper 72 WP. The growing season of 2007 was dry with high temperatures. Disease did not spread out very much during June and July due to low disease pressure. It wasn't epidemic, it was seen only in local areas with a low severity. The harvest was made on 24.08.2007. Tuber blight wasn't the problem.

#### **DISCUSSION AND CONCLUSIONS**

In 2008, very early attacks occurring in April were only reported in England and attacks in the second half of May were reported in France, Belgium, the Netherlands, Germany, Switzerland and The Czech Republic. In Northern Europe the weather in May and June was unfavourable or moderately favourable for disease development and the first attacks were found at the beginning of July (Figure 5 and Figure 7). There seems to be a pattern regarding early attacks in 2008 with outbreaks being one month earlier (early June) in Central and Western Europe compared with Northern and Eastern Europe (Figure 5). A similar pattern was observed in 2007, but was less clear than in 2008.

In 2008 the time between the "Date of first observation of late blight in covered or very early planted potatoes", and the "Date when first infections were reported in more than five conventional, normally planted potato fields" was a month or more for some countries (e.g. England and Sweden) but in most countries was 5-14 days. Due to hot and dry weather in May the first attacks of late blight appeared later than normal in many countries. Only one report of late blight was recorded in the early potato district, Bjäre, in the South of Sweden (Fig. 5). In most European countries, weather conditions in August were very favourable for blight. Late blight epidemics started in many conventional fields and spray schedules were difficult to accomplish due to the rain. The blight risks in September were moderate to high (Figure 7)

The data on late blight appearance (Figs 5 and 6) and the data on weather based risk of late blight development (Figs 7 and 8) is sorted in the same order – from North to South of Europe. There is a relatively good correlation between indications of blight weather and date of first disease appearance

(Figs 5-8). It is a problem that blight weather is given as one single indicator value for the whole country. This might be realistic for small countries like Denmark and Estonia, but even relatively small countries like the Netherlands experienced a very big difference in blight weather conditions across the country as described in the 2008 summary report.

Experiences from the collection and analysis of information from the country reports show that there is a need for a harmonised European approach for calculating blight weather to replace the current method used in Euroblight. For some countries data are "best guess" and for other countries the one character given is based on calculations with a DSS. Another problem is that one single value is not enough to describe the regional blight weather conditions. A new weather based index must be calculated with weather data from 3-5 weather stations in important potato growing areas in each country. Another improvement would be to develop a European warning- and monitoring system for early attacks of late blight, that single countries can upload raw data to the system, or, alternatively export specific monitoring data from an existing national system to the monitoring system displaying all data on a map of Europe in a harmonised way.

The fungicide use in ware potatoes in 2008 ranged from 3-5 applications in Eastern Europe, Russia and Belarus to an average of 12-19 applications in UK, Belgium, the Netherlands and France. The fungicide use was slightly higher in many countries in 2007 compared to 2008 (BE, NL, FR, SE, NO, DK), but slightly lower for other countries (EN, AU, CZ, BY, LT and EE). It is not clear how combinations of blight weather conditions, the importance of different inoculum sources i.e. dumps, volunteers and oospores, the use of resistant cultivars, varying pathogen populations, the use of DSSs and the density of potato fields influence differences in fungicide use as shown in tables 1 and 2.

Comparing 2008 and 2007, the information shows that first infections were later in 2008 than in 2007 in the Northern part of Europe. This coincides with relatively unfavourable weather for late blight in May and June in 2008. There were only few reports of early infections from oospores in 2008. The late blight season in 2007 was extreme with many rainy days during most of the season and the fungicide use was higher in 2007 than in 2008. Problems with tuber blight were low to medium in all Europe in 2007 due to a dry September (Fig. 8). In 2008 the extremely bad weather in August resulted in infections in many fields. On the other hand, weather conditions (dry and warm) in September were relatively unfavourable for infections during harvest in many regions. Problems with tuber blight in 2008 have been reported to be low in UK, FR, BE, NL and medium to high in Northern and Eastern Europe. Data uploaded to the Eucablight database on pathogen characteristics show that the 13\_A2 genotype of *P. infestans* spread during 2006-08 and now compromises about 70% of the UK population. The same genotype has been found in the Netherlands, Germany and France, but not in the Nordic countries. The survey and use of DSS show that most countries build their own DSSs based on known model components. The most widespread single DSS seems to be Plant-Plus from Dacom in the Netherlands.

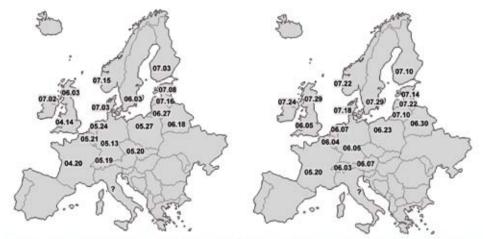


Figure 1. Date of first observation of late blight in covered Figure 2. Date when first infections were reported in more or very early planted potatoes, 2008 than five conventional, normally planted potato fields, 2008

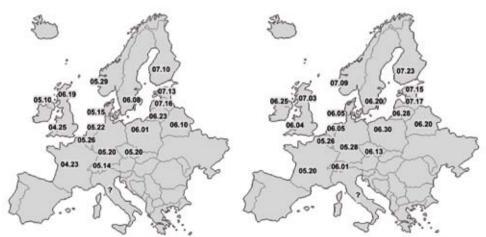


Figure 3. Date of first observation of late blight in covered Figure 4. Date when first infections were reported in more or very early planted potatoes, 2007 than five conventional, normally planted potato fields, 2007

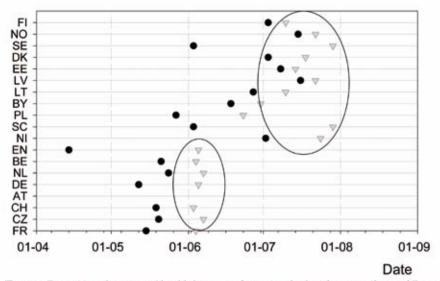


Figure 5. Date of first observation of late blight in covered or very early planted potatoes (dots) and Date when first infections were reported in more than five conventional, normally planted potato fields (triangles), 2008

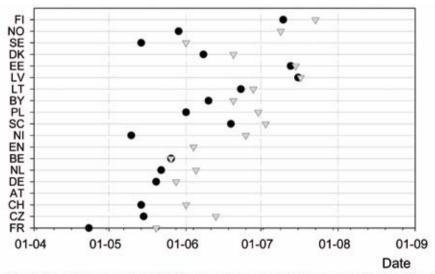


Figure 6. Date of first observation of late blight in covered or very early planted potatoes (dots) and Date when first infections were reported in more than five conventional, normally planted potato fields (triangles), 2007

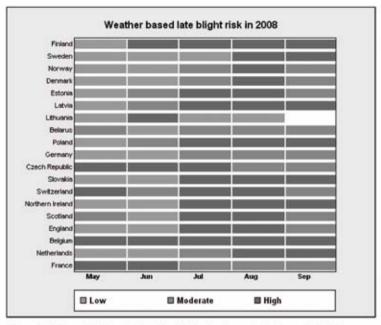


Figure 7. The weather-based risk of late blight development in Europe in 2008.

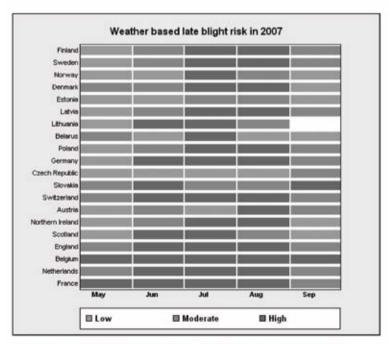


Figure 8. The weather-based risk of late blight development in Europe in 2007.

**Table 1.** Number of fungicide applications used in ware potatoes and all types of potatoes Respectively in 2008

COUNTRY	NUMBER OF FUNGICIDE APPLICATIONS USED IN WARE POTATOES			NUMBER OF FUNGICIDE APPLICATIONS USED IN ALL TYPES OF POTATOES		
	min	max	mean	min	max	mean
Finland	3	9	5	4	9	6
Sweden	4	15	7.5			
Norway	2	8	5.5	2	8	5.5
Denmark	4	9	7	4	14	8.5
Estonia	3	9	5	3	9	5
Latvia	3	5	4	1	5	3
Lithuania	4	10	5	1	4	2.5
Belarus	2	7	4		7	4
Poland						
Germany	4	15	7	4	15	7
Czech Republic	5	12	7	2	8	6
Slovakia	4	8	5			
Russian Federation				1	10	3
Switzerland			8			
Northern Ireland	5	14	10	5	14	10
Scotland						
England	10	12	11	10	12	11
Belgium	17	22	19	10	22	15
Netherlands	8	18	14	6	18	13
France	14	18	15	8	20	15

**Table 2.** Number of fungicide applications used in ware potatoes and all types of potatoes Respectively in 2007

respectively in 2007						
	NUMBER OF FUNGICIDE			NUMBER OF FUNGICIDE		
	APPLICATIONS USED IN WARE		APPLICATIONS USED IN ALL			
COUNTRY	POTATOES			TYPES OF POTATOES		
	min	max	mean	min	max	mean
Finland	3	7	5	2	7	4
Sweden	5	15	8	5	10	6
Norway	2	8	6	2	8	6
Denmark	5	10	7.5	5	16	9
Estonia		6	2.5		6	2.5
Latvia	2	5	3	2	4	3
Lithuania	4	10	5	1	4	2.5
Belarus	2	6	3		6	3
Poland	3	17	5	1	17	3
Germany	4	18		4	18	
Czech Republic	2	7	5		5	4
Slovakia	4	6	5	4	6	5
Russian Federation		9	3	1	11	3
Switzerland						
Austria	3	10	6	3	10	6
Northern Ireland	6	15	10	5	13	9
Ireland						
Scotland						
England	9	12	10	9	12	10
Belgium	18	25	21	10	25	18
Netherlands	14	21	17	12	21	15
France	13	20	17	13	22	18

Table 3. Name of decision support systems used in different EU countries, 2008

	•
COUNTRY	DSS USED IN THE COUNTRY
Belgium	Warning based on Guntz-Divoux
Czech Republic	Negative prognosis
Denmark	PlanteInfo
UK	Blight-Watch and Plant Plus
Estonia	Jögeva PBI web system
France	Mileos (MilPV + MildiLIS)
Germany	PhytophthoraModel Weihenstephan, ISIP
Latvia	Plant Plus
Netherlands	ProPhy and Plant Plus
N. Ireland	DARD Blight-Net
Norway	VIPS
Russia	Plant Plus, VNIIF-3 and SimCast+VNIIF-3
Sweden	(Plant Plus)
Switzerland	PhytoPRE+2000

#### Eleventh EuroBlight workshop Hamar (Norway), 2008

# Results of the Potato Case Study in the EU-network of excellence, ENDURE

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#### **KEYWORDS**

Phytophthora infestans, Best Practices, IPM

#### INTRODUCTION

Late blight (caused by the pseudo-fungus *Phytophthora infestans*) is the most serious potato disease and, when first introduced into Europe in the 1840s, was responsible for the Irish Potato Famine. A conservative minimum estimate of combined losses and costs of control (mainly fungicides) of potato late blight worldwide is 4 billion (4 x 10°) Euros per annum; half of this figure for Europe alone. More fungicide is applied to control blight than is used in any other crop. Integrated management of potato late blight therefore requires a combination of management techniques in order to keep disease levels low and at the same time maintain the quality of the environment. In the Potato Case Study team an inventory was made for the Best Practices for control of late blight in a number of European countries. Four leaflets dealing with these Best practices were published which are available on <a href="www.endure-network.eu">www.endure-network.eu</a> (Figures 1, 2, 3 & 4).

#### BEST PRACTICES

Control measures can be divided in strategic measures and tactical measures. Strategic measures aim at reducing disease pressure up front such as rotation, cultivar choice and measures to prevent primary inoculum sources. These strategic measures are mainly influenced by economical and social factors. Tactical measures include fungicide choice, number of sprays and use of DSS. The advisor/farmer decides on the tactical measures directly related to control of late blight. By restricting the fungicide choice, residues or environmental input consumers/buyers/government can influence the strategic and tactical decisions.

Best Practices are effective measures still under development. For widespread implementation in practice a number of barriers (economic, costs, risk) have to be solved. These measures are being tested by applied research institutes in practice for their effectiveness and further developed.

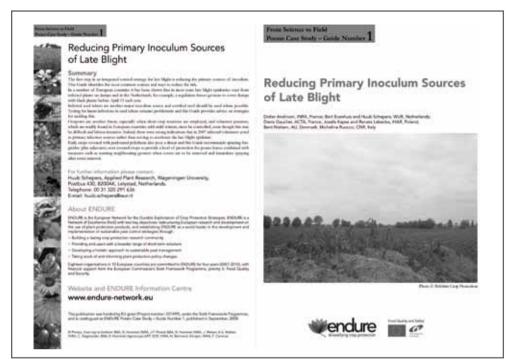


Figure 1. The ENDURE leaflet "Reducing primary inoculum sources of late blight"

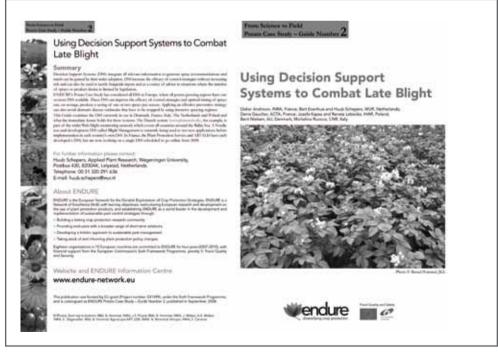


Figure 2. The ENDURE leaflet "Using cultivar resistance to reduce inputs against late blight".

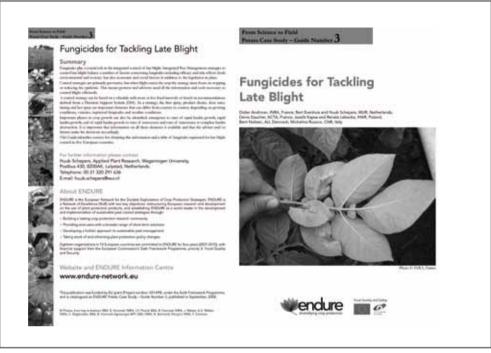


Figure 3. The ENDURE leaflet "Fungicides for tackling late blight"

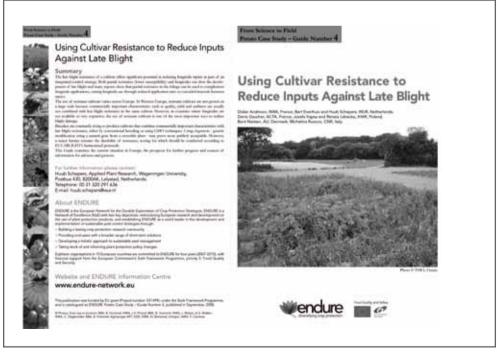


Figure 4. The ENDURE leaflet: Using Decision Support Systems to combat late blight"

#### PRIMARY INOCULUM SOURES

*Dumps*: In The Netherlands a regulation exists that forces growers to cover dumps with black plastic before 15 April. In the UK the influence of dumps on the late blight epidemic is an important part of the Fight against Blight campaign. It is again difficult to quantify the effect of eliminating dumps. Maybe the time between the first appearance of blight in a region and the appearance in production fields could be used as an indication of the influence of the elimination of dumps.

Alternative hosts: In Sweden hairy nightshade (Solanum physalifolium) was observed to be seriously infected with late blight. It is unknown whether this weed is occurring in other countries. Also little information is available about the compatibility of the late blight isolates on this weed and potato.

Oospores: Usually the ratio of the A1 and A2 mating types in the same field is used as an indication for the (possible) occurrence of oospores. Monitoring for both mating types can help to assess the risk for the occurrence of oospores. There are still a lot of questions regarding oospores. What triggers their germination? Probably water, but maybe also the temperature change during winter (freezing/thawing) plays a role. Nothing is known about the effect of organic matter on survival and germination and whether certain crops can be used as a trap plant. It is stated that the best way to reduce the influence of oospores is to prevent the development of late blight in the previous crop and to control volunteers (in which oospores can be formed abundantly). Furthermore crop rotation with longer rotation intervals can be used as an additional measure.

Seed potatoes: It is recommended to use certified seed but this is not a guarantee that the seed will be completely free from blight since blight can be latently present in the seed tubers. In Poland for example the availability of certified seed is limited. It is technically possible (PCR) to detect latent infections in seed tubers. The problem is however that when 1:10,000 tubers is infected it can already create a primary inoculum source. With such a low frequency of occurrence it will be almost impossible to find it in a sample of a reasonable size. It might also be possible that infected tubers not only infect a plant that grows from this tuber but that it can also infect daughter tubers without infecting aboveground plant parts. In order to assess the risk for the occurrence of latently infected tubers it is recommended to survey the history of the growing season in which the seed potatoes were grown. The incidence of late blight in the crop and the choice of the fungicides and their timing will provide information that can be used to assess the risk for the occurrence of latently infected tubers.

Uncontrolled late blight: Volunteers: the number of volunteers is mainly influenced by weather conditions in winter. Milder winters result in more volunteers. Usually volunteers are not a primary infection source. But in 2007 there was a strong indication that infected volunteers also acted as primary infection sources. Depending on the crop in which the volunteers occur, control is usually difficult and labour intensive. A real-time vision detection of volunteers in sugar beet fields is developed in The Netherlands. Early (covered) crops: control must be emphasized either by spraying over the crop cover or directly after removal of crop cover. The cover should be removed on days with weather conditions that are not critical for spreading of viable spores.

Unsprayed/organic crops/allotment gardens: In The Netherlands there is a regulation that forces growers to treat (or destroy) a crop with an excessive infection of late blight. Usually these infected fields do not occur early in the season and are therefore not considered to be important primary infection sources.

#### **CULTIVAR RESISTANCE**

Usually resistance is not the most important characteristic for the choice of a cultivar. In Poland the resistance to viruses is being utilized, while resistance to late blight is not sufficient in widely grown potato cultivars. When there is a strong demand by buyers, super markets or governments for less fungicide input or no input at all (organic), the late blight resistance of a cultivar provides an important tool to achieve this.

The stability of resistance is very important. In each of the countries present the cultivars are tested for resistance to late blight. It is important to know how frequently these tests are updated. It is recommended that the harmonized protocols developed in EUCABLIGHT are used to test the resistance and stability of resistance. Resistance genes used in cultivars are not known. It is also difficult to find information on the use and distribution of resistant cultivars (www.euroblight.net).

In France the resistance is monitored during the season so that information can be applied in IPM control strategies during the same season. In most late blight DSS, cultivar resistance is taken into account. To make a better use of resistance it is recommended that the influence of resistance on the epidemic is described in a better way, so that the IPM control can be adapted accordingly.

Regarding early blight, there are no standardized data available on resistance. Cultivars are **not** rated for resistance to early blight in National lists.

It is technically possible to extract resistance genes from other species (transgene) or from sexually compatible Solanum species (cisgene), combine them into cassettes and insert them into potato cultivars. The acceptance by consumers and governments will be an important barrier for use in practice, but utilization of cisgenesis (a genetic modification of a recipient plant with a natural gene from a crossable –sexually compatible- plant) to introduce high resistance to "market selected" potato cultivars seems to be the most perspective way to reach the goal, as this method may receive public acceptance much easier than transgenesis.

#### **FUNGICIDES**

The threshold for late blight infections is zero. Growers do not and can not tolerate blight and the use of fungicides plays an important role in controlling blight. The efficacy and side-effects but also economic, social factors and legislation will influence the IPM strategies to control late blight.

The control strategy is primarily preventative but when blight enters the crop the strategy will have to focus on trying to stop/reduce the epidemic. It is important that growers and advisors have all the information/tools necessary to control blight efficiently. A control strategy can be based on a schedule with more or less fixed intervals or can be based on the recommendations derived from a DSS. To optimize a late blight control strategy the timing of the first spray, the product choice, the dose rates, the timing and the last sprays are important elements. These elements can differ from country to country depending on growing conditions, varieties, registered fungicides and weather conditions. It is important that information on these elements/bricks is available and that the advisor/farmer can make his own decisions accordingly, depending on his own perspectives. It is important to clarify the relative effect of each of these elements/bricks on late blight control.

The EuroBlight network could play a role in making these data readily available. Euroblight is also a platform for weather based DSS. This platform could be used to calculate blight risk for each country using country specific DSS, thus providing information on number of sprays and justification that can be used to compare and discuss with farmers/advisors.

Product choice & timing: The first priority of farmers/advisors is efficacy. The European network

EuroBlight publishes a fungicide table with all important characteristics of fungicides (<a href="www.euroblight.net">www.euroblight.net</a>). Sometimes dose rates can be reduced in relation to weather conditions and/or cultivar resistance. But in some cases as in Poland it is not allowed to use lower dose rates than those mentioned on the label. In The Netherlands strategies in which efficacy, costs and environmental input are taken into account are tested under practical conditions to convince growers of their robustness. Taking into account the new EC pesticides legislation and consumer attention to environment, the combined use of traditional fungicides and bio-fungicides might in future be an interesting option.

#### DECISION SUPPORT SYSTEMS

These systems integrate all relevant information to generate spray recommendations. There is room for improvement but since the DSS are already technically on a high level we estimate the effect will be small. More can be gained by increasing the use of (parts of) the DSS by farmers/advisors. It is important to convince farmers/advisors that information from DSS will increase the efficacy of their control strategy without increasing the risk. In other words: DSS should primarily not aim at a high reduction in the number of sprays but it should aim at an effective control of late blight (including a large enough safety margin). On average the use of DSS can save 1-2 sprays per season. DSS can also be used to justify the input of fungicides and to advice in situations when the number of sprays (or product choice) is limited by legislation.

# **ACKNOWLEDGEMENTS**

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# Late blight in developing countries and the role of the Global Initiative on Late Blight GILB

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#### **SUMMARY**

This paper describes potato late blight (PLB) in developing countries where the disease is a critical problem related to poverty. This is particularly the case in many highland tropical areas where potato is produced year round. PLB causes severe losses and forces dependence on fungicides, which have negative health and environmental consequences. The primary solutions to the problem appear to host resistance, farmer capacity building and use of the most effective and environmentally friendly control products available. Many research and development projects have been or still are being implemented that address PLB, but these are not coordinated and do not necessarily use standard protocols or measures of progress. This undoubtedly leads to losses in synergy and provides little opportunity for assessment of global impact. A potential role for a the Global Initiative on Late Blight (GILB) could be: i) targeting and baseline identification; ii) monitoring progress and assessing impact; iii) assessing risk of pathogen and climate change; and iv) building a community of practice through better communication among stakeholders in different parts of the world.

#### **KEYWORDS**

Phytophthora infestans, global databases, Phytophthora.exe, networking, Euroblight.

# UNSUSTAINABLE TRENDS IN POTATO PRODUCTION

In the context of soaring staple food prices which impact developing countries hardest, potato is an increasingly important alternative source of food and nutrition for many areas where the crop can be grown<sup>1</sup>. Potato is a very important crop for alleviating poverty in many parts of the developing world, and particularly in many highland tropical ecosystems where the crops is grown year round. In many of these areas, agriculture is the dominant livelihood and thus a major pathway to economic development. Potato plays an important role in this development because of its importance in the cooler highlands, where poverty is common. Growth of potato in sub-Saharan Africa, for example, has increased several-fold in the past decade (Low *et al.*, 2007).

Concomitant to the growth in potato production, information is emerging that indicates that intensive potato production is not sustainable as it is currently being practiced (Sherwood, 2009). Many factors may affect the sustainability of potato production, such as natural resource degradation, poor quality of planting material<sup>2</sup>, poorly developed value chains in emerging markets, and high intensity of pests and diseases, with PLB arguably being the most severe of these. This discussion deals with PLB.

PLB, caused by the oomycete (fungus-like) pathogen *Phytophthora infestans*, is the most devastating biotic constraint to potato production on a global scale. PLB is the world's most important food crop

PPO-Special Report no. 13 (2009), 37 - 44

Why Potato? The International Year of the Potato Webpage http://www.potato2008.org/en/aboutiyp/index.html.

<sup>&</sup>lt;sup>2</sup> Bacterial wilt here is considered on of the problems affecting the quality of planting material.

disease, with global annual losses estimated to be between US\$ 3 and 5 billion (Judelson & Blanco, 2005, Haldar *et al.*, 2006, Haverkort *et al.*, 2008). James (1990), reported that of the world's major food crops, potatoes were subject to the highest percentage loss to diseases (near 20 %) and this is primarily due to late blight. This is set to increase as the spread of new, more aggressive strains of the pathogen is causing increasing damage worldwide (Fry *et al.*, 2009). Furthermore, the consensus of most climate change models is that the highland tropics will get wetter and warmer and these conditions will make it even more difficult for farmers to manage the disease (Forbes & Simon, 2007).

Throughout the developing world, small-scale farming households engaged in many types of agriculture are using *highly hazardous and mutagenic (HHM) pesticides* (Anonymous, 2004), with adverse health and livelihood consequences. Potato is a crop on which many HHM products are used. According to work by CIP and collaborators in northern Ecuador (Yanggen *et al.*, 2003) and elsewhere in the Andes (Orozco *et al.*, 2007), farm children and applicators particularly suffered high rates of poisoning. There is less information about pesticide dependency in potato production in Asia and Africa, but what little is known would indicate that the situation is similar to that of the Andes (Hijmans *et al.*, 2000; Schumann, 2005). Pesticide use on potato in Indonesia is probably higher than in the Andean region (F. Ezeta, personal communication).

There is no indication that pesticide dependency trends will improve. A recent study in Peru indicated that importations of HHM pesticides have increased between 2000 and 2003 (Arica *et al.*, 2006).

# Making potato production sustainable

More sustainable approaches to intensive potato production for developing countries have been developed by collaborating partners, involving the use of new technologies and farmer capacity building. The foremost strategy for managing the PLB economically without negative externalities is via the use of resistant varieties of potato. Host resistance may easily reduce fungicide needs to 1/3 of what is commonly used (Kromann *et al.*, 2009). At this time, highly resistant experimental cultivars exist that could fit into many production systems in developing countries.

This then leads to the question of why susceptible cultivars are still used? There are many socioeconomic factors limiting the adoption and widespread use of a new potato cultivar, particularly market forces (Walker *et al.*, 2003), and the lack of functioning seed systems in developing countries that would produce the planting material needed for diffusion. Since potato has a low multiplication rate, diffusion of new cultivars via informal seed flows takes a long time. Unfortunately, another reason susceptible cultivars are still grown is that many cultivars have been released that had singlegene resistance and pathogen populations quickly adapted to these (Forbes & Landeo, 2006).

# Diffusion and adoption of resistant materials will involve:

Making seed of new candidate cultivars available for distribution to and evaluation by farmers and local late-blight researchers.

Collecting and processing accurate information on local adaption, market acceptance and resistance stability in different locations for improved cultivar selection.

Capacity building among local researchers and extension workers on the factors that often thwart diffusion efforts.

To date, resistance in the cultivars mentioned above is not sufficient for complete control of PLB and supplementary protection is needed. One promising alternative technology at this time appears to be the use of phosphonate (also referred to as phosphites), which are salts of phosphorous acid (H<sub>3</sub>PO<sub>3</sub>) that pose very low risk for human health and environment. The use of phosphonate as safe and effective control for oomycete pathogens, like the one causing potato PLB, has been known for decades (Coffey & Joseph, 1985; Mayton *et al.*, 2008), but patent constraints restricted development of commercial products, and as a consequence there is little agro-industry interest in this approach. The patent constraints no longer exist and many generic products are now available, and some at very low cost<sup>3</sup>. In 2008, CIP and partners had promising results with phosphonate in both Peru and Ecuador; results were presented here at the meeting in Hamar and in another international meeting (Taipe *et al.*, 2008, Forbes *et al.*, 2008). However, the effects of different phosphonate formulations are cultivar specific and adaptive research is needed for site- and cultivar- specific use of products, particularly for determination of dosages and application frequencies.

Weather, pathogen (or pest) aggressiveness and host plant resistance form a complex interaction of factors that affect the final degree of disease or pest severity. This complexity can be grasped at the research level via disease and pest simulation (Andrade-Piedra *et al.*, 2005; Sporleder *et al.*, 2004). Disease and pest simulation is a process by which the infection (or infestation) process can be recreated with critical information about weather, the plant and the pathogen or pest. CIP and partners have been developing PLB simulation capacity for the highland tropics for more than a decade (Andrade-Piedra *et al.*, 2005). The tool has been used for comparative epidemiology in Scandinavia (Hansen *et al.*, 2006) proving its global applicability, and in sub Saharan Africa (unpublished data). Simulation provides a first step analysis into the ways in which a particular cultivar might behave with particular HHM alternative tactics (e.g., phosphonate).

Taking the complex interaction of weather, host resistance and pathogen/pest aggressiveness and rendering it into something farmers can manage is not easy. Toward this end, CIP and partners engaged in 2005 and 2006 in a process of developing competency-based farmer training modules for use in farmer field schools (FFS) or other intensive farmer training programs (Cáceres *et al.*, 2008). These modules help farmers build their competencies to manage LB by increasing their understanding of the factors that affect disease development. This approach to PLB training was based on knowledge management theory, which provides a good framework for development of training materials in general (Zapata Sánchez, 2005).

#### A Role for GILB

In response to this problem, the International Potato Center (CIP) and partners have joined their efforts by creating the Global Initiative on Late Blight<sup>4</sup> (GILB). To date, three international conferences have been held, the last in Beijing in April, 2008. Through these meetings and the Web page, GILB has been a useful tool to help researchers increase PLB management knowledge in developing countries.

In response to problems described above a number of country- or region-specific projects have been or are being implemented that attempt to alleviate negative impacts of blight, although in most cases this is only one component of the project. Furthermore, each project often focuses only on one or more themes mentioned above (host resistance, IDM, capacity building), and also affect one or more regions. For example, one project CIP convenes now that is funded by the OPEC Fund for International Development deals with finding effective PLB control methods and involves Latin America and Asia. Another project CIP convenes has an objective on identifying cultivars resistant to PLB, but it is only focused on S. America.

<sup>&</sup>lt;sup>3</sup> Personal communication, Jose Ochoa, plant pathologist, Quito, Ecuador.

<sup>&</sup>lt;sup>4</sup> GILB began in 1996 and further information is available at the GILB Webpage: http://gilb.cip.cgiar.org/.

As with many development projects, those with a component related to PLB are not necessarily linked or even coordinated, nor do they necessarily used standardized approaches. One can identify several crucial areas where this lack of coordination could limit efficacy at a global level; and certainly make it difficult if not impossible to monitor progress as it is often very difficult to compare results. A more globalized approach to the PLB problem would seem to offer a number of advantages. In a sense, a global effort would make sense when it adds value to the more localized efforts; and this appears to be an ideal role for GILB. However implementing a global effort of this nature would have several objectives reflecting technology needs and knowledge gaps.

Many of the technology and knowledge gaps that require attention for implementation of a global PLB initiative were discussed in the last GILB conference in Beijing and can be summarized in four areas: i) targeting and baseline identification; ii) monitoring progress and assessing impact; iii) assessing risk of pathogen and climate change; and iv) building a community of practice through better communication among stakeholders in different parts of the world. Some of the technology and capacity needed for an effective global PLB program already exist in Europe, within the context of the Euroblight initiative. Other needs would be met by proposed collaborative activities, the results of which could also provide new tools for the Euroblight network.

# Targeting, baselines, objectives and indicators

Targeting and benchmarking will consist of identifying the areas where poverty, potato production and PLB overlap. A preliminary CIP study on poverty and PLB severity identified the following general target regions: S. China and the Himalayas, the northern Andes, and mountainous regions of sub-Saharan Africa, including Ethiopia, Kenya and the Lake Kivu area (Forbes & Lizarraga, 2007). This study should be repeated taking advantage of recent advances in science, including new knowledge on pathogen diversity, and with improved modeling capacity and better weather databases.

# Database development and protocol standardization

To improve the accuracy of the preliminary study, baselines of host resistance and farmer capacity will be described by surveys, analytical approaches and establishment of robust *pathogen and host data bases*, such as those developed by Euroblight (www.euroblight.net). These databases house information about the cultivars currently being used and the pathogen strains in different regions. This information is needed to establish baselines of actual disease severity, measure progress toward adoption of resistant potato varieties and develop accurate assessments of the risk of increased PLB severity due to climate change. Currently, little work has been done on the pathogen in developing countries and there has been little coordination of pathogen characterization methods. Efforts would be made to standardize pathogen characterization using the Euroblight software "Phytophthora. exe" and recently developed quantitative scales for resistance (Hansen *et al.*, 2005).

# Assessing impact and risk

Progress in ongoing and new R&D projects that address PLB will be monitored against baselines using modern IT technology to link project implementers, and by using GIS-based modeling to project expected outcomes and impacts. GIS modeling using information on host and pathogen (Hijmans *et al.*, 2000) will be used to estimate the impacts of resistant cultivars and farmer capacity building. Impact will be assessed using these analytical procedures and cross-referenced with

selected surveys. PLB poses an evolving risk to farmers because of a constantly changing pathogen population and changing climate. Existing technology for estimating this risk (Forbes & Simon, 2007, Hijmans *et al.*, 2000), should be improved to take into account not only climate but the other corners of the disease tetrahedron: host (level of resistance), pathogen (strain aggressiveness) and human (farmer capacity to manage PLB). The *pathogen and host data base* will provide the host and pathogen information. A methodology for classifying the PLB management capacity of the farmer is also contemplated as a joint research activity. Risk analyses will be made using data on host, pathogen, climate and estimated farmer management capacity.

Improving the community of practice: building capacity of researchers and extension agents and farmers

Implementation of databases described above will require capacity building of PLB workers in developing countries. This, together with an improved information access via the GILB Web page will support an invigorated community of practice. Farmer capacity building approaches have recently been developed in the Andes (Cáceres *et al.*, 2008) that appear to be effective in helping farmers learn to manage PLB. These can be adapted and promoted at local levels.

# LONG TERM IMPACT

This project proposes to monitor and enhance the scaling out of technologies that reduce the negative effects of PLB. Enhancement will come through improved knowledge sharing and better standardization of approaches. The positive impacts on resource poor farmers and the urban poor who would have better access to nutritious food, are based on long-term changes: adoption of resistant cultivars and improved farmer knowledge, and should therefore be lasting. Furthermore, research has demonstrated that capacity a farmer gains to manage one constraint is often applied to other constraints (Berg & Jiggins, 2007), so one would expect spinoffs for other aspects of potato production or even greater productivity in other crops.

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# Recent genotypic changes in *Phytophthora infestans* in Japan (1997-2007)

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#### **SUMMARY**

From 1997 to 2003, most isolates of *Phytophthora infestans* in Japan were of the genotypes JP-1, JP-2, JP-3, or JP-4. Subsequently, the prevalence of JP-1 and JP-3 decreased in northern Japan until 2007, while JP-1 persisted in southern Japan. Genotypes similar to JP-2 have been reported in Korea, China, Russia, and the United Kingdom. It has been proposed that JP-2 migrated to Japan, while JP-3 and JP-4 originated within Japan via sexual reproduction. We found most JP-2 isolates to be metalaxyl resistant, whereas >90% of JP-4 isolates were susceptible. Thus, Japan harbors unique populations of *P. infestans*, which are changing continuously and dynamically. It is therefore necessary to monitor the situation in neighboring countries in order to understand the trend among Japanese populations of *P. infestans*.

# **KEYWORDS**

potato late blight, population, genotypes, allozymes, RG57 fingerprints, mitochondrial DNA haplotypes

# INTRODUCTION

Japan began producing potatoes extensively about 150 years ago. Currently, about 2.8 million tons of potatoes are produced in Japan per year. About 80% of the potato crop is produced on the northernmost island of Hokkaido. The first report of late blight in Hokkaido was made by Ideta (1901). Up to 1996, *Phytophthora infestans* genotype US-1 (mating type A1) and JP-1 (mating type A2) were the dominant cause of potato late blight in Japan (Mosa *et al.*, 1989; Koh *et al.*, 1994). Since 1997, however, new A1 mating-type genotypes have been recognized (Kato and Naito 1997), including JP-2, JP-3, and JP-4 (Gotoh *et al.*, 2005), as have other derived genotypes (Gotoh *et al.*, 2007), based on information related to their allozymes, RG57 DNA fingerprints, and mitochondrial DNA (mtDNA) haplotypes. Here, recent changes in the Japanese *P. infestans* population are described in relation to those in other countries.

#### **MATERIALS AND METHODS**

# Genotypes of Japanese isolates

Phytophthora infestans isolates were collected from potatoes grown on Hokkaido, Honshu, and Kyushu between 1997 and 2007. The mating types of the isolates were determined by pairing each isolate with known A1 (DN111, derived from Hokkaido in 1991) and A2 (TB201, derived from Hokkaido in 1992) isolates of P. infestans on 10% clarified V8 juice agar medium (Nishimura et al., 1999). All isolates were genotyped based on their glucose phosphate isomerase (Gpi) and peptidase (Pep) alleles (Goodwin et al., 1995b), RFLP genomic DNA fingerprinting (Goodwin et al., 1992), and mtDNA haplotyping (Griffith and Shaw 1998). To confirm the genetic relationships between isolates DN107 (JP-1), 98F1 (JP-2), and AM-CT1 (JP-3), the isolates were subjected to amplified fragment length polymorphism (AFLP) analysis according to the method described by Vos et al.,(1995). Referring to Abu-El Samen et al.,(2003), four combinations of EcoRI (E-AT) and MseI (M-CAA, M-CAC, M-CAG, and M-CAT) primers were used. The reactions were performed using the AFLP Analysis System I (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions.

# Metalaxyl sensitivity

Metalaxyl sensitivity was assessed *in vitro* by calculating the EC<sub>50</sub> value for each isolate according to the level of growth inhibition on rye-A agar medium amended with metalaxyl at 0, 1, 10, or 100 mg L<sup>-1</sup> (for isolates derived between 2001 and 2004) or the sporulating area on a potato leaf disc treated with metalaxyl at 0, 1, or 10 mg L<sup>-1</sup> (for isolates derived between 2005 and 2007). Based on our results, the isolates were assigned to one of three groups: sensitive (EC<sub>50</sub> < 0.1 mg L<sup>-1</sup>), intermediate  $(0.1 < EC_{50} < 10 \text{ mg L}^{-1})$ , or resistant (EC<sub>50</sub> > 10 mg L<sup>-1</sup>).

**Table 1.** Multilocus genotypes of Japanese *Phytophthora infestans* based on mating type, two allozymes, RG57 fingerprinting, and mtDNA haplotyping.

RG57 type	Mating type	Gpi <sup>a</sup>	Pepª	RFLP <sup>b</sup>	mtDNA haplotype
US-1	A1	86/100	92/100	101 010 101 100 110 100 011 001 101 00	Ib
JP-1	A2	100/100	96/96	100 011 000 000 110 110 001 001 100 01	IIa
JP-2	A1	100/100	100/100	100 010 001 100 110 100 011 001 101 00	IIa
JP-3	A1	100/100	96/100	100 011 001 100 110 100 011 001 101 00	IIa
JP-4	A1	100/100	96/100	101 010 101 100 110 100 111 101 101 00	IIa

<sup>&</sup>lt;sup>2</sup> Gpi, glucose-6-phosphate isomerase, Pep, peptidase

<sup>&</sup>lt;sup>b</sup> Fingerprints were generated using the probe RG57. The presence or absence of bands is indicated by a 1 or 0, respectively. Bands 1-25, 8a, 14a, 23a, and 24a are listed from left to right. Bands 8a and 23a were generated in this study.

#### RESULTS AND DISCUSSION

# Genotypes of Japanese isolates

Table 1 presents the genotypes of Japanese isolates of *P. infestans*. Based on data derived from four tests (mating type, Gpi and Pep alleles, RFLP genomic DNA fingerprinting, and mtDNA haplotyping), the isolates were divided into nine genotypes (data not shown), which were further classified into five groups: US-1, JP-1, JP-2, JP-3, and JP-4. Among the genotypes, only JP-1 was of mating type A2; the others were of mating type A1. We were able to confirm a number of polymorphisms in the Pep allozyme and RG57 fingerprint.

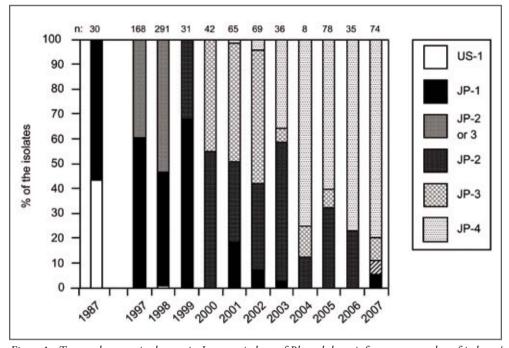


Figure 1. Temporal genotypic changes in Japanese isolates of Phytophthora infestans. n: number of isolates / Column 1987 indicates the previous Japanese situation (Mosa et al., 1989). / Those isolates from 1997 and 1998 that were not identified by RG57 fingerprinting are referred to as "JP-2 or -3."

US-1 was a dominant genotype in the 1980s (Mosa *et al.*, 1989); however, it was not found in Japan after 1998 (Fig. 1). The prevalence of another dominant genotype, JP-1, also declined annually; this genotype is currently found only in small areas in the field. The next generation of genotypes, JP-2 and -3, dominated in the early 2000s; however, these also occur only in limited areas in the field today. The dominant genotype since the mid-2000s has been JP-4. We found that Japanese *P. infestans* has changed dynamically every year.

Across the Japanese Islands, the distribution of each genotype since the year 2000 has been quite distinct from that during the 1980s (data not shown). In particular, of the dominant genotypes in the 1980s, US-1 disappeared while JP-1 was limited to southern Japan. On the other hand, new dominant genotypes have emerged, such as JP-4, which expanded to southern Japan after being restricted to Hokkaido. We assumed that seed potato migration had its production center in Hokkaido, and that this affected the migration of new genotypes from Hokkaido to the southern part of Japan.

The relationship between the Pep allozyme from JP-4 and JP-1/JP-2 and between the Pep and

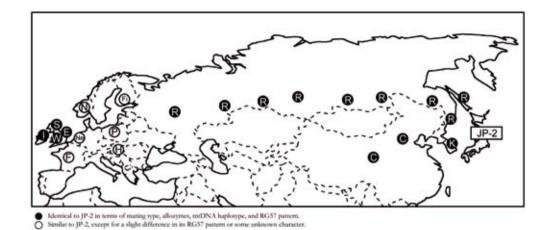


Figure 2. Distribution of IP-2 analogs across Eurasia.

P: Poland 1990 (Sujowski et al., 1994) / F: France 1995 (Lebreton et al., 1998) / N: Norway 1996, F: Finland (Brurberg et al., 1999) / Ne: The Netherlands 1996 (Zwankhuizen et al., 2000) / E: England 1996, W: Wales 1996 (Puvis et al., 2001) / S: Scotland 1996 (Cooke et al., 2003) / I: Northern Ireland 1996 (Carlisle et al., 2001) / R: Russia 1993 - 1998 (Elansky et al., 2001) / H: Hungary 1998 (Bakonyi et al., 2002) / K: Korea 2002 (Personal communication from Dr. K. Y. Ryu) / C: China 1996, JP-2: Japan 1996 (Akino et al., 2004)

RG57 fingerprints from JP-3 and JP-1/JP-2 (Table 1) bore a striking resemblance to the cases of BC-2 (Goodwin *et al.*, 1995a) and US-11 (Goodwin *et al.*, 1998), which are regarded as sexual progeny. Moreover, the AFLP patterns of JP-3 (data not shown) resembled the F<sub>1</sub> progeny of sexual reproduction (van der Lee *et al.*, 1997). JP-1 and JP-2 reportedly coexisted in Hokkaido from 1996 to 1999, whereas US-1 nearly completely disappeared during that period (Kato and Naito 2001). These results suggest that Japanese JP-3 is a product of sexual reproduction between JP-1 and JP-2. In the case of JP-4, the relationship with JP-1 and JP-2 is unclear based on their RG57 fingerprints; however, its *Pep* allozyme indicates the possibility of a connection.

We also compared other published genotypes to JP-2 (Fig. 2). A genotype identical to JP-2 was reported in Nothern Ireland (Carlisle *et al.*, 2001), England and Wales (Purvis *et al.*, 2001) and Scotland (Cooke *et al.*, 2003), which has been identified in other European countries (Cooke *et al.*, 2006). An additional identical genotype (SIB1) was isolated near Moscow, in the trans-Siberian region, and in Sakhalin, which is an island neighboring Hokkaido (Elansky *et al.*, 2001). SIB1 is the dominant genotype in far eastern Russia. The distribution of SIB1 is very important because it connects European and Asian populations of this genotype. At present, JP-2 analogs were probably more widely distributed across northern Eurasia than previously thought. Considering the extent of its distribution and dominance in Japan, Korea, China (Guo *et al.*, 2008), far eastern Russia, and parts of Europe, this genotype may have had an important influence on the population of *P. infestans* in each region.

Based on the data currently available, one may formulate a hypothesis concerning the relationships among the Japanese genotypes. The oldest genotype, US-1, was dominant in Japan until the late 1980s, when it was replaced by new populations of JP-1. Around 1996, a new genotype, JP-2, was introduced into Japan from outside the country. JP-1 and JP-2 are of mating types A1 and A2, respectively; thus, they generated progeny. These progeny, JP-3 and JP-4, subsequently expanded throughout the potato fields of eastern Hokkaido.

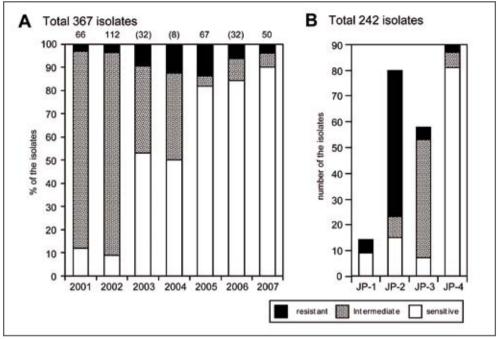


Figure 3. Metalaxyl sensitivity among Japanese isolates of Phytophthora infestans.

A: Annual change in metalaxyl sensitivity. The data for 2001-2004 were produced using the agar plate method, while the data for 2005-2006 were produced using the leaf disc method. B: Metalaxyl sensitivity of the isolates according to genotype (2001-2007). The total number of isolates between plates A and B was unequal because plate A contained unidentified isolates.

# Metalaxyl sensitivity

Figure 3 (A) shows the annual change in metalaxyl sensitivity among the Japanese isolates (as well as data from a number of unidentified isolates belonging to those genotypes). Between 2001 and 2002, most of the Japanese isolates were resistant or moderately resistant to metalaxyl, and the prevalence of these isolates steadily decreased each year. It is important to note the difference between the two methods used to test for metalaxyl sensitivity. The isolates collected between 2001 and 2004 were tested by the agar plate method, whereas the isolates collected between 2005 and 2007 were analyzed by means of the leaf disc method. In most cases, our preliminary data showed that an intermediate response by the agar plate method corresponded to a resistant response by the leaf disc method. Figure 3 (B) shows the level of metalaxyl sensitivity among the Japanese isolates according to genotype. Over half of the JP-1 isolates were susceptible to metalaxyl, whereas most of the JP-2 and -3 isolates were resistant or moderately resistant. In contrast, most of the JP-4 isolates were sensitive.

In recent years, the plant protection office of Hokkaido has called on farmers to voluntarily refrain from using phenylamide fungicides; thus, this increased sensitivity may be a result of the farmers' efforts. Consequently, it is possible that the sensitivity of the JP-4 isolates to metalaxyl is not only a genetic character but also an effect of fungicide use.

# **CONCLUSIONS**

The population of *P. infestans* in Japan is changing dynamically, and it has a close relationship with those in neighboring countries. In future, these populations should be continuously monitored in

order to identify alterations. The mechanism of change in the Japanese *P. infestans* population is unknown; however, we noted a number of differences in metalaxyl sensitivity and aggressiveness against potatoes and tomatoes between JP-1, JP-2, JP-3, and JP-4 (data not shown). The differences in competitive and survival ability between the genotypes under unfavorable conditions are currently under investigation.

In the control of potato late blight in Japan, several new developments have deepened the farmers' awareness of integrated control methods, such as the introduction of the Decision Support System. However, more research is needed to evaluate the effectiveness of these integrated systems, and to provide more complete information about pathogens and fungicides. Moreover, it is important to periodically monitor the population structure in Japan and to collect information about the population structure in neighboring countries.

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# Phytophthora infestans - the next generation: a field trial in Sweden

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#### **SUMMARY**

A field trial was carried out in the middle of Sweden in 2001. The field was planted with certified seed tubers *cv*. Bintje. The field was divided into four blocks. Six weeks later each block was inoculated with six isolates of *Phytophthora infestans*. The isolates were of five different SSR-multilocus genotypes. Four were of the A1 and two of the A2 mating type. Each block was covered with fleece. Oospores were found in blighted leaves collected three weeks after inoculation. At that date the first inoculated plants were totally blighted.

In the middle of June the following year soil samples were collected in three plots from each block. Isolation of viable *P. infestans* isolates from the soil samples was made by a detached leaves baiting technique. Isolates were captured from five of the twelve sampled plots and were characterized by mating type and with six SSR-markers. In the isolates captured from the soil the next season parental SSR genotypes were not present. Hybrid offspring isolates identified by SSR genotyping and mating type determination constituted the major part of the isolates captured. Two of the five genotypes used as inoculum could be excluded as parents.

This study gives direct evidence that *P infestans* can mate, produce oospores that survive Swedish winter, germinate and cause infection the next season.

# **KEYWORDS**

Phytophthora infestans, SSR analysis, oospores

# Phytophthora infestans population changes: implications

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#### **SUMMARY**

This paper describes the genotypic identification of *P. infestans* isolates collected in 2007 and 2008 and the characterisation of the dominant genotype (13\_A2) for a range of properties including aggressiveness on detached leaves, on plants in the field and on tubers. The resistance of various cultivars to isolates of 13\_A2 was investigated and compared with published resistance ratings.

#### **KEYWORDS**

Phytophthora infestans, population changes, aggressiveness, host resistance, late blight

#### INTRODUCTION

Monitoring of isolates of *P. infestans* each year in 2005 and 2006 showed that the proportion of A2 mating type isolates in the population had increased dramatically from their a low occurrence in mid-1990s (Fig. 1). This increase in A2 mating type isolates was associated, through characterisation using SSR markers, with the emergence of a genotype of *P. infestans*, (designated 13\_A2 (sometimes referred to as 'blue 13')), first recorded in the UK in 2005 (Fig 2). There are two main implications for changes in the population: 1) the new population may have traits that differ from the previous population (e.g. aggressiveness, virulence and fungicide resistance) which will impact on late blight management and 2) increase the risk of long-lived soil-borne inoculum (oospores) being deposited in soil when both mating types are present in a crop.

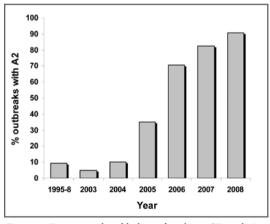


Figure 1. Percentage late blight outbreaks in GB with A2 mating type isolates present

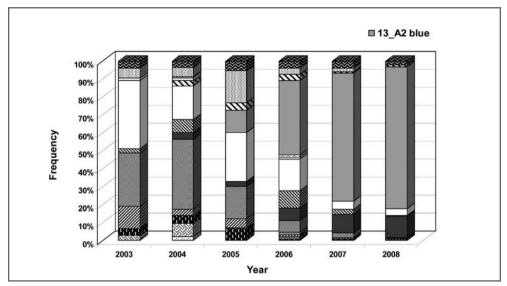


Fig 2. Proportions of the SSR-based genotypes of P. infestans isolates collected each year in GB potato crops.

The objective of this study was to continue to monitor the genotype and mating type of isolates of *P. infestans* occurring in GB in 2007 and 2008. The virulence and aggressiveness of the new genotype 13\_A2 was compared with other genotypes in a series of laboratory and field experiments.

# **MATERIALS AND METHODS**

In 2007, 1400 isolates of *P. infestans* were collected as part of the Potato Council Fight Against Blight survey throughout the growing season and assessed for genotype and mating type as previously described by Cooke *et al.*,(2007).

The aggressiveness of 17 GB isolates selected from those collected in 2007 as described above and a range of control isolates, and 9 foreign *P. infestans* isolates from a range of genotypes was tested on detached leaves of five potato cultivars:

	Resistance rating	
	Foliar Tu	ıber
King Edward	3	4
Maris Piper	4	5
Estima	4	5
Cara	7	7
Lady Balfour	8	7

In brief, isolates were maintained on leaves of cv. Craigs Royal prior to inoculation and 6 replicate detached leaflets of each cultivar were then inoculated with a single drop of inoculum containing 420 sporangia per droplet. Leaflets were incubated at either 13°C or 18°C and the following measurements were made: 1) Incubation Period (IP) = time to first symptoms, 2) Latent Period (LP) = time to sporulation 3) Lesion size (2 measurements) at 6 days after inoculation.

In addition, in order to examine the aggressiveness and fitness of genotype 13\_A2 compared with 4 A1 genotypes under field conditions, sporangia of 5 isolates of *P. infestans* (13\_A2, 2\_A1, 6\_A1, 7\_A1 and 8\_A1), all known to be pathogenic, were mixed in equal proportions and used to inoculate

plants of the five cultivars in a field experiment with 4 replications. Fungicides to control late blight had been applied until one week before inoculation. One plant in the centre of a 25 plant plot of each cultivar was inoculated by spraying the lower leaves. Plants were misted twice daily and the severity of disease was measured on 5 occasions after inoculation. At each date, lesions were sampled onto Whatman FTA® cards and DNA from approximately 800 lesions taken over the course of the epidemic was genotyped using SSR markers.

The aggressiveness of 13\_A2 genotype was compared with 3 A1 mating type genotypes, by inoculating tubers of cvs Maris Piper, King Edward, Lady Balfour and Cara. For each isolate, 2 replicates of 50 tubers were sprayed with an inoculum suspension of each isolate and incubated at 4°C for 8 weeks before the incidence and severity of disease (as a percentage of tuber surface area showing tuber blight symptoms) were recorded.

The virulence of isolates of 13\_A2 was tested on plants of Black's differential set. The ability of the isolates to infect a range of commercially grown cultivars was tested in field trials. The cultivars were selected according to one of the following criteria: occupied a significant area of planting in 2007; previously recorded as being of moderate or high resistance; anecdotal reports of reduced resistance compared with published rating. All tests were conducted according to Eucablight standard protocols (<a href="www.eucablight.org">www.eucablight.org</a>)

# **RESULTS**

Of 1400 isolates genotyped in 2007, approximately 80% were identified as genotype 13\_A2 (Fig. 2). When the proportion of 13\_A2 isolates identified throughout the growing season was analysed, it was found that the 13\_A2 genotype dominated from the earliest outbreak (Fig. 3). However, there is no evidence for increasing levels of recombination in the GB populations and that the *P. infestans* population is made up of relatively few clones (Fig. 2).

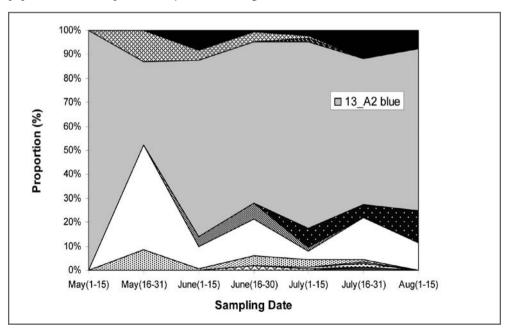


Fig 3. Percentage of isolates which were 13\_A2 genotype between May and August 2007

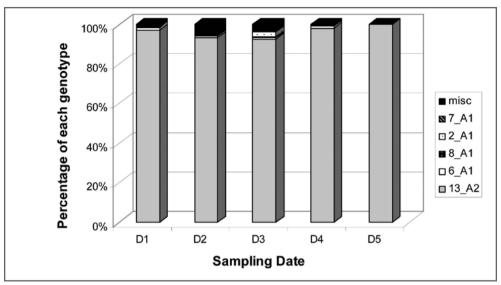


Fig 4. Percentage of isolates of various genotypes, identified in a field trial to assess their relative aggressiveness on 5 cultivars at 5 sampling dates, shown as a proportion of the total.

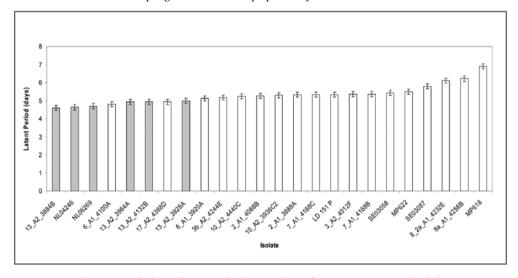


Fig 5. Average latent period (days) of a range of isolates. Isolates of genotype 13\_A2 are shaded in grey.

Aggressiveness tests revealed that, on average, isolates of genotype 13\_A2 produced larger lesions than isolates of other genotypes, particularly at 13°C (data not shown), that their latent period (time until sporulation) was on average shorter (Fig. 5) and that they caused significantly more disease in inoculated tubers of all tested cultivars following incubation at 4°C (data not shown).

Some cultivars showed less resistance than their published ratings would suggest when exposed to infection by isolates of 13\_A2 genotype in field trials carried out by SCRI and SASA in 2008. Examples are given in Figure 6. However, some cultivars e.g. cv. Sarpo Mira showed no apparent change in resistance when challenged by 13\_A2 genotype (Fig. 7). In addition, cv. Sarpo Mira also

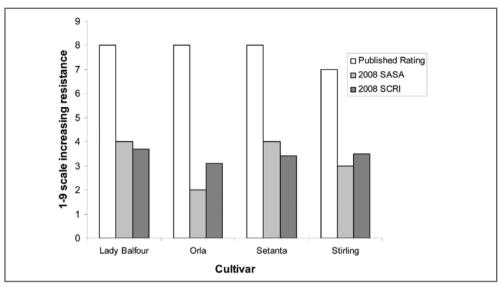


Figure 6. Resistance rating (on a 1-9 scale of increasing resistance) of cultivars tested by both SASA and SCRI in 2008 with an isolate of genotype 13\_A2 as compared with current published resistance rating.

seemed to be resistant in a range of trials across Europe where similar changes in populations of *P. infestans* have been recorded, according to data derived from the Eucablight host resistance database which compares trials carried out under standard protocols. Further testing is necessary to validate the 2008 results and to screen other cultivars that are widely grown and/or have moderate or high levels of resistance to foliage blight according to published ratings before any general conclusions can be drawn. Such information is an essential part in the process of developing strategies to manage late blight control of new populations. Given the dramatic changes to the *P. infestans* population and

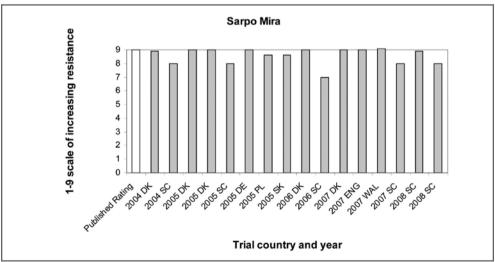


Figure 7. Resistance of cultivar Sarpo Mira on a 1-9 scale of increasing resistance in trials carried out in various countries and years compared with the published disease resistance rating. Data is from the Eucablight database on host resistance.

the potential for increasing diversity in the future, management strategies must take account of the traits of the contemporary population. Control of blight may become more difficult in the UK in future due to increased virulence and aggressiveness of isolates of the 13\_A2 genotype of *P. infestans* resulting in existing cultivars being less resistant in practice than previously.

#### CONCLUSIONS

The proportion of isolates belonging to the 13\_A2 genotype was high (70-80%) in the GB *P. infestans* population in 2007 and 2008

Genotype 13\_A2 survived over-winter, was identified in the earliest disease outbreaks and was dominant throughout the epidemic.

In detached leaf tests isolates of 13\_A2 were more aggressive than isolates of other genotypes, particularly at 13°C, and had a shorter latent period.

Isolates of 13\_A2 were of a complex race structure and were able to overcome host resistance in some previously highly resistant cultivars, but not others and further data sets are needed before general conclusions about the erosion of host resistance relating to dominance of 13\_A2 can be drawn.

In combination, these factors suggest that the dominance of 13\_A2 genotype means that increased vigilance is needed in order to control late blight.

# **ACKNOWLEDGEMENTS**

We acknowledge The Potato Council, The Scottish Society for Crop Research and the Scottish Government for funding and crop scouts for provision of blight samples. Some of this work was carried out as part of a larger Potato Council funded project in which ADAS, SAC, CSL and The Sárvári Research Trust were contributing partners.

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# Resistance of Sárpo clones to the new strain of *Phytophthora* infestans, Blue-13.

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#### **SUMMARY**

Field trials at two sites in England and Wales in 2007 and one site in Wales in 2008 were infected with strain Blue-13 of *Phytophthora infestans*. Foliar resistance, as measured by disease progression and rAUDPC values, showed that Sárpo cultivars and clones displayed a high resistance to this aggressive strain even under high blight pressure conditions. Sárpo Mira had the highest resistance in all trials and only two Sárpo clones had a higher rAUDPC than cv. Robijn, the EUCABLIGHT durably resistant standard. Greenhouse experiments using whole plants grown from stem cuttings supported these results and confirmed that strain Blue-13 was more aggressive than the most frequently occurring genotype of A1 mating type in the UK. The resistance of many commercially available cultivars that had previously shown good resistance to late blight has been severely compromised by strain Blue-13.

# **KEYWORDS**

Sárpo, late blight, resistance, Phytophthora infestans, Blue-13

# INTRODUCTION

Sárpo clones, bred by the Sárvári family in Hungary, have been assessed in several European countries and many clones have shown very high levels of resistance to all strains of *Phytophthora infestans* to which they have been exposed. Two of these clones, Sárpo Mira and Axona, have been accepted as cultivars onto the UK National List.

Current weather patterns and the recent changes in the blight population in Europe have increased the need for blight resistant cultivars. The exceptionally wet summers of 2007 and 2008 in the U.K. have highlighted the shortfalls of relying solely upon fungicides to control blight epidemics. Many commercial growers were faced with conditions that made regular spraying impossible. Phenylamide resistance in the Blue-13 strain (genotype 13\_A2) has compromised control further still. The proposed withdrawal of many blight fungicides under the recent EU Directive, and the phasing out of copper-based preparations relied upon by organic growers, means that cultivars with good, broadspectrum resistance to late blight are set to become the cornerstone of IPM.

Strain Blue-13, detected at a low frequency in UK in 2005 (Shaw *et al.*, 2007), continues to increase in frequency and is now the predominant strain in U.K. and in much of Europe (Cooke *et al.*, 2008).

# **MATERIALS AND METHODS**

#### Field Trials

Two trials were conducted in 2007, one in North Wales and one in Cornwall, England. One trial was conducted at the same site in North Wales in 2008. No irrigation was used in any trial.

#### 2007

The field trial at Llanbedrgoch, Anglesey, North Wales contained 8 Sárpo clones plus the Sárpo cultivars Mira and Axona. EUCABLIGHT standards Robijn and Bintje were included as were Black's "single" R-gene differentials. Further varieties with reputed high resistance to late blight were included, eg Lady Balfour and Orla. The trial consisted of two randomised replicates of 40-tuber plots of each variety. Maincrop varieties were planted on 4<sup>th</sup> May and early cultivars on 1<sup>st</sup> June. Replicate blocks were surrounded by one row of Sárpo Mira. Instead of spreader rows, one plant of cv. Bintje was planted in an outside row of each plot to generate inoculum. Plants of Bintje were sprayed with a suspension of sporangia of strain Blue-13 at 1000 spores/ml on 2<sup>nd</sup> July and were bagged overnight to maintain leaf wetness and encourage rapid infection.

This trial was managed under conventional agronomic practices and had received NPK fertiliser at 350kg/ha on 30<sup>th</sup> April.

The second trial, at Duchy College, Truro, Cornwall, England consisted of 6 Sárpo clones plus Sárpo Mira. EUCABLIGHT standards Robijn and Bintje were included but no R-gene differentials were planted. The cv. Orla was also included as an early variety with reputed high resistance. This trial relied upon natural infection and was surrounded with spreader rows of cv. Charlotte.

The site of this trial was certified as organic and no artificial fertilisers were used. Decayed farmyard manure was applied in the winter prior to planting.

#### 2008

The trial was planted at Llanbedrgoch, Anglesey, North Wales. The design was similar to that used in 2007 but this trial was replicated three times in 25 tuber plots and planted on 29<sup>th</sup> April. Along with previously tested Sárpo clones and cultivars it also included some of the newest Sárpo clones. All EUCABLIGHT resistance standards and the 11 R-gene differentials were included. Also included were a wider range of commercially available blight resistant varieties.

The trial was artificially inoculated using the same method as the 2007 trial and had received similar levels of fertiliser prior to planting.

Scoring of the percentage of foliar late-blight in all trials was done according to Cox & Large (1960). Observations were made at 3-5 day intervals. Relative Area Under Disease Progression Curve (rAUDPC) values for all cultivars/clones in each trial were calculated (Fry, 1978).

# Whole Plant Inoculation Experiment

Plants of five Sárpo clones and cvs Sárpo Mira and Lady Balfour were grown in a greenhouse. Stem cuttings were taken from these plants and planted in sterilised compost. When the plants were well established they were transplanted into five litre compost-filled pots. Approximately six weeks after transplanting four plants of each variety were inoculated with a suspension of sporangia of strain Blue-13 at 1000 spores/ml. An additional four plants were inoculated with strain Pink-6, the predominant genotype of the A1 mating type, at the same spore concentration. The plants were maintained in separate greenhouses and misted regularly. The plants were assessed using Malcolmson's 1-9 scale (Cruickshank *et al.*, 1982) periodically from the appearance of the first disease symptoms.

#### RESULTS

#### Field Trials

# 2007, Wales

Highly blight conducive conditions allowed rapid progression of the disease on plots of susceptible standard Bintje, as well as the supposedly resistant cvs Lady Balfour and Orla (Fig. 1). Sárpo Mira, Axona, Will and Val showed significantly higher foliar resistance to strain Blue-13 than all other cultivars/clones included in this trial.

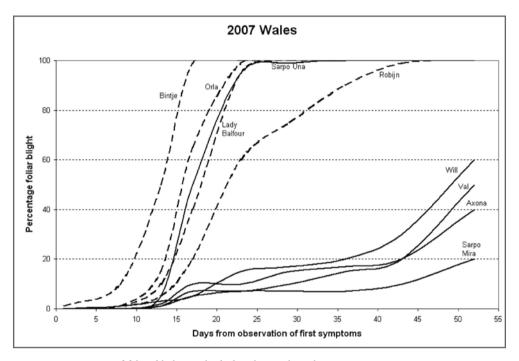


Figure 1. Progression of foliar blight at Llanbedrgoch, North Wales in 2007. Disease progression curves are shown for five Sárpo clones and cvs (solid black lines) and four standard cultivars (broken lines).

# 2007, Cornwall

Natural blight infection was first recorded in one spreader row of cv. Charlotte on 20th June. A leaflet sample of blight from a number of plants was sent to SCRI for genotyping. All samples were identified as strain Blue-13 (genotype 13\_A2). Blight-conducive weather allowed for rapid progression through the spreader rows and the plots of susceptible cv. Bintje. Much lower levels of foliar blight were observed in Sárpo clones Will and Val and in Sárpo Mira (Fig. 2) than at the Llanbedrgoch site. This could in part be due to uneven inoculum spread resulting from natural infection. However, blight progression in cvs Bintje, Lady Balfour and Robijn were very similar to that from the Llanbedrgoch site.

Table 1 shows the rAUDPC values for both trials. It is evident that there was lower blight pressure in the naturally infected Cornwall trial than in the artificially inoculated trial in Wales. Sárpo Mira had the lowest rAUDPC at both sites. Cvs Lady Balfour and Orla, which have foliar blight ratings of 7 and 8 respectively (NIAB potato variety guide 2007), showed no significant difference in resistance from cv. Bintje, used as the highly susceptible standard.

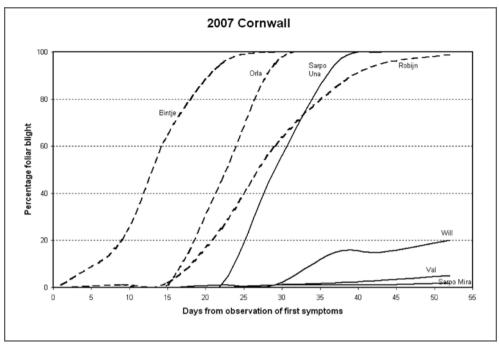


Figure 2. Progression of foliar blight at Duchy College, Cornwall in 2007. Broken lines indicate non-Sárpo varieties.

**Table 1.** rAUDPC values for a selection of Sárpo varieties and standards at both sites in 2007.

Cultivar/clone	Llanbedrgoch, Wales	Duchy College, Cornwall
Sárpo Mira	0.06	0.00
Val	0.10	0.00
Axona	0.14	
Will	0.21	0.14
Robijn	0.66	0.36
Sárpo Una	0.74	0.28
Lady Balfour	0.76	
Orla	0.84	0.50
Bintje	0.88	0.72

# 2008

The weather conditions at the Llanbedrgoch trial site were again highly favourable to the rapid progression of late blight. Infected leaf material was collected mid and late epidemic and sent to SCRI for genotyping to confirm that strain Blue-13 was still predominant in the field.

A clear distinction can be seen between Sárpo and non-Sárpo varieties included in the trial (Fig. 3). Sárpo clones and cultivars, with the exception of Sárpo Una, displayed a much slower progression than the standard cultivars.

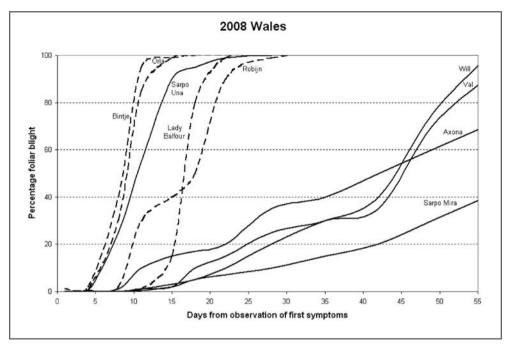


Figure 3. Progression of foliar blight at Llanbedrgoch, Wales in 2008. Progression curves are for the same cultivars/clones as 2007.

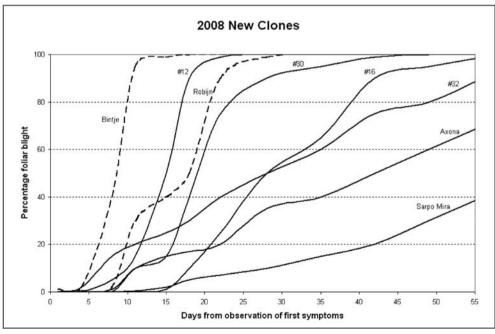


Figure 4. Progression curves of four new Sárpo clones from Llanbedrgoch 2008. EUCABLIGHT standards Robijn and Bintje are included along with Sárpo cvs Mira and Axona to display the range of foliar resistance of the new clones.

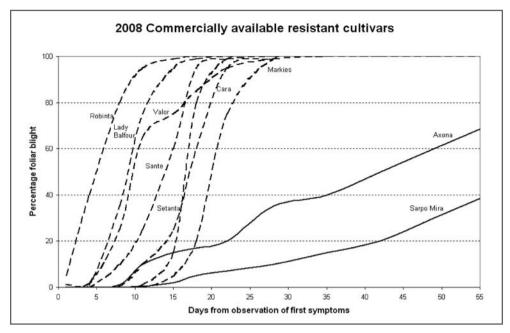


Figure 5. Progression curves showing a range of foliar resistance in seven widely grown blight resistant cultivars and compared to the foliar resistance of the commercially available Sárpo cultivars Axona and Sárpo Mira.

The newest Sárpo clones showed a range of foliar blight resistance (Fig. 4). None displayed the high level of resistance of Sárpo Mira but only clone #12 had a lesser resistance than cv. Robijn which is the EUCABLIGHT standard with the highest resistance.

None of the commercially available blight resistant cultivars included in the 2008 trial showed levels of foliar resistance comparable to Axona and Sárpo Mira (Fig. 5). The majority of these cultivars were totally defoliated by 21 days from observation of first symptoms. Axona and Sárpo Mira were showing 20% and 5% foliar blight respectively at the same observation date.

**Table 2.** rAUDPC values for a selection of Sárpo varieties and resistance standards in 2008.

Sárpo cultivar/clone	rAUDPC	Non- Sárpo cultivar	rAUDPC
Sárpo Mira	0.25	Markies	0.74
Val	0.48	Robijn	0.75
#32	0.48	Orla	0.79
Will	0.50	Cara	0.80
Axona	0.53	Valor	0.80
#16	0.60	Setanta	0.81
#30	0.63	Lady Balfour	0.81
Sárpo Una	0.78	Sante	0.84
#12	0.78	Robinta	0.85
		Bintje	0.88

Table 2 shows rAUDPC values of the cultivars/clones assessed from the 2008 field trial in Llanbedrgoch. Of the non-Sárpo resistant varieties that were included, only cvs Robijn and Markies showed significantly higher levels of resistance than susceptible standard cv. Bintje.

# Whole Plant Inoculations

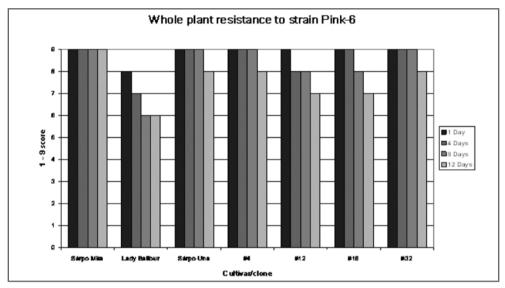


Figure 6. Resistance of a range of cultivars/clones inoculated with strain Pink-6. Values given are a mean score of four plants scored at one, four, eight and twelve days from observation of first symptoms.

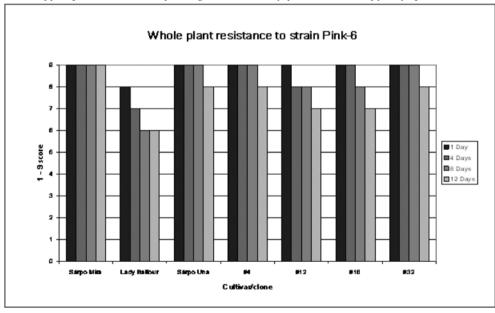


Figure 7. Resistance of a range of cultivars/clones inoculated with strain Blue-13. Values given are a mean score of four plants scored at one, four, eight and twelve days from observation of first symptoms.

Genotypes showed a range of resistance to strain Pink-6 (Fig. 6). Sárpo Mira showed no symptoms after 12 days whereas Lady Balfour showed least resistance. Other cvs/clones showed intermediate levels of resistance. In contrast, all genotypes showed lower resistance with strain Blue-13 (Fig. 7). Again, Sárpo Mira showed the highest resistance and Lady Balfour showed one of the two lowest resistances with other cvs/clones showing intermediate resistance.

#### DISCUSSION

The Sárpo clones and cultivars tested expressed a range of resistance when challenged by strain Blue-13 in field trials. Higher rAUDPC values for all varieties were seen in the inoculated (Wales) trial in 2007. However, the ranking of the Sárpo clones, based on their rAUDPC values, was almost identical at both sites in 2007. Although increased levels of foliar blight were observed in all varieties in the 2008 trial, the ranking of these varieties was very similar to the results from both sites in 2007.

Blight-conducive weather over the growing seasons of 2007 and 2008 promoted rapid progression in all three trials as can be seen from rAUDPC of susceptible standards. As expected, varieties known to have resistance all showed more rapid blighting in these trials than in trials in previous years (Kiezebrink and Shaw, 2005). However, some of these varieties, including Lady Balfour and Sárpo Una, appeared to have retained little or no resistance in the presence of strain Blue-13. The whole-plant inoculations, which compared resistance to Blue-13 with resistance to frequently occurring and aggressive A1 strain, confirmed that Blue-13 overcame some of the resistance in varieties like Lady Balfour and Sárpo Una. The resistance of other Sárpo clones including Sárpo Mira appears durable in the presence of Blue-13.

The range of commercially available non- Sárpo resistant cultivars that were assessed in 2008 all displayed a high level of susceptibility to strain Blue-13 in blight conducive conditions. The breakdown of resistance in many previously resistant cultivars when exposed to the Blue-13 strain has been reported from other trials (Lees *et al.*, 2008).

Table 3 gives the official NIAB foliar blight scores for a range of cultivars and the values calculated from the 2008 Llanbedrgoch trial.

**Table 3.** 1-9 scores for a range of commercially available blight resistant cultivars (9=resistant, 1=susceptible).

Cultivar	NIAB score	2008 trial score
Sárpo Mira	9	8.1
Axona	9	6.3
Lady Balfour	8	3.4
Markies	8	4.3
Setanta	8	3.5
Orla	8	3.7
Robinta	8	2.9
Sante	7	3.1
Cara	6	3.5
Valor	5	3.5

From these data it can be seen that only Sárpo cultivars offer a useful level of resistance when exposed to strain Blue-13 in high blight pressure conditions. Urgent re-assessment of official resistance scores is needed in light of the predominance of this highly aggressive strain.

#### CONCLUSIONS

The results of the field trials in 2007 and 2008 show that the majority of Sárpo clones are highly resistant to the aggressive Blue-13 strain even in very blight conducive conditions. Whole plant inoculations in isolated greenhouses confirm these results and show that Blue-13 is much more aggressive than the Pink-6 strain.

### **ACKNOWLEDGEMENTS**

The work of the Sárvári Research Trust was funded by the Welsh European Funding Office – Objective 1 up until March 2008. We thank all staff at Henfaes Research Centre and at Duchy College who have hosted field trials of Sárpo clones. Particular thanks must go to Mr. Roger Tebbutt for hosting the Llanbedrgoch trials over a number of years. We would also like to thank all EUCABLIGHT partners and in particular Dr. David Cooke at SCRI for SSR analysis of leaf material from field trials.

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# Aggressiveness and transmission: does a correlation exist in *P. infestans*?

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#### **SUMMARY**

A trade-off between pathogenicity and transmission is often postulated in co-evolution models, to explain the persistence of pathogens. We attempted to identify experimentally the existence of such a trade-off in the potato late blight pathogen, *Phytophthora infestans*, in measuring the asexual transmission potential of isolates differing in pathogenicity. Higher aggressiveness reduced the transmission rate from infected tubers to the foliage of daughter plants, by increasing stem mortality pre-emergence. It also led to a high infection rate of small tubers. Therefore, the most aggressive isolates suffer a double penalty for season – to season transmission: they reduced the probability of infected tubers to survive over winter, and they reduce the number of infected stems produced by surviving tubers in the next season. These data strongly suggest the existence of a trade-off between aggressiveness and transmission over seasons in *P. infestans*. This trade-off should result in stabilisation of aggressiveness levels over long periods in the potato late blight pathosystem.

#### **KEYWORDS**

Phytophthora infestans, tuber blight, stem blight, aggressiveness, transmission

# INTRODUCTION

Using resistant cultivars is a useful alternative to pesticides, but only if resistance is durable. This supposes that aggressiveness does not continually increase over time. Many researchers showed that European populations of *Phytophthora infestans* are polymorphic for aggressiveness (UK - Day & Shattock 1997; Ireland - Carlisle *et al.*, 2002 date; F-Lebreton *et al.*, 1999; Pilet *et al.*, 2003; Montarry *et al.*, 2006; NL - Flier *et al.*, 1999). This implies that selection for higher aggressiveness is possible. Furthermore, Andrivon et al (2007) showed that aggressiveness increases during epidemics. One can thus wonder what the consequences of such a selection are in the long term. Two major evolutionary scenarios can be envisioned:

If high aggressiveness does not lead to a decrease in season-to season transmission, a gradual increase in mean aggressiveness over time should be observed, thus leading to resistance erosion;

By contrast, if high aggressiveness involves a decrease of transmission during winter survival, aggressiveness should remain stable.

In *Phytophthora infestans*, asexual transmission between seasons requires three successive steps: 1-transmission from infected leaves to daughter tubers, 2- winter survival of infected tubers, and 3- emergence of diseased stems from such tubers. Our hypothesis is that a high aggressiveness

(severe symptoms) should lead at the end of the epidemic to the production of few, small, severely infected daughter tubers, then to a high rotting rate during winter survival, and finally a high proportion of stems dying before emergence. If these hypotheses are correct, a high aggressiveness to the foliage would result in poor season-to-season transmission. Conversely, a low aggressiveness would lead to the production of many and big tubers with low infection during the epidemic, a low rotting proportion during winter survival, and a low death rate of stems before emergence: a low aggressiveness would then result in good pathogen transmission between seasons. In artificial infection experiments, Montarry *et al* (2007) showed that the survival rate of tubers is not affected by the aggressiveness of isolates infecting them. This paper thus focuses on the two other steps of season-to-season transmission, in an attempt to identify a trade-off between foliar aggressiveness and transmission between seasons. The first part of our work deals with the emergence phase and the second part will focus on daughter tuber contamination.

# **MATERIALS AND METHODS**

# Isolates and cultivar used

We used 3 isolates of *P. infestans* presenting differences in aggressiveness in tests performed under controlled conditions on leaflets of the susceptible potato cv. Bintje. Isolate S1 produces small lesions, whereas isolates S2 and S3 produce large lesions; both S1 and S2 show a low density of sporangia, whereas S3 shows a high density of sporangia (Table 1).

All transmission experiments were performed on susceptible cultivar Bintje with one hundred tubers per isolate and using water as a control.

Table 1. Isolate aggressiveness tested on Bintje leaflets

Isolates	Mean size lesion	Sporangia number/cm <sup>2</sup>	Sporangia number per lesion
S1	11.78	39051	463000
S2	14.39	41986	610766
S3	14.51	67329	977983

# Methods for tubers inoculation to study tuber to foliage transmission

First, a sporangia suspension was produced by washing spores from infected detached leaflets 7-10 days after inoculation. This suspension was sprayed (for a final inoculum density of about  $5.10^4$  sporangia/tuber) onto certified Bintje tubers previously kept for 3 days in humid chambers to allow lenticels to open. After inoculum was sprayed, tubers were kept for 4 further days in the humid chambers before planting in potting mix in 14 cm diameter plastic pots. Plants were grown in a greenhouse with temperature regulated at 15-20°C (night/day) and 16 h daylight for the rest of the experiment.

Different scorings were made during vegetation: percentage emergence, stem number per plant and number of diseased stems per plant. At harvest (fifty days after planting), the number and weight of daughter tubers and the percentage of diseased tubers were scored. This experiment was made in two successive years; only data from the first year are shown in this paper.

# Methods for foliage inoculation to study foliage to tuber transmission

We used the same isolates as in the first experiment, and the same susceptible cultivar Bintje. We produced greenhouse-grown plants at different stages of their growth: D1 (72 days after planting), D2 (56 days after planting) and D3 (42 days after planting). Then, all plants were transferred to cloth-covered structures (here after designated as 'chapels') outside; the cloth was permeable to water and air, but prevented external contaminations. For the inoculation, sporangia suspensions were produced from infected detached leaves for each isolate separately, before depositing twenty drops of 20µl of a given suspension (5.10<sup>4</sup> sporangia/ml) on 10 plants per isolate and growth stage. For each isolate and date, 10 plants were inoculated, with water used as a control. All plants inoculated with the same isolate were placed in the same chapel. Results about differences between inoculation stages are still incompletely analysed, and will thus not be shown or discussed in this paper. After symptoms developed on the foliage, the infection of daughter tubers was favoured by spraying the plants with water to wash sporangia down towards tubers.

Disease severity on the foliage of each plant was monitored at regular intervals during the experiment. At harvest, the total number and weight of daughter tubers and the number and weight of blighted tubers were recorded. Tubers were stored for 2-3 months after harvest, and a second assessment was made to take latent infections into account.

### RESULTS AND DISCUSSION

# Tubers to foliage transmission

Emergence

One month after planting, plants inoculated with the most aggressive isolate S3 showed significantly lower emergence rates than plants inoculated with S1 or S2 isolates (Table 2).

Late blight symptoms, which grew over time, were observed on a low proportion of stems (Figure 1); very premature stem death was also recorded. Infection also changed the physiology of the plants, as shown by the production of aerial tubers in several plants.

Increasing aggressiveness of the infecting isolates resulted in fewer emerged plants and fewer stems per plant; however, isolate aggressiveness did not affect the proportion of blighted stems among those emerged. Overall, transmission of the most aggressive isolates is reduced compared to that of less aggressive isolates.

**Table 2.** Emergence notation one month after planting

% emergence one month after planting					
Control	100				
Isolate 1	51				
Isolate 2	67				
Isolate 3	18				

### Foliage to tubers transmission

Classical Late blight symptoms were observed during vegetation following infection: chloroses, necroses at the plant top, on petioles, or at the tip of leaflets. Rain splashing on infected leaves resulted in small secondary necroses. As expected, the three isolates progressed differently on the foliage, reflecting their differences in aggressiveness. Isolate aggressiveness did not influence the total number of tubers produced, nor their average weight; however, higher isolate aggressiveness resulted in more blighted tubers, and a lower average weight of these compared to lower aggressiveness levels (Figure 2).

## **CONCLUSIONS**

These two experiments provide strong evidence for a trade-off between aggressiveness and transmission in *P. infestans*. We indeed showed that high aggressiveness leads to high death rate of stems before emergence, whereas low aggressiveness leads to low stem mortality rate. The foliage to tubers transmission showed that isolates aggressiveness influences the number of blighted tubers and their average weight. We can thus conclude that high aggressiveness leads to poor transmission over seasons, while low aggressiveness ensures a more efficient transmission.

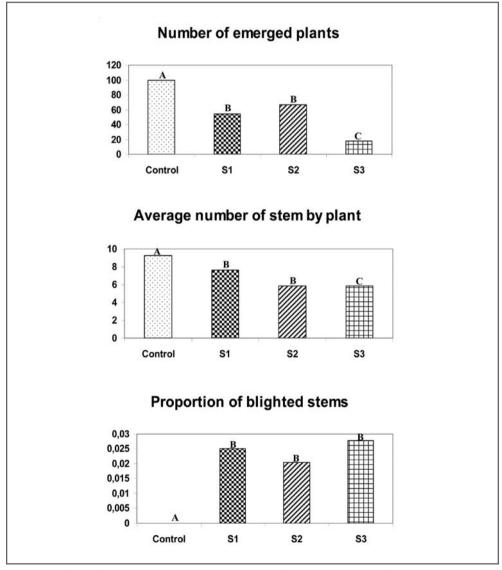


Figure 1. Tuber to foliage transmission: number of emerged plants, average number of stem by plant and proportion of blighted stems

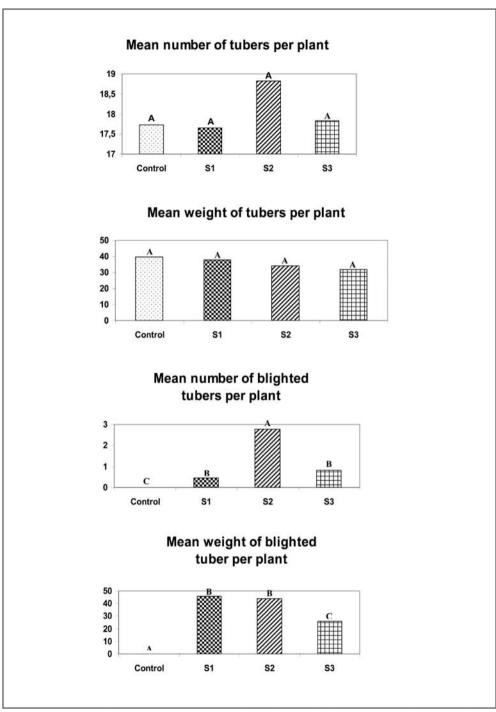


Figure 2. Foliage to tubers transmission: Mean number and weight of tubers per plant and mean number and weight of blighted tubers par plant.

The second experiment showed that aggressiveness affected the mean weight of diseased tubers. It is thus likely that the survival of infected tubers will be affected by aggressiveness. This seems in contradiction with the previous experiment on over-winter survival of infected tubers (Montarry *et al.*, 2007), which showed no differential survival of Bintje tubers artificially infected with isolates of different aggressiveness. This contradiction is however probably only apparent, as the artificial infection experiment used tubers of identical calibres for all isolates. It would thus be useful to check now the survival of tubers of different sizes and with different initial severity of blight.

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# Is oospore production of *Phytophthora infestans* modulated by levels and components of partial resistance?

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#### SUMMARY

Late blight can be controlled by potato cultivars exhibiting partial resistance, which is expressed during the epidemic stage of the disease and acts on parameters related to asexual reproduction rates such as latent period, lesion growth, spore production. During this stage, pathogen strains can meet and mate if compatible; so they produce oospores, which can then constitute a source of primary inoculum able to withstand hard seasons. We want to understand whether parameters of partial resistance impact oospore production. In other terms, the questions we want to address are: is the global resistance level decisive or is one component more casting?

This paper describes our research strategy to tackle these questions, and some preliminary results. These show a trend towards higher oospore production in genotypes with the lowest levels of partial resistance. These conclusions need now to be confirmed through a more extensive statistical analysis, currently underway, of the data from repeated sets of experiments.

### **KEYWORDS**

Partial resistance, oospore, *Phytophthora infestans*, aggressiveness, sexual reproduction, asexual reproduction

### INTRODUCTION

Partial resistance of a host, often also designated as race non-specific resistance or horizontal resistance, allows infection by a parasite but slows down the rate at which disease increases within a single plant or a population of identical plants (Van der Plank, 1968). Partial resistance of potato (Solanum tuberosum) against Phytophthora infestans can result from at least four different changes of life history - traits related to pathogenicity: 1) lengthening the latent period of the parasite, 2) reducing parasite spread within plant tissue, 3) limiting spore production (i.e. pathogen dispersion), and 4) reducing infectivity (i.e. success of infection). Partial resistance can be assessed at different scales, either in the field by measuring foliage disease progress or in the lab by estimating parameters from tests on detached leaflets or whole plants (for example lesion area, spore production...).

All these experimental assessments of partial resistance are performed during the epidemic stage. Thus partial resistance levels are only relative to asexual reproduction of *Phytophthora infestans*, because asexual spores are the only parasitic form which spread during the epidemics. During the intercrop season, the parasite persists either via mycelium in tubers (cf. Pasco & al., this volume) or via oospores, products of sexual reproduction. Because *P. infestans* is a heterothallic species, two compatible mating types, named A1 and A2, are required for sexual reproduction to occur.

Each mating type produces specific hormones, which induce the differentiation of sexual organs in isolates of the compatible mating type (see for a review, Ko, 2007). This particular physiology requires that both mating types meet during the colonisation of host tissue. Hermaphroditism of each mating type results in either outbreeding if both mating types come into physical contact or in self-fertilization.

Because meeting of isolates from opposite mating types during the epidemic phase is required for sexual reproduction to take place, partial resistance could play a significant role in oospore production by *P. infestans*. Some studies have already been dealing with this hypothesis, and tried to link oospore production to field resistance levels of potato cultivars. These experiments led to ambiguous conclusions: in whole plants, more oospores were produced in plants with either medium level (Hanson & Shattock, 1998) or high levels of partial resistance (Strömberg & al., 2001), whereas in leaf discs, the highest amounts of oospores are observed for either medium (Hanson & Shattock, 1998) or low levels of partial resistance (Strömberg & al., 2001). These discrepancies may be due to differences in the protocols used, the pairs of parent isolates tested, but also to the fact that partial resistance was then assessed only as field scores, rather than as components. We thus decided to investigate the links between characteristics of partial resistance (how is resistance expressed?) and oospore production in detached leaflets. Defining the nature of partial resistance (instead of field resistance level) can help us to understand how the interaction between host and parasite can play a role in sexual reproduction investment between two individuals growing on the same host.

Our research strategy involved two different steps. First, we assessed components of resistance in a set of ten potato cultivars to six isolates of *P. infestans* differing for mating type, by measuring pathogenicity parameters on detached leaflet tests. Depending on the point of view we adopt (pathogen or plant), parameters of interaction can be either measured aggressiveness of our isolates or components of partial resistance. In a second step, oospore production was measured for each compatible pairs of these isolates on all 10 cultivars.

# MATERIALS AND METHODS

# EXPERIMENTAL MATERIALS

Cultivars of Solanum tuberosum

Ten cultivars of *Solanum tuberosum* were tested. Two of them are references: Bintje, a susceptible cultivar in France, and Désirée, a susceptible cultivar in Morocco and with a moderate level of partial resistance in France. Seven of them are old cultivars (Robijn, Roode Industrie, Furore, Rosafolia, Möwe, Herbstrote, Noorstar) bred before the introduction of R genes from *S. demissum*; we thus hope to measure only partial resistance in these clones. The last genotype was developed by INRA, and has a high resistance level in the field.

# Isolates of P. infestans

Six isolates of *P. infestans* were chosen within the lab collection. The choice was based on i) aggressiveness measurements on Bintje in previous tests and ii) the presence of isolates from both mating types. Selected isolates differ for lesion area and spore production. Four isolates are A1 and two are A2, giving thus eight compatible pairs.

# TESTS ON DETACHED LEAFLETS

Assessing behaviour of each isolate on the ten cultivars

The protocol was similar to that described by Andrivon *et al.*,(2003) and Montarry *et al.*,(2006), with minor modifications. Spore suspensions of each isolate were prepared by stirring sporulating leaflets in distilled water, and then calibrated to 5.10<sup>4</sup> sporangia/mL. One 20 µl droplet (containing about 1000 spores) was deposited onto the middle of a healthy detached leaflet kept in a Petri dish

containing water agar to favour high humidity. Inoculated leaflets were incubated under a 16h light – 8h dark photoperiod in a climate cabinet; temperature was regulated at 18 °C during the light period and 15 °C during the dark period, respectively.

<u>Latent period</u> LP (= time between inoculation and appearance of first sporangiophores) was scored from daily observation of leaflets under a binocular magnifying glass. Six days post inoculation, we measured the <u>lesion area</u> LA and collected spores by stirring infected leaflets into Isoton II (Coultronics France). The <u>spore production</u> SP per leaflet was then determined by counting the resulting suspension with a Z1 counter (Coultronics France).

The three aggressiveness components were analysed independently, and were also aggregated into a single <u>aggressiveness index</u> AI, calculated as AI = Log (LA\*SP/LP) (Montarry *et al.*, 2007).

# Measuring oospore production

Spore suspensions of each isolate were prepared as described before. The 8 pairs of compatible isolates were inoculated on the 10 cultivars in two ways: either together at the same point, or separately at two different points separated by about 3 centimetres. After 15 days in controlled conditions, leaflets were dipped in bleach for remove chlorophyll and ground. Oospore concentration per leaflet was measured by counting with a haemocytometer.

## **RESULTS AND DISCUSSION**

# AGGRESSIVENESS OF ISOLATES ALONE – COMPONENTS OF PARTIAL RESISTANCE

There were large differences between isolates and cultivars for pathogenicity components, but the ranking of the cultivars (respectively isolates) differed between isolates (respectively cultivars). This resulted in statistically significant interactions between isolates and cultivars in the ANOVAs for each component tested (and for the aggregate aggressiveness index), the results in the ANOVAs, providing further evidence that partial resistance to *P. infestans* in potato genotypes is not necessarily isolate-non specific.

# HOW TO ESTIMATE AGGRESSIVENESS OF A PAIR OF ISOLATES?

The lack of a 'consensus' ranking of cultivars and isolates makes it difficult to link oospore production by pairs of isolates to each component of partial resistance. Indeed each parent of a pair has its own pathogenicity components, which are not necessary the same than the other parent. This raises the question of a measure of aggressiveness for a pair of isolates (rather than for individual parents), and of its relationships with individual performance of each parent isolate. Several options exist, such as considering either the minimum or maximum parent value in each pair, or the mean between parents. In the first analyses, we arbitrarily chose the mean of parent values: for a given pair of compatible isolates and for a given cultivar, mean values between parent isolates were calculated for each parameter of aggressiveness (LP, LA or SP). These calculations were made for the ten cultivars and the eight pairs. We are now also exploring other alternatives.

## OOSPORE PRODUCTION LINKED TO COMPONENTS OF PARTIAL RESISTANCE

Concentrating on aggressiveness index, results show that the more aggressive the pair of compatible isolates (i.e. the more susceptible the cultivar), the higher the number of oospores (Figure 1, where each point corresponds to one pair of isolates on one cultivar). Similar trends were observed for each parameter of aggressiveness (for example spore production, see Figure 2). These trends need now to be confirmed by statistical analyses of the datasets from the two replicate experiments we performed. These analyses are still ongoing, preventing definitive conclusions to be drawn at this stage.

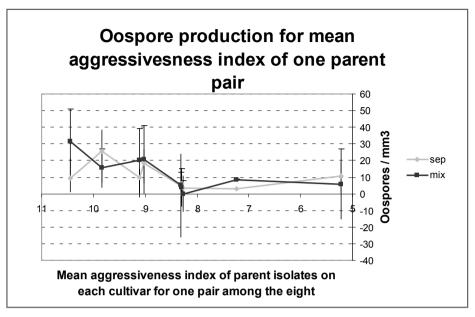


Figure 1. Oospore production according to mean aggresssiveness index of one pair among eight inoculated on the ten cultivars. Each point represente oospore production of this pair on one cultivar as a fonction of mean aggressiveness index of each parent. Grey values represente the separated inocultaion mode, black value the mixed one. Vertical bars show confidence intervals.

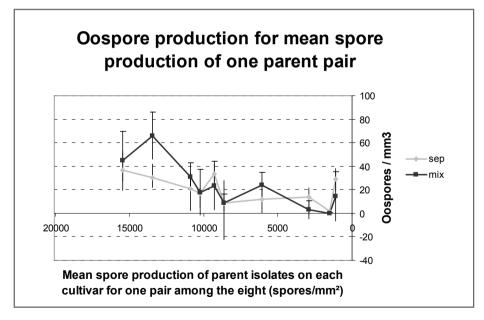


Figure 2. Oospore production according to mean spore production of one pair among eight inoculated on the ten cultivars. Each point represente oospore production of this pair on one cultivar as a fonction of mean spore production of each parent. Grey values represente the separated inocultaion mode, black value the mixed one. Vertical bars show confidence intervals.

# **ACKNOWLEDGEMENTS**

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# Evaluation of spray strategies to control potato late blight with respect to efficacy, economics and the environment

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## **SUMMARY**

Phytophthora infestans has become more aggressive in the last decade. As a consequence growers have to be more alert in controlling late blight. Fungicide choice and timing are key factors to control late blight successfully. An adverse effect of spraying is the environmental risk caused by emissions of fungicides. New strategies to control potato late blight were designed. These strategies have to be effective, cost effective and friendly to the environment. The aim was to show farmers that effective spray strategies under practical conditions, similar to their own situation, can also be friendly to the environment. And thus show that a reduction of the environmental impact of fungicides to control late blight by 75% in 2012 is possible without increasing risks.

The experiment was carried out at 5 locations, coinciding with the main potato growing areas in the Netherlands. Fungicides, which have the capacity to protect newly grown leaves, were used in the early stage of the growing season. Fungicides with tuber protecting characteristics were used in the second part of the growing season. Furthermore spray strategies were linked to cultivar resistance. One strategy focused on control of late blight and early blight, simultaneously. Another strategy was designed to incorporate new, more environmental friendly fungicides.

Late blight and early blight were controlled well by all strategies in 2008, including application of lower dose rates on more resistant cultivars. Compared to a weekly spray schedule, 6 – 9 sprays were saved according to Plant-Plus and 1-2 according to Prophy. Differences were caused by local weather conditions and differences between the Decision Support Systems used. The combination of longer spray intervals and lower dose rates can save the farmer money. However, the net return is predominantly determined by the characteristics of the cultivar grown and not by fungicide choice or number of spray applications.

Environmental impact is based on the characteristics of the fungicide and not solely on the number of applications and dose rate. The used spray strategies fulfilled the requirements on environmental impact, according to the current indicators, but not to a new indicator which is recently developed.

## **KEYWORDS**

Phytophthora infestans, tuber blight, Environmental Impact Points, Environmental Indicator Unit, Spray Index, fungicide

### INTRODUCTION

*Phytophthora infestans* has become more aggressive in the last decade. As a consequence growers have to be more alert in controlling late blight. The potato crop is usually sprayed between 8 and 16

times during the season (Schepers & Spits, 2006). Timing and choice of fungicides are key factors to control late blight successfully. An adverse effect of spraying is the environmental risk caused by emissions of fungicides. The project aims to design new strategies to control late blight. These strategies have to be effective, cost effective and friendly to the environment.

Since 2003 field experiments were conducted to show farmers the developments in controlling potato late blight (Kalkdijk *et al.*, 2007). This article will focus on the results of the 2008 experiments. Key elements of the strategies tested were reduced dose rates based on the level of resistance of the cultivar involved (Fry 1975; Clayton & Shattock, 1995). Furthermore new fungicides on the market were incorporated in the control strategy. Also the control of early blight in combination with late blight was taken into account. The aim was to show farmers that effective spray strategies under practical conditions can also be friendly to the environment, similar to their own situation. And thus show that a reduction of the environmental impact of fungicides to control late blight by 75% in 2012 is possible without increasing risks.

### MATERIALS AND METHODS

# Spray strategies

The experiment was carried out at 5 locations, coinciding with the main potato growing areas in the Netherlands. At each location two local cultivars with different levels of potato late blight resistance were grown (Table 1). To compose a spraying strategy the characteristics of the fungicides have to be adjusted to the growing stage of the potatoes and external circumstances as weather conditions and disease pressure. Fungicides, which have the capacity to protect newly grown leaves, were used in the early stage of the growing season. Fungicides with tuber protecting characteristics were used in the second part of the growing season. Furthermore spray strategies were linked to cultivar resistance (Strategy A and B). The used dose rate of Shirlan was lower when highly resistant cultivars were grown (Spits *et al.*, 2007). Strategy C focused on control of late blight AND early blight. New, more environmental friendly fungicides were used in strategy D. In case of an Alternaria infection risk either Amistar or Signum was used to control early blight in strategy D.

Fungicides were sprayed according to Decision Support System. Plant Plus was provided by Dacom Plant Services and Prophy by Opticrop BV, now part of Agrovision. In general fungicide choice was according to the strategies described (Table 1). However if the Decision Support System advised a fungicide with another mode of action (i.e. curative or systemic) the advice was followed. Advice on adjustment of the dose rate of the fungicides was followed also (Table 2).

**Table 1.** Spray strategies applied at the 5 experimental sites. Between parentheses the resistance value according to the national List (Anonymus, 2007) of the cultivar against foliar and tuber blight is given.

		Lelystad Ware potatoes (	Plant Plus)				
Strategy	rategy Cultivar canopy growing phase tuber filling phase						
A	Bintje (3, 4.5)	Shirlan	(whole season 0.4 l/ha)				
В	Agria (5.5, 7.5)	Shirlan (0.3 l/ha u	ntil flowering and then 0.4 l/	ha)			
С	Agria	Curzate M	Unikat Pro	Ranman 3x			
D	Agria	Revus	Infinito (+Amistar)	Ranman 3x			
		Westmaas Ware potatoe					
Strategy	Cultivar	canopy growing phase	tuber filling pha	se			
A	Lady Olympia (3, 5)	Shirlan (	(whole season 0.4 l/ha)				
В	Agria (5.5, 7.5)	Shirlan (0.3 l/ha u	ntil flowering and then 0.4 l/	ha)			
С	Agria	Curzate M	Sereno	Ranman 3x			
D	Agria	Revus	Ranman 3x				
Valthermond Starch potatoes (Plant Plus)							
Strategy	Cultivar	canopy growing phase tuber filling phase					
A	Starga (5.5, 4.5)	Shirlan (	(whole season 0.4 l/ha)				
В	Seresta (7, 8)	Shirlan (0.2 l/ha u	ntil flowering and then 0.3 l/	ha)			
С	Seresta	Curzate M	Unikat Pro	Ranman 3x			
D	Seresta	Revus	Infinito (+Amistar)	Ranman 3x			
		Vredepeel Ware potatoes					
Strategy	Cultivar	canopy growing phase	tuber filling pha	se			
A	Premiere (2.5, 5)	Shirlan (0.3 l/ha u	ntil flowering and then 0.4 l/	ha)			
В	Hansa (4, 4)	Shirlan (0.2 l/ha u	ntil flowering and then 0.3 l/	ha)			
С	Hansa	Curzate M	Sereno	Ranman 3x			
D	Hansa	Revus	Infinito (+Signum)	Ranman 3x			
		Slootdorp Seed potatoes	(Prophy)				
Strategy		canopy growing phase tuber filling phase					
A	Spunta (5, 4.5)		(whole season 0.4 l/ha)				
В	Agria (5.5, 7.5)	Shirlan (0.3 l/ha u	ntil flowering and then 0.4 l/l	na)			
С	Agria	Curzate M	Unikat Pro	Ranman 3x			
D	Agria	Revus	Infinito	Ranman 3x			

**Table 2.** Fungicides and dose rates used in the experiments.

Fungicide	Active ingredients	Content	Dose rate
rungicide	Active ingredients	active ingredients	(L or Kg /ha)
Amistar	azoxystrobine	250 g / L	0.25
Curzate M	cymoxanil + mancozeb	4.5 % - 68 %	2.0 - 2.5 a
Infinito	fluopicolide + propamocarb HCl	62.5 g / L - 523.5 g / L	1.2 - 1.6 a
Ranman + additive	cyazofamid	400 g /L	0.2 + 0.15
Revus	mandipropamid	250 g / L	0.6
Sereno	mancozeb + fenamidone	500 g /L - 100 g / L	1.5
Shirlan	fluazinam	500 g / L	0.2 - 0.4 <sup>b</sup>
Signum	boscalid + pyraclostrobin	26.7 % - 6.7 %	0.2
Unikat Pro	mancozeb + zoxamide	66.7 % - 8.3 %	1.8

a: Dose rate used depended on advise of Decision Support Systems

b: Dose rate used depending on cultivar, see Table 1.

### Assessments

Disease development of *P. infestans* and *Alternaria spp.* was assessed weekly. Yield and tuber blight were established at the end of the season. At harvest the number of blighted tubers was established. The remaining tubers were incubated for three weeks under for Phytophthora beneficial circumstances. Thereafter the tuber blight incidence was established.

The number of spray applications, the spray index, the amount of active ingredients used and the effect of fungicide sprays on the environment was established. Spray index was calculated by multiplying the number of spray applications with the average relative dose rate compared to the full dose rate as permitted under Dutch legislation.

The costs of the spray applications was calculated based on the number of sprays, the dose rate of the fungicides used and the prize of the fungicides. The gross return was calculated by multiplying the net yield with farmers prizes based on KWIN, which were  $0.10 \in 0.07 \in 1.000$  and  $0.30 \in 1.000$  per kg for ware, starch and seed potatoes respectively (KWIN; Hendriks *et al.*, 2008). The net return was calculated by subtracting the fungicide costs from the financial yield.

The exposure risk to air and water and the environmental risk on water- and soil life of the spray strategies were calculated using the MEBOT farm model (Schreuder et al., 2008).

#### Statistics

The experiments were set up in a randomized block-design with four replicates. Data were analyzed using Genstat 11<sup>th</sup> edition. If necessary data were transformed.

### RESULTS AND DISCUSSION

# Spray applications

The number of spray applications varies with the strategy followed and the location (Table 3). The first spray at Valthermond was carried out on the 30th of June. According to the Decision Support System no infection risk occurred in the early part of the season. To inform the farmers the experiments could be followed on the World Wide Web: www.kennisakker.nl and data were published in farmer's magazines.

**Table 3.** Spray index, number of systemic and curative sprays, number of sprays saved compared to weekly spray scheme and the shortest and longest time interval between spray applications in the 5 experiments.

location	Lelystad	Westmaas	Valthermond	Vredepeel	Slootdorp
D.S.S.	Plant-Plus	Prophy	Plant-Plus	Prophy	Prophy
Spray index strategy A	$8.0^{a}$	$14^{a}$	8	11 <sup>a</sup>	9
Spray index strategy B	7.75	13.75	6.75	8	8
Spray index strategy C	7.9	14.7	8	13.9	8.7
Spray index strategy D	8.0	12.0	6	14	9
Number of sprays with a systemic fungicide in strategies A and B	0 (A) 1 (B)	0	0	0	0
Number of curative sprays in strategies A and B	1 (A and B)	0	1 (A and B)	0	0
Number of sprays saved in comparison to a weekly spray schedule	6-8	0	9	1-2	1
Shortest spray interval (days)	7	5	4	4	5
Longest spray interval (days)	28	10	20	11	12

<sup>&</sup>lt;sup>a</sup>:Cultivar was desiccated one or two weeks earlier than the cultivars in other strategies.

Plant-Plus advised less spray applications than Prophy. This is partly due to weather circumstances at the different locations. But is also caused by differences in the late blight models used and risk perception of the Systems. Prophy emphasises crop protection and Plant-Plus emphasises critical blight weather. Especially in 2008 this led to large differences in the number of spray applications (Table 3). In previous years the differences were in most cases less obvious.

# Disease development

In general the infection risk was low during the rapid growing phase of the crop in 2008. Late blight was found after a high infection risk in week 32 at Lelystad, Westmaas and Valthermond. Strategy D (Revus, Infinito (+ Amistar/Signum) and Ranman) gave the best late blight control, compared to the other strategies (Table 4). No late blight was found during the season at Vredepeel and Slootdorp. Tuber blight was found at Westmaas and Valthermond. Significantly more tuber blight was found in the highly susceptible cultivar Starga than in Seresta at Valthermond. Even though the spray index was 1.25 points higher in strategy A (Starga) than in strategy B. On cultivars with a higher tuber blight resistance level, dose rate reduction is also possible in the second half of the season, which is in accordance with earlier experiments (Spits *et al.*, 2007).

Lowering the dose rate on the less susceptible cultivar (strategy B) did not lead to more late blight compared to strategy A. Dose rate reduction based on the resistance level of the cultivar proved to be possible also in previous experiments (2003-2007). Timing of the fungicide applications however proved to be crucial (Kalkdijk *et al.*, 2007).

**Table 4.** Effect of spray strategies on late blight (%).

location	Lely	stad	West	maas	Valthe	rmond	Vred	epeel	Sloot	dorp
Strategy date	12-	-08	11-	08	2-0	09	sea	son	seas	son
A	0.08	a b	0.06	a b	1.73	. b	0	a	0	a
В	0.10	a .	0.13	a .	0.14	a .	0	a	0	a
С	0.35	. b	0.15	a .	0.05	a .	0	a	0	a
D	0.01	a .	0.01	. b	0.15	a .	0	a	0	a

Early blight was found at each location (Table 5). It was not possible to assess early blight at Valthermond since the crop became senescent before reliable Alternaria readings could be made. In general no significant differences were found in early blight control. Early blight severity was significantly less in strategy D compared to strategies B and C at the last assessment date at Lelystad. Possibly because Amistar targeted at Alternaria was used here.

**Table 5**. Effect of spray strategies on early blight (%).

location	Lely	stad	Westi	naas	Valthermond	Vrede	peel	Sloot	dorp
Strategy date	14-	09	22-	07		5-8	3	7-0	07
A	Dead <sup>1</sup>		0.33	a	n.d.	0.005	a	0.23	. b
В	5.0	. b	0.55	a	n.d.	0.003	a	0.23	. b
С	8.1	. b	0.43	a	n.d.	0.005	a	0.08	a .
D	0.0	a .	0.55	a	n.d.	0.005	a	0.15	a b

 $<sup>^{1}</sup>$ : Early blight rating was significantly higher in strategy A (0.5) than in the other strategies at 4-09

### Economics

In general, spray strategies applied in treatments with the same cultivar (B, C, D) had no effect on yield (Table 5). Probably because late blight severity was low and only small significant differences in late blight control were found between the strategies applied. However at Slootdorp strategy C (60.4 t/ha) had a significant higher yield than strategy D (52.2 t/ha). Differences could not be ascribed to late blight since this disease was not present. Early blight was found, but only marginally and severity was not significantly different between the two strategies. Possibly the use of mancozeb in strategy C gave a nutritional value, which was absent in strategy D.

Yield differences found were associated with the cultivar used and did not depend on the spray strategy.

# Environmental impact

The amount of active ingredients sprayed is not a right indicator for the environmental impact of pesticides. The environmental impact has to be based on the environmental risks of the used fungicides. Toxicological data of fungicides provided for registration are the basis for environmental impact studies. Some of the newly introduced fungicides have less environmental impact than some of the older fungicides. For every spray strategy the Exposure Risk Index to air and water and the Environmental Impact Points on water- and soil life of the spray strategies were calculated using the MEBOT farm model (Table 6). All strategies, except strategy A and strategy C at Westmaas, reached the target for all environmental parameters. In those two cases the number of sprays and the spray index was higher than at the other locations. Data show that it is possible to fulfil the environmental target. However 2008 was not a severe blight year. In previous years, most of the strategies did not fulfil the environmental target, especially at the start of the research period. Due to yearly

adjustments of the spray strategies the environmental risk decreased during the research period. This was partly caused by exploiting the possibility of dose rate reduction in resistant cultivars (Kaldijk *et al.*, 2007) and also because of the introduction of new fungicides. Furthermore the effect of mancozeb on the environment was re-evaluated (based on new research data), which resulted in a reduction of Environmental Impact Points.

Currently a new environmental parameter is being developed, denoted Environmental Indicator Unit (EIU) for surface water; in Dutch: Milieu Indicator Punt (MIP) oppervlaktewater (Van der Linden *et al.*, 2008) This new parameter can be calculated with MEBOT and indicates the chronic impact of active ingredients on water life (Spruijt *et al.*, in prep.). The parameter is strongly influenced by emission by drift. Use of drift reducing nozzles and crop-free zones along ditches reduces the EIU value. However strategy C and D do not fulfil the EIU target. In general strategy A (not at Westmaas) and strategy B (not at Lelystad) fulfil the EIU target (Data not shown). At present it is unclear how EIU is going to be developed further and what the official status will be.

**Table 6.** Effect of spray strategies on economics and environmental impact.

	Yîeld	Gross economic yield	Fungicide costs	Net economic yield	Labour input to control blight diseases	Exposure Risk Index to Air	Exposure Risk Index to soil water	Percentage applications Environmental Impact Points water – life < 10	Percentage applications Environmental Impact Points terrestrials < 100
strategy	ton/ha	€/ ha	€/ ha	€/ ha	h / ha	kg/ha	ppb/ha	%	%
target						0.42	0.5	100	100
Lelystad A	63.1	6310	194	6117	2.4	0.19	0.06	100%	100%
Lelystad B	69.2	6920	198	6722	2.7	0.16	0.06	100%	100%
Lelystad C	68.6	6860	177	6683	2.4	0.03	0.27	100%	100%
Lelystad D	69.4	6940	261	6679	3	0.00	0.00	100%	100%
Westmaas A	64.8	6480	308	6172	4.2	0.43	0.00	100%	100%
Westmaas B	69.6	6960	303	6658	4.5	0.41	0.00	100%	100%
Westmaas C	72.4	7240	315	6925	4.5	0.20	0.58	100%	100%
Westmaas D	69.5	6950	347	6603	4.5	0.06	0.01	100%	100%
Valthermond A	70.0	4620	172	4449	2.4	0.17	0.06	100%	100%
Valthermond B	75.0	4950	133	4817	2.4	0.13	0.06	100%	100%
Valthermond C	72.9	4811	174	4638	2.4	0.01	0.26	100%	100%
Valthermond D	79.9	5273	236	5037	2.7	0.01	0.01	100%	100%
Vredepeel A	65.3	6530	242	6288	3.6	0.34	0.00	100%	100%
Vredepeel B	65.0	6500	209	6291	4.2	0.28	0.00	100%	100%
Vredepeel C	63.9	6390	304	6086	4.2	0.17	0.58	100%	100%
Vredepeel D	65.8	6580	349	6232	4.2	0.06	0.01	100%	100%
Slootdorp A	55.7	16710	198	16512	2.7	0.28	0.00	100%	100%
Slootdorp B	54.7	16410	176	16234	2.7	0.24	0.00	100%	100%
Slootdorp C	60.4	18120	175	17945	2.7	0.11	0.29	100%	100%
Slootdorp D	52.2	15660	195	15465	2.7	0.01	0.01	100%	100%

## **CONCLUSIONS**

Late blight and early blight epidemics were not severe in 2008 and were controlled well by all strategies.

Compared to a weekly spray schedule, 6-9 sprays were saved according to Plant-Plus and 1-2 according to Prophy. Differences were caused by local weather conditions and differences in the late blight models used.

Dose rate reduction based on the resistance level of the cultivar proved to be possible. Timing of the fungicide application however is crucial. In a year like 2008 long spray intervals were possible. The combination of longer spray intervals and lower dose rates can save the farmer money.

The net return is predominantly determined by the characteristics of the cultivar grown and not by fungicide choice or number of spray applications.

Environmental impact is based on the characteristics of the fungicide, and not solely on the number of applications and the dose rate. The used spray strategies fulfilled the requirements on environmental impact, according to the current indicators, but not to a new indicator which is recently developed.

### **ACKNOWLEDGEMENT**

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# Regional spore dispersal as a factor in disease risk warnings for potato late blight: a proof of concept

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### **SUMMARY**

This study develops and tests novel approaches that significantly reduce the fungicide input necessary for potato late blight control while maintaining the required high level of disease control. The central premise is that fungicide inputs can be reduced by reducing dose rates on more resistant cultivars, by omitting applications on days when conditions are unsuitable for atmospheric transport of viable sporangia and by adapting the dose rate to the length of the predicted critical period.

These concepts were implemented and tested in field experiments in 2007 and 2008 in the North Eastern potato growing region in the Netherlands which is known for its high potato late blight disease pressure. Field experiments contained three starch potato cultivars, representing a range in resistance to potato late blight from susceptible to highly resistant, and a series of decision rules determining spray timing and incorporating an increasing number of variables such as: remaining fungicide protection level, critical weather, atmospheric capacity for viable transport of sporangia and the length of the predicted critical period.

The level of cultivar resistance was used to reduce the dose rate of the preventive fungicide Shirlan (a.i. fluazinam) by default.

A 50% – 75% reduction of the fungicide input proved possible in both years without adverse consequences to the crop or yield. The principles can be used in many decision contexts, but further work is needed to test and refine the methods before it can be used in practice.

# **KEY WORDS**

Phytophthora infestans, spores, dispersal, decision support, disease control, environmental impact.

# INTRODUCTION

Potato late blight is an ongoing problem in the global potato industry (Hijmans *et al.*, 2000), demanding grower attention from day-to-day, and still causing losses despite this attention. It is estimated that *P. infestans* is responsible for multi-billion dollar losses annually in global tomato and potato production (Duncan, 1999; Birch and Whisson, 2001; Haverkort *et al.*, 2008). Chemical control is still the most important measure used, and many growers anticipate that disease control requires regular applications of fungicides at high rates and short intervals throughout the growing season. This situation is being questioned, in part due to an increase in environmental awareness

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and public concern about the negative side effects of fungicide use in agriculture, and in part due to concerns regarding the development of pathogen resistance to chemical products. Efforts to reduce the environmental impact of potato cultivation are now driving exploration into new options to reduce the number of sprays, and/or the dosage, while ensuring crop health.

#### MATERIALS AND METHODS

Models and submodels are described in Skelsey *et al.*, (2009). Field experiments were conducted in 2007 and 2008 in Valthermond, the Netherlands in the centre of the Dutch northeastern starch potato growing area. A randomized, split-plot design was used, with three potato cultivars, two (2007) or five (2008) late blight management systems, and four replications. Potato cultivars were used as main plots and potato late blight management systems as sub-plots. Each sub-plot measured 6 m x 12 m. Potato cultivars were Festien (resistant), Seresta (moderately resistant), and Karakter (2007) or Aveka (2008) (susceptible); all starch potato cultivars. Two Curzate treatments were applied on the first two critical periods of the growing season to eliminate latent tuber infections. From then onwards, spray decisions were based on historical and predicted weather and the recommendations of the decision support rules relevant to each specific treatment (Table 1). For a spray advice to be issued all relevant criteria had to be fulfilled. By default, the resistant cultivar was sprayed with 25%, the moderately resistant cultivar with 50% and the susceptible cultivar with 100% of the recommended Shirlan dose rate of 0.4l/ha. Spray treatments were applied maximally one day before the actual start of the critical period.

SIMCAST's (Grunwald *et al.*, 2000, 2002) fungicide units (FU's) were used to determine the remaining level of fungicide protection. Blightdays as described by Skelsey *et al.*, (2009) were used to determine critical days, days with sufficiently long periods of leaf wetness to support infection. The spatial add on determines the daily, cultivar specific, distance weighted infection pressure (DWIP) as described by Skelsey et al (2009) and compares it to a threshold value (the 33 or 50% percentile of a list of DWIP values covering 30 years of relevant critical days). Finally, the length of the predicted critical period is calculated by applying the Blightdays criteria to the seven day weather forecast. When a critical period was predicted, the Shirlan dose rate was reduced proportionally to the length of the predicted critical period with seven days of consecutive critical weather requiring the 'full', cultivar specific, reduced Shirlan dose rate given above.

**Table 1.** Components of the individual decision support systems as applied in the field experiment of 2007 and 2008. All criteria included in a system have to be fulfilled for spray advice to be issued.

Year	System	Spray criteria included*					
		Remaining fungicide protection	Critical weather	Spatial criterium (33 or 50%)	Length predicted critical period		
2007	$\mathrm{FU}_{2007}$	+1	_2	-			
	Spatial33 <sub>2007</sub>	+	7	+	5.75		
2008	$FU_{2008}$	+	-	-	5° <del>e</del> .		
	$CP_{2008}$	+	+	-	-		
	Spatial33 <sub>2008</sub>	+	+	+	-		
	Spatial50 <sub>2008</sub>	+	+	+	-		
	LengthCP <sub>2008</sub>	+	+	+	+		

<sup>\*:</sup> All criteria incorporated in a system have to be fulfilled for spray advice to be issued

<sup>1:</sup> Criterium is included in the system.

<sup>2:</sup> Criterium is not included in the system.

The summer of 2007 was warm and wet. According to KNMI (the Royal Dutch Meteorological Society; <a href="http://www.knmi.nl/">http://www.knmi.nl/</a>), this period saw the second wettest July on record since 1901. In contrast, the summer of 2008 started out relatively dry and was average for the rest of the time.

Disease severity was assessed once or twice per week using the PD scale (de Visser and Meier, 2000). Following completion of the cropping cycle, the plots were desiccated with Reglone.

### RESULTS

# Field experiment 2007

Over the course of the field experiment, FU<sub>2007</sub> recommended a total of 14 fungicide applications. The supplementary spatial component incorporated in Spatial 33,007 modified a total of three of the 14 spray recommendations for the resistant cultivar Festien. On these occasions, cultivars Karakter and Seresta were sprayed whereas cultivar Festien was left untreated. The negated spray recommendation was kept in memory and if in the following days the risk of viable spore transport was high (according to the supplementary spatial component) then fungicides were applied to the untreated plots. This was the case on the day following the first modified spray recommendation, and fungicides were accordingly applied to the untreated Festien plots. On the second and third occasions where a spray decision was modified, conditions continued to be unsuitable for transport of viable spores over distance (according to the supplementary spatial component) and as a result, both chemical treatments were avoided altogether. A high level of disease control was achieved by both DSSs despite (very) favorable conditions for potato late blight and high disease pressure from the direct surroundings of the experiment during large parts of the growing season. Reducing the fungicide dose rates according to resistance level of the cultivar proved to be an effective strategy as there was no significant difference in final disease levels between cultivars. Similarly, the difference in the level of disease control afforded by the two DSSs were not significant. This serves to demonstrate that the supplementary spatial component for DSSs can reduce the number of sprays recommended in a growing season and maintain adequate levels of crop protection.

### Field experiment 2008

The 2008 experiment was designed to give more insight in the individual contribution of a range of "spray triggering events" to the overall performance of the control strategy and the fungicide input (Table 1).

The control strategy based on fungicide degradation alone (FU $_{2007}$  & FU $_{2008}$ ) resulted in a solid control of potato late blight on all cultivars with 16 sprays (Table 2). Including fungicide degradation and critical weather resulted in 8 - 9 spray recommendations. The level of blight control of CP $_{2008}$  was equal to FU $_{2007/2008}$  but required 7-8 spray applications less. Adding the criteria on influx of viable sporangia (33% criterion) reduced the fungicide input by 1 application on the resistant cultivar. Results for the theoretical number of applications are given here because the eradicant treatments became necessary due to an insufficiently strict definition for blightdays. The 50% criterion for influx of viable sporangia resulted in only 3 spray applications on the resistant cultivar Festien (including 2 eradicant applications) but *P. infestans* severity got out of hand indicating this profile incorporates too much risk on the resistant cultivar. On the moderately resistant and susceptible cultivar the number of sprays and the performance of Spatial50 $_{2008}$  was identical to CP2008. When the length of the predicted critical period was taken into account to reduce the recommended Shirlan dose rate, this did not affect the number of sprays or performance but the fungicide input in kg/ha was significantly reduced, especially on the moderately resistant and susceptible cultivar (Table 2).

**Table 2.** Fungicide input in 2008 for the systems included in the experiment and expressed as the number of sprays applied or the number of full dose rate equivalents (0.4 l Shirlan/ha) applied.

	Number of spray applications				
vi	Resistant	Moderately resistant	Susceptible		
FU <sub>2008</sub>	16	16	16		
$CP_{2008}$	11 (8) <sup>1</sup>	9	9		
Spatial33 <sub>2008</sub>	10 (7) <sup>1</sup>	9	9		
Spatial50 <sub>2008</sub>	31,2	9	9		
LengthCP <sub>2008</sub>	9	8	9		
Full dose rate equivalents	3				
$FU_{2008}$	4.75	8.5	16.0		
$CP_{2008}$	7.75 (4.0) <sup>1</sup>	5.0	10.0		
Spatial33 <sub>2008</sub>	7.5 (3.75) <sup>1</sup>	5.0	9.0		
Spatial50 <sub>2008</sub>	5.0 <sup>1,2</sup>	5.0	9.0		
LengthCP <sub>2008</sub>	3.6	3.4	5.3		

An eradicant treatment (2x Infinito + Shirlan tankmix at 100% of the recommended dose rate and a 3 day interval had to be applied. Between brackets the theoretical number of protectant spray applications/dose rate equivalents had the eradicants not been necessary.

P. infestans severity was above the predetermined threshold for premature desiccation.
 This control strategy failed.

#### CONCLUSIONS

Fundamentally, this study is concerned with including more meteorological information in operational decision support. Spatial epidemiology has been scarcely used in operational decision support. Adaptation of the dose rate to the length of the predicted critical period has never been considered for practical reasons. However, when the chemical input and its effects on the environment are questioned, both concepts may play an important role in reducing the environmental burden of potato late blight control.

Development of spatial risk factors for regional spore dispersal from aerobiological models offers a new approach for manipulation of spray intervals in such systems. Previously, the influx component of the infection risk was largely ignored because it is difficult to handle. Instead it was assumed that spore influx always takes place at significant levels so that decisions come to depend on local conditions only. One of the strengths of the approach outlined here lies in the comprehensive use of forecast data already incorporated in many DSSs A further advantage is the applicability of this concept to a range of similar pathosystems characterized by aerial dispersal of inoculum. It has been demonstrated here that spatial risk algorithms are easily incorporated into existing frameworks in the form of a supplementary spatial component, with model results conveniently expressed in the form of a yes-or-no spray modification decision. Results from the field trials showed that in the periods of normal disease pressure approximately 1/3 of the fungicide applications can be saved for the most resistant of the three cultivars tested, which was otherwise sprayed with only 25% of the recommended dose rate of Shirlan. The total reduction of fungicide input for the resistant cultivar (Festien) was high at almost 80%. A high level of protection was maintained in all systems throughout both seasons, except in plots treated according to the Spatial50<sub>2008</sub> system which used an influx threshold which was simply too high. Overall, the results demonstrates that the supplementary spatial component can provide robust control whilst saving approximately 1/3 of the number of sprays.

With respect to the systems that reduce the Shirlan dose rate proportionally to the length of the predicted critical period proved to result in reliable potato late blight control with an additional

significant reduction of the fungicide input, especially on the moderately resistant and susceptible cultivar. In contrast, the influx criteria resulted in savings on the fungicide input especially in the resistant cultivars.

Further field trials are required before definitive conclusions can be drawn as to the utility of the approaches outlined in this paper. Both approaches however are not limited to potato late blight and the methods developed here could also prove to be useful in a decision context for other pathosystems.

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# Invasion of a Virulent *Phytophthora infestans* Genotype at the Landscape Level; Does Spatial Heterogeneity Matter?

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### **SUMMARY**

Proper landscape-scale deployment of disease resistant genotypes of agricultural crop species could make those crops less vulnerable to invasion by resistance breaking genotypes. Here we develop a multi-scale, spatiotemporal model of the potato late blight pathosystem to investigate spatial strategies for the deployment of host resistance. This model comprises a landscape generator, a potato late blight model, and a suite of aerobiological models, including an atmospheric dispersion model. Within individual growing regions, increasing the number of host genotypes caused the greatest reduction in epidemic extent, followed by reduction of the proportion of potato in the landscape, lowering the clustering of host fields, and reducing the size of host fields. Deployment of host resistance in genotype mixtures had a large effect on disease invasion.

The use of space as an isolation barrier was effective in scenarios involving two distinct potato growing regions. It was possible to completely eliminate the risk of epidemic spread from one region to another using inter-regional separation distances ranging from 8 to 32 km. The overall efficacy of this strategy was highly dependent, however, on the degree of spatial mixing of potato genotypes within each region. Deployment of host resistance in genotype mixtures in both regions served to reduce the overall level of incidence in the landscape and the inter-regional separation distance required to eliminate relevant levels of between-region spread of disease.

# **KEYWORDS**

Phytophthora infestans, invasion, Gaussian plume model, landscape design

## INTRODUCTION

Crop heterogeneity can profoundly affect epidemics caused by the transmission of infectious agents (e.g., Zhu *et al.*, 2000). Landscape design and strategic deployment of host resistance therefore emerge as a means to spatially separate aggressive resistance-breaking pathogen genotypes from other susceptible local host populations. In this paper we raise an important question regarding the spatial epidemiology of *Phytophthora infestans* – does spatial heterogeneity in host populations matter, and if so, what scale is relevant?

We describe a simulation framework to investigate which spatial strategies reduce the development of epidemics when a breakthrough of resistance occurs in a proportion of the host population, i.e., when a new, aggressive strain of the pathogen evolves. The framework comprises a recently validated potato late blight model (Skelsey *et al.*, in press) and a suite of aerobiological models, including a long range atmospheric spore dispersion model. Invasion opportunities of virulent pathogen genotypes are assessed under a variety of historical weather conditions and theoretical landscape configurations. The effects of landscape characteristics are studied using scenarios that vary a potato growing region (1 region scenarios) using a limited set of design parameters, and two spatial scales of mixing of host genotypes: genotype mixing *within* a field or *between* fields. Invasion opportunities of virulent pathogen genotypes are also assessed at larger spatial scales using scenarios that vary the separation distance between two distinct potato growing regions (2 region scenarios). In the 2 region scenarios, an additional spatial scale of genotype mixing is simulated; *between-region* mixing, whereby each region is homogeneous for a particular potato genotype.

### MATERIALS AND METHODS

### Simulation framework

Host and pathogen life cycles, host-pathogen-environment interactions, and fungicide applications (protectants and eradicants) are simulated on gridded (raster) landscapes composed of potato and non-potato areas. The number, size, aggregation and classification of potato fields can be varied. In the 1 region scenarios, each potato growing region measures  $6.4 \times 6.4 \text{ km}$ . In the 2 region scenarios, each potato growing region measures  $2 \times 2 \text{ km}$ , with a variable amount of non-potato area between them.

An adaptation of the spatiotemporal/integrodifference equation model of the potato late blight pathosystem originally developed by Skelsey *et al.*,(2005) is used to provide field-scale dynamics. In this study, two potato phenotypes are represented: susceptible and (partially) resistant. The susceptible phenotype represents the genotype with the broken resistance, while the other phenotype represents all other (partially) resistant genotypes. Partially resistant varieties can still become infected, although the rate of infection is far reduced in comparison to the 'broken' or susceptible genotype. Host-pathogen interactions are characterized in the model using quantitative components of resistance (lesion growth rate, sporulation intensity, and infection efficiency) measured in the laboratory on potato leaflets of two cultivars, providing parameter values for the susceptible (broken) and the (partially) resistant interaction (Skelsey *et al.*, in press). The field-scale potato late blight model was recently validated for these and other cultivar-isolate interactions using data from field trials in the Netherlands (Skelsey *et al.*, in press).

Individual fields in the landscape are linked through models describing: spore release from sporangiophores; spore escape from the canopy (de Jong *et al.*, 2002); spore dispersion and deposition (partial-reflection Gaussian plume model - Overcamp, 1976); and spore survival during transportation, according to the dose of global radiation received (Mizubuti *et al.*, 2000).

Within this modeling framework, the composition, configuration and connectivity of host populations are manipulated in order to reveal their influence on epidemic progress. The results of these manipulations are used to develop spatial strategies for the deployment of resistance genes.

### Spatial scenario analyses – 1 region scenarios

Four basic scenario analyses are defined. These analyses address the influence of: (1) the proportion of potato in the landscape; (2) the number of different resistant potato genotypes; (3) the size of fields; and (4) the degree of spatial clustering of potato fields on the rate and extent of invasion of a new, resistance breaking *P. infestans* genotype. In these scenarios, potato genotypes can be deployed at two spatial scales of mixing. Under the first scheme, designated 'between-field mixing,' each

**Table 1.** Spatial parameter values defining the five sets of spatial scenarios

			1 region scenarios		
	ъ.	Proportion of	Susceptible		
Scenario	Region	potato in	proportion of the		Spatial pattern
analysis	size	the region	population	Field size	of fields
	(km)	(-)	(-)	(ha)	(-)
1	6.4 x 6.4	1/64, 1/16, 1/4, 1	1/4	1	Random
2	$6.4 \times 6.4$	1/4	1/64, 1/16, 1/4, 1	1	Random
3	$6.4 \times 6.4$	1/4	1/4	1, 4, 16, 64	Random
4	6.4 x 6.4	1/4	1/4	1	Clustered (scale = 0 to 1
			2 region scenarios		
		Proportion of	Susceptible		Separation
	Region	potato in each	proportion of the		distance
Scenario	size	region	population	Field size	between regions
analysis	(km)	(-)	(-)	(ha)	(km)
5	2 x 2	1	1/2	4	0, 1, 2, 4, 8, 16, 32

individual field in the landscape contains a single host genotype; either resistant or susceptible. Under the second scheme, designated 'within-field mixing,' each field contains a mixture of resistant and susceptible varieties, i.e., a genotype mixture.

# 2 region scenarios

The use of space as a barrier to pathogen invasion from one potato growing region to another is investigated in '2-region scenarios.' This scenario analysis addresses: (5) the influence of the separation distance between two growing regions on epidemic extent. One region is the 'donor' (infected), and the other the 'receptor' (disease-free). In addition to simulating both the between-field and within-field mixing schemes, a third level of genotype mixing is included: 'between-region mixing,' whereby the donor region is entirely composed of the susceptible potato phenotype, and the receptor region is entirely composed of the resistant potato phenotype. The spatial parameter values defining all 5 sets of spatial scenarios are given in Table 1. The parameter setting defining landscape maps, as in Table 1, in combination with the spatial scale of mixing genotypes, defines a scenario.

# **RESULTS AND DISCUSSION**

The results of this study indicate that spatial heterogeneity in host populations does matter for *P. infestans*, and that landscapes can be designed that suppress invasions of virulent pathogen genotypes. The more effective of the strategies tested for individual growing regions were those that increased the number of potato genotypes, and/or increased the degree of spatial mixing of genotypes, i.e. within-field genotype mixing (Fig. 1). Landscape designs that focused on spatial isolation of aggressive pathogen strains through manipulation of field size and clustering of potato fields were found to have limited effect. There was little evidence of thresholds in the response of epidemics to manipulation of spatial landscape variables, i.e., in the 1 region scenarios, no situations were identified in which spatial design had quantum effects on the prevalence of *P. infestans*.

The use of space as an isolation barrier was effective in the 2 region scenarios, but the efficacy of this strategy was again dependent on the degree of spatial mixing of potato genotypes (Fig. 2).

The results of the 2 region scenarios act to confirm the scant information in the literature on the large capacity of *P. infestans* for long distance dispersal (e.g., Zwankhuizen *et al.*, 1998; Mizubuti *et al.*, 2000; Sunseri *et al.*, 2002). Nevertheless, it was possible to completely eliminate the risk of epidemic spread from one region to another. Geographic separation of growing regions according to potato phenotype (between-region mixing) was particularly successful in this respect, with a

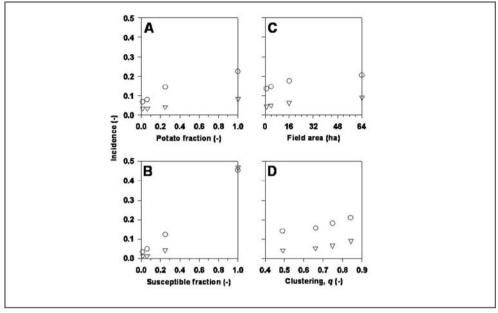


Figure 1. Influence of spatial host population characteristics on the spatial extent of simulated potato late blight epidemics within individual growing regions. Panels A to D correspond to spatial scenario analyses 1 to 4 (Table 1). Incidence is defined as the number of potato hectares infected (disease severity ≥ 1%) relative to the number of potato hectares in the landscape. Circular data markers show predictions for the between-field genotype mixing scheme, and triangular data markers show predictions for the within-field genotype mixing scheme.

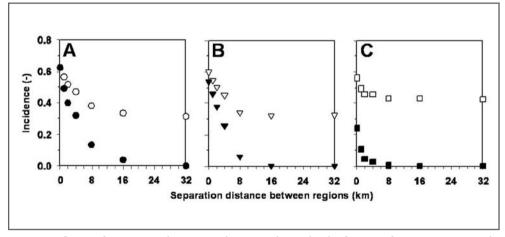


Figure 2. Influence of inter-regional separation distance and spatial scale of mixing of potato genotypes on the spread of potato late blight disease from a 'donor' (inoculated) to a 'receptor' (disease-free) potato growing region (Table 1; scenario analysis 5): (A) between-field genotype mixing; (B) within-field genotype mixing; and (C) between-region genotype mixing. Incidence is calculated in two ways: open data markers show the number of potato hectares infected (disease severity ≥ 1%) relative to the number of potato hectares in the landscape, and solid data markers show the number of potato hectares infected in the receptor region relative to the number of potato hectares in the receptor region.

complete elimination of between-region spread at a separation distance of 8 km. As landscape planners, however, we must concern ourselves with the total amount of disease in the landscape (both donor and receptor regions). Landscape incidence levels were markedly reduced when the degree of spatial mixing of potato genotypes within regions was increased.

# CONCLUSIONS

In combining this knowledge, we arrive at a unified spatial strategy for deployment of host resistance, with a view to suppressing invasions of a virulent strain of *P. infestans*: landscape-wide, homogenous (non-aggregated) deployment of diverse genotype mixtures in small fields. Simulation results suggest that this strategy would be effective in reducing spatial increase in disease within and between growing regions, and thus in minimizing the consequences of a breakthrough in resistance. Secondary effects could include an increase in the performance and durability of resistance, and a reduction in the need for plant protection products.

## **ACKNOWLEDGEMENTS**

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# Improvement of potato late blight forecasting

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## **SUMMARY**

A new potato late blight forecasting model based on hourly weather data is developed based on trials with spore traps and trap plants. The model is built up of sub models for the different steps in the disease cycle, spore production, spore release, survival and infection of spores. Long humid periods are needed for spore production and at moderate humidity the process goes slower. The amount of viable attached sporangia is reduced by drought, and some spores are washed off during rain. Spores are released into the air by a drop in humidity or increased radiation, but the release is inhibited by high leaf wetness. The amount of viable released spores is strongly inhibited by solar radiation. The spore load is also reduced by precipitation. The leaf wetness duration has to be sufficient for the spores to germinate and infect. The risk of blight development is a function of the amount of viable released spores and the duration of leaf wetness.

### **KEYWORDS**

Phytophthora infestans, spore production, release, survival, infection, forecast.

# INTRODUCTION

Potato late blight, caused by *Phytophthora infestans*, is a considerable problem in most potato production areas (Fry *et al.*, 2001). The most common way to control potato late blight is to apply fungicides. To optimize the effects of the fungicide it should be applied shortly before the infection happens. Development of the potato late blight epidemic is highly dependent on the weather. This has been known for a long time and in Norway potato late blight forecasting started in 1957. The *P. infestans* population in the Nordic countries has changed in the last decades. In a Nordic project, called NORPHYT, experiments to study the different factors affecting spore production, release and infection was conducted. The gained knowledge from these experiments in addition to accumulated knowledge from potato late blight research and literature studies has been used in the attempt to improve late blight forecasting (Mizubuti *et al.*, 2000; Schepers, 1998; Hansen, 1992).

# **MATERIALS AND METHODS**

In the NORPHYT trials 2006-2008 a Burkhard volumetric spore trap and trap plants were placed in the centre of a potato field infected with potato late blight to catch *P. infestans* inoculum. Potato plants, of cultivar Bintje, grown in pots in the greenhouse were used as trap plants. One set of trap plants was put out at eight o'clock in the morning and collected at three o'clock in the afternoon and

incubated wet (sprayed with water and covered with plastic). The other set of trap plants was put out at three o'clock in the afternoon and collected at three o'clock the following afternoon and incubated dry. Late blight infection on the trap plants was recorded after one week of incubation at 15-18 °C. Spores on the tape in the spore trap were counted in the microscope and recorded per hour. Infection data and spore catches were analyzed in the relationship to the weather data (temperature, relative humidity, rain, wind, leaf wetness duration and global radiation) to find the factors affecting spore production, release and infection.

### RESULTS AND DISCUSSION

Three years of spore trap and trap plant trials in Norway were used to make the model. *P. infestans* needs a long humid period to produce spores. To initialize spore production the water vapour deficiency has to be below 220 Pa (equivalent to 82.1 %, 87.1 %, 90.6% and 93.1% relative humidity at 10, 15, 20 and 25 degrees °C respectively) for a period of minimum 80 hour\*degrees. The spore production increases with the duration of the humid period. Normally these humid periods occurred during the night. A small pause in the humid period, as long as the water vapour deficiency remains below 520 Pa, reduces the spore production but it can continue if the humidity increases again. The spores are released by a drop in the humidity in the laminar layer around the leaf, either caused by the sunlight heating the leaves or a reduction in the relative humidity. This drop normally occurred during the morning. The spore catch in the spore trap increased after these drops. Rain washes the spores down and the spore catch was low in periods with rain. If the leaf is wet or there is no drop in the humidity the spores seems to remain on the sporangiophores up to two or three days before they perish. The spores need free water from rain or dew to germinate. For the spores to be able to infect the wet period must be minimum 40 hour\*degrees. If the wet period is long a higher proportion of the spores will be able to infect.

On some days more blight was observed on the trap plants that were put out three o'clock in the afternoon and collected at three o'clock the following afternoon (incubated dry) than on those that were put out at eight o'clock in the morning (incubated wet). This happened when spores were released during the morning hours and plants in the field were wet from dew and the dew remained long enough for the spores to infect. The trap plants put out at eight in the morning came from the greenhouse, hence they were dry. Spores landing on these trap plants therefore had to survive until the wet incubation in the afternoon to be able to infect. On sunny days there were nearly no infections on the plants incubated wet. This indicates that survival of the spores must be included in the late blight forecasting model.

Different weather conditions promote the distinct steps in the late blight epidemic cycle. Sun promotes spore release, but inhibits spore survival. Leaf wetness inhibits spore release but promotes spore germination and infection. Late blight will not be a problem if the infection conditions are good at a time when spores are not produced or spores are produced but not viable any more. Every step in the epidemic cycle must be included in the forecasting model and in the right order.

The improved potato late blight forecasting model is based on hourly weather data and predicts the risk of spore production, with subsequent spore release, spore survival and infection, if there is inoculum in the field. For the time being only the algorithm in the model is made. These algorithms will be programmed in a test version to validate the model with field trials. A disadvantage with the model is that it needs leaf wetness duration, and this is not currently available in the prognosis from the meteorological office.

## **CONCLUSIONS**

Spore production happens during long humid periods, which mainly occurs during the nights. Spores are released by a drop in the humidity most often occurring in the morning. If there is dew formation in the haulm that remains a few hours after sunrise the spores can infect. If there is no leaf wetness in the morning, the spores have to survive until the next wet period to be able to do any damage. Survival of spores is strictly restricted by solar radiation. The new potato late blight model predicts the risk of spore production and subsequent spore release, survival and infection. The model should be evaluated with weather prognosis and a leaf wetness prognosis needs to be developed.

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# Climate change impact on late blight development and control in Denmark?

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### INTRODUCTION

Climate has changed in Denmark. The growing season is one month longer, maize is widely grown and brown rust has become a problem in winter wheat (Olesen et al., 2006). The Colorado beetle is spreading from the south and potato volunteer plants are more abundant than 25 years ago. Since 1990, the number of treatments to control potato late blight has increased from approximately 5 to 9 treatments in normal dosage per growing season (Environmental Protection Agency, official pesticide sales statistics). Symptoms derived from soilborne inoculum (oospores) were first time recognised in Denmark in 1997, and early attacks derived from oospores is a problem in the Nordic region (Hannukkala et al., 2007; Andersson et al., 2009). In a Nordic survey on P. infestans in 2003, it was found that both mating types A1 and A2 were present close to 1:1 in Denmark, and it was concluded that sexual recombination is taking place in the whole Nordic region (Lehthinen et al., 2008; Lehthinen et al., 2009) leading to increased variation in genotypes and a suspected high adaptability to cultivar resistance and changes in the physical environment. As the climate and the epidemiological behaviour of P. infestans changes at the same time, it is difficult to evaluate the importance of each factor on the use of fungicide etc. In Finland it has been found that increased and earlier occurrence of epidemics during the period 1933-2002 was associated with a combination of climate change and lack of rotation (earlier attacks from oospores) and that the sales of fungicides used against late blight increased 4-fold from the 1980s to 2002.

Regarding the future climate, the most recent scenario reports from IPCC suggests that Denmark at the end of this century will have similar weather conditions as they have today in central Germany, Holland or Northern regions of France depending on scenario assumptions. (Olesen *et al.*, 2006). In the EuroBlight country reports for 2007 and 2008 it is indicated that the fungicide use to control late blight was approximately 50% higher in the Netherlands than in Denmark, i.e. 13-15 applications per season in the Netherlands compared to 8,5- 9,0 in Denmark (Hansen *et al.*, 2009, this proceeding). Can we expect a future need for a higher level of fungicide input in Denmark due to more conducive blight weather as in The Netherlands and the UK today? To answer questions on future climate impact on late blight development and control in Denmark, we started to compare the weather based risk of blight in the UK, The Netherlands and Denmark.

### **METHODS**

The weather based blight risk was calculated using three different blight indices, over three years

and three countries, and using weather data from two weather stations from each country and each year. The methods were: infection pressure as used in PlanteInfo (BlightMan), DK (Hansen *et al.*, 2006), Smith criteria from Blight Watch, UK (http://www.blightwatch.co.uk/content/bw-Smith. asp) and disease pressure from Prophy, Agrovision, NL (http://www.opticrop.nl). Weather data used for calculations were from the years 2006, 2007 and 2008 from Tylstrup and Flakkebjerg in Denmark, Lelystad and Valthermond in the Netherlands and St Ayreshire and Marham in the UK. In the UK, the Smith rule is currently used in a slightly modified version compared to the original rule: A full Smith Period has occurred if, on each of 2 consecutive days: the minimum air temperature was at least 10°, and there were a minimum of 11 hours with a relative humidity of at least 90%.

Within the calculation there is a provision for a 'near miss'. This occurs when the temperature criterion has been satisfied but the number of hours with a high relative humidity totalled only 10 hours on one or both days. In this exercise we calculated the Smith Criteria on a daily basis (10:00 previous day to 09:00 current day) and then calculated the running sum for two days (current day + previous day). This means that the Smith criteria index ranges between 1 and 4.

In Denmark the infection pressure is calculated as described in Hansen *et al.*, 2006, except that the running sum of calculations is 5 days including two days weather forecast (in this exercise is used + 2 day of measured data). Each day the risk for sporulation is calculated and the number of hours is accumulated for 5 days. The range for 5 days is then 0-120. During a high infection pressure it is expected that there is risk of both sporulation and infection. Infection pressure of < 20 is low; 20 – 40 moderate and > 40 is high.

The method behind the disease pressure from Prophy is not published, but it is based on the criteria for sporulation and infection as described in the literature (Harrison, 1992). The Prophy disease pressure is a daily index accumulated during xx days, but in this exercise not including the weather forecast. Comparing the DK infection pressure and the Prophy disease pressure, one can then expect a two day difference in peaks of infection pressures.

Calculations of infection or disease pressure during the growing season were compared using the three methods. The blight risks in the three countries were compared using the same blight index, and model outputs were related to data from the EuroBlight country reports on early attacks of potato late blight in commercial fields.

#### **RESULTS**

The weather based blight risk was calculated for several combinations of year/station/country /method. The obtained results were quite similar and in this article we concentrate on selected results; comparing the three methods on calculation of blight risk using data from Tylstrup, DK in 2006, and comparing blight risk among countries using infection pressure for the season, 2008. At Tylstrup, 2006, the BlightMan infection pressure and the Prophy disease pressure indicated a shortlasting high infection pressure just before or at crop emergence in the second half of May – low risk in June and then again a peak just before 1 July. July was unfavorable for blight but both methods indicated very high risk for a longer period in August, and the Prophy disease pressure obtained its maximum of 100 (Figure 1). The Smith criteria indicated low and high risk in the same periods as the other two methods. In 2006, only three attacks were recorded in June in the monitoring network in Denmark. Several attacks were recorded in commercial fields in the first week of July and widespread in the country. Then weather turned into dry and warm conditions and the disease development stopped until the first week of August when the epidemic development started. It was difficult to drive in the fields with spraying machines due to heavy rain and the disease developed very fast and aggressive. For some of the starch potato cultivars new leaves were still emerging and heavy attacks were recorded even in fields sprayed intensively. Weather in September was rainy and blight favorable (Figure 1), and tuber blight was a big problem this year.

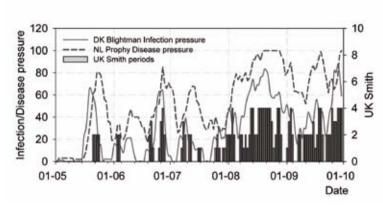


Figure 1. Calculation of blight risk using three different indices: DK Blightman, infection pressure (Red solid line); Prophy disease pressure (Blue dashed line) and Smith criteria (Bars). Weather data are from Tylstrup in Denmark, 2006.

Taking into account that the BlightMan infection pressure includes a weather forecast and the Prophy disease pressure do not, the result from those two indices seems to be very similar - they indicate the same periods of low and high risk for blight. For all three indices there was a good correspondence between observed blight development and the calculation of blight risk. We did not calculate and compare all combinations of years and stations, but similar results were found for other years and stations (not published). For the comparison of blight weather between the countries it was decided to use the BlightMan infection pressure.

To validate the usefulness of the infection pressure to predict early attacks of late blight, we used biological data, 2008, from the EuroBlight country reports, and corresponding weather data from Tylstrup, DK, S. Aireshire, UK and Valthermond, NL (Figure 2). In 2008, the date when "late blight was recorded in more than five conventional fields" was 18 July in DK, 5 June in the UK and 7 June in the Netherlands. Results show that first infections were recorded after a period with moderate to high infection pressure. In Denmark, the situation was extreme as no late blight at all was recorded in June. Late blight did establish in the second part of July and it developed epidemic in August. The situation was similar in parts of the Netherlands (Hansen *et al.*, this proceeding).

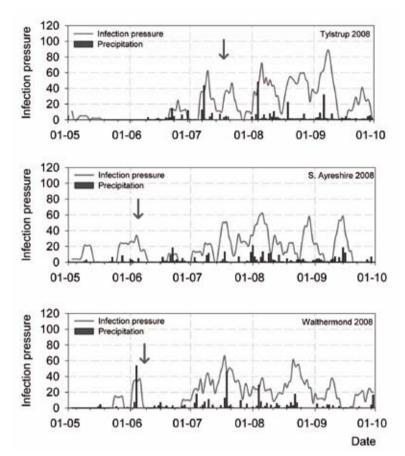
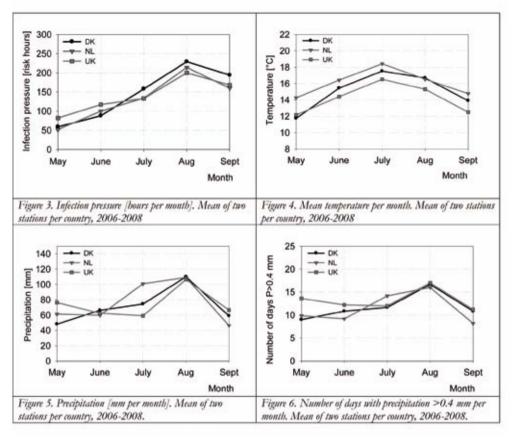


Figure 2. Weather based infection pressure (solid line) and precipitation [mm] [bars] at Tylstrup, DK; S. Aireshire; UK; and Valthermond, NL, 2008. The dates when "more than 5 conventional fields were infected" are indicated with an arrow.

Based on weather data from 2006 to 2008 and two stations per country, the mean values per month for infection pressure, temperature, amount of precipitation and number of days with precipitation, were calculated (Figure 3-6). For all countries, the infection pressure was increasing from May until August with a slightly lower infection pressure in September. For May and June, the infection pressure was the same in DK and NL but slightly higher in the UK. In July, August and September, the infection pressure was the same in the UK and the Netherlands but slightly higher in Denmark. These differences were not tested for statistical significance (Figure 3).

In May the mean temperature was approximately 14° in the Netherlands and 12° in Denmark and the UK. The highest temperatures were recorded in all countries in July. The temperature difference between Denmark and the Netherlands decreased during June and July and ended the same, approximately 16.5° in August. The precipitation amounts and the number of days with precipitation varied considerably between countries and between months, but maximum was always recorded in August (Figure 5 and 6).



#### DISCUSSIONS AND CONCLUSIONS

In the climate change scenarios from IPCC, the climate in 2040-50 in Denmark is expected to be similar to the climate in the Netherlands today (Olesen et al., 2006). We tested the hypothesis that a relatively higher fungicide use in the Netherlands was due to more conducive blight weather conditions in the Netherlands than in Denmark. For the years 2006-2008 the mean infection pressure per month was at the same level or a bit higher for two Danish locations than for two locations in The Netherlands. Interestingly, the simple risk indicators for blight weather conditions used in the UK, DK and NL, pointed out exactly the same periods as favorable/not favorable for late blight. For all countries, the risk for late blight development was increasing from May to August. In August, the potato plants are relatively more (age-dependent) susceptible to blight, and attention should be high on late blight control. Highest mean monthly temperatures were recorded in July, but mean monthly precipitation [mm] and days with precipitation were at a maximum in August. The mean monthly values for infection pressure were correlated with precipitation amounts and days with precipitation and not with temperature (unpublished). If the temperature increases during the summer as expected in the scenarios, this might have no or even a negative influence on blight development. For Denmark less precipitation days and amounts (10-25% decrease) is expected, but on the contrary, 10-20% increase in days with intense showers. Longer dry spells will inhibit blight development, but heavy showers might prevent spraying in the fields for days, and if blight is present, promote inter-field inoculum dispersal and new infections.

Increasing temperatures might influence crop growth considerably. In Denmark potatoes are planted when the soil temperature is higher than 8°C. This date was in 2009 one week earlier (15

April) than in 1988 (22 April) (unpublished data). In Denmark we find first attacks earlier now due to a combination of earlier planting/earlier crop emergence and early attacks from oospores – but only some years. In 2008, we did not record any attacks derived from oospores and first attacks were recorded in the first week of July. This is one month later than in other years. In Denmark, volunteer plants are controlled effectively and especially in seed potato areas. Dump piles exists, but until now we have no indications that early infections from dump piles are important for late blight development (Lars Bødker pers. comm.). Due to climate change the importance of the different inoculums sources for late blight development (dump piles, volunteer plants, oospores, and infected tubers) might change, and there is a need for basic research in this area. During the season the higher temperatures will increase the need for irrigation, decrease the length of the plant growth period, yield might decrease, and problems with Colorado beetle, aphids (virus), nematodes, Erwinia and early blight might increase. In contradiction, the increase in CO<sub>2</sub> will have a positive effect on plant growth and yield. The increase in both CO<sub>2</sub> level and temperature will increase the crop growth rate, and this might influence tactics and choice of fungicides for protection of new growth

In the Netherlands, first fungicide treatment is recommended first time after crop emergence the disease pressure is above a certain level. In Denmark, most growers start spraying before row closing, but if late blight is not recorded in the monitoring network in the region and the infection pressure is low, then they await next blight risk or start with reduced dosages of fungicide. In Denmark we have successfully used reduced dosages in cereals for more than 25 years, and a similar approach has been introduced in potatoes for relevant blight fungicides. Reduced dosages are used mainly in the more resistant starch cultivars. The potato area in DK is 40.000 ha, 20.000 ha is starch potatoes. 60% of the starch potatoes are of the highly resistant cultivar Kuras, and consequently, a major part of the seed potatoes grown are Kuras and other similar resistant cultivars. The tolerance for low levels of blight might be higher i.e in starch producing areas in Denmark compared to areas with mainly ware/fresh/processing potatoes as in the UK. Finally the density of potato fields has great influence on the risk of early attacks, the actual infection pressure during the season, and consequently, most probably also the level of tolerance for blight. The density of potato fields is much higher in the Netherlands than in Denmark.

We conclude that the weather conditions during the season seem not to be more conducive for blight in the Netherlands and in the UK compared to Denmark. The differences in fungicide use as recognized in the EuroBlight country reports are probably due to a range of other factors: use of cultivar types and density of potato fields leading to higher infection pressure, milder winters in the UK and the Netherlands leading to earlier infections in commercial fields from dump piles and infected volunteer plants, different traditions and pressures from pesticide action plans including level of pesticide taxes. One can expect different interpretations of weather based risk calculations in decision rules in DSSs and in general advise schemes on LB control.

#### ACKNOWLEDGEMENT

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

# The impact of fungicide spray order on foliar blight control in two growing seasons

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#### **SUMMARY**

The effect of fungicide spray order and the benefits of blocking or alternating different fungicides were examined in two field trials in Scotland in 2006 and 2007. It was clearly demonstrated that spray order can influence considerably the efficacy of a fungicide programme against foliar blight. In both years blocks of three sprays of the same fungicide gave better control than single alternating sprays of the two fungicides. However, the order of the blocks of Infinito and the other fungicide in the programme had a greater effect on foliar blight severity than whether fungicides were blocked or alternated. The most effective order of the blocks was different in the two years. Blight control was best where Infinito protected the crop during periods of greatest risk of infection. In 2006 when the disease pressure, in terms of favourable weather and available inoculum, was greatest late in the season, positioning the Infinito blocks for the final three sprays was most effective. In 2007 the most effective placing of the three Infinito sprays was the middle three of the nine sprays because severe pressure occurred many weeks earlier. Foliar blight severity at the end of the season was most closely related to the cumulative ratings for the three fungicides applied consecutively during the period of highest blight risk.

#### **KEYWORDS**

Late blight, *Phytophthora infestans*, foliar blight, fungicides

#### INTRODUCTION

It's well known that fungicide products differ in their effectiveness in controlling foliar blight. However, what isn't appreciated as much is that blight control can vary considerably for programmes with exactly the same inputs but with the fungicides sprayed in a different order. Two field trial experiments, funded by Bayer CropScience, at SAC, Auchincruive Estate, Scotland, investigated the positioning of Infinito in spray programmes with other fungicides. The experiments evaluated the effect of spray order and also the relative efficacy of blocking fungicides compared with alternating their application.

#### **MATERIALS AND METHODS**

In both 2006 and 2007 all fungicide programmes started when the leaves were meeting along the rows with three applications of Curzate M WG (cymoxanil + mancozeb). The different fungicide

treatments Infinito (fluopicolide + propamocarb hydrochloride), Shirlan (fluazinam) or Ranman Twinpack (cyazofamid + approved adjuvant) were then applied from application four until haulm desiccation, at 7- to 10-day intervals depending on blight risk. The mean spray intervals were 7.7 and 7.8 days in 2006 and 2007 respectively. Typical block and alternate programmes are illustrated in Table 1. In 2007 the programmes with blocking had the Infinito block as both the middle three and the final three sprays of both the Shirlan and Ranman TP programmes. In 2006 this was only possible where Shirlan was the other fungicide. The fungicide doses (kg or l/ha) were Curzate M WG 2.0, Infinito 1.6, Ranman Twinpack 0.2 + 0.15 and Shirlan 0.3 in 2006 but 0.4 in 2007. The blight susceptible cultivar used was King Edward (foliar and tuber resistance ratings of 3 and 4 respectively).

Table 1. Examples of block and alternate programmes

	Block	Alternate	Block
Spray			
-	Curzate M WG	Curzate M WG	Curzate M
-	Curzate M WG	Curzate M WG	Curzate M
-	Curzate M WG	Curzate M WG	Curzate M
1	Infinito	Infinito	Shirlan <sup>1</sup>
2	Infinito	Shirlan <sup>1</sup>	Shirlan <sup>1</sup>
3	Infinito	Infinito	Shirlan <sup>1</sup>
4	Shirlan <sup>1</sup>	Shirlan <sup>1</sup>	Infinito
5	Shirlan <sup>1</sup>	Infinito	Infinito
6	Shirlan <sup>1</sup>	Shirlan <sup>1</sup>	Infinito

<sup>&</sup>lt;sup>1</sup> Shirlan replaced by Ranman TP in other programmes

#### **RESULTS AND DISCUSSION**

Results are presented in Figures 1 and 2. The most interesting comparisons are between programmes that have the same fungicides applied the same number of times in total, but in different orders. For example the programmes containing Shirlan should be compared with each other. There was no consistent advantage from blocking or alternating the fungicides (Figs 1 & 2). In both years the order of the blocks of Infinito and the other fungicide in the programme had a greater effect on foliar blight severity than whether fungicides were blocked or alternated. Critically, the most effective order of the blocks was different in the two years. Blight control was best where Infinito protected the crop during periods of greatest risk of infection. In 2006 when the disease pressure, in terms of favourable weather and available inoculum, was greatest late in the season (Fig. 3) positioning the Infinito blocks for the final three sprays was most effective. In 2007 the most effective placing of the three Infinito sprays was the middle three of the nine sprays because severe pressure occurred many weeks earlier (Fig. 4). In both 2006 and 2007 alternation gave intermediate control compared with the blocked programmes with different fungicide orders.

Closer scrutiny of the fungicides used when blight risk was high, i.e. weather-based risk was high and the mean severity of foliar blight was sufficiently high for many sporangia to be present, helps to explain the control achieved by the different programmes. Some individual applications of fungicide had a large influence on the final severity of foliar blight (Table 2a). However, in both years foliar blight for the different treatments was most closely related to the cumulative ratings for three consecutive test fungicides (Table 2c). In 2006 applications 4, 5 and 6 accounted for the most variance whereas in 2007 it was applications 1, 2 and 3. Although in 2006 a low severity of foliar blight was present prior to the fourth application (Fig. 3) foliar blight severity was less well related to the cumulative rating for fungicides 3, 4, 5 and 6 than just 4, 5 and 6. In 2006 the key property required for spray 4 was persistence of protection because the plots were challenged 5 and 6 days

after application, during a Smith Period. Curative activity was critical for spray 5 because this spray was applied 2 days after a Smith Period. The ratings of the three different fungicides were reflected in the relative efficacies of the different programmes (Table 3).

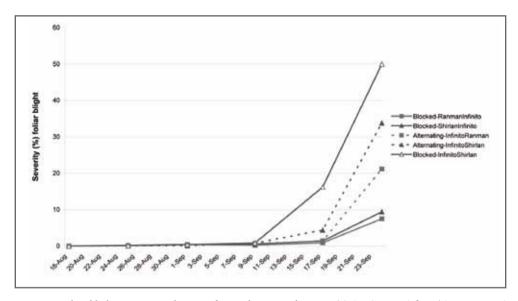


Figure 1. Foliar blight severity in relation to fungicide spray order: 2006 (LSD (P=0.05) for 24 Sep. was 10.3)

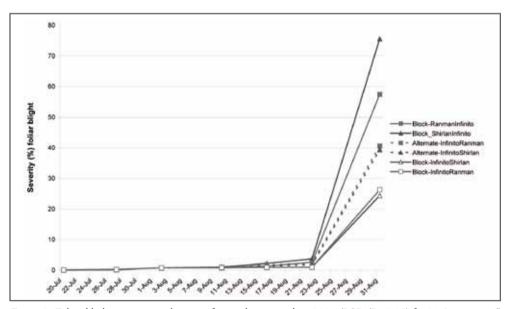


Figure 2. Foliar blight severity in relation to fungicide spray order: 2007 (LSD (P=0.05) for 31 Aug. was 7.6)

**Table 2.** Percentage of variance in final severity of foliar blight accounted for by the ratings of test fungicides used at different times

#### a) Single test fungicide applications

		Test fungicide application				
	1	2	3	4	5	6
2006				80.7		80.7
F pr.				0.024		0.024
2007	66.0		85.6			64.3
F pr.	0.031		0.005			0.034

#### b) Pairs of consecutive test fungicides

	Test fungicide applications				
	1, 2	2, 3	3, 4	4, 5	5, 6
2006				82.1	82.1
F pr.				0.022	0.022
2007	70.1	93.2	58.3		66.0
F pr.	0.023	0.001	0.048		0.031

#### c) Groups of three consecutive test fungicides

		Test fungicide applications				
	1, 2, 3	2, 3, 4	3, 4, 5	4, 5, 6		
2006				90.8		
F pr.				0.008		
2007	95.1	73.4				
F pr.	< 0.001	0.018				

#### d) Groups of four consecutive test fungicides

	<u></u>	Test fungicide applications	
	1, 2, 3, 4	2, 3, 4, 5	3, 4, 5, 6
2006			
F pr.			
2007	80.5	60	
F pr.	0.010	0.043	

Results from non-significant regressions are not shown.

In 2007 foliar blight increased substantially several weeks earlier than in 2006, i.e. between 22 and 31 August (Fig. 4). There is strong evidence that the fungicide applications on 30 July, 8 and 15 August, i.e. sprays 1, 2 and 3 of the programme, were critical for blight control. Foliar blight severities at the end of the trial were most closely related to the cumulative ratings for these three applications (Table 2c). The addition of the rating for application four to those of the first three sprays reduced the variance accounted for (Table 2d). Sprays 1 to 3 were each followed by Smith Periods 6, 4 and 4 days later respectively and therefore persistence of protection was important. For sprays 2 and 3 there was scope for curative activity. The ratings of the three different fungicides were reflected in the relative efficacies of the different programmes (Table 4).

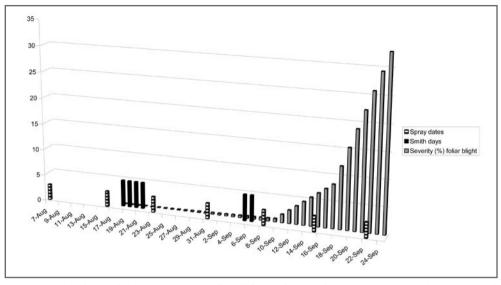


Figure 3. Foliar blight development (average for all fungicide-treated plots) in 2006 in relation to Smith Periods and spray dates

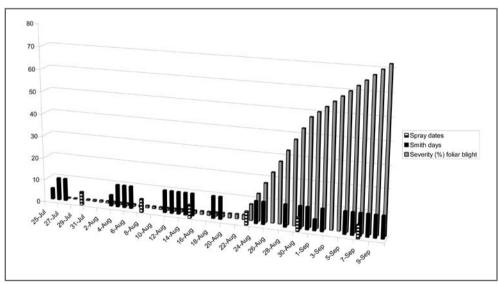


Figure 4. Foliar blight development (average for all fungicide-treated plots) in 2007 in relation to Smith Periods and spray dates

**Table 3.** Cumulative ratings for the fungicides used for sprays 4, 5 and 6 and foliar blight severity (%) in 2006

	_	Fungicide applied on:			Cumulative	% blight on
		31 Aug.	8 Sep.	15 Sep.	Rating <sup>1</sup>	24 Sep.
Block	Ranman then Infinito	Infinito	Infinito	Infinito	13.4	7.5
Block	Shirlan then Infinito	Infinito	Infinito	Infinito	13.4	9.4
Alternate	Infinito and Ranman	Ranman	Infinito	Ranman	13.0	21.2
Alternate	Infinito and Shirlan	Shirlan	Infinito	Shirlan	10.4	33.8
Block	Infinito then Shirlan	Shirlan	Shirlan	Shirlan	6.9	50.0
LSD (P=0.05	)					10.3

<sup>&</sup>lt;sup>1</sup> Sum of the new decimal ratings for leaf blight from the Euroblight Fungicide Table for sprays 4, 5 and 6 plus a rating of 2 for curative activity where fungicide timing allowed curative action. Shirlan applied at 0.3 l/ha in 2006.

**Table 4.** Cumulative ratings for the fungicides used for sprays 1, 2 and 3 and foliar blight severity (%) in 2007

	_	Fung	icide applied	on:	Cumulative	% blight on
		30 July	8 Aug.	15 Aug.	Rating <sup>1</sup>	31 Aug.
Block	Infinito then Ranman	Infinito	Infinito	Infinito	15.4	26.3
Block	Infinito then Shirlan	Infinito	Infinito	Infinito	15.4	24.4
Alternate	Infinito and Ranman	Infinito	Ranman	Infinito	13.2	40.6
Alternate	Infinito and Shirlan	Infinito	Shirlan	Infinito	11.9	39.4
Block	Ranman then Infinito	Ranman	Ranman	Ranman	10.8	57.5
Block	Shirlan then Infinito	Shirlan	Shirlan	Shirlan	6.9	75.6
LSD (P=0.05	5)					7.6

<sup>&</sup>lt;sup>1</sup> Sum of the new decimal ratings for leaf blight from the Euroblight Fungicide Table for sprays 4, 5 and 6 plus a rating of 2 for curative activity where fungicide timing allowed curative action. Shirlan applied at 0.4 l/ha in 2007.

The main conclusion from these trials is that the decision regarding which fungicide to apply when can be critical. The results presented argue against having a blight fungicide programme planned in advance of the season, and in favour of a pro-active approach to target the most effective fungicide when the risk is highest and its key properties will be fully utilized. Two increasingly important principles for potato production are sustainability and the return on the cost of inputs. Matching the most appropriate fungicide to the prevailing risk conditions as they change during the growing season can contribute substantially to sustainability and the economic return from fungicide inputs.

Although the dates of the three most important fungicide applications were very different in 2006 and 2007, the timing of the three sprays in relation to foliar blight development was similar. In 2006 the percentage foliar blight was estimated to be 0.3%, 0.6% and 6.4% when sprays 4, 5 and 6 respectively were applied. In the following year the corresponding values for foliar blight were 0.5%, 0.9% and 1.3% for fungicide applications 1, 2 and 3. As expected, the impact of fungicide application was greater when there were actively sporulating blight lesions

in the vicinity. This confirms the view that estimation of the number of sporangia challenging the crop, either by trapping sporangia or using models to estimate their number, will allow more rationale use of fungicides compared with weather-based blight risk assessment alone.

Three fungicide applications accounted for 90.8% and 95.1% of the variance in final foliar blight severity in 2006 and 2007 respectively. The relatively small contribution of applications 1, 2 and 3 in 2006 was most likely due to the limited disease challenge until late August. In 2007 the rate of epidemic development in the plots during the first 3 weeks of August was sufficiently large that the outcomes for the different fungicide programmes were very much determined by the first three sprays; applications 4, 5 and 6 did little to further influence foliar blight development within plots.

#### **ACKNOWLEDGEMENTS**

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

### Efficacy of different fungicides for the control of early blight

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#### **SUMMARY**

Potato early blight caused by *Alternaria solani* and *Alternaria alternata* is a major disease of potatoes and other Solanaceae. By now, *Alternaria* species have established themselves as destructive pathogens. Due to considerable yield losses early blight is getting a serious problem in many German potato growing areas. Over the past years, early blight has been greatly underestimated although heavy infections can cause yield losses up to 30% and more. As a result of this, early blight is currently in the focus of present fungicide strategies. Up to now, the management of this disease is achieved mainly through protectant fungicide applications. In the course of an early blight research project at the Centre of Life Sciences in Weihenstephan intensive investigations have been conducted concerning the optimization of fungicide strategies. The application of fungicides at different times of the disease progress in combination with potent active ingredients enabled the development of an effective disease control. For this, several field trials were carried out in 2007 and 2008.

#### **KEYWORDS**

Early blight, disease control, fungicide, active ingredient.

#### **INTRODUCTION**

Apart from the widespread potato disease late blight, early blight causes increasing problems for German farmers. Within the last years, an increase in disease frequency has been observed, with early blight becoming an important disease in potatoes (Hausladen et al., 2004). During the last years, a monitoring program has been accomplished in potato culture throughout Germany, which documents the widespread occurrence of early blight in all German potato growing areas. Different factors, such as environmental conditions and plant physiology seem to have a central influence on the disease progress of Alternaria. Recognized primarily as a foliage pathogen, Alternaria epidemics mainly occur when the weather is warm and dry with short periods of high moisture (Venette and Harrison, 1973). As first symptoms predominantly occur on lower leaflets, favourable weather conditions lead to disease spread onto higher leaf levels. If blight is severe, whole leaves can be infected and plants may be entirely defoliated. Heavy infections can cause considerable yield losses up to 30% and more (Johnson et al., 1986). Especially in late maturing potato varieties early blight can reduce tuber weight as well as starch content (Leiminger, 2007). The control of polycyclic diseases like early blight requires multiple applications of fungicides. Up to now the most effective disease control has been achieved by the frequent application of protectant fungicides (Gent and Schwartz, 2003). Targeted applications of potent active ingredients led to a reduction of the number of sprayings.

#### MATERIALS AND METHODS

Two field trials were carried out in 2007 and 2008 to evaluate the efficacy of different active ingredients for the disease control of early blight. Trials were designed as a randomized complete block. Each plot had a size of 32 square meters (6 potato rows in width and 8 m in length). Each trial was replicated four times. The potato culture was fertilized, cultivated and managed according to general agricultural practice. Potato trials were carried out over both years using the variety Kuras. Fungicide trials included a fully untreated control as well as a late blight free variant. To prevent the development of late blight, the fungicide Ranman (400 g cyazofamid/l) was applied as a cover spray every 8 to 10 days at a dose of 0.2 l per ha. As disease progress of early blight was not affected by the use of Ranman, early blight was allowed to develop naturally during the course of the growing season. Different active ingredients were tested in order to investigate disease control against early blight. Several active ingredients like mancozeb, chlorothalonil, fenamidon as well as strobilurines such as kresoxim-methyl, azoxystrobin, trifloxystrobin, pyraclostrobin, pyraclostrobin + boscalid were applied in addition to Ranman. Mancozeb, chlorothalonil, fenamidon as well as pyraclostrobin + boscalid were tested at their recommended dose rate. The strobilurines kresoximmethyl, trifloxystrobin and pyraclostrobin were tested at the same amount of active ingredient as azoxystrobin (125 g/ha). Each of these active ingredients was applied three times in the course of disease development. Fungicide applications were initially conducted, when early blight symptoms appeared for the first time on potato leaves. They were continued in two weeks intervals/spacings. In 2007 the specific early blight application was carried out at July 14th, July 31th and August 14th. In 2008 application was done at July 16th, August 1st and August 16th. Fungicides (in 400 l of water/ha) were applied with a portable backpack-sprayer.

Table 1. Active ingredients against early blight and used dose rate

active ingredient	a.i. (g/l)	applicated amount of active ingredient (g/ha)
Mancozeb	750	1350
Chlorothalonil	500	1000
Fenamidon	500	200
Azoxystrobin	250	125
Kresoxim-methyl	500	125
Trifloxystrobin	500	125
Pyraclostrobin	200	125
Pyraclostrobin	67	16,75
Boscalid	267	66,75

Disease progress was observed weekly from the occurrence of early blight symptoms weekly until the end of the season. For the ratings, potato plants were divided into three levels (lower, middle and upper leaf level) in order to follow up disease development. For each of these leaf levels, the amount of the necrotic leaf area was determined. At the end of the season, both centre rows were harvested and yield as well as starch content was assessed.

#### **RESULTS**

The efficacy of various active ingredients in the control of early blight was determined in two consecutive years (2007, 2008). Concerning early blight symptoms, no difference could be observed in the appearance of first symptoms within the early blight untreated plot and the fungicide treated variants. The onset of early blight was detected in the field already three weeks after crop

emergence. However, in the course of the vegetation period, marked differences were observed in the comparison of fungicide treatments. The strongest increase in disease severity was observed in the early blight untreated plot. Here, the potato plants were totally destroyed by early blight untill the end of August (AUDPC 419). In comparison to this, fungicide application resulted in a slower build-up of inoculum and disease progress was reduced. Apart from the Alternaria-control, early blight disease developed most clearly in the mancozeb treated plots (AUDPC 367). Lower AUDPC values could be recognized in plots treated with strobilurines. Here, disease progress was suppressed most obviously.

**Table 2.** Comparison of the AUDPC and yield in dependence of the applied active ingredient.

applicated active ingredient	AUDPC mean 07/08	yield (relativ) mean 07/08	starch content (relativ) mean 07/08	starch yield (relativ) mean 07/08
untreated against <i>Alternaria</i>	419	100	100	100
mancozeb	367	108	104	112
chlorothalonil	334	107	104	111
fenamidon	283	105	105	110
kresoxim-methyl	300	106	106	113
trifloxystrobin	250	114	105	120
pyraclostrobin	215	113	106	120
pyraclostrobin + boscalid	188	112	106	120
azoxystrobin	115	118	109	129

In 2007 as well as in 2008 the lowest disease progress was observed in plots treated with pyraclostrobin + boscalid or azoxystrobin. Nevertheless, a complete elimination of early blight symptoms was not possible with any of the active ingredients used.

An insufficient early blight control resulted in significant yield losses due to premature defoliation of the potato plants. Plots without any specific application against early blight (e.g. control plot) showed the lowest yield of all. The examinations clearly show that early blight affects all economically significant factors such as tuber yield, starch content as well as starch yield. As a result of fungicide application yield increased significantly. Highest tuber as well as starch yield was obtained by the use of specific treatments with strobilurines. Therefore, disease control as well as increase in yield can be better achieved with strobilurines, which are currently admitted for the specific use against early blight, than with broad-spectrum fungicides.

#### CONCLUSION AND OUTLOOK

Over both years, early blight was monitored as a destructive disease, which caused yield losses due to premature defoliation. Fungicide application enabled an effective disease control. Considering the specific fungicide impact, differently good results could be obtained in the control of early blight. By now, only few investigations have addressed the impact of active ingredient on *Alternaria solani* and *Alternaria alternata*. Actually both pathogens can be found in all German potato growing areas. This indicates that an efficient control of early blight in the field necessitates fungicides effective against *Alternaria solani* and *Alternaria alternata*. Therefore specific investigations are necessary to a better evaluate the effectiveness of active ingredients against early blight.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Effectiveness of some fungicides in control of *Alternaria*alternata and *Alternaria solani*

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#### **SUMMARY**

Chemical protection is one of the methods applied in early blight management programs, particularly in protection of susceptible potato cultivars. Due to increasing problem of early blight on potato crops, potato management programs should also consider effectiveness of fungicides included in control of this disease. Various active ingredients are registered for the control of the early blight in different countries.

Control of the early blight is of economic importance. Unsatisfactory efficacy of some fungicides in early blight control on potato crops could be also a result of application of less efficient products, their late application and may be composition of *Alternaria* populations in different regions.

The results of the experiments carried out in Plant Breeding and Acclimatization Institute in Bonin under laboratory conditions, showed different effectiveness of tested fungicides in limiting development of two species of *Alternaria*, which could confirm dissimilar efficiency of the same fungicides on different areas of their application.

#### **KEYWORDS**

potato, early blight, control, fungicide efficacy

#### INTRODUCTION

Some consequences of the climate change have been already observed on potato fields and they mostly concern changes in development and incidence of major potato pathogens. *Phytophthora infestans* as a causal agent of late blight, very common disease causing losses up to 70% on uncontroled fields (Hoffman & Schmutterer, 1983), and at early infections causing 100% lost of tuber yields (Fry, 1994) should be taken into consideration first.

The recent years have shown an increasing importance of *Alternaria* fungal species, causal agent of a potato early blight. The causal agents of the disease are fungi from *Alternaria* genus: *Alternaria alternata* and *Alternaria solani*. Quantity ratio regarding an occurrence of both species varies and is dependent on the climate conditions (Hausladen & Leiminger, 2007). Both *Alternaria* species differ also in some morphological features such as mycelium color and mycelium growth rate on the media, spore structures and temperature requirements. Spores of *A. solani* are usually borne singly and spores of *A. alternata* are formed in chains. In the course of disease development the morphological symptoms are difficult to distinguish therefore they are evaluated jointly as the early blight.

Factors affecting the early blight development;

- Alternating dry and high humidity periods
- Wind borne spores
- Factors influencing plant weakness (senescent plants, soils with low contents of organic matter, low nitrogen fertilization, other pathogens' infection).

The causal agents of early blight are an example of typical necrotrophic organism i.e. a pathogen infecting weaker and older plants (Rotem, 1966). Potato plants infected with some viruses are more susceptible to the early blight infection (Hooker, 1980). Harmfulness of the disease is estimated differently in various regions of the world (tab.1).

**Table 1.** Estimated yield losses caused by the potato early blight (*Alternaria* spp.)

Country	Yield losses	Source:
USA (Colorado)	10-57 %	Harrison et al (1965)
Russia (Soviet Union)	25%, locally 60%	Reinoch (1974)
USA	20-30%	Johnson <i>et al.</i> ,(1986), Fry (1994)
Netherland	up to 50%	Hadders (2002)
Brasil	up to 73%	Brune, et al.,(1998)
South Africa	up to 50%	Denner & Theron (1999)
Poland	10-32%	Kuczyńska (1992),
Poland	6-45%	Kapsa & Osowski (2004)

Higher regional yield losses caused by the early blight were observed, however, most were related to cultivars with recognized susceptibility to this disease (Kapsa & Osowski, 2004).

#### **MATERIAL AND METHODS**

In the year 2007 studies were conducted at the Plant Breeding and Acclimatization Institute of Bonin with the emphasis on estimation of the efficiency of selected fungicides in limiting the development of *A. alternata* and *A. solani* species under laboratory conditions.

Isolates of *Alternaria* species were collected on the potato fields in Bonin, at the beginning of 2006 growing season. They were isolated from the first typical early blight lesions found on the lower leaves of potato plants. Isolates of *Alternaria* species were maintained on artificial medium (potato dextrose agar) under favorable conditions for their development (temp. 25-27°C, RH 80-90%). Two-weeks old pieces of fungus mycelium (diameter = 5 mm) were cultured in 10 cm Petri dishes on potato agar media containing different fungicides (tab.2).

**Table 2.** Fungicides used in the research:

No	Treatment	Active substance	Mobility*	Dose kg-l /ha
1.	Untreated control	-	-	-
2.	Altima 500 SC	fluazinam	С	0,4
3.	Amistar 250 SC	azoxystrobin	s	0,5
4.	Antracol 70 WG	propineb	С	1,8
5.	Dithane NeoTec 75 WG	mancozeb	С	2,0
6.	Infinito 678,5 SC	fluopicolide+propamocarb-HCl	t-s	1,6
7.	Revus 250 SC	mandipropamid	S	0,6
8.	Ridomil Gold MZ 68 WG	mefenoxam + mzb	s	2,0
9.	Tanos 50 WG	cymoxanil+famoxadone	t	0,5
10.	Unikat 75 WG	zoxamide + mzb	С	2,0

<sup>\*</sup>mobility: c - contact, t - translaminar, s - systemic, t-s - translaminar-systemic

For both the fungus species, 10 Petri dishes in two replications, of each tested fungicide were used. In the experiment, cultures of each fungus species on medium without any fungicides made out unprotected control. Fungicides were applied at two doses – recommended for field application and 10 times lower. Criteria for assessment were: diameter of mycelium (mm), surface of mycelium compared with control (%) and number of spores on 5 mm pieces collected near inoculation point. *A. alternata* and *A. solani* were cultured until the size of control variant was approximated to diameter of Petri dish. Size of mycelium was measured every 3 days. For comparison of isolates sporulation intensity after 21 days was also taken into account. Agar plugs (diameter 5 mm) with fragment of the growing colony were transferred into flasks with 3 ml of the distilled water. Flasks were shaken for a few minutes to suspend spores. Number of spores was counted twice for each isolate using Bürker chamber. The results were expressed in number of spores per 1 mm² of growing colony. The obtained results were analyzed in a 2-factorial ANOVA, the factors being treatments and the fungicide applied.

#### RESULTS

Both species of fungus *Alternaria* differed in some morphological features such as mycelium color and mycelium growth rate on the media (tab. 3).

On control variant final linear growth of *A. alternata* mycelium was insignificantly higher compared with *A. solani*. On the other hand, the development of *A. solani* was much slower and was late by 3-5 days.

**Table 3.** Development of *Alternaria* species mycelium depends on applied fungicide

Treatment	Linear growth of mycelium (mm)		Surface of my	Surface of mycelium (%)	
	A.alternata	A.solani	A.alternata	A.solani	
Control	84,1	83,6	98,9	98,4	
Altima 500 SC	17,6	8,3	20,7	15,2	
Amistar 250 SC	50,0	62,5	58,8	72,7	
Antracol 70 WG	20,6	13,8	24,2	23,4	
Dithane Neo Tec 75 WG	0	0	0	0	
Infinito 687,5 SC	0,0	32,9	0,0	76,9	
Revus 250 SC	41,4	0,0	48,7	0,0	
Ridomil Gold MZ 68 WG	42,7	0,0	50,2	0,0	
Tanos 50 WG	52,0	54,5	61,2	84,1	
Unikat 75 WG	0,0	0,0	0,0	0,0	
LSD (a=0,5)	1,6	2,9	1,9	3,4	

The studies carried out in Bonin revealed that both linear mycelium growth and surface of mycelium differed and were dependent upon *Alternaria* species and the fungicide addition (fig.1).

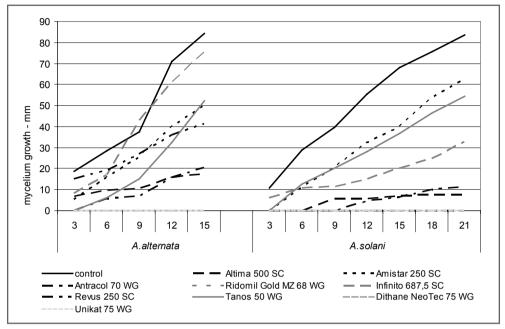


Figure 1. Influence of a fungicide supplement on development of mycelium growth

Tested fungicides showed differences of efficacy in control of two species of *Alternaria* genus (fig.2). Fungicide efficacy in control of *A. solani* balanced between 25,2-100% and *A. alternata* varied between 38,2-100%

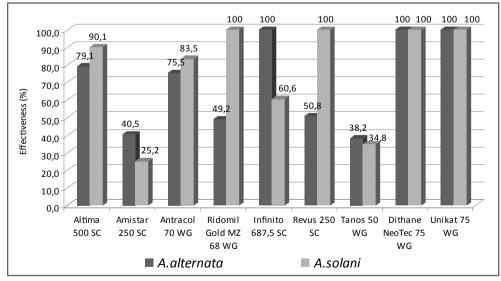


Figure 2. Effectiveness of some fungicides in growth inhibition of A. alternata and A. solani

Some of the fungicides inhibited growth of the both species at the same level. The highest influence on inhibition of development rate was observed after "application" of Unikat 75 WG and Dithane Neo Tec 75 WG (effectiveness = 100%). A few of fungicides showed better effectiveness in inhibition of *A. alternata* (Infinito 687,5 SC - effectiveness = 100% and Amistar 250 SC - effectiveness = 40,5%).

Some of the tested fungicides were more effective against *A. solani* (Revus 250 SC & Ridomil Gold MZ 68 WG - effectiveness = 100%, Altima 500 SC - effectiveness = 90,1%, Antracol 70 WG - effectiveness = 83,5%). Efficacy of the tested active substances is shown in table 4.

**Table 4**. Characteristics of some fungicide active components in inhibition of mycelium growth of *Alternaria* species in laboratory conditions

Efficacy-%	A. alternata	A. solani
75,1 - 100	mancozeb; zoxamide+mzb; fluopicolide+propamocarb- HCl; fluazinam; propineb;	mancozeb; mandipropamid; mefenoxam+mzb; zoxamide+mzb; fluazinam; propineb;
50,1 -75,0	mandipropamid	fluopicolide+propamocarb-HCl;
< 50,0	mefenoxam+mzb; azoxystrobine; cvmoxanil+famoxadone	cymoxanil+famoxadone; azoxystrobine

#### DISCUSSION

Plant protection aiming at prevention of pest occurrence and suppression of disease development faces various problems for instance; pathogen variability often is affected by biological and environmental factors.

Chemical protection is one of the methods applied in early blight management programs, particularly in protection of susceptible potato cultivars. Due to increasing problem of early blight on potato crops, potato management programs should also consider effectiveness of fungicides included in control of this disease. Various active ingredients are registered for the control of the early blight in different countries. In Poland this list includes chlorothalonil, fluazinam, metiram and mixtures famoxadone+cymoxanil, mefenoxam+mzb, fluopicolide +propamocarb-HCl, zoxamide+mzb. It is well known that in case of the early blight, the disease, which is difficult to control the efficiency of applied fungicide (in field conditions) at a level of 60-70% is satisfactory. Zitter (1984), Fry (1994) and Stevenson & James (1997) recommend routine chemical treatments against the early blight.

The proper protection against the pathogen relies on few basics, among other things, the selection of fungicide, an application at appropriate dose, time of season, and correct spraying technique. Protection of potato plants against early blight was started at first symptoms. The causal agent of early blight attacks plants that are weaker or older (Rotem, 1966) so first symptoms of the disease might be missed due to abundant foliage development. Hence, there is a necessity to develop a model of monitoring of early blight development and systems of forecasting that would allow determining more preciously time of first treatment.

Control of the early blight is of economic importance. Unsatisfactory efficacy of some fungicides in early blight control on potato crops could be also a result of application of less efficient products, their late application and may be composition of *Alternaria* populations in different regions. In midwestern regions of the USA the early blight is considered as a disease of more economic importance than the late blight and its causal agent *A. solani* as an endemic species. (Gudmestad & Pasche, 2007). The long lasting observations and research conducted in Poland (Kapsa & Osowski, 2007) or eastern and southern Germany in the years 2002 – 2006 revealed more frequent occurrence of *A. alternata* (Hausladen *et al.*, 2004, Hausladen & Leiminger, 2007). *A. alternata* is also responsible

for potato early blight in Israel and Brasil (Droby *et al.*, 1984; Boiteux & Reifschneider, 1994). In other countries such as the USA and the Netherlands - *A.solani* is a dominating species. (Johnson *et al.*, 1986; Shtienberg *et al.*, 1996; Wiik (2004); Bouwman & Rijkers (2004); Gudmestad & Pasche 2007).

The results of the experiments carried out in Bonin under laboratory conditions, showed different effectiveness of tested fungicides in limiting development of two species of fungus *Alternaria*, which could confirm dissimilar efficiency of the same fungicides on different areas of their application.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Consento: New experiences on the control of late blight 2007-2008

### A summary of recent data with Consento in Europe

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#### SUMMARY

Since its introduction on the European market in 2004, Consento has provided consistent protection against potato late blight with additional early blight efficacy.

Recent laboratory studies demonstrate the benefits of using Consento in the changing context of *Phytophthora infestans* epidemiology in North-European countries. Thanks to the combination of the active substances fenamidone and propamocarb-HCl, Consento proved to be as effective on both A1 and A2 mating types. This includes also metalaxyl-resistant strains and 13\_A2 isolates ("A2 Blue 13") which show high aggressiveness and are now widely distributed in Europe.

In addition, field trials performed in 2007-2008 and the experience from practice in the Nordic countries confirmed the sustainable performance of Consento against potato late blight, especially in the severe weather conditions of summer 2007.

Thanks to its diverse biological features (control of both direct and indirect germination of *Phytophthora infestans*, activity on all phenotypes, protection of new growths, rainfastness, dual late blight and early blight control), Consento is considered today as a solid basis in the fungicide programs of potato protection in Northern Europe.

#### INTRODUCTION

Phytophthora infestans populations have been in constant evolution in Europe since the 1970's. Significant changes have been observed in the recent years with the rapid development of the 13\_A2 sub-population and the more widespread phenylamide resistance. Potato late blight is more than ever a major concern for potato growers who are looking for fungicides offering sustainable disease control even in the most adverse climatic conditions as observed in 2007 in North-European countries.

In the light of this new and changing potato late blight background, recent studies were initiated to demonstrate the value of using Consento (fenamidone + propamocab-HCl), in spray programs.

The first part of this paper summarizes laboratory tests which show the biological profile of the fenamidone component on various A1 and A2 mating types including 13\_A2 genotypes and metalaxyl resistance. In the second part, greenhouse and field trials conducted with Consento are reported with a focus on practical experience with this fungicide in Denmark.

#### REFERENCE FUNGICIDES AND TEST PRODUCTS

Fungicides tested in the different studies are listed table 1.

Table 1. Description of fungicides and rates of use in field trials 2007-2008

		Formulation type Dose rate		Mobility in the plant
fenamidone + propamocarb-HCl (= Consento)	75+375	SC	2.0 L/HA	Translaminar + systemic
fluazinam	200	SC	0.4 L/ha	Contact
zoxamide + mancozeb	8.3+66.7	WG	1.8 Kg/ha	Contact + contact
cyazofamid	400	SC	0.2 L/ha (+ 0.15 L/ ha adjuvant)	Contact
fluopicolide + propamocarb-HCl	62.5+625	SC	1.6 L/ha	Translaminar + systemic
mandipropamid	250	SC	0.6 L/ha	Translaminar + contact
dimethomorph + mancozeb	90+600	WG	2.0 Kg/ha	Translaminar + contact
cymoxanil + 40+400 WG		2.0 Kg/ha	Translaminar + contact	

#### LABORATORY AND GREENHOUSE STUDIES

#### Comparative efficacy of fenamidone against A1 and A2 mating types

No shift in the sensitivity of *Phytophthora infestans* European populations to fenamidone has been detected from 1995 to 2000 (corresponding to the base-line establishment). Since the introduction of QoI based products for potato late blight control, the EC50 value of fenamidone has been determined according to mating type of the isolates tested (EC50 = effective concentration which gives 50% disease reduction in a leaf disc test). In table 2, below, the EC50 of fenamidone calculated for A1 mating type isolates did not differ significantly from the EC50 calculated for A2 mating type isolates. Therefore, as there is no difference in EC 50 values for A1 and A2, it can be concluded that fenamidone is equally effective on both A1 and A2 mating types of *P. infestans*.

Table 2. Fenamidone EC50 values for A1 and A2 mating types of P. infestans

Mating type	EC 50 (mg/l)	
A 1	5.8 – 12	
A 2	3.4 – 9.4	

#### Sensitivity of metalaxyl resistant isolates to fenamidone

Metalaxyl (phenylamide) resistance first developed in the 1980's (Carter *et al.*, 1982). Cross resistance studies have been conducted with metalaxyl resistant isolates, and the EC90 values are summarized in table 3. As fenamidone was equally effective on metalaxyl sensitive and resistant *P. infestans* no cross resistance occurs between fenamidone and metalaxyl.

Table 3. Fenamidone: EC 90 value for metalaxyl sensitive and resistant strains

P. infestans strain	characteristic	fenamidone	metalaxyl
F 19	metalaxyl resistant	EC <sub>90</sub> 20 ppm	EC <sub>90</sub> 100 ppm
F 495	metalaxyl sensitive	EC <sub>90</sub> 15/20 ppm	EC <sub>90</sub> 1 ppm

#### Efficacy of fenamidone to 13\_A2 ("A2 Blue 13") populations

With the increasing spread of 13\_A2 genotypes across Europe (cf. David Cooke), we compared the sensitivity to fenamidone of a 13\_A2 reference genotype with 3 other genotypes.

The fenamidone EC50 value of the strain 13\_A2 is equal to the EC50 calculated for two A1 isolates (table 4). In addition, another A2 isolate which was not identified as 13\_A2, proved to be slightly more sensitive to fenamidone. The results with the four isolates were in line with the fenamidone base-line whatever their mating type or genotype.

**Table 4.** *P. infestans* fenamidone sensitivity related to A1 and A2 mating type and genotype (A2 genotyping by Dr D Cooke, SCRI)

0 11 0 1				
Strain	Mating type	13_A2 genotype (David Cooke)	EC50 fenamidone (mg/L a.i.) in Consento	
Ref A1 from France	A1	-	3.02	
Ref A1 from Belgium	A1	-	3.248	
Ref A2 from Belgium	A2	No	1.439	
French A2 from 2007	A2	Yes	3.27	

#### New growth protection

Greenhouse grown potato plants were marked below the apical part with a red ring to observe the expansion of newly formed leaves between the time of treatment and inoculation. Plants were treated with fungicides in a spray cabinet with a water volume of 300 L/ha at field dose rates and placed at 18°C day / 16°C night and 60% humidity before inoculation. After a 1 or 2 week interval, plants were inoculated by spraying a sporangia suspension of *P. infestans* (40 000 sporangia / ml). Plants were then incubated in climatic chambers under controlled conditions (80 to 90% of relative humidity, 18°C day and 16°C night). Percentage diseased area on new expanded leaves was assessed

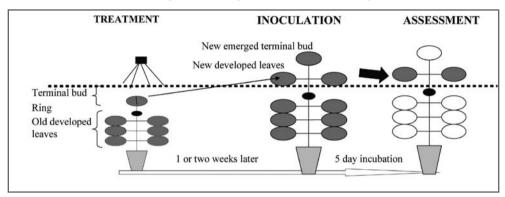


Figure 1. New growth protection methodology developed in greenhouse condition.

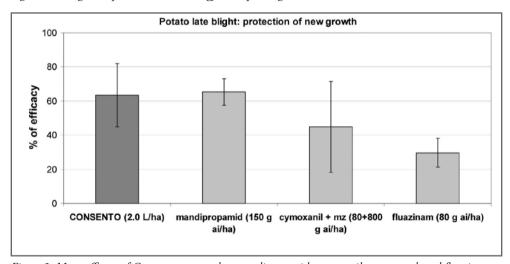


Figure 2. Mean efficacy of Consento compared to mandipropamid, cymoxanil + mancozeb and fluazinam to protect new growth.

6 days after inoculation. The data were converted into % of efficacy following Abbott formula. Figure 1 illustrates the methodology set up and figure 2 shows the results. Consento provided 50-80% control of *P. infestans* on the new expanded leaves. No significant differences were observed with mandipropamid. Both Consento and mandipropamid were more effective than cymoxanil + mancozeb or fluazinam to protect new growth. Consento provided good late blight control on newly developed leaves which can be explained by the re-distribution of the fenamidone component.

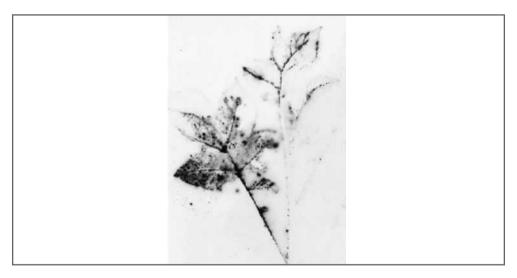


Figure 3. Re-distribution of radio-labelled fenamidone to expanded leaves after terminal bud application

This re-distribution of fenamidone from the treated terminal buds to the expanded leaves was demonstrated by studies using radio-labelled fenamidone. These results are illustrated in figure 3.

#### **EXPERIENCE WITH CONSENTO IN FIELD CONDITIONS 2007-2008**

During the last 2 years, a number of potato late blight trials and field demonstrations were implemented in Northern Europe. Various fungicide programs containing Consento were compared to standard programs.

#### Summary potato late blight efficacy trials in Germany (2007-2008)

Five field trials were implemented by Bayer CropScience in Germany in 2007-2008 in order to compare the preventive activity of Consento to market standards. Three trials conducted in Niedersachsen in 2007 and 2008 were planted according to normal practice (end of April) with the susceptible cultivar 'Bintje'. In 2008, in region Nordrhein-Westphalen, a set of two trials were planted late in the season using the cultivar 'Bintje' and the less susceptible cultivar 'Agria'. Trial design mostly followed EPPO guideline PP 1/2(4) (http://pp1.eppo.org) with complete blocks, full randomization and 3 to 4 replicates.

Fungicide applications were done using conventional sprayers every 7 days (+/- 1d), starting in strictly preventive conditions. Depending on locations, 8 to 10 sprays were applied in total. Disease severity was assessed weekly from the first application until beginning of crop senescence.

In one trial, the field performance of Consento was carefully observed under the rainy conditions of 2007. Records of *Phytophthora infestans* infections and daily rainfall data are presented in the figure below (Fig. 4).

Weather conditions experienced in Germany in 2007 were very favourable for potato late blight development. The first disease outbreak occurred in the first week of June and defoliation was almost complete within less than 3 weeks in non-protected plots. The late blight pressure remained extremely high until the end of the growing season, due to frequent rainfalls: rain events occurred

nearly every day from mid-June to mid-August in Niedersachsen.

In these extremely severe conditions, all fungicides could markedly delay the disease expansion. A differentiation between treatments was however observed from mid-July when plots treated with standard fungicides (cyazofamid, dimethomorph + mancozeb, zoxamide + mancozeb) as well as mandipropamid, rapidly reached 80 % defoliation. In contrast, Consento protected the crop for more than one week longer and the combination fluopicolide + propamocarb-HCl retained 70 % green leaves until the last assessment (Fig. 4).

Similar trials were repeated in 2008, The R-AUDPC values were calculated after the last assessment, based on disease progress through the whole season. Results are summarized and presented in the

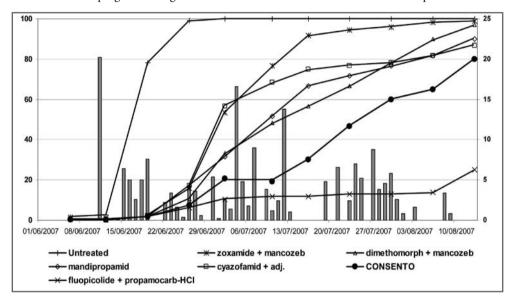


Figure 4. Potato late blight control with Consento under rainy conditions. Assessments of leaf area diseased and precipitation records in Germany, Niedersachsen, 2007

box-plot graphic chart below (Fig. 5).

In contrast with 2007, the late blight pressure was rather low in 2008 in parts of Northern Europe. Although the first disease outbreak was observed mid-May in early potatoes, the late blight risk remained at a low level until end of June and was moderate in July-August. Consento showed equivalent activity compared to the best reference fungicides – fluopicolide + propamocarb-HCl, mandipropamid and cyazofamid – and was markedly superior to fluazinam and zoxamide + mancozeb, with better disease control and higher consistency.

#### Practical experience with Consento in the Nordic countries

Consento is registered in 3 out of 4 Nordic countries (Norway, Finland and Denmark) using the trade name "Tyfon". Registration in Sweden is still pending, to be able to cover the whole Nordic region.

Tyfon is mainly used in the early stages of growth of potato, where the systemic and the translaminar

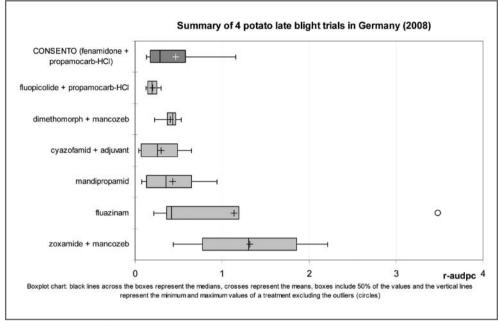


Figure 5. Potato late blight control with Consento (R-AUDPC). Summary of 4 efficacy trials, Germany, 2008.

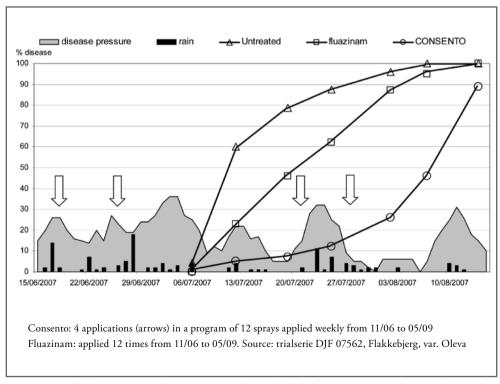


Figure 6. Late blight protection with Consento (=Tyfon) under high disease pressure. % disease on leaves related to rainfall and disease pressure (Denmark, 2007)

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properties can be utilized, and in periods with high risk for a high disease pressure. In addition, the advantage of a good side effect against *Alternaria spp.* is noticeable.

In Denmark several trials demonstrate that Tyfon gives good control when used in periods of high disease pressure. Especially in 2007 under the very high disease pressure which characterized this year, trials demonstrated that if Tyfon was used just before the outbreak of disease, very good protection was obtained (see Fig. 6).

A small survey carried out in the 3 countries (Norway, Finland and Denmark) confirms that farmers which have used the product were very satisfied.

#### CONCLUSIONS

Five years after its introduction on the North-European market, Consento (=Tyfon) confirmed its very consistent potato late blight control in different conditions and changing late blight populations. Experience from farmer practice and from efficacy trials performed during the last 2 years show that Consento meets the efficacy level of common standards, even under extremely high disease pressure of 2007, and provides an additional activity against early blight.

This consistent late blight protection is the result of complementary biological features of fenamidone and propamocarb-HCl combined in a high quality formulation:

Control of *Phytophthora infestans* at all key steps of its life-cycle, especially direct and indirect germination

Effective control of A1 and A2 mating types, including 13\_A2 ("A2 Blue 13") genotypes Control of *P. infestans* metalaxyl-resistant strains

Good protection of new growth

Good resistance to rainfall

In conclusion, Consento is a robust tool to control foliar blight caused by the new aggressive *P. infestans* isolates and to prevent resistance development. It provides potato growers with a sustainable solution for late blight control and a sound foundation for early season management of late blight.

#### **AKNOWLEDGEMENTS**

We would like to thank Bayer CropScience colleagues for their dedicated work with Consento and Dr David Cooke and colleagues at SCRI for their support in the preparation of the present paper.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

# Infinito: A unique fungicide with multiple activity for tuber blight control - EU experiences 2005-2008

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#### **SUMMARY**

Launched in the UK in 2006, and subsequently registered for use in Belgium, Germany, Poland and the Netherlands, Infinito \* combines the novel fungicide fluopicolide with propamocarb-HCl for control of potato late blight *Phytophthora infestans*. This combination provides robust, quick-acting late blight efficacy with strong anti-sporulant activity and long lasting performance, resulting in excellent and consistent control of foliar, stem and tuber blight in late blight programmes. Its multiple action give a robust basis for protection against tuber blight and this paper presents results from trials carried out in Europe from 2005 to 2008, showing the benefits of using Infinito.

#### **KEYWORDS**

Phytophthora infestans, tuber blight, fluopicolide

#### INTRODUCTION

Tuber infection from late blight, *P. infestans* is a threat to all potato crops affecting not only marketable yield but also their storage potential. Infection of tubers can occur when zoospores or sporangia produced on the aerial parts of the plant are washed down the stems or through the soil profile of the ridges. The zoospores are capable of swimming in the soil moisture film and when soils are at or near to field capacity the risk of tuber blight is higher, although good well-formed ridges with few cracks can help reduce tuber infection. Soils artificially or naturally infected with sporangia can remain infective to potato tubers from as early at the first onset of tuber formation for up to 77 days (Andrivon, 1995; Dubey and Stevenson, 1996; Evenhuis *et al.*, 2005; Zan, 1962). Historically, protection of tubers from infection relied on the use of late blight fungicides containing fentin hydroxide or fentin acetate at the end of late blight fungicide programmes, but since the withdrawal of these sporicidal materials new strategies have been developed.

Protection of tubers from late blight infection is usually through the application of foliar late blight fungicides with some or all of the following properties:

- the ability to limit sporulation of the disease on the leaves and stems
- a good sporicide so that sporangia or zoospores are killed by the fungicide before they can infect
  tubers. Rainfall and irrigation are important in the redistribution or dilution of fungicide and
  its movement and hence the viability of sporangia in soil.
- the ability of fungicides to affect viability and infectivity of sporangia during their formation, leading to less infectious inoculum should lesions develop on the plant.

Infinito is active at all stages of the life cycle and its multiple action is key to its activity against tuber blight (Latorse *et al.*, 2006). When used from tuber initiation onwards, the fluopicolide component in Infinito protects tubers by reducing the amount of viable inoculum, its fast sporicidal activity, preventing the release of zoospores from the sporangia (Tafforeau *et al.*, 2005; Latorse *et al.*, 2007).

#### MATERIALS AND METHODS

Replicated small plot trials were carried out by external co-operators and Bayer CropScience in France, Germany, the Netherlands and the UK. Applications were made every 7 days throughout the growing season using pressurised knapsack equipment through flat fan nozzles calibrated to deliver 200-400 l/ha. Infinito was applied either throughout the season or as blocks of sprays in a spray programme of different late blight fungicides. Commercial standards shown in Table 1 were applied at recommended doses for the different countries. Field trials were carried out on potato crops with cultivars sensitive to late blight either in areas at high risk of late blight infection or where artificial inoculation and misting/irrigation could be carried out to stimulate the development of late blight epidemics. Where artificial inoculation was made, it was with isolates recently collected from potato crops and took place in unsprayed guard areas in each trial.

Samples of a minimum of 100 tubers were taken at harvest and stored for at least 6 weeks prior to assessing tuber blight.

Where possible, data were analysed statistically using an analysis of variance and LSD test at the 5% probability level or Duncan's Multiple range test.

**Table 1.** Fungicides and tested dose rates used in the field trials

Treatments	g a.i./ha	L or Kg/ha
<b>Infinito</b> (fluopicolide + propamocarb)	75+750 – 100+1000	1.2 - 1.6 L/ha
cyazofamid + adjuvant	80	0.2 + 0.15 L/ha
fluazinam	200	0.4 L/ha*
metalaxyl-M + mancozeb	76+1216 or 80+1280	1.9 or 2 Kg/ha
mandipropamid	150	0.6 L/ha
cymoxanil + mancozeb	90 +1360	2.0 Kg/ha*
zoxamide + mancozeb	15+1200	1.8 Kg/ha
mancozeb	1687	2.25 Kg/ha

<sup>\*</sup> unless otherwise stated

#### RESULTS AND DISCUSSION

#### Netherlands

Figure 1 shows a box-whisker plot summarizing results from 6 trials carried out by Bayer CropScience in the Netherlands, two in each of three different seasons from 2005 to 2007. Treatments were applied from 7 to 12 times throughout the season at 7 day intervals. Tubers were sampled at harvest and assessed for tuber blight after a period of storage. Results with Infinito at 1.2 or 1.6 L/ha show the very consistent and high level of tuber blight control achieved.

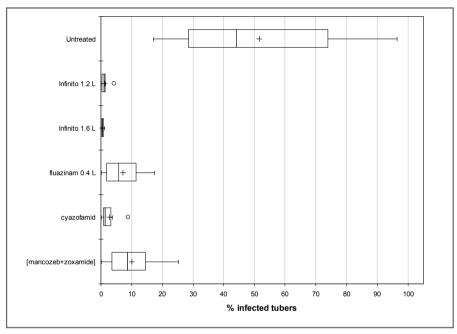


Figure 1. Control of tuber blight (mean of 6 trials Netherlands, 2005-2007)

Table 2 shows results from a trial carried out by Applied Plant Research (PPO) in the Netherlands from 2005. Programmes commenced with 3 sprays of mancozeb, followed by 2 applications of cymoxanil + mancozeb. When the tubers were 28-35 mm in size, the first of six weekly applications of Infinito, cyazofamid, fluazinam or mancozeb was made. After desiccation, tubers were sampled at harvest and assessed for tuber blight both at harvest and 4 weeks later. Untreated plots became heavily infected with foliar blight, which in turn caused heavy tuber blight infection. Infinito showed excellent tuber blight protection in the trial.

**Table 2.** Control of tuber blight (Applied Plant Research, the Netherlands, 2005)

Treatment	AUDPC	% infected tubers
Untreated Control	277.0	16.6 b
mancozeb	77.2 c	18.8 b
fluazinam	62.0 abc	7.5 a
cyazofamid	48.4 ab	6.8 a
_Infinito 1.6 L/ha	41.6 a	6.0 a
LSD (excluding untreated control)	16.97	n/a

Means not followed by the same letter are significantly different (p=0.05)

In 2008 a series of 4 trials was carried out by Bayer CropScience to compare programmes with Infinito positioned mid-season. Applications were made every 7 days from crop emergence and programmes consisted of the following treatments:

Reference 1: 9 x cymoxanil+ mancozeb 2.5 kg/ha, 3 x fluazinam 0.4 L/ha

Reference 2: 4 x cymoxanil+ mancozeb 2.5 kg/ha, 5 x cymoxanil+ mancozeb 2.5 kg/ha + fluazinam 0.3 L/ha, 3 x fluazinam

Reference 3: 4 x cymoxanil+ mancozeb 2.5 kg/ha, 8 x fluazinam

Reference 4: 4 x cymoxanil+ mancozeb 2.5 kg/ha, 5 x cyazofamid, 3 x fluazinam

Infinito 1.2 L/ha: 4 x cymoxanil+ mancozeb 2.5 kg/ha, 5 x Infinito 1.2 L/ha, 3 x fluazinam

Infinito 1.6 L/ha: 4 x cymoxanil+ mancozeb 2.5 kg/ha, 5 x Infinito 1.6 L/ha, 3 x fluazinam Untreated control

Infector plants were placed in the spreader rows after the third spray. Tubers were sampled at harvest and results are shown from assessments made after storage. The results shown in Figure 2 demonstrate that the use of Infinito either at 1.2 or 1.6 L/ha, in a block of sprays mid-programme, gives consistent reduction of tuber blight.

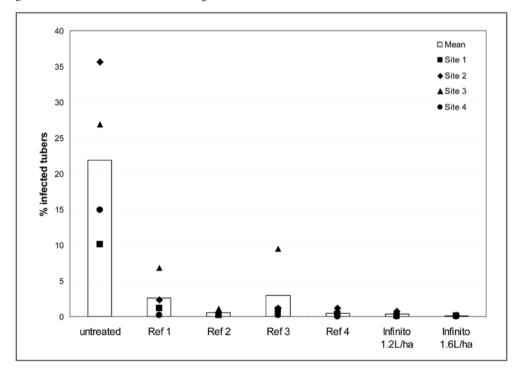


Figure 2. Control of tuber blight in spray programmes (4 trials, Netherlands 2008)

#### France

In 2 trials carried out by Bayer CropScience in France in 2006 (Figure 3), four applications of Infinito 1.6 L/ha were compared with fluazinam and cyazofamid. In both trials, Infinito gave the best reduction of tuber blight.

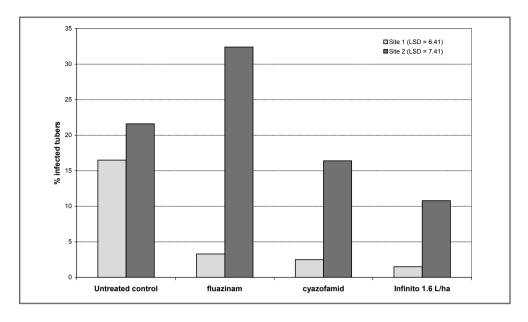


Figure 3. Control of tuber blight (mean of 2 Bayer CropScience trials, France 2006)

#### Scotland

In a trial carried out for Bayer CropScience at SAC, Auchincruive in 2005 (Figure 3), the use of Infinito in programmes was evaluated. Programmes commenced with five or six applications of cymoxanil + mancozeb and were followed by applications of fungicides with activity against tuber blight (Infinito, cyazofamid or fluazinam) as the blight began to establish in the untreated plots (c.1%). The 2005 season was generally low risk for blight at this site and there were only 3 high risk periods recorded. Although there was a lot of blight on the field, all the fungicide treated plots were very well protected during the growing season and had negligible amounts of foliar blight at the final assessment. Applications commenced on 30 June and the final spray was made 3 October 2005.

Table 3 shows the final foliar blight assessment, the blight-free yield measured at harvest, and the incidence of tuber blight, recorded both pre- and post-storage. The untreated control was excluded from the statistical analysis. Results show that despite it being a relatively low blight season, all the programmes gave excellent foliar blight control and increased yields by 24 to 27 tonnes /ha. Tuber blight levels were very low in all treatments. The lower (1.2 L/ha) dose rate of Infinito had slightly more tuber blight than other treatments, but levels in all treatments were less than 2% infection compared with 12% in the untreated control.

**Table 3.** Control of tuber blight and blight-free yield (SAC Auchincruive, Scotland 2005)

Fungicide	Dose (kg or l / ha)	Number of sprays	% Foliar blight at last assessment	Blight-free yield (t/ha)	% blight infected tubers (pre- and post-storage)
Untreated control	-	-	98.0	34.13	12.3
cymoxanil+mancozeb	2.0	6	0.0	60.64	1.8
Infinito	1.2	5			
cymoxanil+mancozeb	2.0	6	0.0	60.80	0.5
Infinito	1.6	5			
cymoxanil+mancozeb	2.0	5			
Infinito	1.6	3	0.1	63.13	1.2
fluazinam	0.3	3			
cymoxanil+mancozeb	2.0	6	0.1	59.39	0.2
cyazofamid + adjuvant	0.2 + 0.15	5			
cymoxanil+mancozeb	2.0	5			
cyazofamid + adjuvant	0.2 + 0.15	3	0.2	58.00	1.0
fluazinam	0.3	3			
F pr.			0.014	0.873	0.852
LSD (P=0.05)			0.131	8.33	2.41

#### Northern Ireland

Following on from 2003-2006 field trials reported by Cooke and Little (2007), a further trial was carried out by AFBI, Belfast using the same methodology in 2007 to compare Infinito programmes. The programmes were:-

A standard programme = 2 x metalaxyl-M+mancozeb followed by 8 x fluazinam 0.3 L/ha Bayer CropScience prog "A" 2 x metalaxyl-M+mancozeb, followed by fluazinam 0.3 L/ha alternating with Infinito (4 sprays of each). First Infinito sprayed 10 July.

Bayer CropScience prog "B" 2 x metalaxyl-M+mancozeb, followed by 1 x fluazinam, 4 x Infinito in a block (1st spray to coincide with visible infection, 10 July), 3 x fluazinam.

Infinito was applied at 1.6 L/ha, and the trial was inoculated 3rd July (3 full Smith periods 1-4 July, Smith criteria met on 5 of first 6 days in July and 10 days in first 3 weeks).

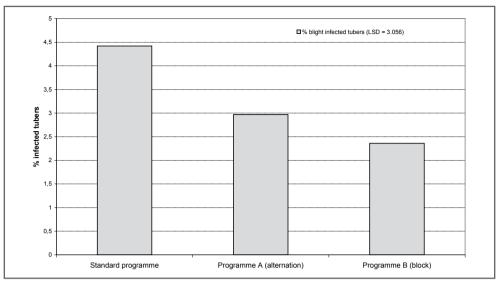


Figure 4. % incidence of tuber blight by weight (all tubers pre- and post-storage assessments -AFBI, Belfast 2007)

The results in Figure 4 show that Infinito based programmes reduced levels of tuber blight compared with a standard fluazinam based programme and the blocked programme B was slightly but not significantly better. Although blight levels were relatively low in all treatments, these results indicate that a block of Infinito applied mid-season when the blight risk was high can be beneficial in the control of tuber blight.

## Germany

Results from a Bayer CropScience trial in Germany in 2008 (Figure 5) demonstrated the benefits of an Infinito programme. Three programmes of 10 spray applications were compared:-

Reference programme 1: 1 x metalaxyl-M+mancozeb, 9 x cymoxanil+mancozeb

Reference programme 2: 1 x metalaxyl-M+mancozeb, 7 x cymoxanil+mancozeb, 2 x fluazinam Infinito programme: 1 x metalaxyl-M+mancozeb, 3 x cymoxanil+mancozeb, 4 x Infinito 1.6 L/ha, 2 x fluazinam

Applications were made at 7 day intervals with the final application made on 12 August 2008, and tuber blight was assessed 12 October 2008.

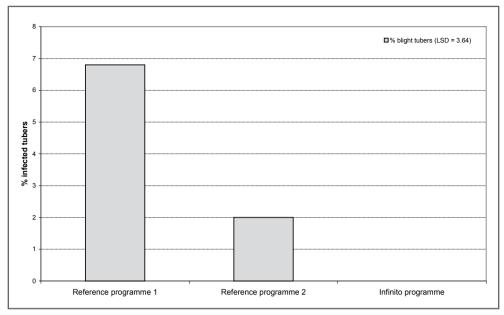


Figure 5. % incidence of tuber blight (Bayer CropScience, Germany 2008)

In two trials carried out by the Amtlicher Dienst (NRW) in the north of Germany, a season-long application programme of Infinito at 1.6 L/ha was compared with programmes of fluazinam and cyazofamid. High levels of tuber blight occurred and all treatments greatly reduced tuber blight Figure 6.

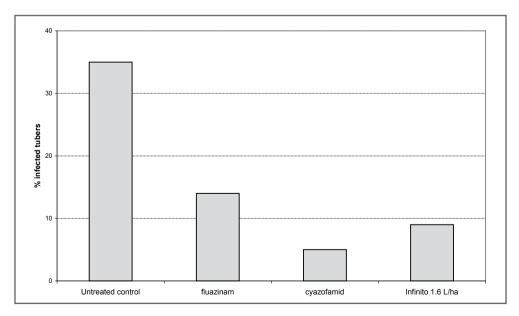


Figure 6. % incidence of tuber blight (Mean of 2 trials, Amtlicher Dienst, NRW, Brentler, Germany 2006)

#### CONCLUSIONS

Control of potato late blight, to protect both tubers from infection and maintain the yield potential of the crop, is of paramount importance for growers. Tuber infection can cause the grower a number of problems which reduce profitability. The crop may be unsaleable or the grower's costs may be increased if blighted tubers need to be removed before sale. Tuber blight can also reduce a crop's storability (by predisposing crops to bacterial soft rots) and in severe situations this can mean that crops are completely lost during storage. The level of tuber blight infection is not always related to the level of foliar blight in a crop, and the use of a fungicide which is effective in both controlling foliar blight and reducing tuber blight can help in protecting the crop not only during the growing season, but also after harvest and during storage.

Infinito's unique multiple action of long-lasting, protectant foliar blight activity; protection of new growth; translaminar, anti-sporulant and sporicidal activity; and the ability to reduce spore viability and germination all contribute to its excellent all-round protection of the potato crop.

In the EU trials reported here, Infinito has given consistent and robust control of tuber blight and has become recognized as an important part of late blight fungicide programmes. Bayer CropScience therefore recommend Infinito at a dose rate of 1.2 to 1.6 L/ha according to blight risk, applied at 7 day intervals, either as a block or in alternation with other fungicides mid-season, when risk of late blight and tuber infection is high.

#### **ACKNOWLEDGMENTS**

We would like to thank colleagues in Bayer CropScience and scientists from AFBI, Belfast, Northern Ireland; Amtlicher Dienst, Germany; PPO-AGV, Lelystad, Netherlands; SAC, Auchincruive, Scotland who contributed to the development of Infinito and the trials reported here.

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# Eleventh EuroBlight workshop Hamar (Norway), 2008

# Mandipropamid - protection of new growth from infection with late blight (*Phytophthora infestans*) in potatoes

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#### **SUMMARY**

Mandipropamid formulated as REVUS SC250 and recommended at 0.6 l/ha or 150 g a.i./ha is a highly effective new fungicide for the control of late blight in potatoes. It is recommended as preventive treatment. Mandipropamid prevents the germination of spores of *Phytophthora infestans* at very low concentrations. Its affinity to the waxy layer of plant surfaces assures long lasting efficacy and excellent rainfastness. Gradual uptake of small amounts of the active ingredient into the plant tissue provides good translaminar and limited curative and antisporulant activity. Results of special field / laboratory tests carried out in 2007 and 2008 also show that mandipropamid provides excellent control of expanding leaves. The amount of mandipropamid absorbed by the buds or small leaves prove to be sufficient to fully protect expanding leaves from disease infection. It can be assumed that mandipropamid absorbed by the buds or small leaves is redistributed with the growing plant tissue. Due to the high intrinsic activity of mandipropamid disease control is maintained in the expanding plant tissue. In these tests mandipropamid was at least as effective in protecting new growth as cyazofamid + adjuvant and clearly better than other reference standards. Rainfastness, long-lasting activity and protection of new growth are key requirements for reliable preventive control of late blight in potatoes under field conditions, especially during the period of rapid vegetative growth.

#### **KEYWORDS**

Mandipropamid, Phytophthora infestans, protection of new growth

#### INTRODUCTION

Mandipropamid is a new fungicide against foliar Oomycete pathogens developed by Syngenta. The chemical and physical properties of mandipropamid, its biological activity and its safety profile were first described in 2005 (Huggenberger *et al.*, 2005; Hermann *et al.*, 2005; Knauf-Beiter and Hermann, 2005). REVUS SC250 containing 250 g mandipropamid per litre formulated product and recommended at 0.6 l/ha was first used commercially for the control of late blight in potatoes in the season 2007.

This paper summarizes results from special field / laboratory tests carried out in the seasons 2007 and 2008 to assess the efficacy of mandipropamid in protecting new growth. Protection of new growth was defined by Bradshaw (2005) as follows: The ratings for the protection of new growth indicate the protection of new foliage due to the systemic or translaminar movement or the redistribution of a contact fungicide. New growth consists of growth and development of leaves present at the time of the last fungicide application and / or newly formed leaflets and leaves that were not present.

#### **METHODS**

In all trials reported here the applications of the different products tested were made in standard replicated field trials.

## Trials carried out by Syngenta

In the trials carried out by Syngenta at Stein in Switzerland in 2007 and 2008 leaves approximately ¼ of the final size were marked just before the first treatment. The day after the treatment (after the first sampling) 15-17 mm precipitation as natural rain or irrigation was applied to the field plots. Marked leaves were sampled at 1, 3, 6 and 12 days after the treatment (DAT). At each sampling a total of 20 leaflets (first pair) from marked leaves were sampled from two different field plots for each product and for untreated controls. The leaves ¼ of the final size just before the first treatment were grown to full size at approximately 6 DAT.

For comparison the protection of already fully grown leaves before the first treatment was also evaluated following the same methodology in the trial in 2008.

Directly after sampling the leaflets were placed lower leaf surface upwards in plastic Petri dishes equipped with a wetted filter paper. A suspension of  $2x10^4$  sporangia/ml of *Phytophthora infestans* (strain no. 96, Syngenta culture collection) in  $H_2O$  dest. was atomized homogenously on the lower leaf surface. Afterwards the Petri dishes were sealed with parafilm and incubated in a climatic chamber at  $16^{\circ}C$  for 24 h in the dark and afterwards at  $16^{\circ}C$  and a light period of 16h/8h until evaluation.

Evaluations were carried out 5 and 7 days after inoculation. The development of the disease was assessed for each leaflet by visually estimating the percent leaf area diseased. The results are expressed relative to the leaf area diseased of untreated leaflets collected from the field.

In these trials no natural infections occurred in the field prior to the last sampling. In both trials the first treatment was made during the rapid vegetative growth phase of the crop (CS 35-59). After the last sampling additional treatments were applied in the field to assess the efficacy of the different treatments to control late natural infections in the field plots.

#### Additional trials

Six additional field / laboratory tests were carried out to further assess the protection of new growth with mandipropamid in comparison to key reference treatments. These trials were carried out and the results reported by the following third party investigators:

Kalkdijk J.R. and Schepers H.T.A.M., PPO, The Netherlands, 2007 & 2008 Bain R., SAC, United Kingdom, 2007 Nielsen B.J., Aarhus University, Denmark, 2008 Hannukkala A. and Laine P., MTT Agrifood Research, Finland, 2008 Heremans B., University College Ghent, Belgium, 2008

The methodology followed in these trials was similar as described above for the Syngenta trials. In these trials a single leaf sampling was carried out 5 or 6 days after the treatment.

#### RESULTS

The results from the trials carried out by Syngenta are shown in Figures 1 and 2.

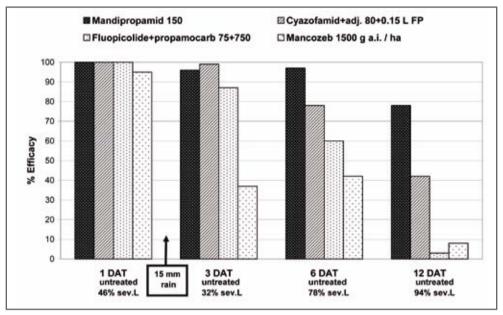


Figure 1. Mandipropamid for the protection of expanding leaves, field / laboratory test carried out by Syngenta in 2007. Leaves, which were 1/4 of the final size at the time of the treatment, were sampled 1, 3, 6 and 12 days after the treatment in the field. The evaluation of inoculated leaves in the laboratory biotest shown here was carried out 7 days after sampling.

In both trials mandipropamid at 150 g a.i./ha provided the best protection of expanding leaves and the best persistence of activity of the products tested. The rain event after the first sampling did not have any influence on the efficacy of mandipropamid. Up to six days after treatment (DAT) mandipropamid provided excellent disease control on the leaves sampled with an average efficacy of more than 90%. The efficacy was clearly lower only at the last sampling at 12 DAT.

Cyazofamid+adjuvant at 80 g a.i./ha + 0.15 litre formulated adjuvant also provided good protection of new growth. There was no obvious influence of the rain event on the efficacy of cyazofamid. However, the efficacy and persistence of this treatment was more variable.

The rain event after the first sampling clearly reduced the efficacy of fluopicolide+ propamocarb at 75+750 or 100+1000 g a.i./ha. As expected the efficacy of mancozeb at 1500 g a.i./ha was also clearly reduced. In these trials the efficacy of these products was clearly weaker than the efficacy of mandipropamid or cyazofamid.

In the trial carried out in 2008 leaves already fully grown at the time of the treatment were also sampled, inoculated and evaluated for comparison. The results (not shown) from these biotests were very similar to the results shown in Figure 2.

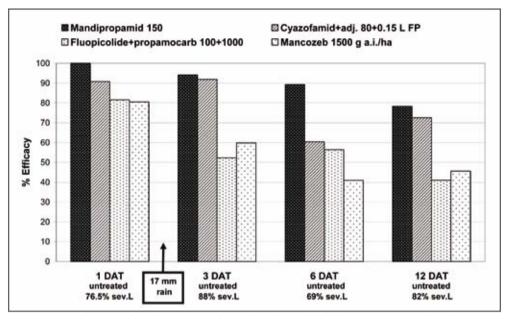


Figure 2. Mandipropamid for the protection of expanding leaves, field / laboratory test carried out by Syngenta in 2008. Leaves, which were ¼ of the final size at the time of the treatment, were sampled 1, 3, 6 and 12 days after the treatment in the field. The evaluation of inoculated leaves in the laboratory biotest shown here was carried out 5 days after sampling.

After the last sampling additional treatments were applied to the field plots to evaluate the field efficacy of the different treatment against late natural infections, which occurred in the field after the last sampling. The ranking of the efficacy of the different treatments based on the field evaluations was consistent with the ranking based on the results of the biotests in the laboratory.

An overview of the results of all trials carried out with mandipropamid for the protection of expanding leaves in 2007 and 2008 is presented in Figure 3.

This summary shows the average % leaf area infected in the laboratory biotests with leaves sampled at 6 (trial in Finland at 5) days after the treatment in the field. Levels of infection are shown for untreated controls and for the different products in the different tests. As indicated in the graph not all products were tested in all trials. The level of infection in untreated controls varied considerably between the different tests. This can be explained by variations in the test conditions of the different laboratory biotests and does not influence the conclusions concerning the efficacy of the different products.

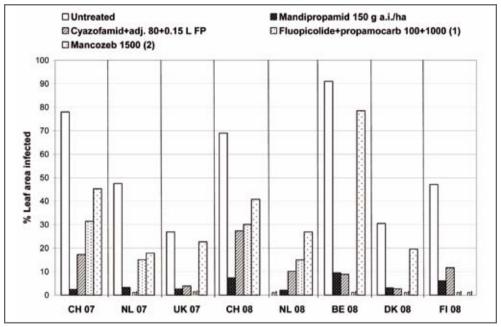


Figure 3. Mandipropamid for the protection of expanding leaves, field / laboratory tests carried out by Syngenta and by third party cooperators in 2007 & 2008. Leaves, which were clearly smaller or present only as buds at the time of the treatment, were sampled 5 or 6 days after the treatment in the field. The efficacy of the different products was evaluated in laboratory biotests.

nt = not tested; (1) fluopicolide+propamocarb tested at 75+750 g a.i./ha in test CH 07 (2) cymoxanil+mancozeb tested at 112.5+1700 g a.i./ha in tests NL 07 & NL 08; the tests were carried out in Switzerland (CH), Great Britain (UK), The Netherlands (NL) Belgium (BE), Denmark (DK) and Finland (FI)

The results summarized in Figure 3 clearly show that mandipropamid provides excellent protection of expanding leaves in all trials. On leaves treated with mandipropamid the leaf area infected was similar or clearly lower than on leaves treated with cyazofamid+adjuvant (7 comparisons). Mandipropamid was clearly better than fluopicolide+propamocarb (4 comparisons). Mancozeb or cymoxanil+mancozeb were consistently the weakest treatments (7 comparisons).

#### **CONCLUSIONS**

Mandipropamid applied as REVUS SC250 at 0.6 l/ha or 150 g a.i./ha provides consistently excellent control of expanding leaves. The amount of mandipropamid absorbed by the buds or small leaves present at the time of the treatment proves to be sufficient to fully protect expanding leaves from disease infection.

It can be assumed that mandipropamid absorbed by the buds or small leaves is redistributed with the growing plant tissue. Due to the high intrinsic activity of mandipropamid disease control is maintained in the expanding plant tissue despite a dilution effect.

In these tests mandipropamid was at least as effective in protecting new growth as cyazofamid+adjuvant and clearly better than other reference standards including fluopicolide+

propamocarb, cymoxanil+mancozeb or mancozeb alone.

Rainfastness, long-lasting activity and protection of new growth are key requirements for reliable preventive control of late blight in potatoes under field conditions, especially during the period of rapid vegetative growth.

The results summarized in this paper further document and explain the consistently excellent disease control observed, when mandipropamid is used under practical field conditions. For the potato grower demanding a high standard of control of late blight, mandipropamid is a new tool providing highly reliable protection from disease infection independent of weather conditions.

#### **ACKNOWLEDGEMENTS**

The authors would like to thank all colleagues and scientists from independent institutes, who have contributed to the studies summarized in this paper.

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# Dithane: Keeping an Old Friend Going

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#### **SUMMARY**

With respect to spectrum, efficacy, crop selectivity, resistance management and affordability mancozeb remains a unique and irreplaceable tool for the potato farmer. Since its first registration in the early 1960's, mancozeb and the Dithane brand has become established as an effective broad spectrum disease management tool for growers across the world for use in both major and minor crops. Mancozeb is registered in over 60 countries and has shown activity on more than 400 fungal species affecting more than 70 different crops. Mancozeb is especially effective against Oomycete diseases and has been particularly popular with potato and tomato growers across the world because of it's proven activity against late blight (Phytophthora infestans) and additional activity against other diseases such as Alternaria spp. In terms of tonnage, mancozeb is still one of the most extensively used fungicides in the world when applied alone or as part of a co-formulation, despite the presence of modern competitors. Mancozeb is a multi-site fungicide with no resistance issues reported since launch and is an essential tool for resistance management of other molecules. Application intervals and rate vary dependent on the crop but in potato at Annex III re-registration the minimal intervals are 7 days and a maximum rate is 1600 g as/ha. On 1 July 2006 mancozeb was included in Annex 1 to Council Directive 91/414, as published Commission Directive 2005/72/EC of 21 October 2005. The current Annex I approval will expire on 30 June 2016. Meanwhile, mancozeb formulations are undergoing re-registration post Annex I and approvals are expected in all MS during 2009 and 2010. The revision of EU 91/414 may threaten the long term future of mancozeb and all associated co-formulations but guidelines are not in place at the time of writing and so the future is difficult to predict. Dow AgroSciences are taking the industry lead to potentially extend the molecule's future availability as a critical tool for EU growers for as long as possible.

#### **KEY WORDS**

Dithane', mancozeb, *Phytophthora infestans*, Annex III re-registration, Dithane' is a trademark of Dow AgroSciences,

## INTRODUCTION

Mancozeb and the Dithane brand remains a critical tool in the hands of EU farmers for managing a wide range of economically important diseases in major and minor crops. Mancozeb's spectrum of disease control is unmatched and the molecule has almost a 50 year history of providing excellent and above all cost effective disease control. Of particular significance is the vital role mancozeb plays in control of the potentially devastating Oomycete diseases inflicting potatoes, vines and tomatoes. Late blight is a fungal disease caused by *Phytophthora infestans* on potatoes and other members of the Solanaceae. It is the most important disease of potatoes, affecting haulm and tubers, with crop

losses in 1846 reported to be about 80%. The socioeconomic impact of the disease is less significant today, though it is generally accepted that yields are reduced dramatically in the absence of disease control and crops need to be sprayed on a minimum weekly basis when the threat of disease is high.

Since it's introduction onto the market in the early sixties, mancozeb and the Dithane brand has enjoyed unparalleled success as a tool for potato and tomato growers through out the world to protect their crops from many diseases. The major reasons for the popularity of the product are presented below and are these characteristics are still highly applicable today:

Mancozeb is a relatively low cost product which provides growers with an economical solution for disease control. This factor permits broad usage in developing countries, where the cost for disease management must be kept low. It is also popular with EU potato farmers as a cost effective protectant fungicide because of the need for multiple applications. When applied correctly and at recommended label rates mancozeb continues to provide reliable and effective disease control year in year out when applied as a protectant fungicide. It is a vital component for many potato blight sprays either as a straight product or when formulated in a mixture with other active ingredients. If the registration of mancozeb is lost then all associated co-formulations with the active ingredient will also be removed from the market and product choice will be limited for the grower. Mancozeb plays a critical role in helping to manage resistance risk when used in combination or alternation with more active systemic products. Despite nearly half a century of intensive use, no incidents of field resistance have been reported for mancozeb formulations. Mancozeb's multi-site activity makes it a desirable blend partner for single site fungicides as part of a resistance management strategy.

Mancozeb is an extremely flexible product with respect to compatibility and safety in tank mix with other fungicides, insecticides and herbicides. In addition, mancozeb can be applied via air, ground sprayers, chemigation or seed treatment providing further flexibility to growers.

In general, crop safety with mancozeb is excellent and there are no rotational issues associated with its use.

Mancozeb is not toxic to bees and has a very good environmental profile with no known issues of leaching.

Mancozeb provides additional control of *Alternaria spp.* which many of the more recently launched late blight actives do not control. Although there are other alternatives such as azoxystrobin for *Alternaria spp.* control in potato and tomato, it does not provide control of late blight. Mancozeb is an essential component of the fungicide spray programme where both diseases are prominent.

Mancozeb supplies micronutrients in the form of Manganese and Zinc to crops. In situations where these elements are limiting crop greening effects are frequently visible.

Breadth of spectrum and registrations in multiple crops around the world. Broad labels are popular with growers looking for single products which can be used on farm to treat an array of disease problems in a range of crops.

Dithane offers regulatory, quality and formulation benefits over other mancozeb products.

This paper charts the history of mancozeb and the Dithane brand. It identifies the uniqueness of the product, why it was essential in the past and is a critical tool for growers today. The journey ahead is uncertain in a highly challenging regulatory environment but Dow AgroSciences will do all it can to extend the product life.

#### THE HISTORY OF ETHYLENEBISDITHIOCARBAMATES (EDBCS)

The active ingredient mancozeb is composed of a mixture of manganese ethylenebisdithiocarbamate and a zinc salt. The chemical class is ethylenebisdithiocarbamate (EDBC). McCallan (1967)

produced an excellent review of this class of products at a time when they were becoming established as key tools for the management of plant diseases. These compounds were among the first synthetic broad spectrum fungicides to be developed. However, it took many other compounds to be developed before mancozeb was finally born in the early 1960's. The first dithiocarbamate commercialised as a fungicide was tetramethylthiuram disulfide, more commonly known as Thiram for which a patent was granted in 1934. Within a few years the next generation of broader spectrum dithiocarbamates followed which included Ferric dimethyldithiocarbamate (Ferbam) and Ziram (zinc dimethyldithiocarbamate). In the early 1940's W. F Hester of the Rohm and Haas company prepared his compound disodium ethylenebisdithiocarbamate (Nabam) which can be considered as the first true EBDC. Nabam's was a relative instable compound until the discovery that adding zinc sulphate to the spray tank had a stabilizing effect on the Nabam and formed zinc ethylenebisdithiocarbamate (Zineb). In 1950 DuPont was granted a patent for manganese ethylene bisdithiocarbamate (Maneb) (Flenner, 1950). In 1961/1962 Rohm and Haas registered the zinc ion complex of maneb (Mancozeb) which was to become the most important and commercially significant of all the EBDCs and the Dithane' brand was born. By the mid 1960's the ethylene bisdithiocarbamate fungicides were considered to be the most important and versatile group of organic fungicides yet discovered. They were the first broad spectrum disease control chemicals available to growers. Mancozeb is now registered throughout the world in over 60 countries and has shown activity on more than 400 fungal species affecting more than 70 different crops. The physical and chemical properties of mancozeb are shown in Table 1

Table 1. Physical and chemical propertie

Common name (ISO)	mancozeb
Chemical name (IUPAC)	manganese ethylenebis (dithiocarbamate) (polymeric) complex with
	zinc salt
Chemical name (CA)	[1,2-ethanediylbis[carbamodithioato](2-)] manganese mixture with
	[1,2-ethanediylbis [carbamodithioato] (2-)] zinc
	(9CI)[ethylenebis(dithiocarbamato)]manganese mixture with
	[ethylenebis(dithiocarbamato)]zinc (8CI)
CAS number	8018-01-7 (formerly 8065-67-5)
CIPAC number	34
Minimum purity	800 g/kg
Technical purity	85 to 90%
Molecular formula	(C4H6MnN2S4)x(Zn)y
Molecular mass	271.3
Structural formula	Mancozeb is a polymeric complex of the monomer illustrated which
	contains 20% manganese and 2.5% zinc
s   S   S   S   S   S   S   S   S   S	- Mn $-$ Zn <sub>y</sub>
	-x
Appearance	Yellowish powder (80%)
Appearance Odour	Yellowish powder (80%) Musty odour
Odour	Musty odour
Odour	Musty odour Density: 1.9938 g/ml at 20°C (81.5%)
Odour Relative density	Musty odour Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%)
Odour Relative density Bulk density	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%)  Relative density: 1.976 g/ml at 22°C. (80%)  0.35-0.60 g/cm³
Odour Relative density Bulk density Melting point	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%)  0.35-0.60 g/cm³  Not measurable, decomposes without melting.
Odour Relative density  Bulk density Melting point Boiling point	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%) 0.35-0.60 g/cm³  Not measurable, decomposes without melting.  Not applicable, decomposes before melting.
Odour Relative density  Bulk density Melting point Boiling point Vapour pressure	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%) 0.35-0.60 g/cm³  Not measurable, decomposes without melting.  Not applicable, decomposes before melting. 1.33 x 10-5 Pa
Odour Relative density  Bulk density  Melting point  Boiling point  Vapour pressure  Solubility in water	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%) 0.35-0.60 g/cm³  Not measurable, decomposes without melting. Not applicable, decomposes before melting. 1.33 x 10-5 Pa 2 - 20 mg/l
Odour Relative density  Bulk density Melting point Boiling point Vapour pressure Solubility in water Solubility in organic solvents	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%)  0.35-0.60 g/cm³  Not measurable, decomposes without melting.  Not applicable, decomposes before melting.  1.33 x 10-5 Pa  2 - 20 mg/l  Mancozeb is practically insoluble in organic solvents.
Odour Relative density  Bulk density Melting point Boiling point Vapour pressure Solubility in water Solubility in organic solvents Flammability	Musty odour  Density: 1.9938 g/ml at 20°C (81.5%) Relative density: 1.976 g/ml at 22°C. (80%) 0.35-0.60 g/cm³  Not measurable, decomposes without melting. Not applicable, decomposes before melting. 1.33 x 10-5 Pa 2 - 20 mg/l Mancozeb is practically insoluble in organic solvents. Mancozeb technical is not flammable.

Source: Dow AgroSciences: An Introduction to mancozeb and the Dithane® brand: Technical Bulletin 2001

#### MODE OF ACTION AND RESISTANCE MANAGEMENT

The direct effect of mancozeb upon core biochemical processes within the fungus results in inhibition of spore germination and hence the classical protectant activity shown by the molecule (Szkolnik, 1981; Wicks and Lee, 1982; Wong and Wilcox, 2001). Mancozeb is a contact fungicide and does not show curative properties when sprayed onto plants where disease has already established. Mancozeb is not translocated through the plant and shows no systemic activity either through the roots or through the leaves of plants. Mancozeb has an excellent record of crop safety over a huge range of crops and environmental conditions.

Mancozeb is classified by the Fungicide Resistance Action Committee (FRAC) in mode of action group M (Multi Site Action). The mode of action of mancozeb and it's contribution to resistance management of diseases and other active ingredients has been a major reason why the compound has stood the test of time and is still essential today. Despite nearly 50 years of use there has been no incidence of fungal resistance recorded to ethylenebisdithiocarbamates (EBDCs) including mancozeb due to their multi-site mode of action. Mancozeb itself is a pro-fungicide which when exposed to water breaks down and gradually releases ethylene bis-isothiocyanate sulfide (EBIS) which is itself converted via the action of UV light into ethylene bis-isothiocyanate (EBI). The toxic effects primarily result from the attachment of both EBIS and EBI to thiol groups, Enzymes such as dehydrogenases, which are rich in thiols, are particularly affected and thus inhibition of respiration commonly occurs in fungi susceptible to ethylenebisdithiocarbamates. Mancozeb break down products disrupt at least six different biochemical processes within the fungal cell cytoplasm and mitochondria (Ludwig and Thorn, 1960; Kaars Sijpesteijn, 1984). Examples are; disruption of lipid metabolism which affects membrane permeability; or disruption of glucides which affect respiration and production of ATP. The multi-site mode of action of mancozeb has clearly been an important characteristic in preventing development of significant fungal resistance in nearly 50 years of commercial use.

Oomycete diseases are notorious for their ability to rapidly develop resistance to single site inhibitor fungicides. In potatoes and tomatoes there is a medium to high risk of resistance developing in *Phytophthora infestans* to single site fungicides. Mancozeb is crucial as a tool both in alternation and formulated mixture to help effectively manage this risk. The same is true of new single site inhibitors entering the Oomycete market where combinations with mancozeb are routinely developed in order to improve spectrum and build in protection against developing resistance. Resistance management is an essential component of effective IPM programmes and for the European potato grower, mancozeb remains an indispensable tool for effectively managing resistance. If the future registrability of mancozeb in the EU is not maintained as a result of regulatory and political challenges, all co-formulation will also be removed from the market and this represents a large proportion of the products growers use for potato blight control.

#### SPECTRUM OF ACTIVITY

The multi site mode of action of mancozeb has demonstrated activity against a wide range of fungal and bacterial diseases groups including ascomycetes, oomycetes, basidiomycetes and imperfect fungi. Nearly five decades of use and continual development around the world have led at one time or another to registrations and or claims of efficacy in over 70 crops and 400 different diseases. The most important uses in the EU include, but are not limited to, control of early and late blights of potato and tomato, control of downy mildews on grapevines and vegetable and salad crops and control of scab on pome fruit. Potato, tomato, vine, and apple represent over 90% of the total sales of mancozeb in Europe. Figure I shows the ten most important disease groups where mancozeb based products are applied globally (Agrobase 2007). Mancozeb also plays an important role in the

production of lower acreage or so called "minor crops" where the range of fungicides available to growers are limited due to a relatively low total market value and high registration costs creating a barrier to entry. In this scenario having an economical and effective fungicide to help manage resistance concerns is vital. Table 2 shows the crops planned to be re-registered in the EU and the associated fungal diseases controlled by mancozeb.

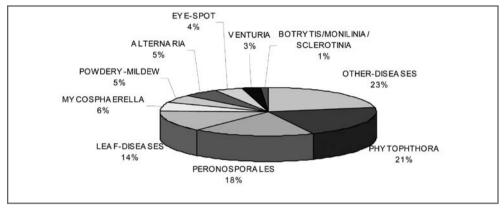


Figure 1. Ten Most Important Disease Groups which represent 96% of Mancozeb Products Value

#### THE MARKET AND WHY MANCOZEB IS STILL USED

As a highly successful commodity product mancozeb is currently produced by numerous generic manufacturers around the world with Dow AgroSciences (DAS) operating as the leading registrant and producer. It has become a truly international product in its reach and DAS currently supports registrations and uses for mancozeb in almost 120 countries worldwide. Market analysis data show that mancozeb was the number one selling fungicide in 1997 with around \$400 million and ten years later in 2007 it is still one of the worlds leading five fungicides with sales in excess of \$500 million. A large proportion of this success can be attributed to the molecule's broad spectrum of activity and favoured choice as a co-formulant with other fungicides. Potatoes and vegetables were the two largest market segments accounting for approximately 29% and 28 % of sales respectively with grapevines (18%) and fruit and nut crops (19%) the two next largest segments. This may explain why mancozeb based products are primarily focused on Oomycete diseases.

A more detailed analysis of mancozeb and competitor multi-site fungicides used on potatoes and tomatoes in the key European fungicide markets of France, Germany, Italy, UK, Spain and Portugal is presented in Tables 3 and 4. The data is drawn from usage statistics reported into Agrobase from 2001-7 and as such is an estimate of relative use patterns rather than absolute figures. Looking at percentage of treated super developed area in potato we see that mancozeb is clearly the leading multi site in this segment which is almost exclusively driven by need to control *Phytophthora infestans* (Table 3). In the three leading Western European countries where potatoes are grown, sprays containing mancozeb (includes co-formulations) represent between 37% and 67% of the treated area and the active is especially favoured in France. On tomato in Italy, copper is used on twice as many hectares as mancozeb (57 vs 26%) whilst on the same crop in Spain, chlorothalonil, mancozeb and copper show equal levels of use at 26%, 26% and 21% of super developed area respectively (Table 4). The usage data suggests the critical importance of mancozeb to potatoes and tomato growers in key EU member states.

Table 2. Crops to be re-registered in the EU and associated fungal diseases controlled by mancozeb

Crop	Disease	Pest controlled
Potatoes	Late blight	Phytophthora infestans
	Early blight	Alternaria solani
Tomatoes	Late blight	Phytophtora infestans
	Early blight	Alternaria solani
	Leaf spot	Septoria lycopersi
Peppers, aubergine (egg	Pepper Blight	Phytophthora capsici
plant) Cucurbits	Downy mildew	Door dot man out out authonois
Cucurbits	Downy mildew	Pseudoperonospora cubensis
Onion (bulb), garlic, shallot,	Downy mildew	Perenospora destructor
leek	White tip of leeks, onion white leaf spot	Phytophthora porri
	Rust	Puccinia allii
Lettuce	Downy mildew	Bremia lactucae
Chicory	Downy mildew	Peronospora spp
•	Rust	Puccinia hieracii
Asparagus	Rust	Puccinia asparagi
Carrot, Salsify	Alternaria leaf blight Downy Mildew	Alternaria dauci
Carrot, Saisiry	Themana rear origine Bowny windew	Plasmoapara crustosa
Cauliflower, Broccoli	Downy mildew	Hyaloperonospa parasitica
Caumower, Broccon	Alternaria blights	Alternaria brassicae
Wheat (winter and anning)		Septoria triticii
Wheat (winter and spring)	Leaf spot	1 ~
T: 111	Brown rust	Puccinia recondita
Field bean,	Downy mildew	Peronospora viciae
Field pea	Downy mildew	Phytophthora phaseoli
Oilseed rape (winter)	Downy mildew	Peronospora parasitica
Apple	Scab	Venturia inaequalis
Pear	Scab	Venturia pirina
Cherry	Cherry Leaf Spot	Blumeriella jaapii
Plum	Rust	Tranzschelia discolor
Peach	Scab	Cladosporium carpohilum
Nectarine	Rust	Tranzschelia discolor)
Apricot		
Walnut	Anthracnose	Gnomonia leptostyla
Grapes (wine and table)	Downy mildew	Plasmopara viticola
	Black Rot	Guignardia bidwellii
	Brenner	Pseudopezicula tracheiphila
	Dead arm disease (only France)	Phomopsis viticola
Olive	Peacock Spot Freckle/Olive	Spilocaea oleaginum
		Gloeosporium olivarum
	Anthracnose (Brown rot)	Capnodium oleaophilum
Citrus	Brown rot	Phytophthora citrophthora
Black currant	Leaf spot	Pseudopeziza ribis
Gooseberry	r	
Ornamentals / Cut flowers,	Japanese Rusts	Puccinia horiana
bulbs	Pelargonium Rust	Puccinia pelargoni zonalis
Roses	Tulip fire	Botrytis tulipae
10303	l . *	1 5 5
	Black spot	Diplocarpon rosae
	Rose rust	Phragmidium tuberculatum
	Powdery mildew	Podosphaera pannosa

**Table 3.** Use patterns for multi site fungicides on potato in France Germany and UK ( % of super developed area (SDA) treated 2001-2007)

Active	% of SDA Ha treated	% of SDA Ha	% of SDA Ha treated
		treated	
Substance	France	Germany	UK
Mancozeb	62	44	37
Metiram	0.60	1.70	0
Maneb	7	8.70	0
Chlorothalonil	3	0	1.70
Copper	1.30	0.30	0
Folpet	0.60	0	0
Propineb	1	0	0
Super developed area treated France 2,664,000 Ha			
Super developed area treated Germany 1,755,000 Ha			
Super developed area treated UK 893,000 Ha			

**Table 4.** Use patterns for multi site fungicides on tomato in Italy and Spain ( % of super developed area (SDA) treated 2001-2007)

Active	% of SDA Ha treated	% of SDA Ha treated		
Substance	Italy	Spain		
Mancozeb	26	26		
Metiram	0.50	9.80		
Maneb	0	3.20		
Chlorothalonil	5.20	26		
Copper	57	21		
Folpet	0.50	0		
Propineb	0	1.60		
Super developed area treated Italy 439,000 Ha				
Super developed area treated Spain 61,000 Ha				

When subjected to severe attacks of late blight , mancozeb (when applied alone) may appear to be outclassed when compared to more recently discovered chemistry, but it remains a highly effective protectant product when used in low to moderate disease risk situations. Dose response data was presented in the Annex III dossier to show the lowest effective dose of mancozeb against late blight (*Phytophthora infestans*) of potato and early blight (*Alternaria solanum*) of tomato and this is shown in Figures 2 and Figure 3 respectively. The data clearly shows that the proposed maximum dose rate of 1600 g as/ha mancozeb (2.0 kg prod/ha Dithane 945 (800 WP)) at 7 day intervals still offers highly effective control of late blight and early blight of potato and tomato and is the minimum effective dose. Against *Alternaria solani* (Figure 3) higher levels of control were achieved with products containing mancozeb as opposed to fluazinam . When formulated with newer actives, mancozeb is a beneficial mix partner adding protectant activity against late blight and early blight as well as resistance management. Table 5 shows examples of fungicidal active ingredients with which a coformulated product containing mancozeb are available globally. This table also further emphasises the potential loss of formulated products available to growers should in the future mancozeb not be Annex I re-registered in the EU.

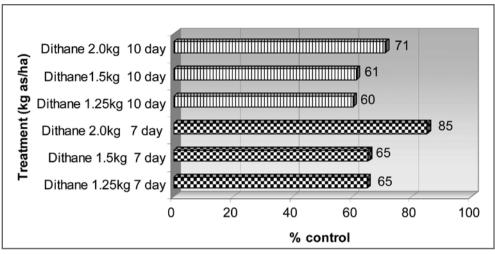


Figure 2. Dithane\* (mancozeb) dose response (7 trials) against Phytophthora infestans of potato in Northern Europe (2002-2006)

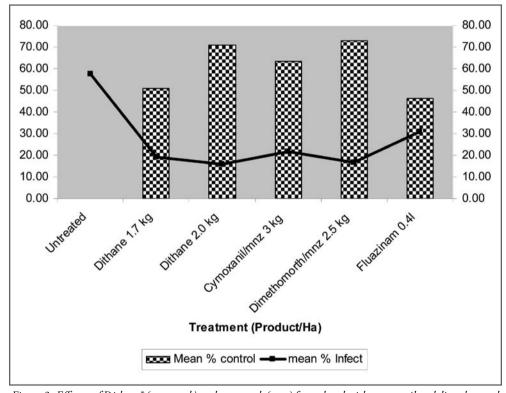


Figure 3. Efficacy of Dithane® (mancozeb) and mancozeb (mnz) formulated with cymoxanil and dimethomorth against Alternaria solani on potato in Southern Europe (mean 6 trials 2000-2003)

**Table 5.** Examples of fungicidal active ingredients with which a co-formulated product containing mancozeb is available.

Benalaxyl	Fenamidone
BenalaxylM (Kiralaxyl)	Folpet
Benthiavalicarb	Fenbuconazole
Copper	Fosetyl-al
Copper+Folpet	Fosetyl+cymox
Copper+Sulphur	Fosetyl-al+Benalaxyl
Cymoxanil	Fosetyl-al+Iprovalicarb
Cymoxanil+copper	Iprovalicarb
Cymoxanil+Copper+	Mandipropamid
Folpet	
Cymoxanil+Folpet	Mefenoxam
Cymoxanil+Fosetyl-al	Metalaxyl
Cymoxanil+Metalaxyl	Myclobutanil
Dimethomorph	Sulphur
Famoxadone	Zoxamide

# REGULATORY CHALLENGES: DITHANE ANNEX III: JOURNEY TO HARMONISATION

The Annex III re registration process for Agrochemicals in the European Union is currently governed by the criteria laid out in directive 91/414. The successful Annex I listing of mancozeb was published in the EU official journal in June 2006. The Dow AgroSciences Annex III re-registration team was now faced with the challenge of submitting modern Annex III dossiers in 26 member states in support of a complicated label encompassing multiple crops (30-40) and diseases. Deadline for this re-registration activity was June 08. Failure to achieve this submission time would lead to loss of registrations and sales by June 2010. The Annex III re-registration of Dithane products in most member states is expected to be completed between 2009 and 2010.

Every product has challenges when progressing through the registration system. Challenges around re-registration of mancozeb at Annex III member state level were many because of the age of the compound, the large number of crops and diseases to support, the variations amongst national registrations and the nature of the active ingredient. More practical regulatory challenges included, old and outdated labels, rate differences for same crop and disease in different countries, variations in water volumes, spray interval and crop registered in each EU country differed greatly with some registrations based on very little data.

Building country specific dossiers is a costly and time consuming process and was particularly challenging with a molecule of mancozeb's complexity. An additional layer of complexity was added with the need of regulatory support Dow AgroSciences provides to its multinational customers who buy Dow AgroSciences mancozeb annually to co-formulate with their own actives (approx. 18 Annex III dossiers). It was essential to align multinational account customers with the strategy to ensure continuity between Dow AgroSciences and their own internal process for re-registration of mancozeb co-formulations. Externally the Annex III process has created many challenges at the European Commission and member state level due to insufficient resources being available to cover

the huge workload generated by the re-registration process. Having considered the challenges posed from both an internal and external perspective the decision was made to the push forward mancozeb as a pilot molecule for zonal registration in the EU with a view to realizing significant reductions in workload and project complexity. The zonal rapporteurs were the UK and Greek regulatory authorities.

Re-registration offered Dow AgroSciences the opportunity to update and unify the labels across Member States with a common GAP. The zonal strategy for mancozeb was to group 26 European Union countries by geographical location and to align GAPs and labels within Northern and Southern EU Member States (Table 6). To enable this data sharing exercise countries were assigned to either the EU Northern Zone or EU Southern Zone. Countries in each zone have been grouped primarily because of residue definition of plant protection products. However, similarities of important crop groups, local growing practices and current label recommendations have been taken into account. Climate comparability has also been used to support efficacy sections of this dossier where appropriate. The zonal approach to re-registration was the most cost effective way to support a label of 33 crops in Northern zone and 40 crops in the Southern zone. Efficacy was still addressed at member state level but it was impossible to produce a country specific efficacy package to support all these uses. It was essential to present new efficacy data to support the proposed GAP and the minimum effective dose required to control important economic diseases in the major crops, namely potato, tomato, grape vine and apple that represent 90% of the mancozeb business It was not possible to present new efficacy data to support all proposed label uses as the molecule is well known and efficacy data have been presented in the past to Member States and so historical data sets were used. Mancozeb is critical to the production of lower acreage and minor crops where specific efficacy data may be hard to obtain in field trials and too expensive to fund. It is proposed that if effective control of the major diseases can be proven in these core crops then control should be expected with similar type diseases in other crops including minor crops.

**Table 6.** The zonal strategy for mancozeb Annex III re-registration in European Union countries by geographical location

Northern Zone (UK rapporteur)	Southern Zone (Greek rapporteur)	Exceptions <sup>1</sup>
Belgium	Bulgaria	Austria
Czech Republic	Croatia	Germany
Denmark	Cyprus	·
Estonia	France	
Finland	Greece	
Hungary	Italy	
Ireland	Malta	
Lithuania	Portugal	
Latvia	Slovenia	
Luxembourg	Spain	
Netherlands		
Poland		
Romania		
Slovakia		
Sweden		
UK		

<sup>&</sup>lt;sup>1</sup> Germany is re-registering outside the EU Annex III timetable and is assessing under local rules (BBA). Austria will mutually recognise decisions made in Germany. If Turkey enters the EU it will be part of the Southern Zone in relationship to climate and crops grown.

#### FORMULATION TECHNOLOGY

Mancozeb's physical properties and method of manufacture make formulating the product in anything other than wettable powder (WP), water dispersible granules (WDG) or simple flowable suspension concentrates (SCs) a significant technical challenge. Mancozeb from Dow AgroSciences is sold globally in all of these formulations under the main trade name Dithane. Pure mancozeb active substance will quickly degrade when exposed to normal environment conditions, therefore during the continuous manufacturing process a stabiliser is incorporated into the powder as well as other inert ingredients. Dithane formulations have incorporated novel formulation technology to enhance active ingredient foliar redistribution, local vapour action to adjoining leaves and to provide unique rainfastness.

The mancozeb in Dithane is formulated to improve rainfastness and also local redistribution on the leaf via dew and light rainfall thus improving spray coverage. Dithane moves in water, taking advantage of the same conditions that promote the germination of fungal spores. The basis for this is the careful incorporation of surfactants and wetting agents into the formulation. Dew cycles or a light rainfall of less than 2.5 cm will trigger the action of the surface active agents and permit Dithane fungicide to redistribute or migrate to parts of the leaf missed by initial spray deposits. Indeed replicated trials by Hislop in 1967 showed that the mean area protected by Dithane extends 13 times from that of the original spray deposit and this is thought to be as a result of diffusion of the fungicidal activity through the vapour phase in the micro environment surrounding the leave surface. Interestingly, Hislop also showed that this phenomenon is much less pronounced with zineb or dithianon, and is not observed at all with fentin acetate, folpet, captan or copper oxychloride.

#### THE FUTURE

On 1 July 2006 mancozeb was included in Annex 1 to Council Directive 91/414, as published Commission Directive 2005/72/EC of 21 October 2005. The current Annex I approval will expire on 30 June 2016. Annex III zonal dossiers were submitted to EU MSs by June 08 and re-registrations are anticipated between 2009 and 2010. Over the years, mancozeb has been subjected to pressure from NGOs, many generic competitors, price pressure and supply pressure. More recently though, the revision of 91/414/EC has changed EU regulatory paradigm. Hazard-based criteria pose new challenges to all of industry and may threaten the long term future registration status of many pesticides. The new EU framework is in place, but several guidelines and implementation rules still need to be established. Without complete knowledge of new rules, it is impossible to predict their impact on future approval of mancozeb, the Dithane brand and all associated co-formulations. It is critical to retain mancozeb as one of the few remaining broad spectrum fungicides for growers of major and minor crops. With respect to spectrum, efficacy, resistance management and affordability, mancozeb and the Dithane' brand remains a unique and irreplaceable tool for EU growers. Probably one of the greatest impacts will be the potential disease resistance issues that may arise as mancozeb is a proven resistance buster. Loss of mancozeb from the market will also mean removal of all mixture products and this will leave a limited number of narrow spectrum active ingredients often with a single site mode of action and the threat of resistance eventually developing. Also, with revisions to the water framework directive and other regulations it may put pressure on other active ingredients and make potential mancozeb replacement co-formulations difficult to register in some MSs. The loss of mancozeb will have greatest impact in major crops but it will be every much as dramatic in lower acreage crops where there are already minimal chemical solutions available to these growers. Dow AgroSciences are taking the industry lead to pro-actively support the molecule's future availability for EU growers for as long as possible.

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# Eleventh EuroBlight workshop Hamar (Norway), 2008

# New Phytophthora populations: A shift from indirect to direct sporangial germination?

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#### INTRODUCTION

Phytophthora infestans, the causal agent of potato- and tomato late blight, remains a serious threat for (commercial) potato and tomato production. In North Western Europe, frequent fungicide applications, mostly aimed to prevent infection, form the back bone of potato late blight control. Modern protectants such as Shirlan (a.i. fluazinam) are highly effective against (germinating) P. infestans sporangia and zoospores. Zoospores in particular are so sensitive to low concentrations that the many applications over the past two decades may well have exerted sufficient selection to pressure against the formation of zoospores. Thus, over the years the balance between direct and indirect germination may have shifted towards direct germination.

This hypothesis was investigated at Bayer Crop Science and Plant Research International.

#### MATERIALS AND METHODS

#### Plant Research International (PRI)

At PRI, 15 *P. infestans* isolates originating from before 1990 and 20 isolates from after 1990 (including 15 from 2000 or later) were selected to test the hypothesis that zoosporulation is more abundant in isolates from before 1990. In the period before 1990, Shirlan and other fungicides highly active against zoospores were not available whereas the more recent isolates have a history of exposure to fungicides highly active against zoospores of 10 years or more.

*P. infestans* isolates were grown on potato leaves, cv Bintje and after 7 days incubation at 15°C, sporangial suspensions were obtained by rinsing sporulating leaves in tap water. The sporangial concentration was adjusted to  $5 \times 10^4 - 1 \times 10^5$  sporangia/ml.

For the indirect germination assay, 1ml of potato extract was added to 10 ml of sporangial suspension. This suspension was incubated at  $10^{\circ}$ C for 3 hours to allow for zoospore release.  $100\mu$ l of this suspension was then examined microscopically for the fraction of empty sporangia (out of 100 sporangia) and the zoospore density in the field of view on a 1-5 scale: 1 = no zoospores observed; 2 = a few zoospores are present, ... 5 = the field of view is swarming with zoospores.

Germination was defined as a sporangium with a germ tube larger than half the diameter of the sporangium or zoospore.

## **Bayer Cropscience (BCS)**

To evaluate the potential for direct or indirect germination of different *Phytophthora infestans* samples as related to the country and year of origin and mating type, a methodology was developed at BCS laboratory to determine in parallel for the same strain both the capacity for direct and indirect germination.

60 *P. infestans* isolates were tested for their capacity of releasing zoospores in water and for their direct germination capacity in potato dextrose broth (PDB). *P. infestans* strains were grown on potato leaflets and after 6 days, sporangia were washed off either in PDB or in demineralized water. Sporangial concentrations were adjusted to 4 x 10<sup>4</sup> sporangia/ml and four 20µl droplets of each suspension were deposited on silanized microscope slide (fig.1.).

20µl droplet deposit on hydrophobic slices

Figure 1. In vitro germination of sporangia A: % of indirect germination in water B: % direct germination in PDB

These slides were then incubated at 16-18°C for 18 hours. Germination was assessed microscopically as described above. The % of empty sporangia and germinated sporangia are used as indicators for the potential for indirect and direct germination respectively.

#### **RESULTS**

#### PRI

Zoosporulation was quantified by the percentage empty sporangia and the zoospore density in the microscopes field of view on a 1-5 scale. Results are given in Figure 2. Both parameters do not

support the hypothesis that the ability for zoospore formation has decreased since 1990.

# **Bayer Cropscience**

In addition to the 59 *P. infestans* strains tested for their capacity for zoospore release in water and capacity for direct germination in PDB, reference strains were included in each experiment. Repeated results on the reference strains were excluded from the statistical analysis to avoid a bias in

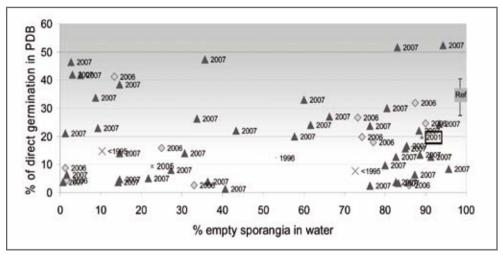


Figure 2. Direct versus indirect germination for individual isolates as influenced by the year of origin of the isolate.

the results caused by the reference isolates.

Results were statistically analysed for the effects of year of origin, country of origin and mating type. As the values studied are percentages, they do not follow a normal distribution and a the non parametric Kolmogovov-Smirnov tests (two samples test) was used to statistically analyze the results.

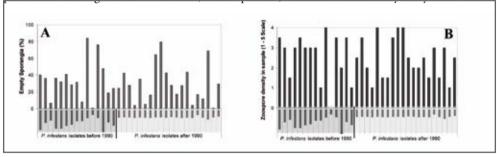


Figure 3. Indirect germination of P. infestans sporangia as influenced by the period of origin of the isolate, before and after 1990. A: the percentage of empty sporangia, as a measure for zoospore release. B: the zoospore density in the microscopes field of view on a 1-5 scale, 1 = no zoospores in field of view, 5 = field of view is swarming with zoospores.

## Influence of the year of origin

Isolates were assigned to 3 classes according to their year of origin:

A: isolates collected before 2001 (including references)

B: isolates collected in 2005 and 2006 (only 1 strain from 2005)

C: isolates collected in 2007

Figure 3 illustrates the results by plotting direct versus indirect germination for each isolate in a scatter graph. In general, direct germination does not exceed 50% whereas indirect germination varies between almost 0% and 100%. All possible combinations between high and low levels for direct and indirect germination occur.

The results of the statistical analysis are given as two box plots (fig 4a and 4b) indicating that the

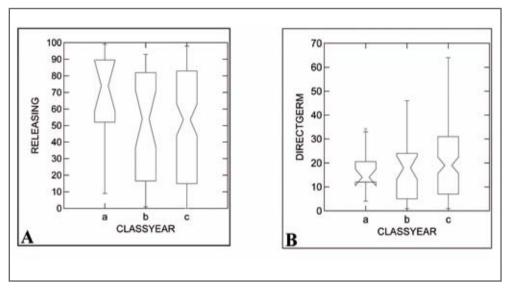


Figure 4. Results from the Kolmogorov-Smirnov two samples test demonstrate that the percentage of indirect germination of sporangia in water (A) and the percentage of direct germination of sporangia in PDB (B) is not significantly influenced by the year of origin of the isolate

year of origin does not significantly influence the isolates capacity for direct or indirect germination. **Influence of the country of origin** 

Although some countries were much more represented than others, figure 5, the strains seem to distribute at random according to the two axes. Unfortunately, a statistical analysis could not be carried out due to the unbalanced composition of the isolate collection with respect to the country of origin.

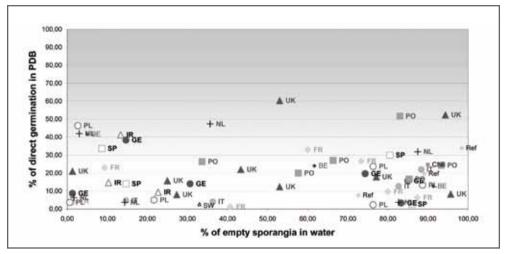


Figure 5. Distribution of European Phytophthora infestans isolates according their ability to germinate directly and indirectly.

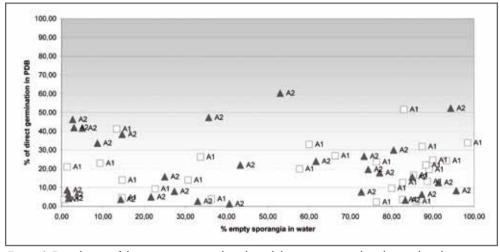


Figure 6. Distribution of the mating type according their ability to germinate directly or indirectly.

## Influence of mating type:

The capacity for direct and indirect germination for twenty one strains of A1 mating type and twenty one strains of A2 mating type was determined. The results are given in figure 6 and figure 7. The percentage of direct germination of sporangia in PDB was not significantly different according to the mating type (Kolmogorov-Smirnov two samples test, figure 7A)

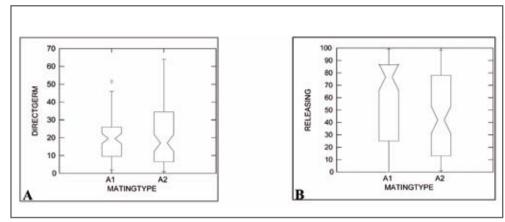


Figure 7. Influence of sexual mating type on direct (A) and indirect (B). Direct germination is not significantly influenced by mating type. Indirect germination on the other hand is significantly influenced by mating type with the A1 mating type displaying a higher capacity for zoospore formation.

Indirect germination on the other hand was significantly influenced by mating type with A1 superior to A2 strains (figure 7B).

# Discussion-conclusions

In this study, the capacity for direct and indirect germination of individual *P. infestans* isolates was studied as related to their period of origin (pre 1990 versus post 1990), the year of origin, the country of origin and the mating type.

At isolate level, the results demonstrate no significant influence of the geographical origin, the year of origin and the period of origin on the isolates ability to germinate directly or indirectly. Within the current set of isolates, A1 mating type isolates were however shown to be slightly better in zoospore formation than the A2 isolates. Thus, at first sight, the hypothesis that modern *P. infestans* isolates may have experienced selection pressure against indirect germination due to the general use of fungicides highly active against zoospores over the past two decades cannot be substantiated.

At population level however Europe has seen a shift from an A1 dominated population before the 1980's to a strongly A2 dominated population at present. On average, the current population may thus have a reduced capability for indirect germination. The cause behind this shift is likely to be related to the driving forces behind the domination of *P. infestans* genotype "blue 13" elucidated elsewhere in this volume.

# Eleventh EuroBlight workshop Hamar (Norway), 2008

# Report of the fungicide sub-group: Discussion of potato late blight fungicides, their properties and ratings

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CHAIRMAN: Huub Schepers (NL)

#### **OBJECTIVES**

The objectives of the sub-group meeting were:

- to review and update the ratings given for the various characteristics of late blight fungicides at the Bologna workshop in May 2007 (PPO-Special Report no. 12 (2007), 107-111)
- to discuss the new decimal ratings for leaf blight control
- to discuss the future use of the harmonised protocols for the evaluation of leaf blight control and tuber blight control

#### DISCUSSION

Before the fungicide ratings and harmonised protocols were discussed the group heard presentations on Consento and Infinito (Bayer), Valbon (Certis), Revus (Syngenta), Dithane NT (Dow) and "New Phytophthora populations" (Kessel *et al.*).

Prior to the workshop Syngenta made available results for mandipropamid covering protection of new growth and curative activity. At the fungicide subgroup meeting it was agreed that Revus was to be included in Table 1 because of widespread use on crops in Europe. A rating of ++ for protection of new growth was agreed to be appropriate based on extensive trial results. However, it was decided that the rating for curative activity should remain at + and not be increased to +(+), even although some trials supported the higher rating.

Nissan Chemical Industries made available, prior to the workshop, results for stem blight control by amisulbrom and rainfastness of this fungicide. The consensus was that amisulbrom should remain in Table 2. The ratings agreed for individual characteristics are presented in Table 2 and are based on the trials data submitted and also trials carried out independently.

Huub Schepers, in a presentation entitled "Testing fungicides for effectiveness against leaf blight using a harmonised protocol in 2006, 2007 and 2008", demonstrated results from some of the trials, made public the new decimal ratings for leaf blight and proposed activities for 2009.

Agreement was reached that the new decimal ratings for leaf blight should replace the plusses scale. The fungicide companies agreed with the ratings. There was also agreement that fungicides not tested sufficiently often would have their plusses ratings removed but ratings for the other characteristics

would remain until replaced by decimal ratings based on trial results.

After some discussion about producing decimal fungicide ratings for leaf blight control during the first half of the growing season the consensus was that there wasn't a large enough number of trials with strong, early epidemics to allow accurate ratings to be calculated.

# GENERAL COMMENTS ABOUT THE RATINGS TABLES FOR LATE BLIGHT FUNGICIDES (TABLES 1 AND 2)

The ratings given in Table 1 are for blight fungicides currently registered in several EU countries and are based on the label recommendations for commercially available products containing one or two active ingredients as a co-formulated mixture. The ratings are NOT for the active ingredients themselves. Table 1 lists the commercially available mixtures of active substances. The ratings given are for the highest dose rate registered for the control of *P. infestans* in Europe. Different dose rates may be approved in different countries.

The ratings given in all columns, except the one for leaf blight, are the opinion of the fungicides sub-group at the Hamar blight workshop, 2008 and are based on field experiments and experience of the performance of products when used in commercial conditions. Ratings for leaf blight are based on results from ten Euroblight field trials during 2006-2008, and only compounds included in a minimum of six of these trials are rated for leaf blight. The scale for leaf blight is a 2-5 scale, to one decimal place. All other ratings are on a 0 to +++ scale, using (+) to indicate half marks. The ratings are intended as a guide only and will be amended in future if new information becomes available. Table 1 is available on the Euroblight website, www.euroblight.net

Table 2 gives provisional ratings for recently introduced products and new fungicide formulations. The inclusion of a product in this table is not indicative of its registration status either in the EU or elsewhere in Europe. These ratings are the consensus view of the fungicide sub-group and are based on information from field experiments or minimal practical experience of a product and will be amended at future workshops, as new information becomes available and the body of experience in commercial use increases.

#### HARMONISED PROTOCOLS AND ACTIVITIES IN 2009

Agreement was reached that there should be three more leaf blight trials in 2009 to allow the rating of products not rated so far and that Dithane NT should be one of the reference treatments. The consensus was that there should be two other reference treatments but no decision was made regarding which two used in the 2006 to 2008 trials were most appropriate. The need to identify which specific formulations were/ are tested in the trials and that this information should be in the fungicide table were agreed.

There was in depth discussion about the harmonised protocol for the evaluation of tuber blight control. It was agreed that the protocol (Version 1.0 dated 24 October, 2007) should be amended, taking account of the comments of the subgroup. The main changes suggested and agreed were that 1600 tubers per fungicide treatment should be assessed for tuber blight, and foliar blight should be a covariate in the analysis of variance.

# DEFINITIONS AND DISCLAIMER (REPRODUCED FROM THE TALLINN 2005 PROCEEDINGS)

#### PHENYLAMIDE RESISTANCE

The ratings assume a phenylamide-sensitive population. Strains of *P. infestans* resistant to phenylamide fungicides occur widely within Europe. Phenylamide fungicides are available only in co-formulation with protectant fungicides and the contribution that the phenylamide component makes to overall blight control depends on the proportion of resistant strains within the population. Where resistant strains are present in high frequencies within populations the scores for the various attributes will be reduced.

#### **NEW GROWTH**

The ratings for the protection of the new growing point (new growth) indicate the protection of new foliage due to the systemic or translaminar movement or the redistribution of a contact fungicide. New growth consists of growth and development of leaves present at the time of the last fungicide application and/or newly formed leaflets and leaves that were not present.

#### PROTECTANT ACTIVITY

Spores killed before or upon germination/penetration. The fungicide has to be present on/in the leaf/stem surface before spore germination/penetration occurs.

#### **CURATIVE ACTIVITY**

The fungicide is active against *P. infestans* during the immediate post infection period but before symptoms become visible, i.e. during the latent period.

#### ANTISPORULANT ACTIVITY

*P. infestans* lesions are affected by the fungicide decreasing sporangiophore formation and/or decreasing the viability of the sporangia formed.

#### STEM BLIGHT CONTROL

Effective for the control of stem infection either by direct contact or via systemic activity.

#### TUBER BLIGHT CONTROL

Activity against tuber infection as a result of fungicide application after infection of the haulm, during mid- to late-season i.e. where there is a direct effect on the tuber infection process. The effect of phenylamide fungicides on tuber blight control was therefore not considered relevant in the context of the table as these materials should not be applied to potato crops if there is blight on the haulm, according to FRAC guidelines. Only the direct (biological) effect of a particular fungicide on the tuber infection process was considered relevant and NOT the indirect effect as a result of manipulation or delay in the development of the foliar epidemic.

#### **DISCLAIMER**

Whilst every effort has been made to ensure that the information is accurate, no liability can be accepted for any error or omission in the content of the tables or for any loss, damage or other accident arising from the use of the fungicides listed herein. Omission of a fungicide does not necessarily mean that it is not approved for use within one or more EU countries.

The ratings are based on the label recommendation for a particular product. Where the disease pressure is low, intervals between spray applications may be extended and, in some countries,

fungicide applications are made in response to nationally issued spray warnings and/or Decision Support Systems. It is essential therefore to follow the instructions given on the approved label of a particular blight fungicide appropriate to the country of use before handling, storing or using any blight fungicide or other crop protection product.

**Table 1.** The effectiveness of fungicide products/co-formulations for the control of *P. infestans* 

	Effectiveness			Mode of Action					
Product 1	Leaf Blight <sup>2</sup>	New growth	Stem blight	Tuber blight	Protectant	Curative	Anti- sporulant	Rain fastness	Mobility in the plant
copper		?	+	+	+(+)	0	0	+	contact
dithiocarbamates <sup>3</sup>	2.0	?	+	0	++	0	0	+(+)	contact
chlorothalonil		?	(+)	0	++	0	0	++(+)	contact
cyazofamid	3.6	++	+	+++	+++	0	0	+++	contact
fluazinam	2.6	?	+	++(+)	+++	0	0	++(+)	contact
zoxamide+ <b>mancozeb</b>	2.6	?	+ 5	++	+++	0	0	++(+)	contact+contact
famoxadone+ <b>cymoxanil</b>		?	+(+)	N/A	++	++	+	++(+)	contact+translaminar
mandipropamid	3.8	++	+(+)	++5	+++	+6	+(+)	+++	translaminar+contact
benthiavalicarb+mancozeb	3.5	?	+(+)5	+(+)	+++	+(+)	+	++(+)	translaminar+contact
cymoxanil+mancozeb		?	+(+)	0	++	++	+	++	translaminar+contact
cymoxanil+ <b>metiram</b>		?	+(+)	0	++	++	+	++	translaminar+contact
cymoxanil+copper		?	+(+)	0	++	++	+	++	translaminar+contact
dimethomorph+mancozeb	2.8	?	+(+)	++	++(+)	+	++	++(+)	translaminar+contact
fenamidone+mancozeb	2.6	?	+(+)5	++	++(+)	0	+(+) 5	++	translaminar+contact
benalaxyl+mancozeb 4		++	++	N/A	++(+)	++(+)	++(+)	+++	systemic+contact
metalaxyl-M+mancozeb4		++	++	N/A	++(+)	++(+)	++(+)	+++	systemic+contact
metalaxyl-M+ <b>fluazinam</b> 4		++	++	N/A	++(+)	++(+)	++(+)	+++	systemic+contact
propamocarb-HCl+mancozeb		+(+)	++	++	++(+)	++	++	+++	systemic+contact
propamocarb-HCl +chlorothalonil	3.4	+(+)	++	++	++(+)	++	++	+++	systemic+contact
propamocarb-HCl+ <b>fenamidone</b>	2.5	+(+)	++	++	++(+)	++	++	+++	systemic+ <b>translaminar</b>
propamocarb-HCl <b>+fluopicolide</b>	3.8	++	++	+++	+++	++	++(+)	++(+)	systemic + translaminar

<sup>&</sup>lt;sup>1</sup> The scores of individual products are based on the label recommendation and are not additive for mixtures of active ingredients. Inclusion of a product in the list is not indicative of its registration status either in the EU or elsewhere in Europe.

**Key to ratings:** 0 = no effect;

+ = reasonable effect;

++ = good effect;

+++ = very good effect;

N/A = not recommended for control of tuber blight;

? = no experience in trials and/or field conditions.

<sup>&</sup>lt;sup>2</sup> Based on Euroblight field trials in 2006-2008.

<sup>&</sup>lt;sup>3</sup> Includes maneb, mancozeb, propineb and metiram..

<sup>&</sup>lt;sup>4</sup> See text for comments on phenylamide resistance.

<sup>&</sup>lt;sup>5</sup> Based on limited data.

<sup>&</sup>lt;sup>6</sup> In some trials there were indications that the rating was +(+).

**Table 2.** Provisional ratings for the effectiveness of new fungicide products /co-formulations for the control of *P. infestans* in Europe

	Effectiveness			Mode of Action			Rainfastness	Mobility in the plant	
Product 1	Leaf blight	New growth	Stem blight	Tuber blight	Protectant	Curative	Anti-sporulant		
amisulbrom	++(+)	?	+2	++(+)3	++(+)	0	?	+++	contact

- 1 The ratings are based on the label recommendation and are not additive for mixtures of active ingredients.

  Inclusion of a product is not indicative of its registration status either in the EU or elsewhere in Europe.
- 2 In one trial there was an indication that the rating was +(+).
- 3 Based on limited data from which the direct effect on tuber blight can be assessed. In some trials there were indications that the rating was +++.

**Key to ratings:** 0 = no effect; + = reasonable effect; ++ = good effect; +++ = very good effect

#### EARLY BLIGHT (ALTERNARIA SOLANI AND ALTERNARIA ALTERNATA)

Fungicide ratings are unchanged from the report of the fungicide sub-group in the proceedings of the tenth workshop in Bologna. It was stated that EPPO approval was expected in 2008 or 2009 for the harmonised protocol for fungicidal control of Alternaria.

#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Recent changes in the *Phytophthora infestans* population in Northern Ireland and first results from a new all-Ireland late blight project

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#### **SUMMARY**

The A2 mating type of *Phytophthora infestans*, first identified in Ireland in 1987, was not detected after the mid-1990s until 2005 when three out of 54 isolates from Northern Ireland proved to be A2. No A2 isolates were identified in 2006, but in 2007, 17% of isolates from Northern Ireland were found to be A2. Intensive sampling of five selected Northern Ireland crops in 2007 showed that in each case both mating types were present within the same crop. RG57 fingerprinting of 26 selected A2 isolates identified two fingerprints, one of which was common to the three 2005 isolates and three of the 2007 isolates. The other 21 A2 isolates from 2007 had the fingerprint associated with the genotype known as 'Blue 13' or 13\_A2, which has been the most common in Great Britain since 2006. This is the first finding of this genotype in Ireland. A new all-Ireland blight project started in the 2008 season and approx. 25% of isolates from extensive surveys throughout the island of Ireland have proved to be A2. These isolates are being further characterised to determine the genotypes present. Both mating types were again found in some crops, so there is a possibility of sexual recombination and the formation of oospores by *P. infestans*.

#### **KEYWORDS**

Phytophthora infestans, potato late blight, mating type, metalaxyl resistance, Ireland

#### INTRODUCTION

The A2 mating type of *Phytophthora infestans* probably arrived in Europe from Mexico in the late 1970s when new strains of both the A1 and the A2 mating types were introduced (Niederhauser, 1991). This gave the possibility of formation of long-lived sexual oospores, which can persist in soil between crops, and of the generation of new recombinant genotypes. The A2 mating type was first found in Northern Ireland in 1987 (Cooke *et al.*, 1995) and in the Republic of Ireland in 1988 (O'Sullivan *et al.*, 1995). It occurred at a low frequency in Ireland in most years until the mid-1990s, but then was not detected again until 2005.

A dramatic increase in the incidence of the A2 mating type in Great Britain since 2005, largely associated with a single genotype known as 'Blue 13' or '13\_A2', resistant to the fungicide metalaxyl

(Cooke *et al.*, 2007; Shaw *et al.*, 2007) prompted investigation of the population in Northern Ireland with the aims of establishing the current frequency of the A2 mating type and whether the 'Blue 13' genotype was present. This led to a proposal for a new four-year all-Ireland project on late blight, which was funded by the Republic of Ireland's Research Stimulus Fund and began in December 2007.

#### MATERIALS AND METHODS

#### Northern Ireland Surveys 2005-2007

Surveys of *P. infestans* in Northern Ireland crops were carried out during 2005-2007. Blighted potato material was collected mainly from commercial crops by members of the Department of Agriculture and Rural Development (DARD) Potato Inspection Service. Each sample consisted of infected leaves and stems from a single crop. Isolates, obtained by bulking together *P. infestans* sporangia from a crop sample, were initially maintained on detached glasshouse-grown leaflets of susceptible maincrop potato cultivars. After testing for metalaxyl sensitivity (below), they were transferred to antibiotic rye agar amended with rifampicin (25 mg L<sup>-1</sup>) and natamycin (25 mg L<sup>-1</sup>).

In 2007, five commercial crops were selected for an intensive survey. From each crop, 120 leaflets bearing single lesions were collected throughout the crop and single-lesion isolates established directly onto antibiotic rye agar.

#### All-Ireland Surveys 2008

The protocol used above was modified for the All-Ireland project to avoid the difficulties of interpretation associated with use of multi-lesion isolates. The survey was carried out at two levels: extensive and intensive, in order to obtain an all-Ireland collection of isolates representative of the current *P. infestans* population. In the extensive survey, it was planned to sample *c.* 50 sites (*c.* 35 in the south, 15 in the north), aiming for 5 single-lesion isolates per site. In the intensive survey, up to 50 single-lesions were collected from each of ten selected crops. Only results for metalaxyl sensitivity (Northern Ireland only) and for mating type from the extensive survey are reported here. Further characterisation is in progress.

#### Phenotypic and genotypic characterisation

Isolates were tested for sensitivity to the fungicide metalaxyl on potato leaf discs floating on 0, 2 and 100 mg metalaxyl L<sup>-1</sup> (Cooke, 1986) and designated sensitive (sporulating on untreated discs only), resistant (isolates sporulating on both metalaxyl concentrations) or intermediate (isolates sporulating on 2, but not on 100 mg metalaxyl L<sup>-1</sup>). Mating type was tested by growing isolates on unamended carrot agar with known reference isolates of the A1 or A2 mating types. The dual cultures were incubated at 15-18°C in darkness for 5-10 days, then examined microscopically for the presence of oospores where the two colonies interacted. DNA fingerprinting using the moderately repetitive probe RG57 was carried out on selected isolates of the A2 mating type using a modification of the method described by Goodwin *et al.* (1992).

#### **RESULTS**

#### Northern Ireland Surveys 2005-2007

The number of single-crop isolates established in the surveys ranged from 20 in 2006 to 54 in 2005, depending on the seasonal incidence of potato blight (Table 1).

**Table 1.** Metalaxyl resistance and mating type of *Phytophthora infestans* isolates in Northern Ireland surveys, 2005-2008

Year	No. of isolates	Metalaxyl resistant (%)	A2 (%)
2005	54	20	6
2006	20	25	0
2007	47	43	17
2008	28ª	54	26

<sup>&</sup>lt;sup>a</sup> only 27 isolates tested for mating type

**Table 2.** RG57 fingerprints of A2 mating type isolates of *Phytophthora infestans* identified in Northern Ireland in 2005 and 2007

Year	Survey	Number of isolates with	RG57 fingerprint	Туре
		fingerprint		
2005	Extensive	3	100 010 000 000 110 100 011 001 1	NI-6
2007	Extensive	1	100 010 000 000 110 100 011 001 1	NI-6
2007	Extensive	7	110 000 010 100 110 010 111 101 1	Blue 13
2007	Intensive	2	100 010 000 000 110 100 011 001 1	NI-6
2007	Intensive	13	110 000 010 100 110 010 111 101 1	Blue 13

**Table 3.** Mating type of *Phytophthora infestans* isolates in Northern Ireland intensively sampled crops, 2007

Site	Cultivar	Blight (%)	No. of isolates	A2 (%)
1	Kerr's Pink	1-5	82	18
2	Saxon	5-50	119	18
3	Maris Piper	1-5	113	1
4	Santé	1-5	98	94
5	Kerr's Pink	<1	119	18
<u>Total</u>			531	28

Three A2 isolates were identified in 2005, from two fields in the south of Co. Down. Two isolates were from a field containing crops of cvs Orla and Santé, which were sampled separately, while the third was from a crop of cv. Premier about 8 km away. All three 2005 A2 isolates were metalaxyl-resistant and shared a common RG57 fingerprint (Table 2) which was not one of those found among the A2 *P. infestans* isolates from Great Britain in that year (Shaw, D.S., personal communication). Only A1 isolates were found in 2006.

In 2007, eight A2 isolates were obtained from the general survey, again all from Co. Down, from crops of seven different cultivars (Désirée, Lady Claire, Kerr's Pink, Marfona, Maris Piper, Milagro, Saxon). Six of the A2 isolates were resistant and two were initially thought to be sensitive. However, re-testing after isolation into agar culture indicated that all were resistant. Of these eight A2 isolates, one had the same fingerprint as the three 2005 A2 isolates while the other seven shared the same fingerprint which was that of the 'Blue 13' genotype (Table 2).

Five crops with widespread infection were selected for intensive sampling during July-August 2007 (Table 3). The first four were in Co. Down, while the last was in Co. Antrim. All five sites had mixed populations with both A1 and A2 mating types present, the percentage A2 ranging from 1-96%. Fifteen of the 150 A2 isolates from these crops were fingerprinted (1-5 isolates per crop); 13 had the fingerprint associated with 'Blue 13' while the other two had the 2005 A2 fingerprint (Table 2).

**Table 4.** Mating type of *Phytophthora infestans* isolates in extensive survey, Ireland in 2008

Collection	Number of isolates	A2 (%)	
Republic of Ireland			
Total	203	25	
<u>Variety</u>			
British Queen	3	0	
Cultra	15	7	
Golden Wonder	40	40	
Kerr's Pink	75	8	
Rooster	37	38	
Unknown	20	20	
Other	13	69	
County			
Carlow	3	100	
Cork	34	0	
Donegal	78	24	
Dublin	9	100	
Kerry	32	3	
Kilkenny	6	0	
Limerick	5	100	
Meath	9	78	
Waterford	9	0	
Wexford	15	40	
Wicklow	3	0	
Northern Ireland			
Total	27	26	
Variety			
Avalanche	5	100	
Dunbar Standard	2	2	
Home Guard	6	0	
Kerr's Pink	9	0	
Other	5	40	
County			
Antrim	12	50	
Down	8	12	
Londonderry	2	0	
Tyrone	5	0	

#### **All-Ireland Surveys 2008**

Only results of the extensive surveys are reported here.

In Northern Ireland, there were unusually few infected crops in the early part of the season due to dry weather in May and the first two weeks of June. As a result only 12 sites were sampled and 28 isolates obtained (1-7 isolates per site). Of 27 isolates from 11 sites tested for mating type, 20 were A1 and 7 A2 (26% A2, Table 2). Eight sites yielded only the A1 mating type and three only the A2. As in 2007, A2 isolates were found in Cos. Antrim and Down. The absence of the A2 mating type from Cos. Londonderry and Tyrone may well reflect the small number of isolates obtained from these counties rather a real difference in mating type distribution. For metalaxyl sensitivity, 28 isolates from 12 sites were tested; 13 were metalaxyl-sensitive and 15 metalaxyl-resistant (54% resistant, Table 1).

In the Republic of Ireland, 43 sites were sampled and 203 isolates obtained (1-16 isolates per site). Of these, 153 were A1 and 50 were A2 (25% A2). At 28 of the sites only the A1 mating type was obtained, while nine sites yielded only A2 isolates. Both mating types were detected in six of the crops sampled. Crops which yielded A2 isolates were located throughout the country, although A2 isolates were detected at a greater frequency in eastern counties.

#### DISCUSSION

After its first finding in Ireland in 1987, the A2 mating type was detected in most years up to the early 1990s, but then virtually disappeared for about 10 years, although it may have been present at a very low frequency. Thus, in Northern Ireland, a single A2 isolate was obtained from a commercial crop in 1995, but no further A2 isolates were found until 2005 (Cooke & Deahl, 2005), while in the Republic of Ireland four A2 isolates were obtained from breeder's plots at Oak Park Research Centre, Carlow in 1996, (Griffin *et al.*, 2002) but no further A2 isolates were confirmed until 2008 (L.J. Dowley, unpublished data). The occurrence of the A2 mating type in 2007 in Northern Ireland

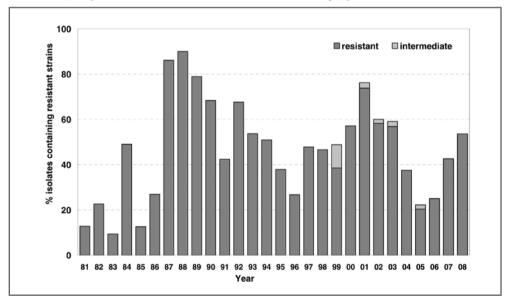


Figure 1. The proportion of Northern Ireland Phytophthora infestans isolates containing phenylamide-resistant strains, 1981-2008

and in 2008 across the whole island of Ireland suggests that changes in the pathogen population are occurring which are similar to those in mainland Europe (Détourné *et al.*, 2006) and Great Britain (Cooke *et al.*, 2008). However, so far the increase in the A2 frequency has not been as marked as has occurred in Great Britain where it went from 10% in 2004 to 35% in 2005 and over 70% in 2006, largely associated with the phenylamide-resistant 'Blue 13 genotype'. The finding of this genotype in Northern Ireland in 2007 has yet to be confirmed for Ireland as a whole in 2008, but further characterisation is in progress to determine the genotypes present and their association with phenylamide resistance. Although there has been an increase in the incidence of phenylamide-resistant strains of *P. infestans* in Northern Ireland since 2005, it is not as marked as that which occurred in the late 1980s (Figure 1). Since both mating types were found at some sites, there is a possibility of sexual recombination of *P. infestans* with the risks of oospore-initiated soil-borne infection and of greater pathogen variation.

#### **ACKNOWLEDGEMENTS**

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Phenotypic characteristics of Finnish and North-Western Russian populations of *Phytophthora infestans* in 2006-2007

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#### **SUMMARY**

Potato late blight caused by the oomycete *Phytophthora infestans* is a severe disease on potato. Under favourable conditions for the disease it can kill unprotected potato haulm in a couple of weeks. In addition rain can spread sporangia formed in potato leaves to tubers and cause tuber blight (Hannukkala *et al.*, 2007). The main initial sources of late blight inoculum are considered to be infected tubers which survive to the next season in cull piles, storage or soil (Zwankhuizen *et al.*, 1998). A new primary source of inoculum appeared in Europe when the old clonal lineage of *P. infestans* was replaced by a new more diverse population during the 1980s (Goodwin, 1997). The new population possesses both mating types and is able to reproduce sexually in potato in the Nordic countries (Brurberg *et al.*, 1999; Hermansen *et al.*, 2000; Lehtinen *et al.*, 2008). Sexual reproduction results in oospores, the resting bodies of *P. infestans*, which can overwinter in soil (Andersson *et al.*, 1998; Lehtinen & Hannukkala, 2004).

#### **KEYWORDS**

Phytophthora infestans, potato, mating type, fungicide resistance, pathotype

#### INTRODUCTION

Potato late blight caused by the oomycete *Phytophthora infestans* is a severe disease on potato. Under favourable conditions for the disease it can kill unprotected potato haulm in a couple of weeks. In addition rain can spread sporangia formed in potato leaves to tubers and cause tuber blight (Hannukkala *et al.*, 2007). The main initial sources of late blight inoculum are considered to be infected tubers which survive to the next season in cull piles, storage or soil (Zwankhuizen *et al.*, 1998). A new primary source of inoculum appeared in Europe when the old clonal lineage of *P. infestans* was replaced by a new more diverse population during the 1980s (Goodwin, 1997). The new population possesses both mating types and is able to reproduce sexually in potato in the Nordic countries (Brurberg *et al.*, 1999; Hermansen *et al.*, 2000; Lehtinen *et al.*, 2008). Sexual reproduction results in oospores, the resting bodies of *P. infestans*, which can overwinter in soil (Andersson *et al.*, 1998, Lehtinen & Hannukkala, 2004).

The changes in phenotypic and genetic properties in *P. infestans* populations have been extensively studied in Central and Western Europe (Knapova Gisi 2002; Day *et al.*, 2004; Cooke *et al.*, 2006; and in the Nordic countries (Brurberg *et al.*, 1999; Hermansen *et al.*, 2000; Lehtinen *et al.*, 2007; Lehtinen *et al.*, 2008). Population studies have been carried out at Moscow region and Far East of Russia (Elansky *et al.*, 2001) but no published data is available about blight populations in North

Western parts of Russia. The blight populations in Koala and Karelia are of special interest for Finland because these regions are relatively close to the eastern border of Finland and late blight has only very recently started to cause severe epidemics at the Northern regions close to the Polar Circle (Hannukkala *et al.*, 2007). The aim of this study was to collect *P. infestans* isolates from Koala, Karelia and Finland and compare the phenotypic traits of these populations and their impact on blight epidemiology and disease management practises.

#### MATERIALS AND METHODS

*P. infestans* isolates for this study were collected in 2006 and 2007 from Murmansk region in Koala, Petrozavodsk region in Karelia, Central and Northern regions of Finland. Totally 163 isolates from Russia and 200 from Finland were obtained. The Russian isolates were sampled within few days' periods in 2006 and 2007, transported across the border to Finland and mailed to MTT, Agrifood Research Finland to be tested and maintained. The Finnish isolates were collected and mailed to MTT individually during August in 2006 and 2007.

The mating type of the isolates was determined on agar, response to fungicides metalaxyl-M and propamocarb-hydrochloride, and pathotype on floating leaf disks as described by Lehtinen et *al.*, (2007). Most of the isolates were transferred into liquid nitrogen for further studies.

#### RESULTS AND DISCUSSION

Both mating types were present at close to 50:50 proportions in both countries. This means that there is potentially sexually reproducing populations both in Russia and Finland. In Finland and Scandinavia both mating types have been present since early 1990s (Hermansen *et al.*, 2000, Lehtinen *et al.*, 2007, 2008). Both mating types have been found at St Petersburg region in Russia but no previous data is available from Koala and Karelia regions in Russia.

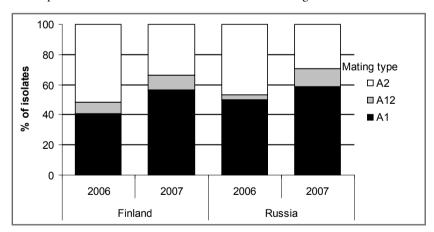


Figure 1. Occurrence of mating types A1 and A2 in Finnish and Russian P. infestans populations in 2006 and 2007. A12 types of isolates produce oospores in pairings with both A1 and A2 tester isolates.

#### Fungicide resistance

The response of blight isolates against systemic fungicides metalaxyl-M and propamocarb-hydrochloride was studied with floating leaf disk method. For metalaxyl-M concentrations of 1, 10 and 100 mg/l and for propamocarb 10, 100 and 1000 mg/l were used. Control was distilled water. Isolates growing only at water were classified sensitive, those growing at the highest concentration were resistant and others intermediate.

In 2006 metalaxyl resistant isolates were not detected and percentage of intermediate isolates varied between 10-20 % in Finland and Russia respectively. In 2007 metalaxyl resistant isolates appeared into the population. Their proportion in Russia was 32 % and in Finland 23 %. In Finland the blight population during 1990s was totally metalaxyl resistant but resistant isolates disappeared at the beginning of 2000s, when the use of metalaxyl fungicides was stopped (Hermansen *et al.*, 2000; Lehtinen *et al.*, 2007, 2008). The increase of metalaxyl resistant isolates in Finland in 2007 might be an indication of increased use of metalaxyl fungicides in 2005 and 2006. The appearance of metalaxyl resistance in the Russian blight population is difficult to explain, because metalaxyl has not been used for blight control in fields where samples were collected.

There were no isolates resistant to propamocarbHCl in Finland or Russia. The proportion on isolates tolerating low concentrations of the fungicide was approximately the same in both countries and both years.

#### Pathotypes based on major gene virulence

Pathotypes based on virulence genes were determined by floating leaf disk method. Potato clones R1-R11 (Black's differential set) possessing all known eleven single resistance R-genes from wild potato, *Solanum demissum*, were grown in greenhouse. R0-clone containing no resistance genes was used as control. Disks 1,5 cm in diameter were cut from fully expanded leaflets, 6 leaf disks of each eleven different clones were floated on distilled water in Petri dishes. Each leaf disk was inoculated with 20 µl droplet of spore suspension of late blight isolates. Leaf disks were incubated for 7 days at 15 °C hereafter sporulating area of each disk was visually scored.

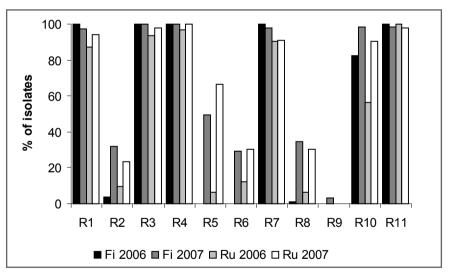


Figure 2. Proportion of Finnish and Russian Phytophthora infestans isolates overcoming major resistance genes R1–R11 in 2006 – 2007.

The populations in both Finland and Russia were changed considerably more divergent in pathotypes from 2006 to 2007. In 2006 only four different pathotypes were found in Finnish blight population while 10 different pathotypes were present in the Russian one. The population structure in 2007 was totally different from 2006. There were 23 different pathotypes in Finnish and 45 in Russian blight population. In the average Finnish isolates contained 5.9 virulence genes in 2006 and 7.5 virulence genes in 2007. The Russian isolates possessed 5.6 virulence genes in 2006 and 7.2 in 2007.

The most common pathotype in both years and countries contained virulence genes 1,3,4,7,10 and 11. The second common pathotype (1,3,4,7,11) in 2006 had been replaced by more complex pathotype (1,3,4,5,7,10,11) in 2007. In 2007 one Finnish isolate contained all eleven virulence genes. Two percentage of isolates in 2007 in both countries contained all virulence genes except virulence 9. The proportion of isolates containing rare virulence genes 2,5,6 and 8 was considerably increased from 2006 to 2007 in both countries. Virulence gene 9 was present for the first time in Finnish population.

#### **CONCLUSIONS**

The Finnish and Russian potato late blight populations in 2006 and 2007 were very similar in phenotypic traits. Both countries have sexually reproducing populations, which means increasing genetic diversity in population and increasing risk of oospore derived late blight attacks very early in the season. High incidence of very complex pathotypes may result in a very rapid breakdown of leaf blight resistance among current potato cultivars. Indications of increased metalaxyl resistance must be taken into account in future choice of fungicides. It is important to be aware of changes in population properties when planning future actions in potato late blight management in both countries.

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### Characteristics of *Phytophthora infestans* isolates from China

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#### **SUMMARY**

In several of the main potato production area's in China the climate is favorable for *P. infestans* and consequently, potato late blight is one of the most serious threats to potato production in China. As part of a comprehensive monitoring program that we could establish for China we started genotypic studies on the current *P.infestans* population in China. We found much more genetic variation than previously reported. Nevertheless, the three mitochondrial haplotypes (Ia, IIa, IIb) and the SSR genotypes found in Chinese isolates were all strongly related to the region of origin. Many genotypes, as determined by multilocus SSR markers, are unique to specific regions and only two genotypes were found in multiple provinces located in the North of China. The genotyping analysis shows strong restriction of the spread of *P. infestans* but this could change radically if infected seed potatoes are transported between the different regions in China. Therefore we are currently intensifying our sampling and aim to set up a monitoring program similar to Euroblight.

#### **KEYWORDS**

Phytophthora infestans, late blight, population diversity, clonal lineage, China

#### INTRODUCTION

The late blight caused by *Phytophthora infestans* is one of the most devastating diseases of potato. In many potato growing area's the climate in China is favorable for *P. infestans* and, as a result, potato late blight is one of the most serious threats for potato production in China. The A2 type was firstly detected in northern China in 1996 (Zhang *et al.*, 1996), and has since spread as far south as Yunnan (Zhao and Zhang, 1999), near the border with Vietnam. Both the A1 and A2 pathogen exist in China, although as yet there is no evidence that they have recombined into a more adapted or more aggressive variant of the pathogen (Yang, Zhu, and Zhang, 2008).

The potato agribusiness is booming in China in recent years (Chen and Qu, 2008; Qu et al., 2005). The national-wide commercial transportation and other trade activities are expected to increase in coming years consequently; the migration of P. infestans in China may be national-wide as well. Understanding the current situation of the P. infestans population in China, as well as monitoring shift and trends in the future is important for rational short- and long-term resistance management and potato breeding. Unfortunately, the current population diversity of P.infestans in China is not well known. In recent years Late blight epidemics in China turned to be more difficult to forecast and to manage possibly because of changes in the P. infestans population. Management strategies and forecasting systems depend on an understanding of its population genetics for instance the occurence of a sexual cycle. It is also important to know which virulence factors are present in the regional pathogen populations, because this will identify which potato varieties will be susceptible to the prevailing P. infestans population in a particular region. The most recent study on P. infestans isolates collected between 1997 and 2003 from Northern China showed that all these isolates belonged to the same clonal lineage (Guo et al., 2009). However, virulence identification indicated highly diverse within this clonal group.

The objective of this study was to explore the level of genetic diversity within the modern *P. infestans* population in China using both classical approaches and highly-informative molecular markers (Table 1). A comprehensive survey of *P. infestans* isolates in whole landscape of China was performed. *P. infestans* isolates from six potato regions were mainly sampled in 2006 and 2007. Finally, 118 isolates were obtained providing an adequate view on the *P. infestans* population in order to understand the current diversity and trends of the *P. infestans* population in China.

#### **MATERIALS AND METHODS**

#### Isolate sampling

Potato leaves with a single lesion were collected from five regions in China (Figure 1), placed the leaves individually in plastic bags or 9cm Petri plates containing 1.5 % water agar, and incubated the leaves until sporulation at 15°C at a light intensity of 12 Wm<sup>-2</sup> and 16 hours. Infected leaves were sectioned into 0.5 cm<sup>2</sup> pieces, placed under tuber slices. After 5-7 days at 20°C, hyphe were transferred to pea agar (Goodwin, Drenth, and Fry, 1992; Shattock *et al.*, 1990). In total, 118 isolates were obtained and cultured (Table 1).

Table 1. Origins and characteristics of Phytophthora infestans isolates collected in China

Region	Year	Isolates	Mating type	MtDNA	SSR
Northeast	2004-2006	29	18	17	17
Inner Mongolia	2006	25	14	14	14
Hebei	2006-2007	15	4	14	14
Sichuan	2007	18	15	16	18
Yunnan	2004-2006	15	8	10	10
Fujian	2007	16	10	14	15
Total		118	69	85	88

#### **DNA** extraction

Isolates were grown in pea broth at  $20^{\circ}$ C for 5 days and after 5 days the mycelia were collected into 8-strip tubes (Genomic DNA was isolated from 20 mg of lyophilized mycelium using the PUREGENE DNA isolation kit (Gentra, Minneapolis, MN) following the manufacturer's instructions and eluted with 50  $\mu\lambda$  ultra-pure water. DNA extracts were stored at -20 °C.

#### Haplotype test

Mitochondrial haplotypes were determined using the PCR-RFLP method of Griffith & Shaw (Griffith and Shaw, 1998). Restrict digestions of amplified regions P2 (MspI) and P4 (EcoRI) allowed differentiation of four mitochondrial (mtDNA) haplotypes Ia, Ib, IIa and IIb.

#### Microsatellite analysis

Eight microsatellite markers were selected for microsatellite analysis of all isolates (using fluorescently labeled primers, from PRI). The forward primers (of 8 primer pairs) were labeled with either FAM or HEX. Amplification reactions consisted of 10 ng template DNA, 200 μM dNTPs, 0.8 U *Taq* DNA polymerase (Roche, Indianapolis, IL), 1.5 mM MgCl<sub>2</sub>, and different volume of each primer in a 20 μl reaction volume. Amplifications were run in a PTC200 thermocycler (MJ Research, Waltham, Massachusetts, USA), with initial denaturation at 94°C for 2 min, followed by 13 touchdown cycles of 94°C for 30 seconds, from 66 to 53°C for 30 seconds, and 72°C for 30 seconds, then by 28 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 min. 1-2 μl of PCR product from successful amplifications were added to 1 μl de-ionized formamide loading buffer, denatured at 92°C for 3 min. The resulting amplification products were sized by capillary electrophoresis on an automated ABI 3730 using the molecular standard GeneScan-500 ROX and GeneMapper4.0 software (Applied Biosystems). The genotyping cluster was generated with TREECON software developed by University of Konstanz.

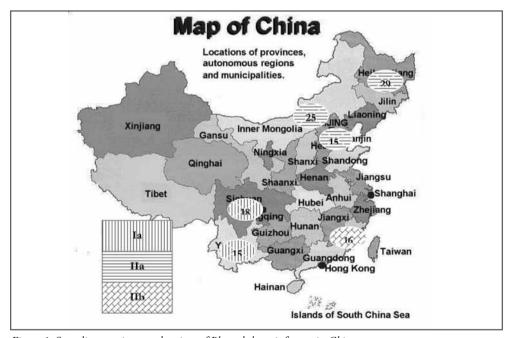


Figure 1. Sampling provinces and regions of Phytophthora infestans in China.

Numbers on the map correspond to isolate number finally obtained from different provinces and regions. Three haplotypes, Ia, IIa and IIb

#### RESULTS

#### Genotyping analysis (haplotyping and microsatellite analysis)

mDNA haplotype test was a classic genotyping approach of *P. infestans*, which can differentiate four alleles (Ia, Ib, IIa, IIb). Three haplotypes (Ia, IIa, IIb) were found and and all correlated strongly to the region of origin. In Northern China all isolates had the IIa haplotype, in southeast China all isolates had the Ia haplotype, while in southwest China all isolates had the IIb haplotype (Figure 1, Table 1). No Ib strain (US-1 clonal genotype) was found among Chinese isolates.

The eight SSR markers showed a high level of differentiation and finally 43 alleles and 40 genotypes were concluded among Chinese isolates. A genotyping cluster was calculated among Chinese isolates (Figure 2). The occurrence of variable multilocus genotypes was clearly depending on the origin of the isolate and many genotypes are unique to a particular region. The genotyping analysis clearly demonstrates that the current population is not a single clonal lineage. Many genotypes are unique to specific regions. The cluster is divided into three subgroups. The isolates from Northeast, Inner Mongolia Province, Hebei Province and one province Yunnan located in Southwestern China were clustered into Subgroup I, which included the clonal group from the previous study (Guo et al.,

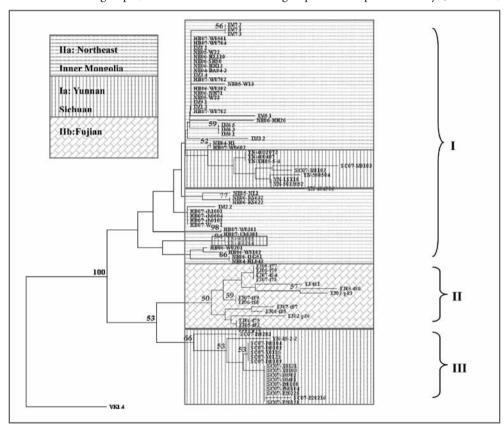


Figure 2. The genotyping clusters by SSR markers.

2009). Three different backgrounds present three haplotypes of isolates. The cluster is divided into three subgroups. Subgroup I contains the isolates from Northeast, Inner Mongolia, Hebei and Yunnan provinces; Subgroup II has the isolates from Fujian province; Subgroup III has the isolates

from Sichuan province. Subgroup II only has the isolates from Fujian province; Subgroup III has the isolates with A2 mating type from Sichuan province and one isolate with A2 mating type from Yunnan Province. Inside one region, genetic structure showed very variable (Figure 2). Isolates from Fujian and Sichuan were clustered to individual subgroups, which showed P.infestans did not crossly migrate between Fujian and Sichuan regions. In Northern regions and Yunnan, it was not surprising that those isolates were together in one subgroup, who were the early potato regions in China and where the potato trade exchange would be more frequent than in other regions.

#### DISCUSSION

Potato late blight has been through 20 years in China, both mating types were found only since last century, in 1996 (Zhang et al., 1996). Few detail reports has been published on whole country-wide P. infestans population (Yang, Zhu, and Zhang, 2008), especially modern population structure before this survey. The migration and diversity of P. infestans population in China was a mystery, which made very difficult for potato resistance management in China. Since potato agribusiness is stimulated by Chinese government and is developing very fast in recent years, more and more commercial transportation and other potato trade activities are carrying on with national-wide, which could move P. infestans around China for instance by infected seed potato's. Meanwhile, potato late blight is not effectively controlled and much more fungicides are used during the potato season in China. The monitoring of P. infestans population structure in China is becoming increasingly important to assist forecasting the occurrence of late blight and guide the resistance management in the field.

This survey was performed to unveil the population diversity and forecast the migration of *P. infestans* in China for efficient and long-term potato resistance strategies. Our SSR analysis does not show clear evidence of sexual reproduction. However, more research should be carried out to assess whether the sexual reproduction is a potential threat in China. In the genotyping cluster and haplotype results, Chinese isolates did not belong to the same haplotype or one clonal lineage anymore. In this modern population of *P. infestans*, isolates from Fujian had one unique haplotype, IIb. Ia haplotype was found in Yunnan and Sichuan provinces, but isolates from these neighbor provinces were genetically grouped to individual clonal subgroups, which showed *P.infestans* did not nationally migrate in these provinces. In Northern regions (Northeast, Inner Mongolia and Hebei provinces) and Yunnan, all isolates were together in one subgroup, who were the early potato regions and where the potato trade exchange would be in more frequency than other regions (Yang, Zhu, and Zhang, 2008).

This study gave a full picture of *P. infestans* modern population in China, which will help to understand the population diversity and migration of *P. infestans* in China. For further research on population diversity, we firstly need to set up a storage system for long-term storing the isolates. Many accidents occurred during storing the isolates and in short period all isolates from different years were lost. To learn from those lessons, a long-term storage system with liquid nitrogen would be set up at Institute of Vegetables and Flowers in Beijing. After this general study of *P. infestans* population in China, a deep sampling action to cover all potato regions in China will be successively carried on in coming years. The successive monitoring of population diversity would provide a dynamic trend of *P. infestans* migration in China and help to make the management decision for late blight resistance, and give an advice on policy makers.

The whole sampling work is done with national-wide researchers on potato late blight in China. Based on the current achievements and collaborations, we are going to organize a late blight initiative in China and start up an umbrella project to support of researchers, technology developers

and agricultural knowledge agents to improve short- and long-term resistance management of late blight in China. We started a comprehensive analysis using the most up to date tools and standards but we would like to extend our characterization using more isolates collected from more regions and different years. We also would like to raise awareness on the current *P. infestans* population and the potential risks of latent infection in seed tubers. Therefore we welcome collaboration and seek joined projects to understand the population structure and its application in forecast the occurrence of late blight and guide the resistance management in certain regions similar to what was achieved by Euroblight for Europe.

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# Host-pathogen interaction between *Phytophthora infestans* and *Solanum tuberosum* under different photoperiods

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#### SUMMARY

Components of potato resistance to *Phytophthora infestans* were screened for the leaflets of Black's differentials grown in different photoperiods. Stronger infection, expressed as Lesion Growth Rate, and stronger sporulation, was observed on potato leaflets of potato plants grown in short day conditions. Latent Period did not differ on plants from both growing conditions. The influence of short day and long day conditions was not observed for the infection of the *R2* Black's differential with each virulent or avirulent *P. infestans* isolates. Some avirulent isolates caused symptoms of infection on leaflets of differentials *R5*, *R6*, and *R8* grown in short day conditions.

#### **KEYWORDS**

Phytophthora infestans, quantitative resistance, qualitative resistance, light

#### INTRODUCTION

Two major kind of resistance is expressed in potato plants to *Phytophthora infestans*. Resistance governed by R-gene(s), which leads to hypersensitive reaction in the incompatible interaction with pathogen isolate possessing avirulence gene(s), or so called "field resistance", when no R-gene(s) are involved, but many genes, and the effect of these genes action can be measured. From our previous observations the virulence of *P. infestans* isolates assessed when plants were grown in short day (SD) conditions was more complex than those assessed on plants from long day (LD) growing conditions. Effect of different photoperiod conditions was evaluated in two type of interactions. "Field resistance", quantitative resistance, was evaluated by measuring Lesion Growth Rate (LGR), Sporulation Intensity (SI) and Latent Period (LP) after inoculation of six genotypes of potato in compatible interaction with three *P. infestans* isolates. Incompatible interaction, qualitative resistance, was screened by using six different genotypes of potato with 18 *P. infestans* isolates.

#### MATERIALS AND METHODS

#### Growing conditions of potato plants

Potato plants were grown in the greenhouse for three weeks and then were transferred to climatic chambers with constant temperature  $20^{\circ}$ C and two different photoperiods. Plants were acclimated for three weeks before the first test was done: in SD conditions – 8 hours of light a day, and in LD conditions, 16 hours of light a day. The intensity of light was measured at the bottom of the plant and at 32 cm of plant height and was equal to  $11\text{-}30~\mu\text{mol}~\text{m}^{-2}\text{s}^{-1}$  respectively.

#### Inoculum preparation and leaflet inoculation

Inoculum consisted of a sporangial suspension that was prepared as described by Zarzycka (2001) from sporulating lesions of potato leaflets and adjusted using a haemocytometer to a concentration of 50,000 sporangia ml<sup>-1</sup>.

Detached leaflet assay. In all experiments fully developed leaflets were detached from the middle part of the plants grown in SD and LD conditions. Leaves were placed on wet cellulose wadding in a plastic tray. Each leaf was inoculated by depositing one 30  $\mu$ l droplet of the inoculum on the abaxial side of the leaf. The trays with leaves were covered with glass. The inoculated leaves were incubated for 7 days at 16°C with a constant illumination of 11.5  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>. After the first 24 h of incubation the leaves were turned abaxial side down.

#### METHODS OF EVALUATION

#### Quantitative resistance

Six potato genotypes were used: Black's differentials R1, R3, R4, R7, R11 and cultivar Craigs Royal. For inoculation three virulent P. infestans isolates were used from IHAR Młochów collection: MP 832, MP 585 and MP 674. 20 leaflets per each potato genotype, per each P. infestans isolate, were inoculated in 3-5 tests performed. The leaflets were detached from plants acclimated for 3-7 weeks in SD or LD conditions. "Field resistance" was assessed by estimating three components: LGR was estimated based on linear regression of mean diameter of lesion as a function of days after inoculation, y = ax + b, where y - mean diameter of lesion ( $\sqrt{ncd}/4$ , where y - mean diameters of lesion), y - mean diameters of days after inoculation, y - mean diameters of lesion increase of the lesion size in mm/day. LP was also defined as y - mean by y - mean diameters of the lesion size in mm/day. LP was also defined as y - mean diameters of sporangia. SI was estimated as a number of sporangia. SI was estimated as a number of sporangia. Black's differentials and the isolate MP 585.

#### Qualitative resistance

In compatible and incompatible interaction, six potato genotypes were used: Black's differentials: *R2*, *R5*, *R6*, *R8*, *R9*, *R10*. For inoculation 18 *P. infestans* isolates were used from pathogen collection at IHAR Młochów Center: MP 585, MP 674, MP 750, MP 832, MP 833, MP 834, MP 840, MP 842, MP846, MP847, MP851, MP866, MP868, MP870, MP872, MP873, MP879, MP880. 20 – 24 leaflets per genotype, per isolate, were inoculated in 3-5 tests performed on leaflets detached from plants acclimated for 3-7 weeks in SD or LD conditions. Symptoms were assessed in two grade scale, 0 – for lack of symptoms or nonsporulating lesions, 1 – for sporulating lesions.

#### **RESULTS AND DISCUSSION**

#### Quantitative resistance

Lesion Growth Rate

A significant influence of the growing conditions, genotype and genotype by growing conditions interaction on expression of LGR was revealed. In general LGR was higher for plants grown in short day conditions. Tested genotypes differed in LGR from each other and also different reaction of genotypes was observed depending on growing conditions. The influence of *P. infestans* isolate and isolate by growing conditions and isolate by genotype interactions as well as the interaction of isolate by genotype by growing conditions was not found.

#### Latent Period

Analysis of variance for LP revealed statistically significant but with small effect of isolate and

genotype on this trait. The effect of growing conditions was not significant.

#### Sporulation Intensity

The method used for estimating number of spores in 1 ml of water from 1 cm<sup>2</sup> of lesion was affected by a large error. Nevertheless, the significant differences were observed for sporulation intensity for plants grown in different photoperiods. Nevertheless, the significant differences, higher sporulation, were observed for SI on leaflets detached from plants grown in short day conditions for Craigs Royal, *R*1, *R*4, and *R*6. For the differentials *R*5, *R*9, *R*10 and *R*11 sporulation was observed only on leaflets grown in SD conditions. Differences in sporulation on *R*2 and *R*3 were not significant.

#### Qualitative resistance

For Black's differentials R1, R3, R4, R7, R11 only three virulent isolates were tested, because avirulent races to those specific R-genes were not found in IHAR's collection of *P. infestans*, and all these isolates caused symptoms of disease on 20 leaflets of each differential from both short and long day conditions.

The influence of growing conditions were not observed for the *R2* plants. The leaflets detached from these plants showed symptoms of infection after inoculation with three virulent isolates and did not show any symptoms of infection after inoculation with 15 avirulent isolates of *P. infestans*.

The R5 plants did not show any symptoms of infection after inoculation with 6 P. infestans isolates. For 8 P. infestans avirulent isolates leaflets originated from SD conditions showed symptoms of infection significantly more often than leaflets from LD conditions. After inoculation with the isolate MP 832 symptoms were observed on leaves from both growing conditions. Two avirulent isolates cause sporadically symptoms of infection either on leaflets from plants grown in SD or in LD, respectively.

Similar reaction was observed for the *R6* and *R8* plants, but not for the *R9* and *R10*.

#### **DISCUSSION**

The potato resistance to *P. infestans* depend on many factors. Among them the lower light intensity or shorter photoperiod increase susceptibility of potato plants (Colon, 1994; Victoria and Thurston, 1974). The conclusion was that the photoperiod is a significant factor in the expression of quantitative resistance to *P. infestans* of some potato genotypes, but not all genotypes of potato react the same way. These findings were confirmed in this study for different potato genotypes.

For the first time it was observed that there was a group of avirulent isolates of *P. infestans*, evaluated previously on leaflets from plants grown in a greenhouse, which can behave as virulent for *R*5, *R*6, *R*8 plants grown in short day conditions, while they remain avirulent or virulent only sporadically for plants grown in long day conditions. Similar phenomenon was reported by Ward and Buzzel in regard to the light influence on the interaction between soybean genes for resistance and *Phytophthora megasperma* f. sp. *glycinea* (Ward and Buzzel, 1983). In their studies several *Rps* genes incompatible with race 4 failed to condition resistance to this race in intact etiolated hypocotyls.

#### **CONCLUSIONS**

In general short day growing conditions of potato plants influence on stronger infection, expressed as higher lesion growth rate, and stronger sporulation, but not for latent period, after inoculation with virulent *P. infestans* isolates. Potato genotypes differ in sensitivity to different photoperiods. The influence of short day and long day conditions was not observed for the infection of the *R2* Black's differential with each virulent or avirulent *P. infestans* isolates. But surprisingly some avirulent isolates caused symptoms of infection on leaflets of differentials *R5*, *R6*, and *R8* grown in short day conditions. Further studies are needed to understand the role of light conditions in qualitative resistance of potato to *P. infestans*.

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## Aggressiveness differences between A1 and A2 isolates of *Phytophthora infestans* from France

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#### **SUMMARY**

The frequency of A2 mating type isolates of *Phytophthora infestans* dramatically increased in France since 2003. This fast invasion of A2 isolates was first observed in Northern France, then in all potato production areas. To understand these population changes, we investigated the hypothesis that A2 isolates could be more aggressive than A1 isolates. To this end, 238 isolates (111 A1 and 127 A2 mating type) were collected in the three main potato areas (North, West and Center) from 2004 to 2007. Experiments under controlled conditions were performed to determine aggressiveness of A1 and A2 isolates, by comparing lesion size and sporulation of each isolate inoculated on leaflets of Bintje. Our results revealed that A1 populations were more aggressive than A2 populations, especially A1 isolates collected in 2007. Therefore, aggressiveness (as measured under our experimental conditions) does not explain the fast expansion of A2 isolates in France. Furthermore, populations collected in the three main potato regions showed differences in aggressiveness. A1 isolates from Northern France had a greater sporulation than A1 isolates from Western France; A2 isolates from Northern France were more aggressive than A2 isolates from Central. Finally, isolates collected from refuse piles in May showed a lower sporulation capacity than those sampled from fields in June, providing further evidence that aggressiveness increases during epidemics.

#### KEYWORDS

Phytophthora infestans, mating type, invasion, aggressiveness components, geographic scale, populations

#### INTRODUCTION

Drastic changes in *Phytophthora infestans* populations have been observed in the 2000s in France. The frequency of A2 mating type remained at very low levels until 2003, and French populations were thus almost formed of A1 mating type isolates. However, a substantial increase in A2 mating type was first observed in North of France during 2003. A very fast and steady progression of this mating type was then detected among Northern isolates, as the proportion of A2 isolates increased from 6% in 2003 up to 76% in 2007 (Montarry *et al.*, 2006a; Dubois and Duvauchelle, 2007; Duvauchelle *et al.*, 2008). In 2007, most *P. infestans* isolates were A2 mating type all over France, with some regional differences in A2 frequency. In Western France (Brittany), A2 mating type isolates were first detected later (2005) than in Northern France, but their progression was similar,

with frequencies A2 isolates from 2005 to 2008 of 5%, 10%, 42% and 73% respectively. In this region, a sampling conducted in 2008 showed local variations, with 84% of A2 isolates in central Brittany, but only 64% in the North-Western part of the region. This invasion by the A2 mating type is concommitant with the increase in the A2 frequency that has been taken place in some other European countries with oceanic climate, such as the UK (Cooke *et al.*, 2007; Cooke *et al.*, 2009, this volume; Lees *et al.*, 2009, this volume).

The aim of this study was to determine whether A2 mating type isolates were more aggressive than A1 isolates, to explain the fast invasion of A2 isolates in France. To this end, comparisons were made with A1 and A2 populations sampled from the three main potato production areas during 2004 to 2007. Each isolate was characterized phenotypically for its aggressiveness, which is considered as the resultant of quantitative pathogenicity traits.

#### MATERIALS AND METHODS

#### Phytophthora infestans isolates

A total of 238 isolates (111 A1 and 127 A2 mating type) were sampled from 2004 to 2007 from naturally infected potatoes from three main production areas in France (North, West [Brittany] and Center). In 2004-2005, 81 field isolates (43 A1 and 38 A2) were all collected in Northern France, on the susceptible cultivar Bintje, on 2<sup>nd</sup> August 2004 and 25<sup>th</sup> July 2005. In 2006, 54 isolates (25 A1 and 29 A2) were also sampled in Northern France, but they came from refuse piles (12 A1 and 6 A2, collected in May) or from several cultivars of commercial fields (13 A1 and 23 A2, sampled on June, until 6<sup>th</sup> July). In 2007, *P. infestans* populations were collected in the three regions: in the North, 39 isolates (13 A1 and 26 A2) from commercial fields of Bintje on 28<sup>th</sup> June; in the West, 30 A1 isolates from experimental trials on Bintje in one location, Ploudaniel (29) on 19<sup>th</sup> June; in Central France, 34 A2 isolates from fields of cv. Agata, the dominant cultivar of this region, on 14<sup>th</sup> June. For this last year, the proportion of A1 and A2 isolates was different according to the regions. In fact, both mating types were recovered from the Northern samples, but only one mating type was found in the other two regions (A1 mating-type in Ploudaniel and A2 mating type in Central France). These single-lesion isolates were maintained as axenic cultures on pea agar medium.

#### Mating type determination

We determined the mating-type of each isolate by pairing on pea agar with known A1 and A2 testers, incubating in the dark at 15°C for 10-14 days, and observing cultures for oospores formation under a microscope.

#### Aggressiveness tests

#### Potato plant material

Bintje plants were grown from certified seed tubers in pots (one tuber per pot) filled with 1:1:1 sand-peat-compost mixture, in a glasshouse regulated at 15-20°C and 16 h of photoperiod. Leaflets were collected for experiments on 6-8 week-old plants.

#### Inoculum preparation

Each isolate was first multiplied separately on detached Bintje leaflets. The leaflets were infected by deposing a drop of suspension of *P. infestans* sporangia collected by flooding a 3-week-old culture with 5-6 mL sterile distilled water and gently scraping the colony surface to remove sporangia. After seven days of incubation in humid chambers under controlled conditions (15°C/18°C night/

day temperatures, 16h daylight), the sporangia produced on infected leaflets were collected in sterile water; the resulting suspensions were adjusted to  $5 \times 10^4$  sporangia mL<sup>-1</sup> and used for aggresiveness experiments.

#### Aggressiveness quantification

Aggressiveness of each isolate was tested on six leaflets of cv. Bintje. Each leaflet was placed abaxial face up on the lids of inverted Petri dishes containing 10 g  $L^{-1}$  water agar (two leaflets per dish), and inoculated by depositing a 20  $\mu L$  drop of sporangial suspension at the leaflet center. Infected leaflets were incubated six days as described before. Lesion size (area in cm²) was then measured by using a image analyser, with Histolab software (Microvision Instruments, Evry, France). Each leaflet was washed in 10 mL saline buffer (Isoton II); sporangia production per lesion was determined with a particle counter (Beckman Coulter Z2, Villepinte, France), and sporulation capacity was calculated as sporangia production per cm² of lesion. In 2004-2005, the latency period was also determined by observing daily the apparition of sporangia on the inoculated leaflets. Data were submitted to analyses of variance (ANOVA) using the general linear models (GLM) procedure of the SAS statistical software (SAS Institute, Cary, NC). Whenever significant effects were detected, means were compared using the Student-Newman and Keuls test.

#### **RESULTS**

Aggressiveness of A1 and A2 isolates from North of France, collected from 2004 to 2007
For the three years 2004 to 2006, the comparison between aggressiveness of A1 and A2 populations of Northern France indicated that A1 isolates were slightly more aggressive than A2 isolates (Figure 1). However, differences are not very important with all aggressiveness components, as shown for lesion size in Figure 2. On the contrary, ANOVA revealed significant effects between A1 and A2 isolates collected in 2007 in North. Comparison of mean values showed that A1 isolates produced larger lesions (9.6 cm²) than A2 isolates (8.3 cm²), and sporulation of A1 isolates was significantly higher than that of A2 isolates (Figure 3). These results indicated that A1 populations from Northern France were more aggressive than their A2 counterparts, on Bintje, under our experimental conditions.

Aggressiveness of A1 and A2 isolates collected from three French regions in 2007

A significant effect of mating type was detected on aggressiveness components of isolates from

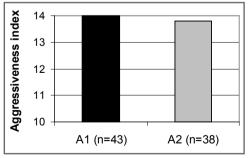


Figure 1. Comparison of aggressiveness of A1 and A2 isolates of P. infestans collected in Northern France, in 2004 and 2005.

Aggressiveness index is expressed as ln (area lesion x spores per lesion x 1/ latency period).

Statistical difference with p = 0.016

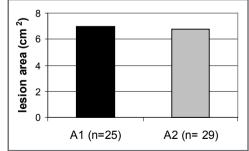


Figure 2. Comparison of lesion size produced by A1 and A2 isolates of P. infestans, collected in 2006, in Northern France. Isolates were inoculated on Bintje leaflets and incubated during 6 days.

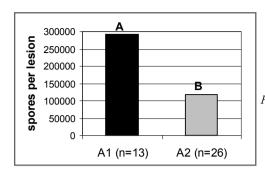


Figure 3. Comparison of number of sporangia per lesion produced by A1 and A2 isolates of P. infestans, collected in 2007, in Northern France. Isolates were inoculated on Bintje leaflets and incubated during 6 days.

Northern, Western (Ploudaniel) and Central France. The A1 isolates collected from North and West produced larger lesions and a higher number of sporangia on leaflets than A2 isolates from North and Center (Figure 4). Among the isolates coming from these three regions, A1 isolates from Northern France were significantly the most aggressive ones. Although lesion size was similar between A1 isolates from Northern France and Ploudaniel, those from Northern France produced twice as many sporangia per lesion than A1 isolates from Ploudaniel (29x10<sup>4</sup> spores/lesion and 15x10<sup>4</sup> spores/lesion, respectively). The A2 populations from Northern and Central France produced similar numbers of spores per lesion (11x10<sup>4</sup> spores/lesion). The A2 isolates from Central France showed a slightly higher capacity of sporulation than A2 from Northern France, but they produced smaller lesions (6.5 cm² vs 8.3 cm², respectively). Therefore, A2 isolates from Northern and Central France were the least aggressive among all isolates sampled in 2007, but differences appeared between populations of these two regions, according to the aggressiveness components considered.

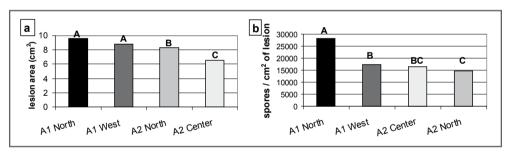


Figure 4. Comparison of A1 and A2 isolates of P. infestans collected in three French regions (North, West and Center) in 2007, based on: **a)** lesion size and **b)** capacity of sporulation on Bintje leaflets, 6 days after inoculation

#### Aggressiveness of isolates collected from refuse piles and fields

In 2006, isolates were collected from Northern France at two different periods: early from piles and later in fields. Sample from refuse piles contained more A1 than A2 isolates, whereas there were more A2 than A1 isolates in field samples. We thus expected that isolates from piles would be more aggressive than isolates from fields. Our data showed no difference for the lesion size between these two samples, but ANOVA revealed a significant effect of sampling date on sporulation capacity (Figure 5). The comparison of sporulation capacity indicated that isolates collected from piles were less aggressive than those sampled from fields. This result suggested that isolates collected at the start of the epidemic were less aggressive than those sampled later in the season.

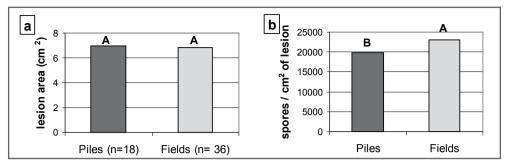


Figure 5. Comparison of P. infestans isolates collected from refuse piles and fields in Northern France during 2006, based on: **a)** lesion size and **b)** capacity of sporulation on Bintje leaflets, 6 days after inoculation

#### DISCUSSION AND CONCLUSIONS

The objective of this work was to investigate one possible explanation for the rapid invasion of A2 isolates all over France. Our results showed that A1 isolates were more aggressive than A2 isolates on Bintje. Therefore, aggressiveness (as measured in our conditions) did not explain the fast expansion of A2 populations. Moreover, our data showed that aggressiveness of the populations was different in the three main potato regions of France. The A1 isolates are most aggressive in Northern France, where Bintje has been cultivated for long periods on large areas. This result is consistent with the hypothesis of adaptation of *P. infestans* populations to the dominant host genotype Bintje (Montarry *et al.*, 2008). We have also observed that capacity of sporulation is smaller in isolates collected from piles than those from fields. This result suggests that aggressiveness increases during the season, which confirms previous data obtained on *P. infestans* populations from Western France (Andrivon *et al.*, 2007).

Our experiments were done on a susceptible cultivar and under controlled conditions optimal for the development of the pathogen. It would be worthwhile to conduct them under other conditions (cooler temperatures, different cultivars). Indeed, these parameters may be favorable to A1 populations, but "new" A2 populations could have an advantage in tests performed in another manner (Lees *et al.*, 2009, this volume). In the same way, we can do the hypothesis that A2 isolates exhibit particular characteristics; they could thus be adapted to climatic changes which could lead to best survival or development (especially sporulation) under extreme weather events in fields, or they could be able to overcome previously resistant cultivars.

Since A1 and A2 isolates were simultaneously detected in some fields, formation of oospores is now possible in France. The presence of these sexual spores could lead to a higher genotype diversity in *P. infestans* isolates in which some of them could be able to overcome formerly resistant cultivars. These oospores represent also a potential risk as primary source of inoculum, because they can survive in soil for several years.

It is now necessary to analyse interactions between aggressiveness and other traits, such as the genetic structure of these populations. Neutral molecular markers have proved particularly valuable to understand the changes of *P. infestans* populations (Lees *et al.*, 2006). Previous data revealed that the Northern France population was more diverse than populations from Western France, and that there were important variations in genotype frequencies over successive seasons (Lebreton *et al.*, 1998; Montarry *et al.*, 2008). The high genotype diversity observed in the Northern France population could be the effect of its geographic situation, at the confluence of flows from other large neighbouring potato production areas (Belgium, The Netherlands, UK). Little is known about

repartition of the 13\_A2 genotype in France and whether this genotype is responsible of the fast A2 expansion, as observed in Great Britain (Cooke *et al.*, 2007; Lees *et al.*, 2009, this volume), although A2 isolates from France fell into several genotypic clusters (Montarry *et al.*, 2006b, 2008). It is thus important to have information on population structure and their evolution according to geographic scales in different French regions, according to time scales on several years and during a crop season.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Influence of recent climate change on late blight risk in the UK

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#### **SUMMARY**

The influence of recent climate change in the UK on potato late blight pressure was assessed by analysing the total annual number of Smith Days from four sites from 1998 to present. At each site regional (synoptic) and local data was used to review the number of annual risk periods. Also reviewed was the number of late blight outbreaks and fungicide use from 2003-2008.

Analysis of regional data showed an increasing trend in blight risk in the last 10 years. The number of risk days recorded in 2008 was over 70% higher than the days in 1998 at all sites. Analysis of local data, in the last 7 years, also showed the increasing trend of risk days. The number of risk days recorded in 2008 was around 70% higher at coastal sites (South West & East Anglia) than in 2002, however the increase in risk days was lower at sites further inland (North East & East Midlands). In the period since 2003 there were increases in the number of blight outbreaks recorded (up by 245%) and number of fungicide treatments applied (up by 42%).

The specific climatic factors causing the increases in UK blight risk, in the last 10 years, requires further investigation. Higher temperatures may be creating more risk periods earlier and later in the season. More frequent extreme rainfall events may be causing longer periods of high humidity.

If the occurrence of late blight risk continues to increase or remain high, there will be continued reliance on fungicide input, and therefore DSS's will be important to monitor and manage this effect of changes in the climate.

#### **KEY WORDS**

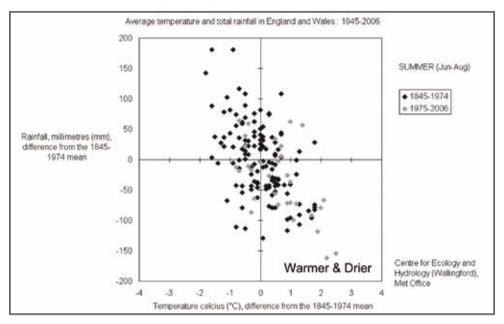
Climate Change, Smith Days, Regional & Local Data, Blight Risk, Outbreaks, Fungicide Use, DSS

#### INTRODUCTION

By 2050 it is predicted that as a result of climate change potato growing regions in Europe will shift. As summers become hotter and drier, around the Mediterranean Sea, conditions will marginalise for potato growing because of water shortages, and in Eastern Europe growing will get more difficult as conditions become too dry. Whereas, in North West Europe, conditions will become more favourable, and in the Scandinavian regions, new areas of production will be created (source NL press article). An unknown at present will be the effect of these more favourable conditions in the north of Europe on late blight risk in the future. A clue to answering this question could be in analysing recent changes in late blight risk.

Records in the last 30 years (1975-2006) from the UK, show summers have been warmer and drier

compared to the previous 140 years (1845-1974), see chart 1 (Centre for Ecology and Hydrology 2007). The Late Blight pathogen (*Phytophthora infestans*) requires relatively wet, cool conditions for optimum development. It could therefore be assumed that UK conditions in recent years will have been less favourable for late blight development and that this trend would continue in the future.



However summer weather in the UK in the last 5 years appears to be becoming more erratic, with extreme events occurring such as the floods of 2007 in June and July, which caused prolonged late blight infection periods.

To evaluate the influence of recent climate change on late blight, risk from 4 regions in the UK is compared over a number of years, based on data from local and regional weather stations. Also compared are blight outbreaks and fungicide use in recent years.

#### **METHOD**

#### Meteorological data and blight risk calculation

Four potato growing regions in the UK (Fig 1) from south to north were analysed for blight risk days annually. Two sites were coastal, South West and East Anglia, whereas two were inland sites, East Midlands and North East. The South West area is predominantly an early fresh market potato growing region, whereas the sites further north, grow mostly maincrop potatoes for both the fresh and processing market.

Blight risk was analysed using both regional data and local data.

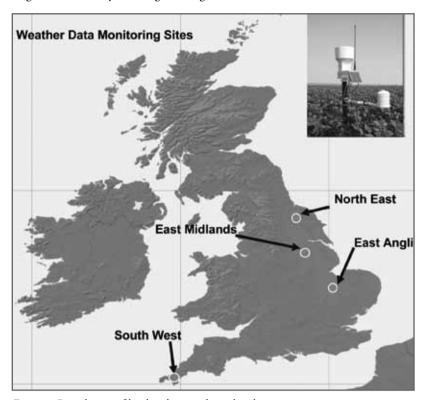


Figure 1. Distribution of local and regional weather data monitoring sites

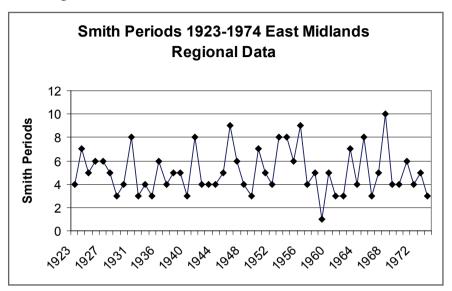
Local data is shown from 2002-2008 using data from Adoon weather stations owned by growers near the sites selected. The annual data calculated for each season was from May 1st to September 30th.

The regional data was taken from the nearest synoptic station as supplied by CSL 1998-2003 and BlightWatch data 2004-2008. Some data is also shown from an earlier study (Croxall, 1975) from one site, the East Midlands, with data from 1923-1974. Generally the regional data available was for a shorter period, from early June to mid September.

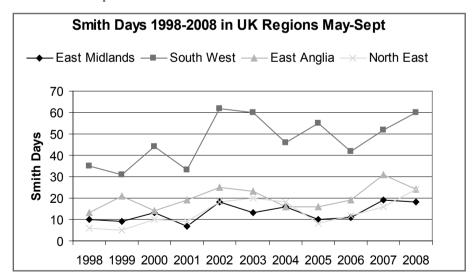
For the purpose of this comparison, blight risk was calculated using a basic decision support system, Smith (Smith, 1956), where 11 hours of temperature at or above 10°C and relative humidity is at or above 90% equates to a critical period (Smith Day), and a Smith Period is 2 consecutive Smith Days. Although more advanced DSS's are used in these regions, such as Plant-Plus (Hadders, 1996), Smith was chosen because it requires only meteorological data, unlike Plant-Plus which also integrates host and pathogen information into the calculation.

#### RESULTS

#### 1. Late Blight Risk Historical Trends

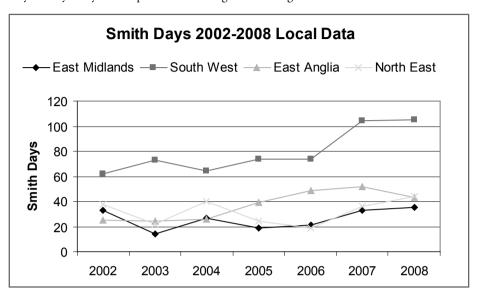


In the East Midlands from 1923 to 1974, Chart 2 shows that late blight risk has fluctuated from year to year, however apart from a few rises in the mid 1950's and 1960's, in general risk has followed a level trend in this period



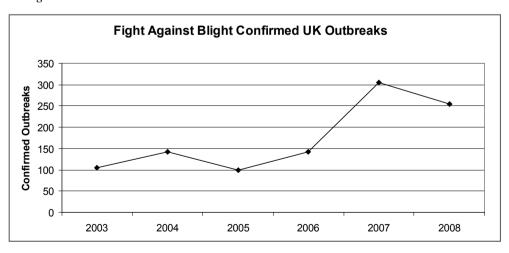
Since 1998 regional risk from all sites has shown an upward trend towards 2008 (Chart 3). The increase in risk was most pronounced in the South West from 2001 to 2002 in other regions the increase has been more gradual, however over the ten years the number of Smith Days has increased at the sites by 80% in the East Midlands, by 71% in the South West, by 85% in East Anglia and by

400% in the North East. Chart 3 also highlights the difference between the South West and the other regions in terms of blight risk, with the South West giving over twice the number of Smith Days in any one year compared to an average of other regions.



The risk trend from the same sites using local data (Chart 4), follows a similar upward path as risk from regional data, albeit the overall increases are not as large. The largest changes were seen in the South West and East Anglia where the number of Smith Days increased by 69% & 72% respectively over the 7 year period. At both sites the stations were close to the coast. Smith Day increases in the East Midlands and in the North East were more modest at 6% & 16% respectively.

#### 2. Blight Outbreak Trends

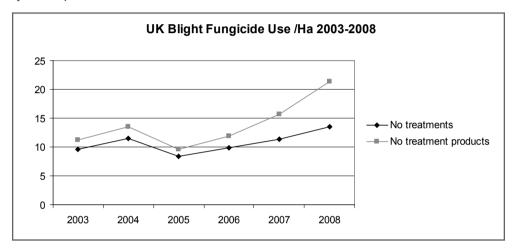


Outbreaks confirmed from the Potato Council (formerly British Potato Council) Fight Against Blight scouting system also shows an upward trend in the last 6 years since the project was initiated.

In 2008 there was an increase of 245% on confirmed outbreaks compared to 2003. The results represent samples taken by over 300 registered scouts from the main UK potato growing regions. Sources of outbreaks include infections from potatoes in crops, dumps, volunteers, organic crops and gardens.

#### 3. Fungicide Use

Fungicide use data from the UK (source Bayer Crop Protection) has shown an increasing trend over the last 6 years of both number of treatments/ha and number of products applied/ha. In that time the number of treatments/ha has risen by almost 4, from 9.5 to 13.5. The rise in the number of products applied/ha has been even greater, increasing from just over 11 to 21 per hectare in the space of 6 years.



#### **CONCLUSION AND DISCUSSION**

From the data presented the influence of recent climate change in the UK has been a trend of increasing blight risk in all regions reviewed from both regional and local data. It is likely that the trend of increasing numbers of outbreaks recorded and fungicide treatments in the last 6 years are linked to the higher levels of disease risk in this period. Other factors contributing to the increase in blight outbreaks could be the emergence of the more aggressive A2 - 13 strain in the UK (Lees, 2008) and the fact that scouts recording risk have become more familiar with the procedure in sending isolates for testing. The increase in fungicide products used in recent years could also be linked to the wider choice of products which have come onto the market in the last few years.

The reason behind the increase in late blight risk has not been analysed in this review. It could be connected to the frequency of extreme rainfall events which has appeared to increased in recent years. Another factor could be that increasing temperatures are creating more optimal risk periods at the start and end of the season. A warmer climate may also be linked to increasing levels of humidity, especially around coastal areas, where sea temperature rise could be creating longer periods of fog and mist. The effect of all these factors on blight risk would merit further investigation, to establish what the future influence of climate change will have on disease risk.

If future risk in North West Europe is to continue to increase, the role of DSS's in this region is likely to change. In the short term they will be important to assess the rate of increasing risk and

manage the higher fungicide inputs required in such conditions. In the longer term, if there is more pressure/legislation to reduce fungicide treatments they may also be required for a more integrated approach to blight management, perhaps in using variety resistance or biological control to reduce the reliance on fungicides.

#### **ACKNOWLEDGEMENTS**

Morray Taylor from CSL for providing regional Smith data (1998-2002). Potato Council Ltd for Flight Against Blight outbreak data. Blightwatch for regional Smith data 2003-2008. Bayer Crop Science for fungicide use data. Dacom for local weather data 2003-2008.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

### Phosphorylation in Plant-Phytophthora interactions

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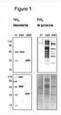
## Phosphorylation in Plant-Phytophthora interactions

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#### INTRODUCTION

Different *Phytophthora* species are among the most devastating pathogens of agronomically important dicot plants. *Phytophthora infestans* caused the Irish potato famine some 160 years ago, and has since then been a major problem in potato production. *Arabidopsis* exhibits non-host resistance to *P. infestans* and is therefore an attractive source of resistance to investigate. Protein phosphorylation is a key biological process regulating many reactions in plant-microbe interactions. We are interested in identifying *P. infestans* induced phosphorylation of Arabidopsis proteins, including determination of phosphorylation sites, in order to obtain a better understanding of the biological role of these modifications. This knowledge could serve as a basis for developing new methods of reducing the effects of pathogen attack such as phospho-mimic mutation of signaling intermediates.



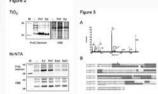
A method to enrich phosphoproteins on TiO<sub>2</sub> resin in native and denaturing buffers was developed using a standard mixture of phosphorylated and unphosphorylated proteins (Figure 1). Both buffers removed unphosphorylated proteins from the mix. Binding under native conditions recovered 50% of the 45kDa protein, while 33% of the 23-kDa protein was recovered using denaturing conditions, indicating that stochiometric changes in phosphorylation might be detected. Enrichment of Arabidopsis cell culture phosphoproteins were evident using both buffers, as compared to a total cell extract. The phosphoproteins patterns were only partially overlapping, indicating that both native and denaturing conditions could be used in order to et at more comprehensive phosphoprotein analysis.

Figure 1. Phosphoprotein enrichment using TiO2 reain under native and denaturing conditions. TiO<sub>2</sub> enriched proteins were separated with SDS-PAGE and the gels analyzed for phosphoproteins by staining with Pro-O Diamond (Molecular Probes), upper panel. Lower panel shows Reside staining of the same gels. Left panel shows results using a standard mit of its proteins, of which two are phosphorylated. Right panel show TiO<sub>2</sub> purification of phosphorylated proteins from Arabidopsis thaliana. In, injust, nat, native conditions; dee. detenting conditions.

Phosphoprotein enrichment identified two proteins that were phosphorylated after elicitation of Arabidopsis cells with Phytophthora infestans zoospores and xylanase, but not with salt or osmotic stress (Figure 2). Both identified proteins contain a universal stress protein domain (USP) of unknown function with conserved residues for ATP binding (Figure 3), (Lenman, Sörensson, Andreasson; Molecular Plant Microbe Interactions; in press).

Figure 2. Two proteins are phosphorylated after elicitation of Arabidopsis thaliana cells. Upper panel,  ${\rm TiO_2}$  purification: lower panel,  ${\rm Ni-NTA}$  purification. Unbreaded (-) Arabidopsis cells, and cells elicitated with either P-infostanz zoopsores (Pirf.), systanze (Xyl), sorbitol (Sonb.) or sodium chloride (NaCI).

Figure 3. A, MALDI MS spectra of trypsin-digested lower band that was phosphonylated after P. infestans elicitatios. B, Protein alignment of USP domain containing proteins (AHQ27230.1 and A15g34430.1). Pepides identified by MALDI MS are shaded. The USP domain is underlined. The asternisk denotes phosphorylated residues.





The determination of phosphorylation sites in the two USP proteins was facilitated by development of a method to derivatize  ${\rm TiO_2}$  purified phosphopeptides with SPITC.

Figure 4. A, MALDI MS/MS spectra of the Al4g27320.1 m/z 1010 phosphopeptide before derivatization. B, MALDI MS/MS spectrum of SPITC derivatized phosphopeptide (m/z 1225).



#### SUMMARY AND CURRENT PROJECTS

We demonstrate the use of TiO<sub>2</sub> resin for enrichment of phosphoproteins as well as a method to derivatize TiO<sub>2</sub> purified phosphopeptides to facilitate the determination of the exact site of phosphorylation. The use of these methods is exemplified by the identification of two USP proteins that were shown to be phosphorylated after elicitation of Arabidopsis cells with *Phytophthrae infestans* zoospores and xylanase.

- 1. Phosphoproteomics on Arabidopsis genotypes with altered signal transduction activities will be conducted.
- Other new putative resistance factors have been identified by bioinformatics. For one of these a phospho-mimic mutant has been created and transformed into potato with the goal of increasing the resistance against *Phytophthora infestans*.
- Secretome analysis of potato breeding material and wild relatives treated with Phytophthora and resistance inducing agents are underway.

#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Potatobreeding in Norway

K. BUNDGAARD

## Potetforedling i Graminor

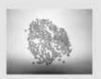




Tradisjonell foredling starter med en kryssing mellom to potetsorter.



Etter befruktning dannes et frøeple. Det likner en grønn tomat, men er giftig.



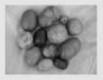
Alle frøene fra en krysning er søsken, men er genetisk forskjellige. Hvert enkelt frø er en ny sort.



Utspaltingen av egenskaper i potet er stor. Bildet viser kryssing mellom to røde sorter.



I de neste 10 år går alle de nye potetsortene i en seleksjonsprosess hvor de vurderes og kasseres etter hvert som de viser egenskaper som ikke er gode nok til endelig godkjenning.



I de første år vurderes fysiske egenskaper som: Form, knollfarge og kjøttfarge.



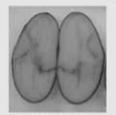
Kunden i butikk fristes lettere av en skinnene potet som er lett å skrelle



I årene deretter vurderes resistens mot tørråte, foma og fusarium (bildet)



Resistens mot tørråte på riset.



Det testes for resistens både mot rattlevirus og mopptoppvirus.



Flatskurv (bildet) samt andre skurvtyper testes det for.



Koketype er viktig for å bestemme sortens anvendelsesområde



For sorter til chips og pommes frites er tørrstoffinnholdet og fargen viktig.



Nye sorter må ha høy avling for å bli populære hos bonden.



Der går 15 år fra kryssning til den nye sort er i butikk og der er kastet 35.500 før den beste er valgt ut.

## osters

## Potato Breeding in Norway



ARIO-2085 8.1 Tohica 8.1 Excert 7.8 NCE-92-22-14 7.8 POL-92-22 14 7.8 POL-92-22 17,6 Innovator 7.2 Roblin 8.8 Rovella 6.6 Rovella 6.6 Roblin 8.8 Roblin 8.		Scale
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Folva 2,9 Van Gogh 2,9 Van Gogh 2,9 Aslak 2,8 Canberra 2,8 Canberra 2,8 Cinchestra 2,8 False 2,7 Redistar 2,7 Redistar 2,7 Congo 2,6 Ludy Amarilla 2,6 Musica 2,6 Ostara 2,6 Ostara 2,6 Robella 2,6 Bruse 2,5 Robella 2,6 Bruse 2,5 Robella 2,6 Bruse 2,5 Robella 2,6 Bruse 2,5 Robella 2,6 Loris (1972) 2,5 Rether 2,4 Loris (1972) 2,5 Rether 2,4 Loris (1972) 2,4 Loris		2,9
Van Gogh         2.9           Aslak         2.8           Canberra         2.8           Orchestra         2.8           Drichestra         2.7           Fakce         2.7           Redstar         2.7           Congo         2.5           Lady Amarilla         2.6           Musica         2.6           Ostara         2.6           Pirol         2.6           Robella         2.6           Bruse         2.5           Gloria (1972)         2.5           Berber         2.4           Chopin         2.4           Chopin         2.4           Juno         2.4           Saline         2.4           Secura         2.4		2.9
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Canberra         2,8           Orchestra         2,8           False         2,7           False         2,7           Redistar         2,7           Congo         2,5           Ledy Amarilla         2,6           Ostara         2,6           Pirol         2,5           Robella         2,6           Bruse         2,5           Gloria (1972)         2,5           Berber         2,4           Birrije         2,4           Chopin         2,4           Eersteling         2,4           Juno         2,4           Saline         2,4           Secura         2,4	Van Gogh	
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Lady Amarilla         2,6           Musica         2,6           Ostara         2,6           Pirol         2,5           Robellia         2,6           Bruse         2,5           Gloris (1972)         2,5           Berber         2,4           Bintje         2,4           Chopin         2,4           Juno         2,4           Saline         2,4           Secura         2,4		2.6
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Ostara         2,6           Pirol         2,6           Robella         2,6           Bruse         2,5           Gloria (1972)         2,5           Berber         2,4           Birnje         2,4           Chopin         2,4           Lersteling         2,4           Juno         2,4           Saline         2,4           Secura         2,4	Musica	2,6
Pirol         2,6           Robella         2,6           Robella         2,6           Bruse         2,5           Gloria (1972)         2,5           Berber         2,4           Birtije         2,4           Chopin         2,4           Eersteling         2,4           Juno         2,4           Saline         2,4           Secura         2,4		2.6
Robella         2,6           Bruse         2,5           Gloris (1972)         2,5           Berber         2,4           Birtje         2,4           Chopin         2,4           Eersteling         2,6           Juno         2,4           Saline         2,4           Secura         2,4		2,6
Bruse 2,5 Gloris (1972) 2,5 Berber 2,4 Birtje 2,4 Chopin 2,4 Luno 2,4 Juno 2,4 Saline 2,4 Saline 2,4 Secura 2,4		2.6
Gloria (1972) 2,5 Berber 2,4 Birtje 2,4 Chopin 2,4 Eersteling 2,4 Juno 2,4 Secura 2,4 Secura 2,4		2.5
Berber         2,4           Bintje         2,4           Chopin         2,4           Eersteling         2,4           Juno         2,4           Saline         2,4           Secura         2,4		
Bintje         2,4           Chopin         2,4           Eersteling         2,4           Juno         2,4           Saline         2,4           Secura         2,4		
Chapin         2,4           Eersteling         2,4           Juno         2,4           Saline         2,4           Secura         2,4		24
Eersteling 2,4 Juno 2,4 Saline 2,4 Secura 2,4		
Juno 2,4 Saline 2,4 Secura 2,4		
Saline 2,4 Secura 2,4		
Secura 2,4		
Secura 2,4 Marylin 2,3		
Marylin 2,3		2,4
	Marylin	2,3

Porter main varieties grown in Norwey. Now brodge varieties taking over, Now brodge varieties taking over, Now brodge varieties/clames brood Now furnige varieties/clames brood

for resistance to late blight

Potato breeding in Norway was started in 1920 by Aksel P. Lunden at the Norwegian University of Life Sciences (UMB), Nineteen varieties were breed and accepted on the national list before the breeding program was moved to Bjørke Forsøksgård and fully taken over by the private company Graminor AS.

In 2008 61 different lines were tested for resistance to late blight in the foliage in a trial on the experimental farm "Staur". The lines were clones/varieties originating from Norway and several other countries, both new and old. The trial was planted on the 4th of June in a randomized block design with 4 plants in each plot in four replicates. The Eucablight standard varieties were included in the trial. Every third row was Redstar, which were inoculated with a mixture of 7 late blight isolates on the 1th of August. Infection was recorded 12 times until the 3th of October.

Breeding for resistance to diseases always had a high priority in the program. Special focus has been on late blight (Phytophthora infestans), phoma (Phoma foveata) and common scab (Streptomyces scables). Sources for late blight were Solanum stolaniferum and European varieties with high resistance.

The spread of late blight in the trial was very slow in September as the mean temperature was between 5°C and 10°C from the 4th of September. Breeding lines and varieties with high resistance of both Norwegian and foreign origin were found in the trial. The old varieties Pimpernel, Kerrs Pink and Beate still have quite high resistance, but these varieties are also very late maturing. Some of the new foreign varieties like Saline and Marylin were recorded to be very susceptible, but these varieties are very early and will therefore be attacked faster then later maturing varieties.



Odin is a coming Norweglan consumption variety with a resistance score of 6,3 to late blight on the foliage and 6,5 to late blight on the tubers.





Rustique is a new Norwegian crisp/french fry variety. Resistance to late blight is 5,6 and 6,2 on the foliage and tubers respectively.

Growing potatoes in the very north of Norway is a challenge in the cool and short season. Summer temperatures are often too low for late bilight and spraying is done only a few times even in the variety Asterix, which is quite susceptible.

#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## The effect of mandipropamid on infection of potato leaves by *Phytophthora infestans*: an SEM study

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#### **SUMMARY**

The activity of mandipropamid against *Phytophthora infestans* on potato leaves was studied using scanning electron microscopy. Mandipropamid prevented infection by either zoospores or sporangia of *P. infestans* through both the adaxial and abaxial leaf surfaces. Zoospores on mandipropamid-treated leaf surfaces failed to germinate and appeared to lose their structural integrity and disintegrate, or occasionally produced short, malformed germ tubes, while sporangia simply failed to germinate. Mandipropamid showed translaminar activity against *P. infestans*, preventing development of visible lesions when it was applied to the adaxial surface only and the abaxial surface was inoculated with *P. infestans*. However, this occurred after sporangial germination and leaf penetration, which appeared normal.

#### **KEYWORDS**

Phytophthora infestans, potato late blight, mandipropamid, scanning electron microscopy.

#### INTRODUCTION

Mandipropamid is a new Syngenta fungicide specifically active against oomycetes (Huggenberger et al., 2005; Huggenberger and Knauf-Beiter, 2007). In 2007, it was approved as the formulation 'Revus' (250 g mandipropamid/l, SC, Syngenta) for the control of potato late blight in the UK. Mandipropamid is structurally unrelated to other fungicides, but on the basis of cross-resistance patterns in Plasmopara viticola, is considered by FRAC to be a member of the CAA (Carboxylic Acid Anilide) fungicide group, to which dimethomorph and benthiavalicarb also belong. The precise mode of action of mandipropamid has not been reported, but it inhibits phospholipid biosynthesis and cell wall assembly. After foliar application, mandipropamid binds to leaf surface wax and moves into plant tissue (Hermann et al., 2005). It has been shown to have a strong effect on intercellular mycelial growth and sporulation. In this study, the activity of mandipropamid against P. infestans on potato leaves was studied using scanning electron microscopy (SEM). The protectant effect of the compound against infection by sporangia and zoospores through both the adaxial (upper) and abaxial (lower) leaf surfaces was evaluated using a range of P. infestans genotypes. Translaminar activity of mandipropamid was also investigated.

#### MATERIALS AND METHODS

**Protectant activity of mandipropamid against infection of potato leaves by** *Phytophthora infestans* Potato plants of the late-blight susceptible maincrop cv. Up-to-date were grown from tubers planted individually in 8 litre pots of sterilised potato compost (7 parts steam sterilised loam, 3 parts peat and 2 parts grit). Plants were reduced to a single stem and maintained in the glasshouse under natural daylight for 8 weeks, when they were *c.* 1 m tall. Leaflets attached to the plants were individually sprayed with mandipropamid (A14926B; 100 mg/l) or water using a Hozelock Spraymist hand sprayer, the remainder of the plant being screened by plastic to avoid contamination.

**Table 1.** Phytophthora infestans isolates used in the SEM studies

Isolata designations	Multi-locus	Mating	Metalaxyl	Allozyme genotype		mtDNA
isolate designation	Isolate designation <sup>a</sup> genotype <sup>b</sup> type resistance <sup>c</sup>		Gpi Pep		haplotype	
41/02	NI-1	A1	R	100/100	100/100	IIa
62/02	NI-1	A1	S	100/100	100/100	IIa
21/02	NI-2	A1	R	100/100	100/100	Ia
47/02	NI-3	A1	S	100/100	100/100	Ia
6/01	NI-4	A1	R	100/100	96/100	Ia
Remarka BL2/02	NI-5	A1	R	100/100	83/100	Ia
06 BPC 4540B	13_A2	A2	R	nd	nd	Ia
Pi02-007	US-8	A2	R	100/111/122	100/100	Ia

a isolates 06 BPC 4540B and Pi02-007 were obtained from potato crops in Wales, UK in 2006 and Michigan, USA in 2002, respectively. All other isolates were obtained from Northern Ireland potato crops in 2001 (6/01) or 2002 b as defined by Cooke *et al.* (2006)

c isolates were defined as metalaxyl-resistant if they sporulated on potato leaf discs floating on 100 mg metalaxyl/litre nd = not determined

After 24 h, the sprayed leaflets were excised, placed in clear plastic boxes lined with moist paper towelling either abaxial or adaxial side up. Spore suspensions of known *P. infestans* genotypes (Table 1) were prepared as follows. Zoospore suspensions of *P. infestans* were prepared by harvesting sporangia from a culture of the appropriate isolate into chilled, sterile water, incubating at 5°C for 2 h to encourage zoospore formation and then allowing warming to 15°C. Sporangial suspensions were prepared by harvesting sporangia into sterile water at 23°C. Each leaflet was inoculated with one 20 µl droplet of the appropriate suspension (*c.* 10<sup>5</sup> sporangia/ml) placed on either the abaxial or adaxial surface to the side of the midrib. After inoculation, leaflets were incubated with illumination at either 15°C for 24 h (zoospore inoculation) or 22°C for 48 h (sporangial inoculation). Samples of leaf tissue excised from the inoculated area were chemically fixed in 4% glutaraldehyde, serially dehydrated in ethanol, critical point dried in an EMscope CPD 750 and sputter-coated with platinum using a Polaron E5100 series II cool. They were then viewed on a FEI Quanta 200 Scanning Electron Microscope.

Additional inoculated leaflets were incubated for up to 7 d after inoculation and the presence or absence of infection, as indicated by the presence of sporulating lesions, was recorded.

## Translaminar activity of mandipropamid against infection of potato leaves by *Phytophthora infestans*

Potato plants were grown and mandipropamid or water sprayed onto selected leaves as above, except that mandipropamid was applied at 30, 70 or 150 mg/l. Care was taken to apply the spray to the adaxial leaf surface only, while protecting the abaxial surface. After 24 h, leaflets were excised as above and placed in clear plastic boxes abaxial side up. The leaflets were then inoculated with a sporangial or zoospore suspension of *P. infestans* and incubated for 24, 48 or 120 h before preparing samples of excised tissue for SEM as above.

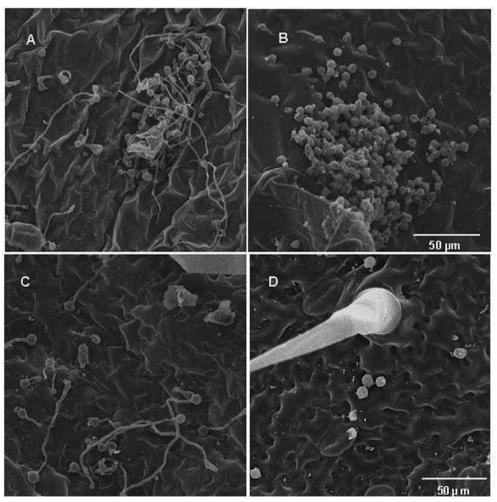


Figure 1. Potato leaves inoculated with Phytophthora infestans zoospores (isolate Remarka BL2/02), water-treated (A, C) and mandipropamid-treated (B, D), 24 h after inoculation, adaxial (A, B) and abaxial (C, D) surfaces. Zoospores have well developed germ tubes on the water-treated leaves (A, C), while they have failed to germinate on the mandipropamid-treated ones (B, D).

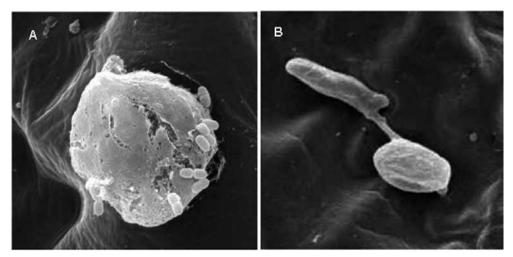


Figure 2. Effects of mandipropamid treatment on Phytophthora infestans zoospores, 24 h after inoculation. A zoospore is disintegrating and bacteria are present on the surface, B zoospore has produced a short germ tube with a malformed appressorium.

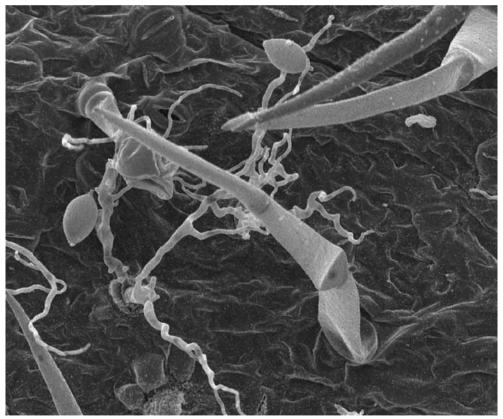


Figure 3. Sporangiophores emerging from stomata on adaxial leaf surface, 120 h after inoculation of water-sprayed leaves with zoospores of Phytophthora infestans.

#### **RESULTS**

#### Isolates used for scanning electron microscopy studies

The majority of the images shown here are from inoculations using Northern Ireland *P. infestans* isolate Remarka BL2/02 (Table 1), which was obtained from a natural infection in a field trial in 2002 and belongs to the NI-5 genotype (Cooke *et al.*, 2006). It was selected because tests on detached leaflets have shown it to be among the more aggressive isolates and it is able to overcome most R-genes (Young, 2007).

## Protectant activity of mandipropamid against infection of potato leaves by zoospores of *Phytophthora infestans*

When either the adaxial or abaxial surfaces of potato leaves, which had been sprayed with water (control) were inoculated with suspensions containing zoospores of *P. infestans*, the zoospores germinated rapidly and after 24 h extensive development of germ tubes could be seen (Fig. 1). There was evidence of the formation of appressoria and of penetration of the leaf tissue. In contrast, on leaf surfaces which had been sprayed with mandipropamid, the zoospores failed to germinate. In

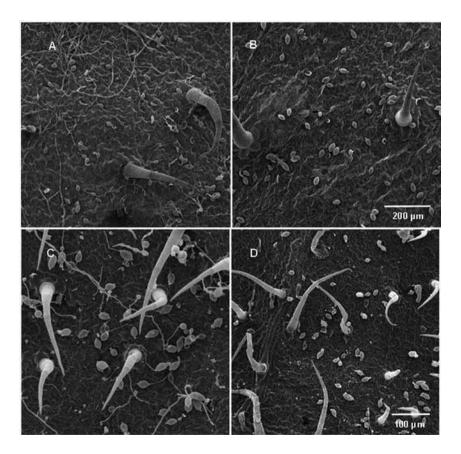


Figure 4. Potato leaves inoculated with Phytophthora infestans sporangia (isolate Remarka BL2/02), untreated (A, C) and mandipropamid-treated (B, D), 48 h after inoculation, adaxial (A, B) and abaxial (C, D) surfaces. Sporangia are developing germ tubes on the water-treated leaves (A, C), while they have failed to germinate on the mandipropamid-treated ones (B, D).



Figure 5. Potato leaves treated with mandipropamid on the adaxial leaf surface only and inoculated with sporangia of Phytophthora infestans on the abaxial leaf surface (translaminar activity test) 24 h later. Germinating sporangia are visible 48 h after inoculation.

some cases, malformed zoospores, which appeared to be disintegrating, were seen and in one case a zoospore with a misshapen appressorium was observed (Figure 2).

By 120 h after inoculation, on water-sprayed leaves sporangiophores could be seen emerging through the stomata on the leaf surface (Figure 3), while there was no evidence of infection on the mandipropamid-treated leaves. The remaining inoculated, water-sprayed leaflets developed visible lesions with sporulation by 4-5 d after inoculation, while the mandipropamid-treated ones developed no lesions and appeared healthy.

## Protectant activity of mandipropamid against infection of potato leaves by sporangia of *Phytophthora infestans*

Germination of sporangia was slower than that of zoospores on water-sprayed leaf surfaces, but by 48 h after inoculation germinating sporangia were observed on both the abaxial and adaxial surfaces. Sporangia on the mandipropamid-treated surfaces, whether abaxial or adaxial, failed to germinate, but did not disintegrate (Figure 4). The remaining inoculated, water-sprayed leaflets developed visible lesions by 5 d after inoculation, while the mandipropamid ones remained healthy.

## Translaminar activity of mandipropamid against infection of potato leaves by sporangia of *Phytophthora infestans*

After 48 h, sporangia were observed to be germinating on the abaxial leaf surfaces regardless of whether the adaxial surface had been sprayed with water or mandipropamid (Figure 5). However, while sporulating lesions developed on the remaining water-sprayed leaflets, lesions did not develop on those where mandipropamid had been applied to the opposite surface.

#### DISCUSSION AND CONCLUSIONS

Tests of selected isolates of *P. infestans* for their sensitivity to mandipropamid showed that none grew on potato leaf discs floating on 50 mg /l or above; all were completely inhibited by either 50 or 10 mg mandipropamid/l (data not presented). At lower concentrations (1, 5, 10 mg/l), growth was greatly reduced compared to that on control discs. All isolates, regardless of genotype, were therefore very sensitive to mandipropamid. Observations in the SEM study indicated that the isolates of different genotypes behaved similarly when exposed to mandipropamid and the only differences between them were in their rates of development on the water-treated leaves.

It was concluded that mandipropamid inhibits infection of potato leaves by *P. infestans* regardless of whether this occurs through the abaxial or the adaxial leaf surface. Where mandipropamid is present on the surface, germination of both zoospores and sporangia is prevented. The SEM study showed that zoospores generally fail to develop walls during encystment and often disintegrated, while the sporangia simply failed to germinate. Mandipropamid also has translaminar activity against *P. infestans*, but this was shown to occur post-infection after the sporangia and zoospores had germinated and penetrated the leaf tissue.

#### **ACKNOWLEDGEMENTS**

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# A2 mating type, metalaxyl resistance and complex virulence profiles: common features in some *Phytophthora infestans* isolates from Algeria

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#### **SUMMARY**

Phytophthora. infestans isolates were collected on potato in the main production areas of Northern and Central regions of Algeria in 2007 and 2008. They were characterized for mating type, sensitivity to metalaxyl and virulence. Metalaxyl resistance was assessed with a floating leaf disk method and virulence patterns were determined using Black's differential set of potato clones. All ten Algerian isolates tested proved to belong to the A2 mating type and to be resistant to metalaxyl. They have highly complex virulence spectra: six of them overcame the eleven specific resistance gene of the differential set, and the remaining four isolates overcame all the specific genes but R9. The high level of metalaxyl resistance in Algerian populations of P. infestans probably makes this active ingredient now inefficient against late blight on potato. Since most of the Algerian isolates tested had the eleven virulence factors, the Solanum demissun genes would probably not be of substantial use to control current P. infestans populations. These results are the first report about Algerian isolates and constitute a first step for the development of better late blight control strategies.

#### **KEYWORDS**

Phytophthora infestans, late blight, mating type, metalaxyl resistance, virulence

#### INTRODUCTION

Algeria, with a population of 35 millions, is an important country for potato production; indeed, potato is one of the priorities of the Algerian National Plan for Agricultural Development (PNDA). Potato crops occupy 90 000 ha, for a total production of two millions tons. In several regions, two main potato crops are grown during a calendar year: the first crop is planted from December to April, with harvest in May to July, while the second crop is planted in July / August and harvested in November to January. Moreover, crop rotations are not always practiced, and potato and tomato fieds are often close to each other. Therefore, *Phytophthora. infestans* inoculum is present in these regions throughout the year. Late blight is thus a permanent worry: in 2007, serious epidemics led to shortage on Algerian markets. The predominant cultivar is Spunta, suceptible to late blight, and metalaxyl is now widely used to control the disease.

Knowledge of *P. nfestans* populations is thus essential to elaborate control strategies against late blight in Algeria, but has been missing so far. The aim of this study was therefore to collect *P. infestans* isolates from main potato production areas in Algeria and compare their phenotypic traits, to provide insights into their impact on blight epidemiology and disease management practices.

#### MATERIALS AND METHODS

#### Origin of Phytophthora infestans isolates

Ten Algerian isolates were collected in Western and Central wilayas (Algerian regions) between Algiers and Oran (distant from 360 km), during 2007 and 2008, from fields on different potato cultivars (Table 1). Two additionnal French isolates were used in the metalaxyl test; they were sampled in INRA experimental trials of Ploudaniel (29). These single-lesion isolates were maintained as axenic cultures on pea agar medium.

Table 1. Origin of Algerian isolates collected in 2007 and 2008 and of two French isolates

Name of isolate	Year of isolation	Location	Potato cultivar
Z0	2007	Ain Delfa	Spunta
Z1	2007	Ain Delfa	Martina
Z3	2007	Mostaganem	Spunta
Z5	2007	Mostaganem	Spunta
Z12	2007	Oran	-
Z13	2007	Tiaret	Atlas
Z18	2007	Tiaret	Atlas
Z21	2007	Chlef	Désirée
Z32	2008	Staouali	-
Z33	2008	Tipaza	Kondor
06-P33	2006	Ploudaniel (France)	Bintje
07-P13-04	2007	Ploudaniel (France)	Bintje

#### Mating type determination

The mating-type of each isolate was determined by individually pairing them on pea agar with known A1 and A2 testers. After 10-14 days incubation in the dark at 15°C, the presence or absence of oospores was recorded under a microscope.

#### Metalaxyl resistance test

The sensitivity to metalaxyl of the isolates was assessed using a floating leaf disk method. Four leaf disks, taken from leaves of potato cv Bintje (6 to 8 week-old plants), were used per isolate (two disks per Petri dishes). Leaf disks (21 mm in diameter) were floated abaxial side up in Petri dishes (55 mm diameter) each containing 7 mL sterile distilled water, metalaxyl (Ridomil 25 WP, Novartis experimental compound) at concentrations of 10 and 100 mg L<sup>-1</sup>. For each isolate, a suspension of sporangia was prepared by flooding a 2 to 3-week-old culture with 6 mL sterile distilled water and gently scrapping the colony surface to harvest sporangia. Each disk was inoculated with a  $20\mu L$  droplet of sporangial suspension that has been induced to release zoospores by chilling at 4°C for 2 h. After seven days incubation at 15°C night, 18°C light with a 16h photoperiod, sporulation of the pathogen was recorded visually. Isolates sporulating on the disks floating on water containing 100 mg L<sup>-1</sup> metalaxyl were rated as resistant, those on 10 mg L<sup>-1</sup> were rated as intermediate and those that sporulated only on water were rated as sensitive.

#### Assessment of virulence spectrum

#### Potato plant material

Plants were grown in pots (one tuber per pot) filled with 1:1:1 sand-peat-compost mixture, in a glasshouse regulated at 15-20°C and 16 h of photoperiod, and were watered with a nutrient solution (Hakaphos, NPK 15/10/15) once a week. Virulence patterns were determined using Black's differential set of potato clones, each having one of the R1-R11 race-specific resistance genes, and Craig's Royal as susceptible cultivar. This set was originally provided by Scottish Agricultural Science Agency (SASA, Edinburgh, UK) and seed tubers were multiplied by INRA (UMR APBV, Ploudaniel, France). Leaflets were picked from 6-8 week-old plants, for experiments.

#### Inoculum preparation

Each isolate was first multiplied separately on detached Bintje leaflets. The leaflets were infected by deposing a drop of suspension of *P. infestans* sporangia collected as described above. After seven days of incubation in humid chambers under controlled conditions (15°C/18°C night/day temperatures, 16h daylight), the sporangia produced on infected leaflets were collected in sterile water; the resulting suspensions were adjusted to 5 x 10<sup>4</sup> sporangia mL<sup>-1</sup> and used for virulence experiments.

#### Virulence phenotype determination

Each leaflet was placed abaxial face up on a moist filter paper in a clear plastic dish, and inoculated by depositing a 20  $\mu$ L drop of the sporangial suspension on each side of the midrib. Two leaflets per isolate and differential host were inoculated. After incubation as described in the metalaxyl test above, each inoculation site was scored for the presence or absence of a sporulating lesion and interaction was considered compatible if sporangiophores were visible.

#### RESULTS AND DISCUSSION

The Algerian isolates tested are all A2 mating type and insensitive to metalaxyl which is consistent with recent changes in many European populations (Cooke *et al.*, 2007; Corbière *et al.*, 2009, this volume, Lees *et al.*, 2009, this volume).

Among *P. infestans* populations from Maghreb, some studies have been conducted between 1996 and 2001, especially in Morocco where A1 and A2 mating types were detected. In this country, regional structure was often noticed with a predominant mating type in some locations (Hammi *et al.*, 2001; Andrivon *et al.*, 2007). According to Sedegui *et al.* (2000), there was mating type shift within the *P. infestans* population in Morocco on potato and A2 frequency went from 0% in 1996 to 74% in 1998. Moreover, both mating types were identified in some fields by all these authors,

which indicates the potential for sexual reproduction of the pathogen. Sexual reproduction would then generate greater genetic diversity in the populations, which may lead to genotypes that are more aggressive and difficult to control. This sexual population also means increasing risk of oospores derived late blight attacks very early in the season. This situation had maybe existed in Algeria, afterwards most A1 isolates have disappeared or they are still present in some regions. Moreover, all A2 isolates tested by Sedegui *et al.* (2000) were intermediate or resistant to metalaxyl. In Tunisia, A2 mating type was also detected, but in a smaller proportion (Jmour and Hamada, 2006). The origin of A2 isolates is discussed because A2 mating type was identified from imported potato seed from Europe, which suggests the possible introduction of A2 mating type from Europe (Sedegui *et al.*, 2000). In Algeria, seed tuber importations are important (100 000 tons in 2007-2008), mainly coming from The Netherlands (58%), France (16%) and Denmark (13%) (Bourget, 2008; Rousselle, 2008).

All ten Algerian isolates were resistant to metalaxyl, whereas French isolates were A1 mating type and sensitive to metalaxyl. None of the tested isolates were rated metalaxyl-intermediate. The high frequency of resistance in Algerian isolates is understandable given the widespread use of phenylamides against late blight in Algeria. It makes this class of fungicides virtually useless for successful control of the disease in this country. Metalaxyl resistance must obviously be taken into account in future choice of fungicides.

The Algerian isolates have highly complex virulence spectrum. Only two virulence complex profiles were found into the ten Algerian isolates. Most of them overcame the eleven specific resistance genes of the differential set and four of them (Z1, Z12, Z13, Z21) were not virulent to R9, but they overcame all the other specific genes. Some isolates collected on the same year and in the same location did not show a simlar spectrum as isolates Z0 and Z1 from Ain Delfa or Z13 and Z18 from Tiaret. All isolates thus overcome specific resistance genes as R2, R5, R6 and R9 against which virulence is still unfrequent in most parts of Europe (Andrivon et al., 2004; Lehtinen et al., 2008). Such complex virulence patterns are however been noticed in some of the most recent studies from northern Europe. In Poland, in 2006, the average number of virulence factors per isolate was eight, the highest of the European countries. Increase of virulence to some genes was observed in Polish isolates, but frequency of virulence factor 9 was still around 30% (Lebecka et al., 2007). A similar situation was observed in Finland where average number of virulence was more than seven in 2007, whereas, in earlier studies, five to six virulences per isolate have been found in the Nordic countries where sexual reproduction was suspected (Lehtinen et al., 2008; Hannukkala et al., 2009, this volume). None of the Nordic isolates possessed virulence to R9 in 2003, but some rare Finnish isolates containing all 11 virulences were found in 2007. Interestingly, most of the Algerian isolates possessed virulence R9, although R9 has to our knowledge never been introduced into commercial cultivars. In Algeria, the Solanum demissum genes would thus probably not be of substantial use to control current P. infestans populations, as the 11 resistance genes were broken very often. It is important to be aware of this phenotypic trait in Algerian populations when planning future actions in potato late blight management. High incidence of very complex pathotypes may result in a breakdown of resistance among current potato cultivars.

This study provides us the first data on *P. infestans* populations in Algeria and its results are worrying for late blight control and cultivars management. Due to the limited number of isolates tested in this study, further investigations would be worthwhile to establish the current structure of Algerian *P. infestans* populations and to have a better understanding of pathogen-host coevolution. This should be considered to develop integrated management strategies which is crucial in the coming years for Algerian potato production.

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

## Efficacy of the VNIIFBlight Decision Support system in the control of potato late blight in Russia

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#### **SUMMARY**

Based on the experimental results, a mathematical model, which identifies the image of the weather, favorable or unfavorable for the outbreaks in the potato late blight development, has been developed. This model and the 5-day weather forecast have been included into DSS VNIIFBlight. Field trials showed that the scheme of sprayings, based on the VNIIFBlight, makes it possible to reduce the number of fungicide applications, as compared with a routine scheme.

#### **KEYWORDS:**

potato, late blight, forecast, decision support system, fungicide

#### INTRODUCTION

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is considered to be the most important and devastating potato disease in Russia. To control late blight, two main strategies, the breeding of resistant cultivars and/or the use of fungicides, are applied. Nowadays, fungicides play the key role in the late blight control, though the frequency and timing of their application depend on a number of factors. It is known that fungicides necessary for the late blight control are effective only if they are applied before and very close to the time of infection (Bødker and Nielsen, 2001).

The most important question, usually asked by potato producers, is "Which terms are the most suitable for fungicide treatments?" Because of different reasons, many Russian farmers often make two serious mistakes: they carry out the first treatment too late, and the intervals between the treatments are too large. As a result, the efficacy of the late blight control decreases.

The decision support systems (DSSs), developed to provide the late blight control, allow the user to identify the most suitable dates for the spraying. The DSS calculations for the need of the fungicide treatment are based on the knowledge of the weather influence on the pathogen development and on the mechanism of action of fungicides. At present, about 20 DSSs are used in the western countries. The most popular ones are NegFry, SimPhyt, Plant-Plus, ProPhy, Guntz-Divoux/Milsol, and PhytoPre+ 2000 (Schepers, 2004; Wander, Spits, Kessel, 2006). According to Schepers (2004), the use of DSSs in some European countries reduced fungicide input by 8-62%, as compared with a routine scheme of treatments, in 26 of 29 validations. In this paper we report about an attempt to work out a similar system for the Russian conditions.

#### Theoretical promises and approaches

It is known that the epidemic frequency of the late blight depends on climatic conditions. There

are three zones with different epidemic frequencies of the late blight in Russia (Spiglazova, 2004; Filippov, 2007). Late blight is worst in the West and North-West of European Russia and in parts of the Far East; less severe in the Central, Northern, and some southern regions of European Russia and some parts of the Far East; and least severe in the South-East European Russia and Siberia. The most of potato-producing areas in Russia are located in climatic zones, where the epidemic frequency is less than 50%. Therefore, it is clear that the routine scheme with fixed intervals between fungicide treatments is justified only in climatic zones with a high epidemic risk. Fungicide treatments in the regions with a low disease development level are unprofitable. The practical use of a DSS, based on the weather factors, would allow a Russian user to increase significantly the efficacy of fungicides applied against the late blight.

To determine meteorological conditions, important for the disease development, the following experiment was carried out within many vegetation seasons in the Moscow region, the Primorsky Kray, Georgia, and Estonia.

Within cereal sowings, we planted 20-30 plots of a potato cultivar, susceptible to the late blight (each plot consisted of 12 plants). Starting from the stage of appearance of 5-6 leaves, every day we inoculated one plot with *P. infestans* zoospores. For each plot, in the evening we placed 3 drops of a zoospore-containing suspension on the foliage of each plant. The drops were covered with moist microchambers for 12 hours (Fig. 1).

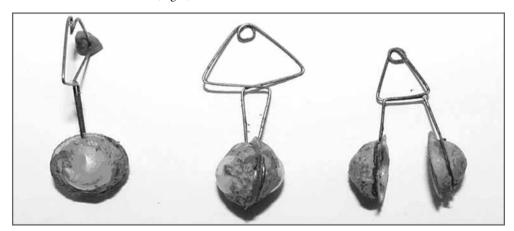


Figure 1. Moist microchambers, used for the infection of potato leaves with the late blight pathogen.

After the inoculation, we every day inspected experimental plots to register the date of the first appearance of primary necroses on the inoculated leaves, and the date of appearance of secondary necroses, caused by the re-infection. It was shown that the late blight development within a vegetation season consists of some separate (sporadic) outbreaks, each representing the result of the re-infection of plants; such re-infection took place in the days, when meteorological conditions promoted the fruiting of primary necroses, spreading of spores within the plot, their survival, and the further infection of leaf tissues. It was quite simple to reveal such days, since we had the data on the disease manifestation, starting from each date of the artificial infection. After the calculation of secondary necroses, all plants on the plot were destroyed. The experiment was stopped, when the late blight symptoms, caused by the natural infection background, were registered on the plots which still had not been inoculated.

According to the results obtained, all days were divided into two groups. The first one included the days, when a re-infection provided an outbreak of the disease (136 cases). The second group included the days, which did not cause such outbreaks (150 cases). Basing on these results, a mathematical

model was developed, which identified the "image" of the weather, favorable or unfavorable for the outbreaks in the late blight development.

To provide the preliminary determination of disease outbreaks and the dates for the treatment of potato with fungicides, we offered to use a standard 5-day weather forecast. The forecast weather conditions are evaluated using the following equations:

$$Y_{1} = -32.47 + 0.75x_{1} + 0.41x_{2} + 0.41x_{3} + 0.27x_{4} + 0.74x_{5} + 0.30x_{6} - 0.07x_{7} - 0.16x_{8} + 0.06x_{9} + 0.01x_{10} + 2.88x_{11} + 1.98x_{12} + 1.98x_{13} + 1.79x_{14} + 0.53x_{15}$$
 (1)

$$Y_2 = -31.34 + 0.63x_1 + 0.37x_2 + 0.419x_3 + 0.22x_4 + 0.65x_5 + 0.24x_6 - 0.06x_7 - 0.15x_8 - 0.135x_9 + 0.15x_{10} + 4.88x_{11} + 3.55x_{12} + 3.34x_{13} + 2.50x_{14} + 2.29x_{15}$$
 (2)

where  $x_{1,2,3,4,5}$  are daily temperatures (°C),  $x_{6,7,8,9,10}$  are night temperatures (°C), and  $x_{11,12,13,14,15}$  represent a rain falling in  $1^{st}$ ,  $2^{nd}$ ,  $3^{rd}$ ,  $4^{th}$ , and  $5^{th}$  days, respectively.

The situation, describing by the equations 1 and 2, are unfavorable and favorable for the re-infection of plants, respectively. If  $Y_1 < Y_2$ , then the spraying is recommended (Starodub, Gurevich, 1989).

The forecast for the first treatment starts when the height of plants achieves 20-30 cm. In the case of cultivars, which late blight resistance is less than 5 scores, it is recommended to repeat the treatment in accordance with the weather forecast, but at least 6-7 days after the previous treatment; in the case of cultivars, which late blight resistance makes 5 scores or more, the repeated treatment should be carried out at least 10 days after the previous one.

The developed model was called as VNIIFBlight. The calculator for a valuation of a weather conditions (Fig. 2) is available to assist in the late blight control (<a href="www.phytonet.ru">www.phytonet.ru</a>, <a href="www.www.kartofel.org">www.kartofel.org</a>).

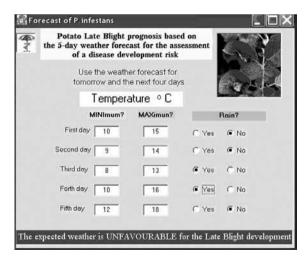


Figure 2. The working window of the VNIIFBlight program for the calculation of favorable/unfavorable conditions for the late blight development.

The purpose of the presented study was to evaluate the efficacy of the VNIIFBlight model under field conditions. We also included in our test program one modification of the SimCast DSS and its combination with VNIIFBlight. Unlike VNIIFBlight, SimCast uses current meteorological information. The weather is evaluated in blight scores, determined by the periods, when the relative

humidity (RH)  $\geq$  90%, and the average temperature during these periods. According to SimCast, it is necessary to apply a fungicide, if 5 or more days have passed from the moment of its previous application, and the cumulative blight score since the last spraying exceeded a critical level. The system starts at the plant emergence.

The field trials were performed during four seasons (2004, 2005, 2007, and 2008) at the experimental field of the All-Russian Research Institute of Phytopathology (Moscow region).

In 2004 and 2005 we compared four schemes of protective treatments:

- 1. Routine fungicide application (the treatment is carried out every 7-10 days).
- 2. VNIIFBlight scheme (the treatments are carried out, basing on VNIIFBlight recommendations).
- 3. SimCast scheme (the treatments are carried out, basing on SimCast recommendations).
- 4. Control (without any fungicide applications). In 2007 and 2008 we applied the following schemes:
- 5. Routine fungicide application.
- 6. VNIIFBlight scheme.
- 7. Combination of SimCast and VNIIFBlight recommendations.
- 8. Control.

In the case of the variant 3, each spraying was carried out when (1) according to SimCast, the sum of blight scores exceeded the critical level and (2) according to VNIIFBlight, meteorological conditions were critical.

In 2004-2005 and 2007-2008 we used in our trials cv. Sante and cv. Red Scarlett, respectively. For all seasons we sprayed potato plants with Shirlan (dose rate 0.4 l/ha). The treatment program for the variant 1 started, when the height of plants achieved 30 cm; the programs for the variants 2 and 3 started at the emergence of the crop.

Field trials were organized using a completely randomized block design, in four replications. The size of each plot was 36 sq. m. The experimental field was fertilized in accordance with a good agricultural practice. During the growing season the disease levels were assessed at weekly intervals up to the desiccation using the British Mycological Society foliage blight assessment key (Cox and Large, 1960).

The severity of the foliar blight infection is expressed as the percentage of yield losses, caused by a destruction of infected leaves (Gurevich, Filippov, and Tverskoy, 1977). This method of analysis is based on the well-known Van der Plank hypothesis, assuming a direct relation between the area under the curve, describing a seasonal dynamics of the disease, and the yield loss (Plank, 1963). It can be expressed by the following equation:

$$\omega = \frac{S}{q} \cdot 100,$$

where  $\omega$  is a yield loss (%), caused by the premature leaf decay, S is the area under the curve, describing the late blight dynamics, and q represents the number of days passed between a bud formation phase and the decay of non-infected leaves. The average q value for early, intermediate, and mid-late potato cultivars is 46, 52, and 84 days, respectively. If the foliage is killed by frost or the harvesting is carried out before its natural death, q is considered as a number of days, passed between the bud formation stage and the moment of the foliage death.

The severity of a tuber blight infection was assessed for the marketable tubers (> 35 mm), taken from each plot during harvesting and after one-month storage. The total severity of the tuber blight infection is expressed as the percent of infected tubers (by weight).

#### RESULTS AND DISCUSSION

The compared growing seasons provided clear contrasts in the disease development. In 2004 and 2008 there was a very high late blight infection pressure. Due to the rainy weather, a rapid disease development took place in the untreated control plots; the total necrosis of the foliage was registered long before the natural leaf decay.

As a result, the yield loss in the untreated control was 62% and 39% in 2004 and 2008, respectively. However, in 2004 the very rapid destruction of infected foliage resulted in the low level of tuber infection. On the contrary, the incidence of the tuber blight for control plots in 2008 was rather high (16.4%).

**Table 1**. The effect of different schemes for a fungicide application against potato late blight

Year	Fungicide application scheme	Rated yield loss due to the foliar late blight, %	% of infected tubers	Number of fungicide applications	Reduction of the number of fungicide applications as compared with the routine scheme, %
	Routine	2.6ª*	1.4ª	8	-
2004	VNIIFBlight	8.2 <sup>ab</sup>	2.9ª	3	62.5
	SimCast	15.7 <sup>b</sup>	2.2ª	7	12.5
	Control	62.0°	2.1ª	0	-
	Routine	>1ª	0	6	-
	VNIIFBlight	>1ª	0	3	50.0
2005	SimCast	>1ª	0	2	66.7
	Control	2.1ª	0.1	0	-
	Routine	>1ª	0.5ª	6	-
2007	VNIIFBlight	>1ª	0.6ª	3	50.0
	SimCast+VNIIFBlight	>1ª	0.3ª	2	66.7
	Control	2.7ª	0.1ª	0	-
2008	Routine	>1ª	1.6ª	6	-
	VNIIFBlight	1.0ª	1.5ª	5	16.7
	SimCast+VNIIFBlight	3.2ª	4.2 <sup>b</sup>	4	33.4
	Control	39.0 <sup>b</sup>	16.4°	0	-

<sup>\*</sup> For each year, the values in vertical columns followed by the same letters are not significantly different at  $P_{0.05}$ .

It is obvious from the Table 1 that, in general, the tested treatment schemes ensure an acceptable level of the late blight control in the case of a severe disease development. One can expect that in the case of field trials, the inoculum pressure from untreated plots and untreated plots of the experimental field is usually much higher than in practice. Table 1 also shows that VNIIFBlight, SimCast, and SimCast+VNIIFBlight programs allow a user to reduce significantly the number of fungicide treatments as compared with the routine plant protection program. However, the most promising results were obtained, when we used only VNIIFBlight or the SimCast + VNIIFBlight combination. In 2008 the percentage of blighted tubers on the plots, treated in accordance with the SimCast+VNIIFBlight program, was a little higher than on the plots, treated in accordance with only VNIIFBlight or the routine scheme.

In 2005 and 2007 the weather was hot and dry. Therefore, the situation with the late blight was about the same for both seasons. We did not reveal any significant disease occurrence neither in the experimental field, nor in the regional potato-growing areas at all. Due to the late blight depression, all fungicide applications were ineffective. However, the use of both tested schemes saved the considerable number of sprayings as compared with the routine scheme.

The analysis of the yield loss percentage, number of infected tubers, and number of fungicide treatments showed for all years that the better spray schemes were VNIIFBlight and the SimCast+VNIIFBlight combination, since they allowed us to reduce the late blight severity in the same extent as a 7-10-day routine scheme, and, simultaneously, to save about 39.6% and 50% of fungicide products, respectively, compared to the routine scheme.

The VNIIFBlight program is more reasonable for Russian farmers, because to use this program, one should have an access to the Internet to obtain a 5-day weather forecast. Also, a simple computer program is necessary to determine the dates of fungicide treatments. In the case of the SimCast+VNIIFBlight combination, one should also have a meteorological station to monitor the current weather situation. Russian potato producers do not have such possibilities.

#### **CONCLUSIONS**

The VNIIFBlight and SimCast+VNIIFBlight schemes of treatment allow a user to control potato late blight in the same extent as a 7-10-day routine scheme and, at the same time, to save about 39.6% and 50% of fungicide preparations, respectively, compared to the routine scheme.

#### ACKNOWLEDGEMENTS

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#### Eleventh EuroBlight workshop Hamar (Norway), 2008

# Use of phosphite to manage foliar late blight in developing countries

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### Is phosphite an alternative for managing late blight in developing countries?

#### Background:

•Mancozeb is economical and effective, and is currently the workhorse of developing country (DC) late blight (LB) control, but as an EBDC, mancozeb poses health and environmental risks in DC (Fig. 1).

•Phosphite (phosphonate) is used on a number of comycete diseases. As a highly systemic compound of low toxicity, phosphite could be an interesting alternatives for LB control in DC, where spraying is done by hand and often with poor quality equipment (Fig. 1).

#### Pending issues:

- Are phosphite formulations effective and cost competitive with mancozeb?
- ·Are phosphites cultivar specific?
- •Are all salts (K+, Ca+, Cu+), all formulations, of similar efficacy?

Table 1. Phosphites used in field trials in Ecuador - 2008

Treatment	Sprays	rAUDPO
Phosphite K	10	0.03a
Phosphite Ca	10	0.02a
Mancozeb	10	0.12b
Untreated	0	0.49c

Table 2. Phosphites used in field trials in Peru - 2008

Treatment	Sprays	rAUDPC
Conventional*	9	0.03a
Phosphite	6	0.04a
Phos. + Fung.	6	0.04a
Untreated	0	0.15b
*Application of several fungicides	C:	



Figure 1. Man spraying potato in Papua New Guinea

#### Results and discussion

➤Overall, there is mounting evidence that phosphite is effective in controlling potato late blight both in foliage and tubers (Tables 1,2 and 3).

>So far, there is little information on economics relative to mancozeb – costs of products vary among locations.

>Some work (1) would suggest there are cultivar specific effects.

➤ Previous work (1) and recent research at CIP would indicate that not all formulations are of similar efficacy. The reasons for this are not clear.

>Several "pending issues' remain and require further research.

Table 3. Previous work on phosphites is generally positive

Ref. (see below)	Foliage efficacy	Tuber blight efficacy	Tomato foliage
1	1	1	
2			1
3	1	Î	
4		Î	
5	Î	Î	

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#### Epidemiology and yield loss of Alternaria spp. in potatoes

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#### **SUMMARY**

Potato early blight occurs worldwide and is prevalent wherever potatoes are grown. Early blight of potato, caused by the fungi *Alternaria solani* and *Alternaria alternata*, can be found in all German potato growing areas. The disease is a risk to crop productivity in the field and results in significant yield losses. An early blight research project at the Technische Universität München conducted a German wide survey accompanied by field frials concerning epidemics and fungicide strategies for the disease control. The aim was to get further information about the interaction of maturity group, disease progression und yield loss. Early blight susceptibility was higher in early maturing potato varieties than in late maturing varieties. Yield losses were greater on late maturing varieties than on early maturing varieties.

#### **KEYWORDS:**

Alternaria solani, Alternaria alternata, early blight, fungicide strategy, chemical control

#### INTRODUCTION

For agriculture potato is still an important crop. In 2008 over 270 000 hectares potatoes were cultivated on German agricultural land (2.3% of total German arable farm land). Despite declining cultivation, potato is the second most important staple food in Germany after grain. Apart from the widespread potato disease late blight, early blight is causing increasing problems for German farmers in the last years. Since the last six years a monitoring has been accomplished in potatoes throughout Germany, which documents the occurrence of early blight in all German potato growing areas. Within the last years an increase in disease frequency was observed and disease has established as a relevant and destructive pathogen (Hausladen *et al.*, 2004). Early blight became a more and more important disease in most of the potato growing areas.

Early blight of potato, caused by the fungi *Alternaria solani* and *Alternaria alternata*, results in significant yield losses. Different factors, depending upon environment and plant physiology, have a severe influence on the disease progress of *Alternaria*.

The disease is still primarily managed by the application of foliar fungicides. Therefore a fungicide spray programme should be used from early in the growing season to vine kill (van der Waals *et al.*, 2001). An early blight research project at the Technische Universität München deals with the basics of the epidemics as well as fungicide strategies in disease control. Field experiments were carried out in 2004 and 2005 to evaluate the efficacy of different fungicide concentrations and the influence of plant age on early blight.

#### Disease cycle:

The fungus survives the winter as conidia or mycelium on infected plant debris in soil or on seed (Rotem, 1994). At favourable conditions spores are formed and are either wind blown or splashed onto plant surfaces where infection occurs at first on lower leaves. On potatoes *Alternaria* causes big brown and necrotic lesions, clearly angular and bounded by the nerves (Picture 1). Initial infections are most frequent on older leaflets. Lesions begin as dark brown to black spots, about 1 mm in diameter. The spots develop in a somewhat irregular shape and usually show concentric rings ("target spot") (van der Waals *et al.*, 2004). As disease progresses infected leaflets may turn yellow and either dry up or fall off. In some years stem infections can occur with symptoms similar to those on the leaves.

Alternaria solani belongs to the large-spored group within the genus, and produces simple, single-borne conidia that contain long and occasionally branched beaks (Ellis and Gibson, 1975). By contrast Alternaria alternata is one of the smaller-spored species, whose conidia are multiseptate,



Picture 1. Early blight symptoms

darkly pigmented and found as short chains borne on conidiophores, rather than single conidia (Petrunak and Christ, 1992).



Picture 2: Single borne conidia of Alternaria solani



Picture 3: Conidia of Alternaria alternata formed in chains

Recognized primarily as a foliage pathogen, epidemics occur when the weather is warm and dry with short periods of high moisture (frequent rain and abundant dew) (Venette and Harrison, 1973; Chelkowski and Viskonti, 1992). Only by observing symptoms it is not possible to distinguish if the necrotic spot is caused by *Alternaria solani* or *Alternaria alternata* (Hausladen, 2004). Morphological analysis of the formed conidia or PCR-technique are required for a clear differentiation of both pathotypes. For this reason in field trials early blight is imputed to *Alternaria spp*.

In their requirements concerning growth conditions both pathotypes are very similar. Already three to four weeks after crop emergence first symptoms can be found in the field on older leaves in the lower leaf levels of the canopy (Johanson and Thurston, 1990). Under favourable weather conditions disease progresses and lesions are also formed on leaves at higher leaf levels. If blight is severe whole leaves can be infected and plants may be entirely defoliated. Heavy infections can cause yield losses

over 30% (Johnson *et al.*, 1986). Especially in late maturing potato varieties early blight can reduce starch content through premature defoliation. Because *Alternaria* produces toxins that diffuse into host tissues, enlarging lesions are often surrounded by a narrow chlorotic halo (Rotem, 1994).

The most effective disease control has been the frequent application of protectant fungicides. Therefore applications have to start at an early stage of the disease (van der Waals, 2001). At this time early blight is still inconspicuously in the occurrence of symptoms and is found only on the lowest leaves. Control of this polycyclic disease depends primarily on multiple fungicide applications.

#### MATERIALS AND METHODS

In 2006 and 2007 seven different varieties of three different maturity groups were planted to check the interaction of maturity group and disease progression. A further field trial was carried out in 2007 to evaluate the economic relevance of early blight in potatoes. Trial was designed as a randomized block. Each plot had a size of 32 square meters (6 potato rows in width and 8 m in length).

This trial was carried out in four different late-maturing starch varieties (Albatros, Maxilla, Logo, Kuras)

The different potato cultivars was fertilized, cultivated and managed according to general agricultural practice.

Two different fungicide strategies were carried out:

- Early blight control, weekly spray of RanMan (cyazofamid)
- Specific treatment against early blight by the use of a mixture RanMan (cyazofamid) and Ortiva (125 g/ha Azoxystrobin)

#### RESULTS

First early blight symptoms could be monitored already two to four weeks after crop emergence. Although, there was no correlation between maturity group and disease infestation, maturity group and disease progress seem to be linked. Early maturing varieties showed an earlier increase in disease severity than late maturing varieties

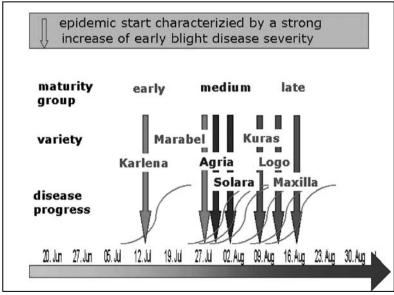


Figure 1: increase in epidemic in different maturity groups

Apart from the reduction of tuber yield, the starch content was also affected negatively by early blight. As a result of this, starch yield was significantly reduced. Fungicide strategies provide a highly effective protection against early blight, whereby infections can be controlled adequately.

Disease intensity was reduced in particular in late-maturing varieties. As a consequence, the assimilating leaf area was kept photosynthetically active for a longer time and yield formation was adhered. Starch yield increased in all varieties examined by 27% to 45%

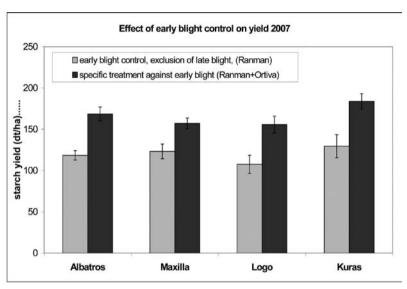


Figure 2: economic relevance of early blight in different late-maturing starch varieties ,location Weihenstephan, Bavaria, 2007

#### **CONCLUSIONS**

Early blight susceptibility was higher in early maturing potato varieties than in late maturing varieties. Yield losses were greater on late maturing varieties than on early maturing varieties. Besides the starch content, tuber size as well as tuber grading were reduced. In order to prevent early blight disease, fungicide strategies, which were applied according to disease specific thresholds, provided a highly effective protection.

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# DuRPh: Sustainable Resistance against *Phytopthora* in potato through cisgenic markerfree modification

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## DuRPh: Sustainable Resistance against *Phytopthora* in potato through cisgenic marker-free modification

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#### Project background

The DuRPh project aims to develop potato varieties with a sustainable resistance against late blight caused by *Phytopthora infestans*. For this challenge, Wageningen UR researchers jointly work on five themes in a 10 year project ending in 2016.

Farmers in the Netherlands generally need to spray their fields 10 to 16 times against late blight. A resistant potato will have great advantages:

- Reduced use of fungicides and less contamination of the environment.
- Lower production costs and a better competitive edge for the seed industry.
- Enhanced food security in resource poor countries where potato is a staple food.

#### Cisgenic genetic modification

In DuRPh, well established cultivars are relatively quickly provided with several additional resistance genes (gene stacking) originating from wild relatives. In principle, these genes could be inserted into potato cultivars via crossing. However, during crossing many unwanted 'wild' properties come along with the desired property,

such as small, malformed tubers, or a bitter flavour. As a result, it takes breeders decades of backcrossing with the cultivated potato to obtain a new cultivar suitable for commercial use. With genetic modification this problem does not occur. The incorporation of genes from related, crossable species is called cisgenic modification or cisgenesis. This is different from transgenic modification where the plant receives exogenous genes, e.g. from bacteria.

#### Project overview

DuRPh consists of five themes:

- 1. Detection and cloning of natural R-genes.
- Transforming cassettes of single or multiple R-genes into varieties.
- Selection of resistant plants with exact appearance and quality as the original variety.
- Spatial and temporal resistance management deploying different cassettes of Regenes to minimise the risk of Phytophthora infestans breaking the resistance and to reduce the build-up of epidemics.
- Communicating en interacting transparently with relevant stakeholders in society.



Seedlings from new crosses



Flowering potato plants.



Field trials with genetically modified potat



Wild relative with late blight resistance

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# Effect of adjuvants on the efficiency of benthiavalicarb plus mancozeb (Valbon 1.6 kg/ha) on the control of late blight in potato

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#### **SUMMARY**

Industrial adjuvants were tested in combination with benthiavalicarb plus mancozeb (Valbon 1.6 kg/ha) in the field to investigate their efficacy on foliar late blight caused by *Phytophthora infestans*. The tested adjuvant-fungicide treatments for late blight control were applied 6 times at 7-day intervals. The effect of the adjuvant-fungicide treatments on epidemic development, tuber blight and tuber yields were determined. Because of the favourable weather conditions a high disease pressure could be observed. The incidence of foliage blight was scored and at the end of the growing season the disease level was lower in plots sprayed with the Valbon-adjuvant combinations than in plots treated with only Valbon. The addition of an adjuvant had a clearly positive effect on the tuber yield although the differences were not significant. In the plots treated with Valbon 6.9 % infected tubers were observed. The mean tuber infection of plots sprayed with the Valbon-adjuvant combinations fluctuated between 2.3 and 15.6 %.

#### **KEYWORDS**

potato, late blight, *Phytophthora infestans*, fungicide adjuvant combination efficacy of adjuvants on fungicide efficiency

#### INTRODUCTION

An adjuvant is broadly defined as any substance added to the spray tank, separate from the pesticide formulation, that will improve the performance of the pesticide or the physical properties of the spray mixture, or both. The right adjuvant may reduce or even eliminate spray application problems, thereby improving overall pesticide efficacy. Adjuvants are designed to perform specific functions, including wetting, spreading, sticking, penetrating, reducing evaporation, reducing volatilization, buffering, dispersing,.... No single adjuvant can perform all these functions, but different compatible adjuvants often can be combined to perform multiple functions simultaneously.

Within the scope of a research project financed by the Institute for the Promotion of Innovation by Science and Technology in Flanders a range of industrial adjuvants were screened for possible application in agriculture. They were tested in different model systems. One model system was potato-*Phytophthora infestans* since potato late blight remains one of the most serious constraints to potato production world wide. To control *P. infestans* and to protect the potato crop, potato

plants are sprayed preventively with fungicides. Therefore, successful production of healthy potato crops relies on repeated applications of several fungicides during the potato growing season. A good rainfastness, spreading and sticking of a fungicide are important characteristics to improve the efficacy of fungicides.

The objective of this study was to investigate the efficacy of Valbon (benthiavalicarb plus mancozeb) in combination with adjuvants to control late blight during the growing season. Benthiavalicarb is a translaminar fungicide while mancozeb is a contact fungicide.

### MATERIAL & METHODS Field trial

A field experiment was carried out on the experimental farm of the 'University College Ghent' at Bottelare during the growing season 2008. The first two treatments were the same for the different objects (mancozeb 2.2 kg/ha). Later on the different adjuvants were compared in a spray system based on 7-day intervals to test their effect on the efficiency of benthiavalicarb plus mancozeb (Valbon). The experiment was set up with the variety 'Bintje'. The different objects were separated by two rows of the resistant variety Gazoré. Treatments were carried out with a AZO sprayer to 3 m wide and 10 m long plots. The spray boom was equipped with TeeJet nozzles (Teejet XR 11003 VK: 300 l water/ha or XR 110015 VK: 150 l water/ha) spaced 50 cm apart. The water volume was always 300 l/ha with the exception of Magic Sticker for which a water volume of 150 l/ha was used. The tested adjuvants and the applied doses are summarized in table 1.

Table 1. Adjuvants tested and applied dose

Adjuvant		Dose
Actirob B	methyl ester rapeseed	500 ml/ha
Magic Sticker	styrene acrylate copolymerxypropoxy polyether	500 ml/ha
FullStop	styrene acrylate polymer	250 ml/ha
G850	fatty amido alkyl betaine	500 ml/ha
Softanol 70	alkyloxypolyethylene oxyethanol	30 ml/ha
AE 5	fatty alcohol ethoxylate	30 ml/ha
Famee 5	methylester ethoxylate	30 ml/ha
Zipper	trisiloxane ethoxylated propoxylated ethoxy-propoxy polyether	100 ml/ha
TB5031	block copolymer	30 ml/ha
Purasolv BL	n-butyl lactate	150 ml/ha
P05	Abion	1 l/ha
BC02	green oil	500 ml/ha
Bond	synthetic latex plus alcohol alkoxylate	42 ml/ha
Certain S	polisiloxane	45 ml/ha
Certain ES	polisiloxane plus trisiloxane	30 ml/ha
Trend	idodecyl alcohol ethoxylate	100 ml/ha
PBG		1,4 l/ha
Break Thru Vibrant	alkoxylated alcohol	250 ml/ha
Break Thru In & Go	polyether-modified polisiloxane	75 ml/ha
GW1380		200 ml/ha
BIOT 1		30 ml/ha
BIOT 2		30 ml/ha
BIOT 3		30 ml/ha

Due to low temperatures and rainy weather the potato field was infected naturally by *Phytophthora*. Those weather conditions favoured the development of late blight all over the plots so that no artificial infection was necessary.

The experimental design was a completely randomised block design with four replicates. The adjuvants fungicide treatments were randomised within the blocks.

Following crop husbandry measures were taken: planting date of certified seed potatoes: 7 May 2008; row distance: 0.68 m; fertilisation 6 May 2008: 240 kg/ha N, 140 kg/ha  $P_2O_5$  and 180 kg/ha  $K_2O$ . Herbicide treatment 9 May 2008: linuron + pendimethalin + prosulfocarb: 750 g + 800 g + 3,2 kg/ha (Luxan Linuron 1,5 l/ha + Stomp 2 l/ha + Defi 4 l/ha); control of Colorado potato beetle 31 July 2008: lambda-cyhalothrin: 7,5 g/ha (Karate 75 ml/ha).

Diquat 600 g/ha (3 l/ha Reglone, Zeneca) was used to dessicate leaves and stems on 13 September. Potatoes were harvested on 6 October.

#### Disease estimates

To measure the intensity of foliage blight caused by *P. infestans* the foliar blight assessment key of the Blight Workshop in Tallinn was used: 0.0 % blight: no disease observed; 0.1 %: more than 1 lesion in a plot of 100 plants; 0,2 %: up to 25 lesions in a plot of 100 plants; 0,3 %: up to 50 lesions in a plot of 100 plants; 0,4 %: up to 75 lesions in a plot of 100 plants; 0,5 %: up to 100 lesions in a plot of 100 plants or 1 lesion per plant; 0,6 %: 2 lesions per plant in a plot of 100 plants; 0,7 %: 4 lesions per plant in a plot of 100 plants; 0,8 %: 6 lesions per plant in a plot of 100 plants; 0,9 %: 8 lesions per plant in a plot of 100 plants; 1 %: 10 lesions per plant in a plot of 100 plants; 5 %: 1 lesion per compound leaf or 50 lesions per plant in a plot of 100 plants; 10 %: 2 lesions per compound leaf or 100 lesions per plant in a plot of 100 plants; 25 %: nearly every leaflet with blight lesions, but plants retain normal form, plants may smell of blight, 75 % of plot leaf area remains green; 50 %: about 50 % of leaf area destroyed by blight; 75 %: about 75 % of leaf area destroyed by blight; 95 %: only a few leaves on plants, but stems green; 100 %: all leaves dead, stems dead or dying.

The overall amount of percentage blight was assessed at regular intervals for all the plots.

Data were analysed by performing analysis of variance (SAS 2.0). The One-sample Kolmogorov-Smirnov test was used to analyse the normal distribution of the obtained results. The Tukey test was used to compare treatment means when data were normally distributed. The non parametric test Kruskall-Wallis was used when the data were not normally distributed.

#### Harvest

Tubers were harvested mechanically. Two rows central of each plot were harvested over a distance of 7 m. All tubers were washed, weighed after grading and assessed for blight within 2 months after harvest. Washed tubers were examined visually for the presence or absence of lesions symptomatic of late blight. Furthermore, infected tubers were cut longitudinally to confirm the presence of dry brown corky rot in the tuber beneath the lesion, a symptom typical of late blight tuber infection. The diagnosis of tuber blight was further confirmed by observing sporangia production after incubating tubers with characteristic lesions in plastic containers containing moist paper towels.

#### **RESULTS & DISCUSSION**

The growing season 2008 was characterized by a cloudy, rather cold and wet summer. In June the mean temperature was 16.2 °C and 76 mm of rain was recorded. In July the mean temperature was 18.2 °C and in total 61.4 mm rain was recorded. In August the mean temperature was 17.5 °C and 103.2 mm rain. These weather conditions were very favourable for late blight development.

The incidence of foliage blight was scored weekly from 23 June until 16 September. The field experiment in 2008 indicated that Valbon (benthiavalicarb plus mancozeb) and the Valbon plus adjuvants combinations had a significant suppressive effect on established epidemics compared to untreated plots. The differences in control efficiency for the fungicide combinations tested were rather small and statistically not significant. Only at the end of the growing season differences in efficiency between the tested adjuvants were observed (Fig. 1). On the first of September a foliage

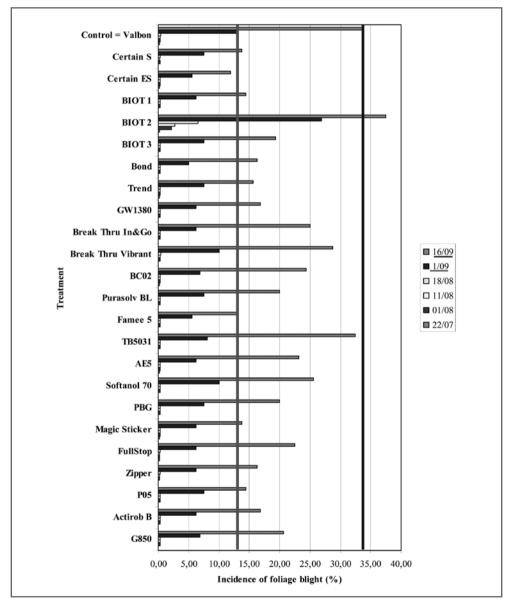


Figure 1: Influence of the fungicide-adjuvant combinations applied on foliage blight of 'Bintje' at the end of the growing season 2008.

incidence of 13 % was observed for Valbon (benthiavalicarb plus mancozeb). For the treatments of Valbon (benthiavalicarb plus mancozeb) combined with an adjuvant the percentage foliage blight fluctuated between 5 and 10 % with a mean infection of 7 %. On 16 September the foliage incidence reached 34 % for Valbon (benthiavalicarb plus mancozeb). For Valbon in combination with adjuvants the infection fluctuated between 12 and 33 % with a mean infection of 19 %.

**Table 2.** Influence of the fungicide-adjuvant combinations applied on tuber yield of 'Bintje' during the growing season 2008.

Treatment	Yield/plant	Total yield (ton/ha)	Yield < 32 (ton/ha)	Yield > 32 (ton/ha)
G850	1,72 ± 0,21	55,49 ± 8,87	4,07 ± 0,40	49,62 ± 8,79
Actirob B	$1,81 \pm 0,10$	$57,93 \pm 1,96$	$3,81 \pm 1,25$	$50,85 \pm 3,03$
P05	$1,78 \pm 0,18$	$59,37 \pm 6,54$	$4,46 \pm 0,30$	$52,63 \pm 7,29$
Zipper	$1,89 \pm 0,06$	$61,21 \pm 2,41$	$5,05 \pm 0,59$	$53,06 \pm 3,19$
FullStop	$1,84 \pm 0,20$	$59,68 \pm 7,19$	$3,67 \pm 0,67$	$54,32 \pm 8,20$
Magic Sticker	$1,65 \pm 0,15$	$54,58 \pm 5,00$	$4,01 \pm 1,17$	$48,51 \pm 4,91$
PBG	$1,93 \pm 0,10$	$63,05 \pm 3,68$	$4,00 \pm 1,57$	$57,04 \pm 3,32$
Softanol 70	$1,95 \pm 0,13$	$62,75 \pm 4,17$	$4,49 \pm 0,65$	$56,82 \pm 4,51$
AE5	$1,76 \pm 0,23$	56,40 ± 9,19	$4,01 \pm 1,09$	$51,63 \pm 6,74$
TB5031	$1,62 \pm 0,15$	$52,32 \pm 5,64$	$3,77 \pm 0,54$	$51,15 \pm 6,30$
Famee 5	$1,69 \pm 0,22$	$55,44 \pm 5,85$	$4,14 \pm 0,76$	$49,32 \pm 6,19$
Purasolv BL	$1,80 \pm 0,13$	$59,43 \pm 3,49$	$3,99 \pm 0,68$	$52,31 \pm 2,55$
BC02	$1,77 \pm 0,13$	$58,38 \pm 2,30$	$3,81 \pm 1,28$	$53,18 \pm 1,87$
Break Thru Vibrant	$1,82 \pm 0,13$	$59,35 \pm 3,95$	$2,87 \pm 1,61$	$54,14 \pm 3,50$
Break Thru In&Go	$1,70 \pm 0.08$	$55,93 \pm 3,49$	$3,45 \pm 0,66$	$50,88 \pm 3,51$
GW1380	$1,74 \pm 0,18$	$55,00 \pm 6,47$	$3,56 \pm 0,90$	$49,29 \pm 6,21$
Trend	$1,78 \pm 0,20$	$57,01 \pm 5,16$	$4,07 \pm 0,44$	$50,19 \pm 6,08$
Bond	$1,85 \pm 0,16$	$60,33 \pm 3,97$	$4,21 \pm 0,97$	$54,33 \pm 4,22$
BIOT 3	$1,88 \pm 0,17$	$60,49 \pm 5,22$	$4,13 \pm 0,91$	$54,90 \pm 5,29$
BIOT 2	$1,77 \pm 0,30$	$55,02 \pm 8,05$	$4,55 \pm 1,35$	$48,05 \pm 7,66$
BIOT 1	$1,84 \pm 0,16$	$59,19 \pm 5,85$	$2,86 \pm 0,69$	$53,75 \pm 6,05$
Certain ES	$1,81 \pm 0,26$	$57,38 \pm 8,72$	$4,17 \pm 1,12$	$51,31 \pm 8,09$
Certain S	$1,87 \pm 0,16$	$59,14 \pm 6,25$	$3,93 \pm 1,01$	$53,60 \pm 6,38$
Control = Valbon	$1,65 \pm 0,21$	$52,18 \pm 7,27$	$3,92 \pm 1,59$	$47,61 \pm 7,93$
Untreated	$1,18 \pm 0,31$	$38,26 \pm 9,17$	$4,19 \pm 1,27$	$28,49 \pm 4,53$
Kolmogorov-Smirnov	0,1021	0,1181	0,0929	0,1269
	NN	NN	NN	NN
Kruskall-Wallis (p = 0	0,05) 0,1434	0,1509	0,5176	0,2057
	NS	NS	NS	NS
NN: not normally dist				
N: normally distribute				
S: significantly differe				
NS: not significantly of	lifferent			

The addition of adjuvants to Valbon (benthiavalicarb plus mancozeb) resulted in a better foliage protection compared to Valbon without adjuvants. Bond, Famee 5 and Certain ES had a clearly positive effect on the efficiency of Valbon (benthiavalicarb plus mancozeb).

No significant differences in yield per plant were observed for the Valbon combinations tested (Table 2 and Figure 2). For Valbon (benthiavalicarb + mancozeb) the mean yield per plant was 1.7 kg/plant. For the different adjuvants combinations applied the mean yield per plant was 1.8 kg/plant. No significant differences in total yield, yield of grade lower than 32 mm and yield of grade higher than 32 mm were observed for the different treatments applied (Table 2 and Figure 2). The total

than 32 mm were observed for the different treatments applied (Table 2 and Figure 2). The total yield of the untreated plot was 38,3 ton/ha and the yield for Valbon (benthiavalicarb + mancozeb) was 52,2 ton/ha. For the treatments of Valbon in combination with an adjuvant the yield fluctuated between 52.3 and 63.0 ton/ha with a mean yield of 55.7 ton/ha. Valbon combined with TB5031

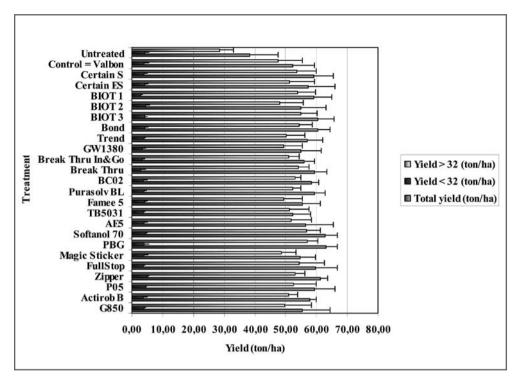


Figure 2: Influence of the fungicide-adjuvant combinations applied on tuber yield of 'Bintje' during the growing season 2008.

had the lowest yield: 52.3 ton/ha. The addition of an adjuvant had in general a clearly positive effect on the tuber yield: a mean increase of 3,5 ton/ha was obtained. A yield of more than 60 ton/ha was obtained for the adjuvants Zipper, PBG, Softanol 70, Bond and BIOT 3. The lowest yield was obtained with Magic Sticker: 54.6 ton/ha.

In the control plots 6.1 % infected tubers were observed (Table 3). The plots sprayed with Valbon (benthiavalicarb + mancozeb) had a tuber incidence of 6.9 %. The mean tuber infection of plots sprayed with the Valbon-adjuvant combinations was 9.3 %: the percentage diseased tubers fluctuated between 2.3 and 15.6 %. The adjuvants Softanol 70 and BC02 in combination with Valbon (benthiavalicarb + mancozeb) had a distinctly positive effect on tuber protection: only an infection of respectively 2.3 and 5.0 % was observed against 6.9 % diseased tubers for Valbon (benthiavalicarb + mancozeb) without adjuvant.

#### **CONCLUSIONS**

The growing season 2008 was characterized by a cloudy, rather cold and wet summer. These weather conditions were very favourable for the development of late blight. In a field experiment the effect of the tested adjuvants on the efficiency of Valbon (benthiavalicarb plus mancozeb) on incidence of foliage blight, tuber yield and tuber blight was investigated. From this field trial can be concluded that the tested adjuvants, with the exception of BIOT 2, had a positive effect on foliage protection in combination with Valbon (benthiavalicarb plus mancozeb). The addition of an adjuvant had in general a clearly positive effect on the tuber yield: the yield fluctuated between 52.3 and 63.0 ton/ha while the yield for Valbon (benthiavalicarb plus mancozeb) was 52.2 ton/ha. The adjuvants Softanol

70 and BC02 in combination with Valbon (benthiavalicarb + mancozeb) had a distinctly positive effect on tuber protection: only an infection of respectively 2.3 and 5.0 % was observed against 6.9 % diseased tubers for Valbon (benthiavalicarb + mancozeb) without adjuvant.

**Table 3.** Influence of the fungicide-adjuvant combinations applied on tuber blight in 'Bintje' during the growing season 2008.

25	
Treatment	Tuber blight %
G850	13,6
Actirob B	9,5
P05	8,0
Zipper	7,5
FullStop	8,2
Magic Sticker	8,4
PBG	6,3
Softanol 70	2,3
AE5	11,8
TB5031	8,7
Famee 5	10,4
Purasolv BL	15,6
BC02	5,0
Break Thru Vibrant	12,9
Break Thru In&Go	7,2
GW1380	6,7
Trend	6,3
Bond	9,4
BIOT 3	8,0
BIOT 2	10,1
BIOT 1	11,0
Certain ES	14,3
Certain S	13,7
Control = Valbon	6,9
Untreated	6,1
Kolmogorov-Smirnov	0,0350
<del>-</del>	NN
Kruskall-Wallis ( $p = 0.05$ )	0,2865
*	NS
NN: not normally distribute	ed
N: normally distributed	
S: significantly different	
NS: not significantly differ	ent

#### **ACKNOWLEDGEMENTS**

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### Characteristics of amisulbrom as a foliar use fungicide on Oomycetes disease

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#### **SUMMARY**

Amisulbrom 200g/L SC has shown its high level of efficacy on foliar late blight in potato through the extensive field trials across Europe and Japan. This paper summarizes the key characteristics of amisulbrom supporting such efficacy in the field. With application on potato leaves the concentration of amisulbrom in drop water on leaf remained at 0.1 ppm or more, and inhibition of zoospore release was significantly high up to 28 days after the application. Also amisulbrom quickly penetrated into wax layers of plant leaf, which is a strong reason why the effect of amisulbrom was not affected by rainfall. In addition to the characteristics, amisulbrom proved a significant effect on the viability of zoosporangia at 4 days curative application, which indicates the inhibition of secondary infection.

#### **KEYWORDS**

Amisulbrom, Phytophthora infestance, late blight, rainfastness

#### INTRODUCTION

Amisulbrom is a new fungicide for control of Oomycetes and Plasmodiophoromycetes disease developed by Nissan Chemical's. This paper summarizes results from the laboratory and greenhouse tests to evaluate the key characteristics of amisulbrom supporting its field performance against late blight in potato.

- Concentration of amisulbrom in drop of water on leaf and inhibition activity on zoospore release.
- 2. Rainfastness
- 3. Distribution within potato leaf
- 4. Inhibition of secondary infection

#### MATERIALS AND METHODS

Amisulbrom was formulated as a solo product of 200 g/L suspension concentration (SC) with adjuvant incorporated, and used for greenhouse tests.

#### Plants and fungal strain

Potato plants (cv. Danshuku) were grown in a greenhouse in 10x10x13 cm pots for 21 days. *Phytophthora infestance* (strain TK-301) was grown on rye A agar plates at 20°C for 10 days. Zoosporangia suspension was perpetrated by pouring distilled water over the fungal colonies and

gently rubbing the surface with a brush. The suspension was adjusted to  $10^5$  sporangia/ml with a haemocytometer.

#### Efficacy of inhabitation on zoospore released in the water on treatment leaf

Pots of potato were sprayed with amisulbrom 20SC at 100 ppm. Four leaves per pot were sampling at 7, 14, 21 and 28 days after treatment and then 20  $\mu$ L distilled water was dropped on the sampling leaves (five drop/ leaf). The treatment leaves were kept in a dew chamber for 24 hours, and then water on treatment leaves was collected.

Zoosporangia were collected by rubbing the surface of the fungal colonies with a stick, and were put in the  $50 \,\mu\text{L}$  water collected on the treatment leaves.

Zoospore release (% sporangia releasing zoospores) was determined after 2 hours incubation at 4°C by microscopically assessing approximately 100 zoosporangia for the release of zoospores.

#### Measuring method of amisulbrom density included water dropped on treatment leaves

Water samples collected on the treatment leaves were added same volume of acetonitrile prior to quantitation by LC-MS/MS.

Water samples collected on the treatment leaves were added same volume of acetonitrile prior to quantitation by LC-MS/MS.

#### Rainfastness

Potato plants were sprayed with amisulbrom SC at 100 ppm and reference products at doses for the following rainfastness experiments. Two hours after fungicide application, the pots were subjected to 40 mm/hr of artificial rain for 3hrs. One day after rainfall, the plats were inoculated and kept in a dew chamber for 1 day and then placed in a greenhouse for 5 days. Efficacy was evaluated on the basis of disease severity.

#### Distribution within potato leaf

 $10 \,\mu l$  of amisulbrom at  $100 \,ppm$  was dropped on surface of potato leaf, and the leaves were collected at 6 hours, 4 days and 7 days after treatment. Proportions of amisulbrom remaining on the leaf surface, adsorbed into the wax and penetration into the leaf tissue were measured.

#### On the leaf surface

The leaves were cut out by the circle of 2 cm in diameter. The 5 pieces of leaves were transferred into a 10 mL test tube and washed for 30 seconds with 5 mL of water. The washing solution was diluted with acetonitrile prior to quantitation by LC-MS/MS.

#### Adsorbed into the wax

The water washed leaves were transferred into a new 10 mL test tube and washed for 30 seconds with 5 mL of acetone. The washing solution was diluted with acetonitrile:water (50:50, v/v) prior to quantitation by LC-MS/MS.

#### Penetration into the leaf tissue

The acetone washed leaves were transferred into a new 50 mL test tube and homogenized in 20 mL of acetonitrile:water (80:20, v/v) for 1 minute. Solid-phase extraction (Supelclean ENVI-Carb, SUPELCO) clean-up was performed prior to quantitation by LC-MS/MS.

The treatment points were cut out in a circle form the leaves (2 cm in diameter). The 5 piece of cut out leaves were transferred into a 10 mL test tube and washed for 30 seconds with 5 mL of water. The wash was diluted with acetonitrile prior to quantitation by LC-MS/MS.

The water washed leaves were transferred into a new 10 mL test tube and washed for 30 seconds with

5 mL of acetone. The wash was diluted with acetonitrile:water (50:50 v:v) prior to quantitation by L.C.-MS/MS.

The acetone washed leaves were transferred into a 50 mL test tube and homogenized for 1 minute with 20 mL of acetonitrile:water (80:20 v:v). The extract was cleaned up using SPE cartridge (Supelclean ENVI-Carb, 500 mg/6 ml, SUPELCO) prior to quantitation by LC-MS/MS.

#### Inhibition of secondary infection

Pots of potato plants were inoculated with *Phytophthora infestans* and maintained in a dew chamber. Four days after inoculation, the pots were sprayed with amisulbrom SC at 100 ppm and the back of the leaf was sprayed emphatically. After 1 hour, two leaves were collected from each pot. The leaves were put in a dew chamber to promote spore formulation. After 24 hours, zoosporangia were collected by rubbing the surface of fungal colonies on leaf with a thin toothpick ahead, avoiding the contact with leaf, and then the zoosporangia were put in 50 µL distilled water.

Zoospore release (% sporangia releasing zoospores) was determined after 2 hours incubation at 4°C by microscopically assessing approximately 100 zoosporangia for the release of zoospores.

#### RESULTS AND DISCUSSION

### Concentration of amisulbrom in drop of water on leaf and the inhibition activity on zoospore released

Inhibition of zoospore release was significantly high up to 28 days after treatment (Table 1,). After 7 days treatment, concentration of amisulbrom was 0.29 ppm, and the density was a tendency to fall little by little with the passage of days, however the density was 0.12 ppm after 28 days treatment (Table. 1). MIC of this strain against inhibition activity on zoospore released was 0.1 ppm, witch indicates this result showed that the amisulbrom density included in the drop of water on the leaf after 28 days treatment, maintained the density in which was possible to inhibit zoospore release.

#### Rainfastness

Efficacy of amisulbrom 20SC against late blight was not affected by rainfall at 40 mm/hr for 3 hr, whereas disease control by mancozeb reduced from 82.9% to 75.5% at the same condition (Figure. 1). This performance was supposed due to preferable formulation besides chemical and physical properties of the compound.

#### Distribution within potato leaf

Proportions of amisulbrom after 6 hr, 4 and 7 days applications, remaining on the potato leaf surface, adsorbed into the wax and penetration into the leaf tissue were measured. This test showed that amisulbrom has high affinity to wax layers and quickly penetrated from surface of leaf to wax layers (Figure 2). It was thought that this performance was one of the reasons why the effect of amisulbrom against late blight was not affected by rainfall.

#### Inhibition of secondary infection

The Percent of zoospore release of zoosporangia at 4 days curative application was 20%, whereas control was 80.5%. And the zoospore was not possible to swim normally, whereas in control was swimming, and it stopped before long (Table 2). In this result, amisulbrom proved a significant effect on the variability of zoosporangia at 4 days curative application, which indicates the inhibition of secondary infestation.

#### CONCLUSIONS

Amisulbrom 200g/L SC has shown its high level of efficacy on foliar late blight in potato through the extensive field trials across Europe and Japan.

In the presented tests, long lasting preventative effect and quick penetration into leaf supporting the excellent rainfastness were demonstrated. In addition to the characteristics, amisulbrom proved a significant effect on the viability of zoosporangia at 4 days curative application, which indicates the inhibition of secondary infestation.

These unique biological properties support the actual performance of amisulbrom in the field, and it is also suggested that such outstanding activity on spores is the ground of tuber blight control.

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**Table 1.** Concentration of amisulbrom in drop of water on leaf and the inhibition activity on zoospore released

	day(d) after tretment			
7d 14d 21d 28				28d
Concentration of Amisulbrom(ppm)	0.29	0.24	0.16	0.11
Inhibition of zoospore release(%)	84.5	99.1	92.9	83.5

**Table 2.** Inhibition of secondary infection

Concentration of	Zoospore	Inhabitation	Zoospore
amisulbrom release (%)		(%)	motility
100 ppm	20.0	75.1	stop
50 ppm 21.3		73.6	stop
Cont.	80.5	-	swimming



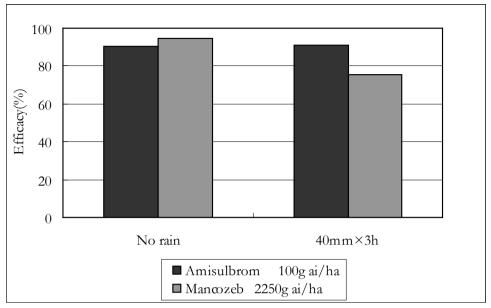


Figure 1. Rainfastness-greenhouse test

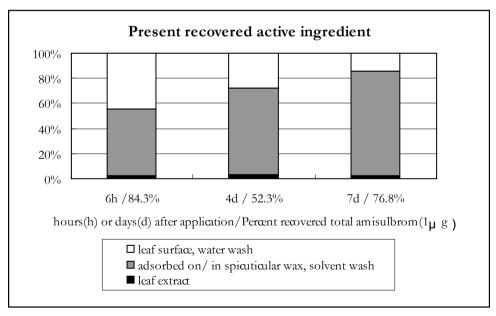


Figure 2. Percent recovered active ingredient

# Effect of Quadris applied as an in-furrow spray against the late blight and early blight on a potato foliage

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#### INTRODUCTION

The oomycete *Phytophthora infestans* and fungi *Alternaria solani* and *A. alternata*, causing potato blights, are considered to be among the most important pathogens of this crop. The increased problem of the *P. infestans* control coincides with the replacement of the "old" clonal lineage by a new, more aggressive population in the most potato-growing regions of the world (Spielman *et al.*, 1991; Vorobjova et al, 1983). Last year the appearance of more aggressive strains led to the shortening of the infection cycle and the more early and rapid epidemic development of the disease; in some regions of Russia, the first late blight symptoms were registered shortly after the shoot appearance. Very early start of the epidemics leads to the necessity of early fungicide treatments and the reduction of intervals between sprayings. It was assumed that the in-soil application of some fungicides during the potato planting is able to suppress the foliar infection at the early phases of the plant growth, to decrease the early epidemic risk, and, as a result, to decrease the number of foliar sprayings. The similar problems also arise regarding the early blight of potato. In recent years the severity of this disease increased, especially in the eastern part of the European Russia and in the Siberia.

We investigated Quadris fungicide (a trade name for azoxystrobin), which belongs to the chemical class of strobilurins, as a potential fungicide for such treatment.

The antifungal potential of azoxystrobin covers all important classes of plant pathogens including *Alternaria* and *Phytophthora spp*. This fungicide was used for more than 10 years to protect a variety of crops, including vegetables and potato, which made it now to be one of the most popular fungicides on the market (Goodwin *et al.*, 1992; Balwin *et al.*, 1996).

Azoxystrobin is a water-soluble fungicide (log  $P_{ow}$  = 2.64) that allows its molecules to be translocated into the plant. They are taken up by roots and translocated acropetally into the shoot and leaves using a water flow (Gisi, 2002).

It is known that the basic target objects for azoxystrobin on potato are the soil-born pathogens (*Rhizoctonia solani* and *Colletotricum cocoides*) – in the case of a soil application, and *Alternaria* spp. – in the case of a foliage spraying. The aim of our study was to obtain data about the level and character of the effect of this substance, applied as an in-furrow treatment, on the development of *Phytophthora infestans* and *Alternaria alternata* on the potato foliage.

#### MATERIAL AND METHODS

The experiments with *P. infestans* were carried out in 2006-2008, and the experiment with *Alternaria alternata* was carried out only in 2008. Each year potato was planted on the field of the All-Russian Research Institute of Phytopathology. The soil type was a medium loam (pH 4). We used cv. Sante (2006) and cv. Red Scarlett (2007-2008), which are very susceptible to both diseases studied.

We used two schemes of treatment:

- Quadris applied during planting as an in-furrow application (3 l/ha);
- Untreated control.

Quadris was applied using a two-row potato-planter with a sprayer, equipped with two nozzles per row, one located at the front side of the planter and directed downwards, into the furrow, and the second one located at the rear side of the planter and directed to spray the soil as it closes around the planted tubers.

In our experiments we used a randomized complete block design with four replications; each plot consisted of 4 ten-meter rows.

After the emergence of shoots, every 7-10 days we took 30 detached potato leaves from each plot and sprayed them with the sporangial suspension of *Phytophthora infestans* (15000 sporangia/ml) or with the conidial suspension of *Alternaria alternata* (50000 conidia/ml). Then the leaves were incubated for 24 h in the dark at 20 °C and RH 98%. Then leaf petioles were put into water, and the further incubation took place under normal light conditions. After 6 days of such incubation, we calculated the number of necroses per each leaf.

#### RESULTS AND DISCUSSION

For each of three seasons the program of a Quadris application resulted in the less severe late blight development on the leaves, than in the case of untreated control (Fig. 1, 3). At the very early stage of a shoot development, the infection severity does not differ from that of the untreated control. Then, after 6-8 days (35-38 days after the planting), the severity of the late blight significantly decreased. The most effective inhibition of the late blight development was registered in 40-60 days after the planting, shortly before the flowering stage. During this period, the number of necroses on leaves, inoculated with *P. infestans*, was 15-50% of the untreated control. The positive effect of Quadris continued for 23-38 days, up to the beginning of foliage senescence. Taking into account the data obtained, one can assume that the absence of any Quadris effect on the results of first inoculations was determined by the weak development of roots in this period and therefore by the weak diffusion of the fungicide from soil to the plants. Quadris, applied as an in-furrow fungicide, also demonstrated a remarkably long-lasting protective effect on leaves inoculated with *Alternaria alternata* (Fig. 2, 4). However, additional experiments are necessary to reveal the influence of the at-planting treatment with Quadris on this pathogen in different seasons.

It is known that azoxystrobine is a mono-site fungicide, and there are some problems, connected with a high risk of the development of a fungicide-resistant strains of *P. infestans* and *A. alternata* (Gudmestad, Pasche, 2007). Now we do not know about the consequences of the soil application of Quadris. However, we suppose that the risk of the development of resistant strains can be reduced, if Quadris will be mixed with another fungicide, having a differing mechanism of action, for example with preparations containing phosphorous acid.

Quadris is not included into the list of pesticides allowed to be used on potato on the territory of the Russian Federation. Therefore, our study pursues only a scientific goal.

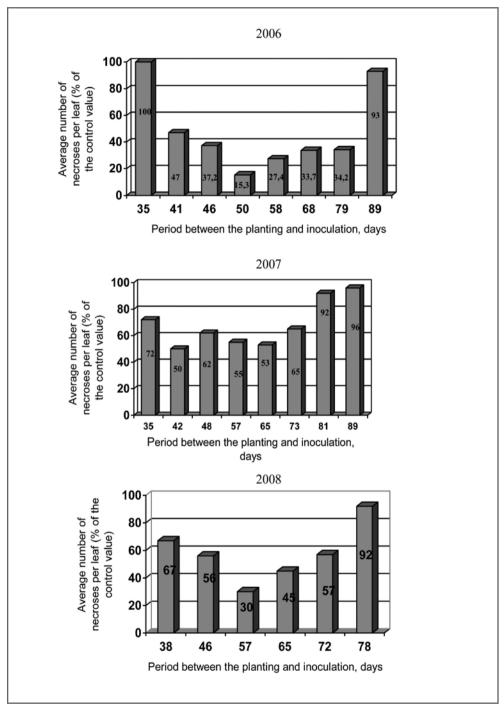


Figure 1. Effect of Quadris application as an in-furrow fungicide on the susceptibility of potato leaves to P. infestans infection.

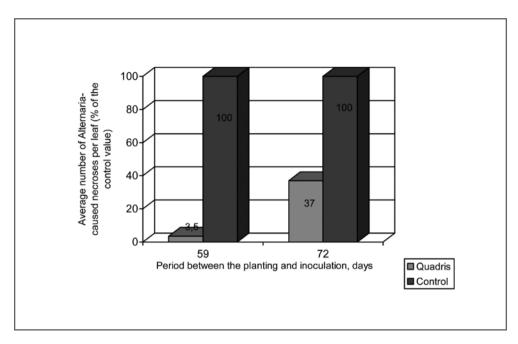


Figure 2. Effect of Quadris, applied as an in-furrow fungicide, on the susceptibility of potato leaves to A. alternata.

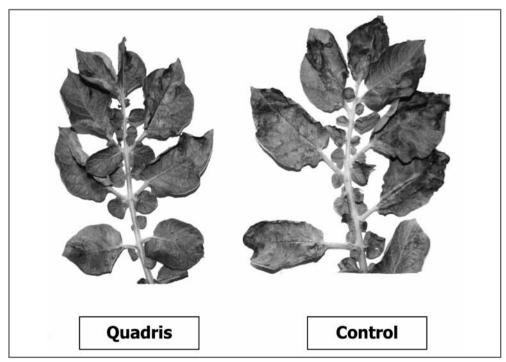


Figure 3. Effect of Quadris applied as an in-furrow treatment on the susceptibility of potato leaves to P. infestans infection at artificially inoculation, (cv. Sante, 2006).

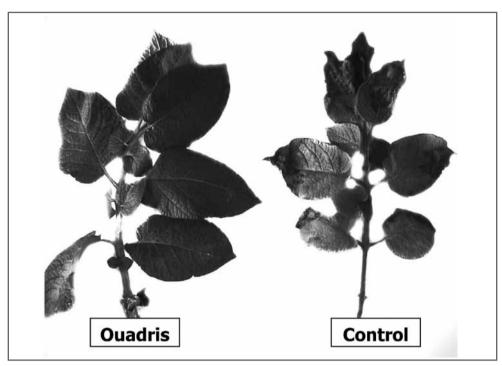


Figure 4. Effect of Quadris applied as an in-furrow treatment on the susceptibility of potato leaves to A. alternata infection at artificially inoculation, (cv. Red Scarlett, 2008).

#### **ACKNOWLEDGEMENTS**

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### Secondary metabolites in potato plants

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#### AARHUS UNIVERSITY

#### Secondary metabolites in potato plants

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### The project

we want to investigate the production of a broad spectrum of secondary metabolites in potato plants in

metabolites in potato plants in response to infection by Phytophthora infestans and their possible role in the defense of the plants against the pathogen

#### Research questions

- Are there any clear differences between the production of secondary metabolites in the leaves in a susceptible and a moderately resistant cultivar at infection by Phytophthora infestans?
- How does a beneficial microorganism below the soil surface (mycorrhiza) affect the production of secondary metabolites in the leaves at pathogen attack?



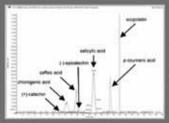
#### Metabolite profiling using LC-MS-MS



The Applied Biosystems 3200 Q Trap LC-MS-MS system in our lab

We use LC-MS-MS (Liquid Chromatography tandem Mass Spectrometry) to analyze the content of secondary metabolites in plant samples.

- Amany compounds can be quantified in the same sample.
- ⇒ compounds can be quantified even if they are only present in a limited concentration



with seven different secondary metabolites known from potato.

Faculty of Agricultural Sciences

# Matching fungicide inputs to cultivar resistance for the control of *Phytophthora infestans* on potato

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#### **SUMMARY**

The feasibility of integrated control under GB conditions using cultivar resistance to reduce fungicide inputs was investigated in 2007. The efficacy of reduced fungicide rates at 7- and 10day intervals on foliar late blight (Phytophthora infestans) severity on Shepody (foliar resistance rating 2), Maris Piper (4) and Lady Balfour was investigated at two sites - Auchincruive, Scotland (SAC) and Llanilar, Wales (ADAS). Active ingredients tested were mancozeb (as Dithane NT WG), fluazinam (as Shirlan) and fluopicolide + propamocarb HCL (as Infinito). The efficacy of integrated control treatments (lower fungicide rates applied to Maris Piper and Lady Balfour) were compared with the benchmark combination of Shepody treated with the highest fungicide rate at 7-day intervals (1.6 l/ha Infinito, 0.4 l/ha Shirlan and 2.0 kg/ha Dithane NT applied to Shepody). The effectiveness of increased cultivar resistance and reduced fungicide inputs on late blight control was found to differ by site and product applied. Significantly better control was achieved in Ayrshire where reduced Shirlan rates had been applied to Maris Piper and Lady Balfour compared with the benchmark treatment. This was similar for Dithane NT, however, only two of the integrated control treatments using Infinito gave significantly better control. At Llanilar, all Dithane NT integrated control treatments gave significantly better control of foliar blight than the benchmark treatment. Only one Shirlan treatment was significantly more effective over the benchmark treatment and no Infinito-based integrated control treatments were significantly more effective. Reduced fungicide rates applied at shorter intervals were generally more effective than higher doses at longer intervals at Auchincruive and marginally more effective at Llanilar.

#### **KEYWORDS**

Late blight, Phytophthora infestans, foliar blight, cultivar resistance, fungicides, integrated control.

#### INTRODUCTION

Growers in GB are facing increasing pressure to reduce production costs and to protect crops from late blight at a time when consumers and retailers want to see a reduction in the environmental impact of fungicides. At the same time, there is increasing evidence showing that the population of *P. infestans* in GB has changed in recent years, with a shift towards more aggressive strains and the widespread dominance of *P. infestans* genotype 13\_A2 over other A1 and A2 genotypes in 2007 (Cooke *et al.*, 2008). Using cultivar resistance offers the potential to reduce fungicide inputs, whilst still achieving adequate control of *P. infestans* in GB and is a viable option for the future. Research conducted throughout Europe and the US has shown reduced fungicide inputs can successfully

reduce foliar blight severity when used on potato cultivars with good foliar blight resistance (Nielsen, 2004; Kirk et al., 2005; Kessel et al., 2006; Naerstad et al., 2007).

This work was carried out as part of the Potato Council's Fight against Blight campaign which aims to contribute to the understanding and development of disease management strategies. One of the objectives was to investigate interactions between cultivar resistance, fungicide and application intervals for GB cultivars and determine the scope for utilising existing cultivar resistance to improve the targeting of fungicide spray programmes.

#### MATERIALS AND METHODS

In 2007 at SAC, Auchincruive Estate, Ayrshire, Scotland and Llanilar, near Aberystwyth, Ceredigion, Wales, fungicide sprays were applied to cultivars Shepody (foliar blight resistance rating 2), Maris Piper (4) and Lady Balfour (8) (Anon, 2006). Treatments were replicated in a split plot design with fungicide programmes as the main plots and cultivars as sub plots in four replicate blocks. Plots at both sites were four rows wide and 7.5 m to 9.0 m in length. Each cultivar sub plot contained 32 plants and was separated by 0.9 m unplanted row length at Llanilar and 1.1 m at Auchincruive. Main plots were separated by 1.5 m unplanted row length. At both sites a single row of King Edward was planted between each of the blocks as a spreader row and these unsprayed rows were inoculated.

The Llanilar and Auchincruive sites were inoculated on 5 July and 16 July respectively using a mixture of representative *P. infestans* isolates. At Llanilar, fungicides were applied in 250 litres of water per hectare using a hand held Oxford Precision Sprayer operating at 200 kPa through 110° flat fan nozzles. At Auchincruive, fungicides were applied in 200 litres of water per ha using a tractormounted, modified AZO compressed air sprayer, operating at 3.5 bars to give a medium/fine spray quality using Lurmark F03-110 nozzles.

All spray programmes started with three applications of propamocarb HCL + mancozeb (as Tattoo; Bayer CropScience) to protect plots during rapid haulm growth. From application four to haulm desiccation, different fungicide treatments were applied at 7 or 10 day intervals. Rates and intervals are shown in Table 1. The label restriction on the number of Infinito applications per season was exceeded to allow scientifically valid comparisons between treatments. Percentage leaf area destroyed by foliar blight was assessed regularly during the epidemic using a modified version of the keys Large (1952) and Anon (1976).

The Area under the Disease Progress Curve (AUDPC) was calculated for each sub-plot and subjected to analysis of variance. The untreated control data were not included in the analyses because of the skewed distribution of the data. To aid interpretation, the statistical significance of differences between treatment means was determined using the Least Significant Difference test at P<0.05 (5%).

#### RESULTS AND DISCUSSION

At both sites, the disease pressure was exceptionally high and this should be taken into consideration when interpreting the results. The 13\_A2 genotype of *P. infestans* dominated at both sites. To assess the efficacy of the integrated control treatments, they were compared with the benchmark combination of Shepody treated with the highest rate of fungicide at 7-day intervals. The three benchmark treatments were 1.6 l/ha Infinito, 0.4 l/ha Shirlan and 2.0 kg/ha Dithane NT. Integrated control treatments were the two more resistant cultivars, Maris Piper and Lady Balfour, treated with less fungicide input than the highest rate at the shortest interval.

OSTATS

**Table 1.** Fungicides, rates and intervals applied to all cultivars.

Product	Active ingredient(s)	g/kg or L product	Concentration of a.i.(a.i./ha)	Rate/ha	Intervals (days)
Infinito SC	fluopicolide + propamocarb HCl	62.5 + 625/L	100 +1000	1.6 (L)	7 and 10
Infinito SC			75 + 750	1.2 (L)	7 and 10
Infinito SC			50 + 500	0.8 (L)	7 only
Shirlan SC	fluazinam	500/L	200	0.4 (L)	7 and 10
Shirlan SC			150	0.3 (L)	7 and 10
Shirlan SC			100	0.2 (L)	7 only
Dithane NT WG	mancozeb	750	1500	2.0 (kg)	7 and 10
Dithane NT WG			1125	1.5 (kg)	7 and 10
Dithane NT WG			750	1.0 (kg)	7 only

The AUDPC values for foliar blight infection for Shirlan, Dithane NT and Infinito are shown in Figures 1 to 3. At the Scottish site all treatments with reduced Shirlan rates applied to Maris Piper and Lady Balfour gave significantly better control than the benchmark treatment (Shirlan 0.4 L/ha on Shepody at 7 day intervals) (Figure 1). This was the same for Dithane NT, except where the three-quarter dose was applied at 10-day intervals (Figure 2). Only two of the integrated control treatments using Infinito, (1.2 and 0.8 L/ha applied every 7 days to Lady Balfour) gave significantly better control than the benchmark treatment (Figure 3).

At the site in Wales all of the Dithane NT integrated control treatments gave significantly better control of foliar blight than the benchmark treatment (2.0 kg of Dithane NT applied every 7 days to Shepody) (Figure 2). In contrast only one of the Shirlan integrated control treatments gave significantly better control than the benchmark treatment, i.e. Maris Piper treated every 7 days with 0.3 L (Figure 1). None of the Infinito-based integrated control treatments were significantly more effective than the benchmark (Figure 3).

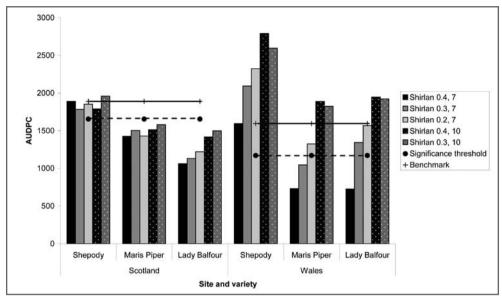


Figure 1. Foliar blight severities (AUDPC) for different combinations of cultivar resistance and fungicide input in relation to the benchmark treatment of 0.4 L of Shirlan applied every 7 days to the cultivar Shepody. AUDPC values below the significance cut-off line indicate significantly better control than the benchmark treatment.

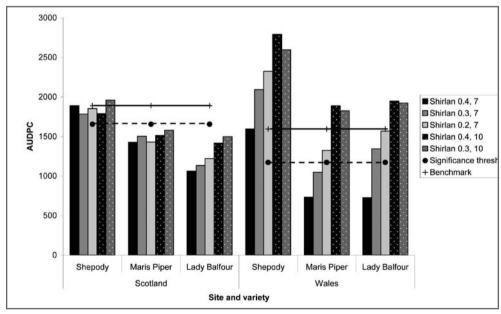


Figure 2. Foliar blight severities (AUDPC) for different combinations of cultivar resistance and fungicide input in relation to the benchmark treatment of 2.0 kg of Dithane NT applied every 7 days to the cultivar Shepody. AUDPC values below the significance cut-off line indicate significantly better control than the benchmark treatment.

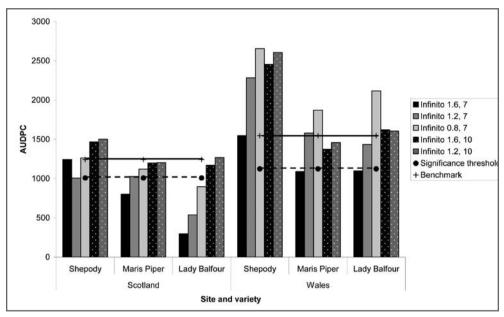


Figure 3. Foliar blight severities (AUDPC) for different combinations of cultivar resistance and fungicide input in relation to the benchmark treatment of 1.6 L of Infinito applied every 7 days to the cultivar Shepody. AUDPC values below the significance cut-off line indicate significantly better control than the benchmark treatment.

As part of an integrated control strategy the same reduction in fungicide input can be achieved in two ways: to apply a lower dose of fungicide at 7-day intervals or a higher dose at 10-day intervals. At Auchincruive lower doses applied every 7 days were generally more effective than higher doses at longer intervals (Table 2). At Llanilar, a reduced dose applied more frequently was marginally more effective than a higher dose applied every 10 days (Table 2). It should be noted that the 7-day programmes at Llanilar were adversely affected by the timing of a high rainfall event, with 45 mm of rain the day after the first of the 7-day treatments was applied.

**Table 2.** Number of times (out of 18 comparisons) that the combination of lower fungicide dose at 7-day intervals was more or less effective compared with a higher dose at 10-day intervals in controlling foliar blight.

Site	Significantly more effective	More effective	Less effective	Significantly less effective
Auchincruive	7	10	1	0
Llanilar	6	3	7	2

The principle of reducing fungicide inputs on potato cultivars with good resistance to foliar blight has been established for many years and previous work demonstrated the potential for adjusting fungicide input depending on cultivar resistance (Fry, 1978; Gans *et al.*, 1995; Clayton & Shattock, 1995; Kirk, 2005). Previous research on foliar late blight control found that lower fungicide application rates were less effective on moderately susceptible varieties where disease pressure

was high, but successful control was possible with lower rates where conditions were moderately conducive (Kirk *et al.*, 2001). This work has confirmed findings from an earlier study (Bradshaw & Bain, 2007) that cultivar resistance could be used in GB as part of an integrated control strategy to reduce fungicide inputs for the control of foliar blight, and has shown these strategies to be effective even where the disease pressure was high. The results from these two experiments in 2007 should be regarded as preliminary. However, they do suggest that an integrated control approach based on cultivar resistance can be effective even under very high disease pressure and where the 13\_A2 genotype dominates the population.

A pre-requisite for the successful use of cultivar resistance in integrated control is reliable, up to date information about foliar resistance ratings. In both trials the "resistance" of Lady Balfour in relation to the two other cultivars was less than expected from this cultivar's official foliar resistance rating of 8 (Anon, 2006). Changes in the resistance ratings of other cultivars challenged recently in GB with genotype 13\_A2 have been reported (Lees *et al.*, 2009). It is interesting to note that in the two 2007 trials the differences in AUDPC values for Shepody, Maris Piper and Lady Balfour were frequently related to the amount of fungicide applied. In general cultivar differences were smaller in untreated plots compared with fungicide-treated plots (Table 3). At Auchincruive the differences between cultivars generally increased as the amount of fungicide applied was increased. This observation was not as consistent at Llanilar; however the same trend was clear for Dithane NT. Further work in this area is required to establish the implications of this result for the contribution of cultivar resistance to blight control in conventional potato production.

**Table 3.** Effect of fungicide input on the size of differences in AUDPC values between cultivars.

	Auchir	ncruive	L	lanilar
Fungicide input		Con	nparison¹	
	1	2	1	2
Shirlan @ 0.4 L, 7 days	827	463	867	861
Shirlan @ 0.2 L, 7 days	631	423	756	1000
Shirlan @ 0.3 L, 10 days	462	379	674	771
Dithane NT @ 2.0 kg, 7 days	1165	576	1111	985
Dithane NT @ 1.0 kg, 7 days	912	633	757	835
Dithane NT @ 1.5 kg, 10 days	430	601	668	893
Infinito @ 1.6 L, 7 days	945	441	448	456
Infinito @ 0.8 L, 7 days	366	143	539	783
Infinito @ 1.2 L, 10 days	234	301	1000	1145
Untreated	-203	144	487	567

<sup>&</sup>lt;sup>1</sup> Comparison 1: Shepody and Lady Balfour; Comparison 2: Shepody and Maris Piper.

#### **ACKNOWLEDGEMENTS**

Funding from Potato Council is gratefully acknowledged.

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# Quantitative trait locus on potato chromosome V influencing late blight resistance and earliness in IHAR's diploid and tetraploid breeding lines

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#### Quantitative trait locus on potato chromosome V influencing late blight resistance and earliness in IHAR's diploid and tetraploid breeding lines FNP

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Potato cultivars resistant to Phytophthora infestans (Mont.) de Bary are in vast majority of late maturity type. This is either due to the effect of the same genes influencing both traits or due to closely linked genes encoding the two traits. In both cases the genes of interest are located on potato chromosome V around R1 locus which in numerous linkage mapping studies was shown to be the most prominent Quantitative Trait Locus (QTL) for earliness as well as an important QTL for late blight resistance.

In next stage it was shown that the association between resistance to late blight and maturity type is not only detectable in different mapping populations but also in wide collection of 600 potato cultivars. Moreover, the markers linked to the QTL on chromosome V in previous linkage studies were also significantly associated with both traits in this collection. The marker alleles associated with increased resistance and later plant maturity were traced to an introgression from the wild species Solanum demissum (Gebhardt et al. 2004).

Within the collection assessed by Gebhardt et al. (2004) there were only a few old Polish cultivars. The goal of our study is to assess IHAR's breeding materials for the genetic variation in this crucial region of chromosome five. We would like to address the question if our modern cultivars and breeding lines differ significantly from the common tetraploid gene pool and if the linkage between higher resistance and later maturity type is detectable in all of them. We have chosen for this purpose a representation of a 200 2x and 4x lines differing in resistance levels, maturity and with many wild species introgressions. S. demissum is present in those materials only via the early introgression into 4x S. tuberosum cultivars. All chosen lines were assessed for resistance to late blight (LB) in detached leaflet test and for maturity type in a field test for vegetation length period. DNA Markers were applied as described by Gebhardt et al. (2004).

Table 1. Marker allele frequencies observed in IHAR's material and compared to data obtained by Gebhardt et al. (2004) (\*).

	Cos A 210	R1 1400	R1 1800	GP179 570	GP76 500	GP76 450
chromosome	v	v	unknown	v	VI	unknown
Allele 1, number scored	9	8	93	0	27	1
Observed frequency	0.05	0.06	0.67		0.27	0.01
Reference frequency*	0.31	0.33	0.57	0.52	0.29	0.00
Allele 0, number scored	162	130	45	48	73	99
Observed frequency	0.95	0.94	0.33		0.73	0.99
Reference frequency <sup>a</sup>	0.69	0.67	0.43	0.48	0.71	1.00
Total number of lines scored	171	138	138	48	100	100

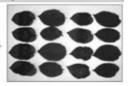
Table 2. Mean LB resistance (in 1-9 scale, 9 = most resistant) and vegetation period length (in days) in groups of IHAR's material divided according to the presence (allele 1) or absence (allele 0)of marker alleles and compared to data obtained by Gebhardt et al. (2004) (°).

	Cos A 210	R1 1400	R1 1800	GP179 570	GP76 500	GP76 450
chromosome	v	٧	unknown	v	VI	unknown
Allele 1	•	•	**		*	1000
Mean LB resistance	5.67	5.77	6.69		4.34	
Reference mean LB resistance <sup>a</sup>	5.60	5.60	4.97	5.13	5.00	
Allele 0	- 11.5	7,044,17	10.00	-12-7		
Mean LB resistance	6.03	6.48	5.88		5.75	*
Reference mean LB resistance <sup>a</sup>	4.93	4.92	5.51	5.16	5.14	
Allele 1						
Mean vegetation period (days)	156.6	158.8	152.8	12	154.4	
Allele 0						
Many unpertation period (days)	1524	152.0	152.0	52	152.7	-217

Mean vegetation period (days) 152.6 152.8 153.8 - 152.7 
\* Marker allele associated significantly with increased LB resistance and later maturity (Gebhardt et al. 2004); \*\* marker allele significantly with increased LB susceptibility (Gebhardt et al. 2004); - not calculated.

Cristianie C., Ballvora A., Walkemeier B., Oberhagemenn P., Schüler K., 2004. Assessing genetic potential in germplasm collections of crop plants by marks-trait association: a case study for potatoes with variation of resistance to late blight and maturity type. Mol Bread 3: 93-102

The preliminary results obtained so far indicated that indeed IHAR's breeding lines differ from the 4x cultivar pool tested by Gebhardt et al. (2004) with regard to allelic freguncies observed for the loci on chromosome V. The alleles descending from S. demissum (Cos A 210, R1 1400) and associated with increased LB resistance and later maturity were rare in our material (Table 1) while frequencies of marker alleles from other loci (R1 1800, GP76) were similar in both studies. Due to big difference in number of individuals with or without certain marker allele (9: 162) we couldn't confirm the statistical significance of differences between the means of LB resistance and vegetation length period in both groups (Table 2). However, we plan to increase the number of tested lines to enable such analyses in the future. We couldn't compare maturity results of the two studies due to different scoring methods.



# Evaluation of lowering fungicide dose rates to control potato late blight 2008

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#### PPLIED PLANT RESEARCH

#### WAGENINGENTE

# Evaluation of lowering fungicide dose rates to control potato late blight 2008

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#### Introduction

Cultivar resistance combined with reduction of chemical input is currently very promising to reduce fungicide input. For implementation in practice, reliable levels of late blight resistance per variety in combination with fungicides dose rates are needed.

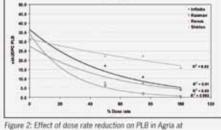
Research was carried out by request of the Dutch Ministry of Agriculture Nature and Food Quality

#### Field trials

Fields trials were carried out at two of the research units of Applied Plant Research, near Lelystad and Valthermond, in the Netherlands, in 2007 and 2008 (Figure 1).

Potato cultivars with different resistance levels to late blight. were used in the experiment. Different dose rates of various fungicides were applied throughout the season, based on a

Spreader rows were inoculated with a mixture of 15 current Phytophthora infestans isolates. Disease severity was assessed.



Lelvstad in 2008



Figure 1: Overview of the field experiment at Lelystad showing distinct differences in potato late blight severity between cultivars, fungicides and dose rates.

Figures 2 and 3 show the effect of lowering the dose rate of various fungicides on potato late blight infection. The average stAUDPC value was smaller on more resistant cultivars (Figure 2) than on susceptible cultivars (Figure 3).

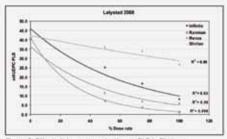


Figure 3: Effect of dose rate reduction on PLB in Bintje at Lelystad in 2008

Dose rate reduction of fungicides seems to be promising. A better understanding of the possibilities in relation to weather circumstances, crop growth and cultivar resistance is still needed. Therefore the experiment is repeated in 2009. Based on data 2007 - 2009 an advice to growers will be given.

#### Applied Plant Research

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# Variation of the foliar aggressiveness of *Phytophthora infestans* from different potato-growing regions of Russia

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#### SUMMARY

The aggressiveness of *Phytophthora infestans* isolates from different regions of Russia (Moscow, Tula, Stavropol, Bryansk, Leningrad, Murmansk regions, and Sakhalin Island) was studied. Potato cultivars with different level of partial resistance to the late blight were used in this study. The results showed that isolates, from different regions, demonstrated various aggressiveness levels on the same potato cultivars. The isolates of the same races, isolated from different regions, also differed in their aggressiveness level.

#### **KEYWORDS**

Phytophthora infestans, potato cultivars, races, aggressiveness

#### INTRODUCTION

Potato late blight (LB) disease, caused by the oomycete *Phytophthora infestans*, is one of the most devastating potato diseases in Russia. To date, the new strains of the pathogen appeared in all potatogrowing regions. This fact increases the possibility of any additional variability in this permanently changing pathogen, making the late blight control more difficult. Our study showed that complex races are typical for all Russian regions (Fig. 1). Therefore, the vertical resistance of potato plants to the simple races can not be used in the LB control. It is known that the growing of potato cultivars with a partial LB resistance is one of the most important components of the integrated pest management of this crop (Colon *et.al.*, 1995).

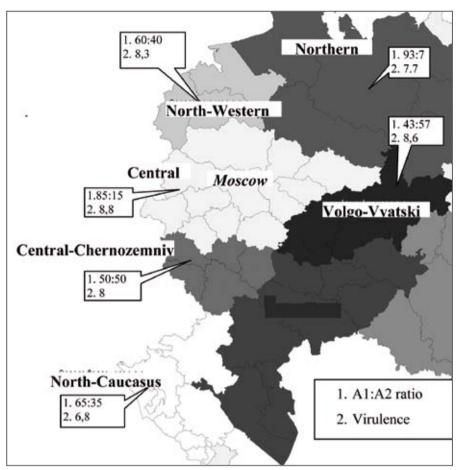


Fig. 1. Average indices of the virulence and compatibility types of the late blight pathogen in European part of Russia.

The objective of this study was to estimate the stability of the partial resistance of potato cultivars as well as the stability of the aggressiveness of *P. infestans* populations in some potato-growing regions of Russia.

#### **MATERIALS AND METHODS**

Experiment 1. Evaluation of the aggressiveness level of *P. infestans* isolates using potato leaves To solve this task, we studied the level of the partial LB resistance of 40 potato cultivars using their artificial infection with *P. infestans* isolates, collected in the different regions of Russia: Moscow, Tula (Central region), Bryansk (Western region), Leningrad (North-western region), Murmansk (Northern region) and the Stavropol territory (North-Caucasus).

To determine the aggressiveness of isolates, the express evaluation method was used (Filippov *et al.*, 2004).

The cultivars tested were grown under field conditions, and the next stages of tests were carried out under laboratory conditions. Detached leaves of each cultivar were inoculated with the mixture of

10 P. infestans isolates, collected in each region studied.

To determine the aggressiveness level, the following tests were performed:

#### Determination of the inoculation efficiency

Ten leaves of each cultivar were sprayed with a conidia suspension (300000 per m²). After the inoculation, leaves were incubated at 18°C in a moist chamber. After the 3-day incubation, the total area of leaves and the number of necroses per cm² of a leave surface were determined.

#### Measurement of the necrosis diameter

Potato leaves were inoculated with separate drops of conidia suspension (1-2 drops per leaflet). The concentration of conidia was the same as above. Inoculated leaves were incubated in a moist chamber for 18 hours at 18°C in the darkness. Then the residuals of the suspension were removed from the leaves using a filter paper, and the leaves were incubated for 4 days at 20°C. Then the diameter of necroses was measured.

#### Measurement of a sporulation capacity

The leaves from the afore-mentioned test were used. The sporulation capacity can be estimated in two ways. The first way is to count the number of conidia per one spot using the Goryaev chamber (hemocytometer). Ten leaflets with necroses were put into 15 ml of distilled water and shaken, then leaflets were removed, the volume of suspension was measured, and the number of conidia per one spot was counted using the Goryaev chamber. The second way represents a visual estimation in scores.

The rated yield loss was calculated and converted into scores using the 9-score scale, where 9 means the highest level of a cultivar resistance. This scale can be used for the estimation of the partial resistance of potato cultivars and the aggressiveness of *P. infestans* strains.

Table 1. Scale for the determination of cultivar resistance and *P.infestans* aggressiveness

Rated yield losses	Level of cultivar resistance to the late blight according to 1-9 scale	Level of <i>P.infestans</i> aggressiveness
< 5%	9-8 (Resistant)	Non-aggressive (NA)
5-15%	7-6 (Moderately resistant)	Weakly aggressive (WA)
16-35%	5-4 (Moderately susceptible)	Moderately aggressive (MA)
> 35%	3-1 (Susceptible)	Highly aggressive (HA)

Experiment 2. Comparison of the level of aggressiveness of *P.infestans* isolates (race 1.2.3.4.5.6.7.8.10.11), collected in different regions of Russia.

#### Determination of P.infestans races

To identify *P. infestans* races, the set of differential plants, obtained from IHAR (Mlochow, Poland), was used.

The differential plants were grown in a climate chamber in two terms of planting: April – August and July – November (until a seed formation). The conditions of growing were the following: 16-hour photoperiod, daily/night temperature 22/18°C, relative air humidity 60-70%, lightness 8,000-10,000 lux, lamp DNAT-400. Races were identified at the temperature 18°C and a high level of a relative air humidity.

The determination of the isolate aggressiveness was carried out according to the express-method.

#### Isolates and cultivars

In these experiments we used isolates, collected in Moscow, Bryansk, and Leningrad regions, Mordovia and also the Stavropol territory. All isolates were identified as a complex race 1.2.3.4.5.6.7.8.10.11, which is widely represented in many regions of Russia. The following potato cultivars with a different level of the LB resistance were used: Sante (MS), Bryanskaya novinka (MS) and Russian Souvenir (MR).

#### RESULTS AND DISCUSSION

#### Experiment 1.

Evaluation of the aggressiveness level of *P. infestans* isolates using potato leaves.

It was shown that the clear gradation of the population aggressiveness depends on the origin of isolates (fig. 2). The number of resistant cultivars for every tested group of isolates was used as the indicator of this gradation. Bryansk and Tula isolates demonstrated high level of aggressiveness, because the total number of susceptible cultivars in these cases exceeded 50%. The isolates from Moscow, Murmansk and Stavropol regions showed a low level of aggressiveness (the number of susceptible cultivars was 30-35%).

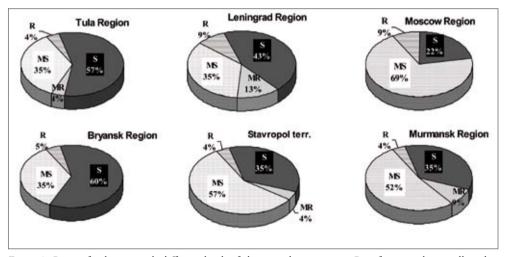


Figure 2. Ratio of cultivars with different levels of the partial resistance to P. infestans isolates, collected in different regions of Russian Federation.

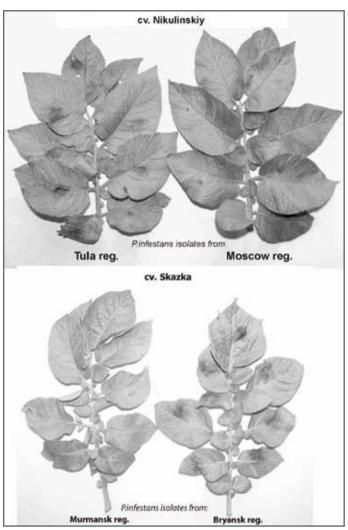


Figure 3. Demonstrates different reactions of some cultivars to the infection with P. infestans isolates from different regions of Russia.

#### Experiment 2.

Comparison of the level of aggressiveness of *P. infestans* isolates (race 1.2.3.4.5.6.7.8.10.11), collected in different regions of Russia.

The results of this experiment (Fig. 4) demonstrate the differences in the reaction of potato cultivars to the infection with the race 1.2.3.4.5.6.7.8.10.11, represented by isolates from different regions of Russia. The isolate from Bryansk, in comparison with other isolates, showed a high aggressiveness level for all potato cultivars. The isolate from Leningrad region showed the lowest level of aggressiveness. It was shown that the cv. Russian Souvenir demonstrated the highest resistance to all isolates used. The revealed variations in the pathogen aggressiveness mean that in different regions the same potato

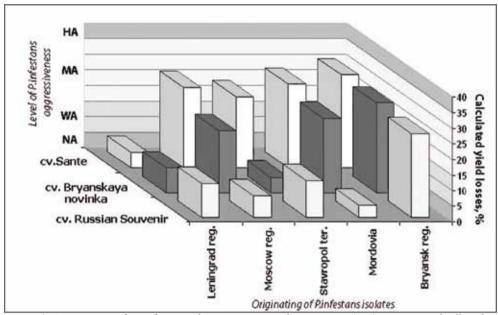


Figure 4. Aggressiveness of P. infestans isolates, representing the race 1.2.3.4.5.6.7.8.10.11 and collected in different regions of Russia.

cultivar can demonstrate the different levels of a partial resistance. Therefore, we conclude that the partial resistance is not a stable parameter and depends on the aggressiveness level of the local *P. infestans* population.

#### **ACKNOWLEDGEMENTS**

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### Forecasting potato late blight in Norway

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#### **SUMMARY**

Forecasting of potato late blight (*Phytophthora infestans*) epidemics in Norway started in 1957 and is now part of the internet based warning system VIPS (Acronym for pest warnings). The late blight warning system has three elements. Early infections of the pathogen are surveyed by local Extension groups and are displayed on a map (web-blight). The Negative prognosis, is used for timing of the first spray. Førsund rules, indicating high risk periods for infection, are used for timing consecutive fungicide treatments. This simple model has been tested and used for many years and seems to be robust. In this paper a practical experiment in farmer fields is presented. Conventional routine fungicide treatments at fixed intervals were compared to treatments according to late blight forecasts in 12 years from 1996-2007. The experiment demonstrated that it was possible to save about 30% of the fungicide applications when the fungicide was applied according to the warnings in VIPS related to routine treatments. Possible improvements of the forecasting system are also discussed.

#### **KEYWORDS**

Phytophthora infestans, potato late blight, forecasting, practical experiments

#### INTRODUCTION

Potato late blight is caused by the oomycete *Phytophthora infestans* and is one of the most important potato diseases worldwide. The total annual costs in Norway caused by potato late blight are about 60 million NOK. These costs include fungicide application, yield and quality loss, cost of inspection, research, advisory service and warnings (Sæthre *et al.*, 2006).

To protect the crop against late blight infections, potato growers need information related to the environment, the host and the pathogen. In Norway information and decision support aiming at late blight control is disseminated via the Internet in a system called VIPS (<a href="www.vips-landbruk.no">www.vips-landbruk.no</a>). However forecasting of late blight epidemics in Norway started back in 1957 (Førsund and Flaaten, 1958 and 1959; Førsund, 1983). Bioforsk is responsible for dissemination of agrometeorological and related information to the agricultural sector. This is done in close collaboration with the Norwegian meteorological institute and the Norwegian Agricultural Extension Service. Weather data are available from Bioforsks weather station network established in important agricultural areas in Norway in the 1990s (Magnus et al., 1991). All information is disseminated via the Internet and increasingly via SMS to Mobile phones.

The late blight warning criteria used in the system are based on empirical data from the old population of *P. infestans*. The new pathogen population has displaced the old one and epidemiological changes might occur. Experiments from 1994-1999 showed that the old forecasting rules were still valid (Hermansen and Amundsen, 2003). The aim of this study was to continue the evaluation of the

available forecasting system in Norway. Data from 1996-2007 is presented. In addition the different elements of the late blight warning system are explained.

#### MATERIALS AND METHODS

#### The warning system

The late blight warning system in VIPS has three elements. Monitoring of first attack of late blight in important potato growing areas is carried out by the advisory service and are displayed in VIPS. This element is a part of a collaborative Internet application established in 1998 by the development of the Internet based Web-blight system, http://www.web-blight.net (Hansen *et al.*, 2001). The monitoring service gives a general warning about early attacks of late blight and collect background information about variety, field types etc. for these early attacks. The very first late blight findings each year are particularly examined regarding type of initial inoculum (tuber-born inoculum or oospores).

The Negative prognosis, developed in Germany by Ullrich and Schrödter (1966) is used to time the first fungicide application. The model is a mathematical-statistical model, developed from empirical data of temperature and humidity at ordinary meteorological stations in Germany in the middle of the 1960's. The model calculates the daily risk value from the date 50% emergence in the conventional fields (early plastic covered potatoes are not included). When the accumulated risk value reaches 150 it is recommended to start application of fungicide according to the Førsund rules.

Førsund rules (Førsund, 1983), indicating high risk periods for infections, is used for timing of the consecutive fungicide applications. The Førsund rules are a simple mathematical rule based model, developed on empirical date of temperature, humidity and rain at ordinary meteorological stations in Norway in the middle of the 1950's (Førsund and Flaatten, 1958, 1959). The temperature criteria were adjusted in 1994 (Hermansen and Amundsen, 2003).

The warnings in VIPS are based on historical weather data obtained from about 80 automatic weather stations operated by Bioforsk and 48 hours weather prognosis calculated and provided by Norwegian meteorological institute as 3- hour's values.

#### Field experiments

The Late blight warning system in VIPS has been compared with conventional "routine" fungicide application and control (the control was treated as the conventional strategy after blight was found in the field) in a large scale field trial every year from 1996 to 2007 in Vestfold County. The experiment has been carried out in cooperation with Vestfold Extension group and a potato grower. The cultivar used were either Beate with foliar resistance score 6 and tuber resistance score 7 (scale from 1 - 9 where 9 is the most resistant) or Oleva with resistance score 4 and 5, respectively. Fertilization and non-experimental pesticide applications were applied according to farmers practice. The trial had a randomised complete block design with four replications. Experimental subplots received 20 m of one spray-width of the equipment used by the farmer (normally a 12 m spray boom). The fungicide Shirlan (fluazinam) at a rate of 300 ml/ha was used in most applications. The first fungicide treatment in conventional plots was applied at row closure in the potato field, and further application intervals were chosen according to normal practice in the area (normally weekly sprays). The warning plots were treated according to the warnings given for area Tjoelling in Vestfold. The first fungicide application was carried out at the first Førsund warning after the ARV had reached 150. After a protection period of 7 days it was sprayed again at the next Førsund warning. The fungicide was applied at the day of the warning or the following day. If there was more than 2 weeks from the last fungicide application to the planned date for haulm killing, it was recommended to apply fungicide 7 days before planned haulm killing. Disease assessments were made every week starting from row closure in the untreated control. The registrations were done on two centre rows of each plot. After disease onset all the plots were assessed for disease severity (scale from 0-100) several times using the B.M.S. key (Anonymous, 1947). Local personnel from the Extension group assessed the plots during the season, but staff from Bioforsk normally carried out the final assessment. A 5 kg potato sample from each subplot was stored at about 15 °C for at least 14 days before assessment of tuber blight.

#### Statistical analyses

Data were subjected to analysis of variance using the GLM procedure in MINITAB® release 15 (www.minitab.com). Sources of variation used to explain the results were year (random), blocks (random) and treatment strategy (fixed). In years with significant differences between the treatment strategies, treatment means were separated using Tukey simultaneous test at the 5 % significant level

#### RESULTS AND DISCUSSION

Two different strategies to control potato late blight were compared, conventional fixed spray intervals and sprayings according to VIPS warnings. There were no significant differences in the severity of foliar or tuber blight between the conventionally treated plots and those treated according to the VIPS warning system any of the years, not even when all the years were pooled together. Hence, compared to conventional treatments it was possible to save in average 31 % of the treatments (1 to 4 treatments) by using the VIPS warning system to time the fungicide applications without significantly increasing the late blight severity (Table 1). However, there was a tendency to a bit more disease using VIPS as a trigger of sprays compared to conventional routine treatments. Fungicide treatments were saved by use of the VIPS warning system compared to conventional fungicide application because the first application was delayed or the intervals between treatments were extended.

Normally late blight is found in conventional potato fields one or more weeks after the accumulated risk value (ARV) of the Negative prognosis has reached 150, but in 2003 late blight was found five days before ARV reached 150 (Table 2). None of the early attacks was recorded at growth stages earlier than closing within rows, not even in the plastic covered crops. There have been no indications of early late blight attacks derived from oospores (numerous infections on leaves touching the ridge), volunteers or dumps up to now in Norway in the Web-blight registrations. This indicates that infected seed potatoes are probably still the most important initial inoculum source for P. infestans in Norway. However, oospores still might play a role in later infections of the crop.

Use of the old simple forecast models in VIPS for timing of fungicide applications against potato late blight gave acceptable results in the period 1996-2007. Blight was found in the foliage 6 of the 12 years in conventional treated plots compared to 10 of the 12 years in the plots treated according to the warning system. However, some disease in the haulm can be accepted if it causes minimal problems with tuber blight. A medium to high level of tuber blight resistance is an essential criterion for a safe use of the warning system to decrease the fungicide input. The most important potato cultivar used until recently in Norway, Beate, has a medium level of resistance to late blight in the foliage and in the tubers. However in recent years more late blight susceptible cultivars are getting popular and thus the potential for reduction of fungicide input is less.

Fungicide treatments according to the Negative prognosis and the Førsund rules worked relatively well but the models have their weak points. The algorithm used to calculate the daily risk values in the Negative prognosis does not use data from a specific temperature interval, 12-14 °C. These are frequent temperatures at some situations in the growing season in Norway. The Førsund rules

**Table 1.** Late blight infection in foliage and tubers at the end of the season and number of fungicide applications in field trials from 1996 –2007 in Vestfold. Two different strategies were compared, conventional and VIPS forecasts. In addition there was an untreated control. In some of the years the "control" was treated as conventional after blight was found in the field.

Year (cultivar)	Control			Conventional			VIPS forecast	s	
	Number of fungicide treatments	% foliage affected by late blight	% tubers affected by late blight	Number of fungicide treatments	% foliage affected by late blight	% tubers affected by late blight	Number of fungicide treatments	% foliage affected by late blight	% tubers affected by late blight
1996 (Beate)	0	25,0 b	2,4	5	0,1 a	1,6	3	0,3 a	0,0
1997 (Beate)	0	0,3 b	0,0	6	0,0 a	0,0	5	0,0 a	0,0
1998 (Beate)	5	2,1 b	1,0	9	0,0 a	0,0	5	0,1 a	0,0
1999 (Beate)	3	20,1	1,6 b	8	0,1	0,1 a	4	0,8	0,2 a
2000 (Beate)	3	0,9 b	1,6	6	0,1 a	0,2	5	0,1 a	0,1
2001 (Beate)	0	22,5 b	4,5	7	0,7 a	2,6	6	0,7 a	2,8
2002 (Oleva)	0	0,3	0,0	9	0,0	0,0	7	0,1	0,0
2003 (Oleva)	0	1,2 b	2,9 Ь	8	0,0 a	0,7 a	5	0,1 a	0,0 a
2004 (Oleva)	0	1,8	0,0	9	0,0	0,0	5	0,0	0,0
2005 (Oleva)	0	0,8 b	30,2	8	0,1 a	5,5	7	0,1a	10,1
2006 (Beate)	4	3,3 b	1,5	8	0,1 a	1,3	5	1,0 ab	4,7
2007 (Oleva)	7	0,8	5,7b	8	0,0	0,0a	6	0,1	1,4a
All years	1,8	6,6b	4,3	7,6	0,1a	1,0	5,3	0,28a	1,6

Treatment responses with the same letter (or with no letter) within year are not significantly different according to Tukey simultaneous test (P<0.05).

are not fulfilled if it does not rain. However, a long period of dew is probably also sufficient for the pathogen to be able to infect the plants. The VIPS warning system needs to be improved to catch such incidents. The relative humidity at 12.00 a.m. must be 75% or higher for the Førsund rules to be fulfilled. This is probably a condition of great importance for survival of released sporangia. However the Førsund rules do not include any specific criteria for spore production, like the long humid periods in the Negative prognosis. The reason why Førsunds rules works relatively good is probably a natural climatic correlation between long humid periods and Førsunds rules criteria.

**Table 2.** Dates at which the accumulated risk value (ARV) for the area Tjoelling in Vestfold had reached the 150 threshold in VIPS, dates for first observation of late blight in Vestfold and in the field trials and the ARV (for the trial) at that date during 2001 – 2007.

Year	ARV > 150	The two first recordings of late blight in conventional potato fields in	Late blight is found in the field trial
		Vestfold county, date and variety (first recording of blight in plastic covered	
		early potatoes)	(ARV at that date)
2001	11/7	23/7 Rutt, 30/7 Troll	2/8 (239)
2002	24/6	12/7 Ostara, 18/7 Troll (4/6 Aksel)	26/7 (237)
2003	19/7	14/7 Troll, 15/7 Ostara (20/6 Rutt)	16/7 (133)
2004	17/7	20/7 Mandel, 4/8 Troll	17/8 (291)
2005	15/7	22/7 Troll, 2/8 Beate (1/7 Rutt)	8/8 (272)
2006	7/7	27/7 Rutt, 30/7 Astrix (28/6 Ostara)	15/8 (329)
2007	9/7	13/7 Laila, 19/7 Oleva (5/7 Rutt)	19/7 (189)
		I .	1

Both the Førsund rules and the Negative prognosis are based on empirical observations of the old P. infestans population. The genetic composition of P .infestans populations has changed over the years. A new population of both mating type A1 and A2 has displaced the earlier population in most of Europe (Fry et al., 1993) including Norway (Hermansen et al., 2000). The high genetic variability in the Norwegian pathogen population (Brurberg et al., 1999) and the mating type distribution and findings of oospores in leaves indicates that P. infestans is able to sexually reproduce here. A Nordic project was started in 2003 to evaluate and quantify epidemiological parameters of the new populations of P. infestans in the Nordic countries. Data from this project is the base for making a new late blight forecasting model (Nærstad et al., 2009). If a validation of this model gives good results it will probably replace Førsund rules in VIPS in the future.

#### **CONCLUSIONS**

Late blight forecasts in Norway are available using the web based system VIPS. Practical experiments using the system shows acceptable results, and 31 % of the treatments were saved compared to routine treatments without significantly increase the late blight severity. However, development of a new late blight model is underway to make more accurate forecasts.

#### **ACKNOWLEDGEMENTS**

We wish to thank the farmer Georg Solberg and the personnel in Vestfold Extension group for nicely taking care of the field experiments. We are also thankful to Andrew Dobson for assessing tuber blight from the experiments.

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# Solar radiation and spore survival in *Phytophthhora infestans*, *Bremia lactucae* and *Pseudoperonospora cubensis*

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### Solar radiation and spore survival

in Phytophthhora infestans, Bremia lactucae and Pseudoperonospora cubensis



V.H. Le', B. Nordskog', R. Nærstad', D.M. Gadouryi and A. Hermansen'

#### Introduction

Aerial dispersal of inoculum is critical to the spread of many plant diseases; including potato late blight (Phytophthora Infestans (Pi)), lettuce downy mildew (Bremia loctucae (Bl)) and cucurbit downy mildew (Pseudoperanospora cubensis (Pc)) Fig. 1. In addition to relative humidity and temperature, spore survival during aerial dispersal is affected by solar irradiation, in particular during long-distance transport at higher altitudes.



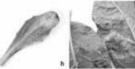


Fig. 1. a) Cucurbit downy mildew, b) Lettuce downy mildew and c) Potato late blish.

#### Materials and methods

We evaluated the potential survival of spores in air by placing detached spores of Pi, Bl and Pc on filter paper in either direct sun or shade at time intervals from 0.5 to 3 h (Pi and Bt), or up to 42 hours (Pc) as shown in Fig. 2. Thereafter, the filter papers were placed in moist chambers for 15 min prior to incubation on pea agar (Pi) or water agar (Bl and Pc) for 24 h, before the viable spores were enumerated. Spores were considered viable if they exhibited a germ tube or released 200spores as shown in Fig. 3.







Fig. 2. a) Spores fixed on nylon filter paper: b-c) racks for exposure in either direct radiation or shadow.

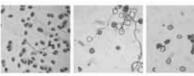


Fig. 3. Germination of a) R infestors b)  $\delta$ . (octuces and c) R cubensis (plate b and c are fixed with lacto fuchsin).

#### Results

Preliminary results show that no spores of Pi, Bl and Pc germinated after 1, 3 and 30 h exposure to direct sun, with critical solar irradiation doses near 700, 2000 and 8500 Wm², respectively. In shade, no Pi spores germinated after 3 h, while spores of Bl and Pc were still viable after 3 and 42 h, respectively.

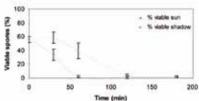


Fig. 4. Germination rate of spores of R Infestons exposed to sun or shadow.

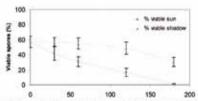


Fig. 5. Germination rate of spores of 8. lactucoe exposed to sue or shadow.

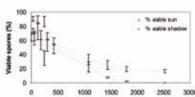


Fig. 6. Germination rate of spores of R cubmsls exposed to sun or shadow.

#### Concluding remarks

In Norway, the potential for long distance distribution of viable Pi spores is restricted, but more likely for Bl and Pc spores. Further experiments will be conducted to find the maximum survival time for spores of these pathogens under Norwegian climatic conditions.

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# Induced resistance against *Phytopththora infestans*– synergy with fungicides?

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# Induced resistance against *Phytopththora infestans* – synergy with fungicides?

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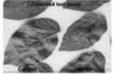
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#### Introduction

Late blight caused by the comycete, Phytophthons infestians, causes every year large yeld losses for Swedish potato growers. Today the only efficient management to control potato late blight is the frequent use of fungicides. Hence it is of a great importance to find new methods with minimized use of functionals.

One method of choice is instuded resistance. BABA (DL-(r-amino butyric acid) is the most promising agent and has been shown to reduce potato late blight also under field conditions.





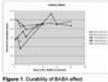


Figure 2. Dose-response of BASA treatmen

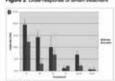


Figure 3 A. B. Effect of BABA in combination with fungicides

#### Aim

To investigate if there was any synergy effect between BABA and fungicides in greenhouse and in Swedish field experiments.

#### Greenhouse experiments

Detached leaf assay was carried out according to Eucablight protocol.

After treatment excised leaves were inoculated with P. Infestors. Lesion sizes were measured 6 days after inoculation.

Fig. 1. Durability of BABA effection ox. Birtle

Fig. 2. Dose-respons effect of BABA on cvs Birtle (sus.) and Ovatio (partial res.). Bar is SD of overall mean.

Fig. 3. Effects of BABA (B) alone and in combination with the fungicides Shrifan (\$) and Electis (B) two days after treatment. Recommended concentrations in field treatments are 0,1% and 0.45 % (v/v) for Shrifan and Electis, respectively.

- A. Effect of different combinations two days after treatment. In the figure the tesion sizes are plotted against the fungicide concentration, in the combined treatment BABA concentration was 0.08 (left point) and 0.02% (right point)(eVs).
- Concentrations in spray solutions were 0.05% for BABA (w/v). 0.02% for Shirlan (w/v) and 0.09% for Electis (v/v).

#### Results

- BABA reduced the lesion sizes with 40-50% up to 4 days after treatment. Leaf spray had a better effect than soil drench.
- A clear dose-response effect of BABA was found on both cultivars.
   BABA had a better effect on the partial resistant cv. Ovatio. GLM showed significant effect of dose (p<0.001), variety (p<0.001) and dose"variety interaction (p=0.02).</li>
- The effect of the combination of BABA and fungicide was better than BABA or fungicide alone.

#### **Field experiments**

Effects of fungicide treatment (Shirian) in different doses in combination with the induces BABA on the development of late blight infection. The fields were treated with Shirian andlor BABA once per week.

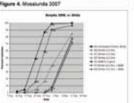
Fig. 5, 6, 7, Effect of BABA in combination with Shirlan in field. Different letters indicate sign. difference (p=0.05). Vertical comparisons, within date.

#### Results

- BABA alone had a weak but significant effect during the early progress of the disease.
- A reduced dose of Shirtan (75% of recommended) in combination with BABA had an equal or better effect on late blight than full dose Shirtan alone.

The project was supported by SLF and Formas. Field experiments were surried out by the Deedigh Farmer's association.





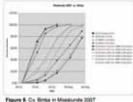


Figure 6. Cv Brite in Borgeby 2008

Figure 7. Cv. Superb in Mossiumda 2008



# Field trials with Cyazofamid and additives against *Phytophthora infestans* in Norway

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# Field trials with Cyazofamid and additives against Phytophthora infestans in Norway



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#### Summary

Ranman plus the adjuvant -Additive for Ranman- has shown good effect against potato late blight in the efficacy trials conducted in Norway in 2001-2003. The effect has been on the same level as other tested fungicides with no significant difference between fungicides. In case -Additive for Ranman-will not be approved in Norway, trials from 2008 have shown that the adjuvant Renol is a possible alternative.

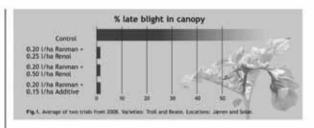
#### Introduction

Norway has its own rules for the approval of new pesticides. The rules are to some extent corresponding to the EU set of rules, but are also in some areas more restrictive, for instance when it comes to the approval of adjuvants. This is why there are only very few approved adjuvants for pesticides in agriculture in Norway, in 2008 Ramman was technically speaking approved for the control of potato late blight in Norway, but not the "Additive for Ramman" due to the special Norwegian rules. For that reason it became relevant to examine another adjuvant as an alternative. This poster presents the results of all official experiments with Ramman in Norway and the testing of the adjuvant Renol as an alternative adjuvant for Ramman.



Relations	% little	Mend		
On the Control	Carrety	tiben	<b>Magita</b>	
Control	86	5.0	401	
0.2 L/Nx Ranman + 0.15 L/Nx Addition	- 1	2.5	***	
0.3 Line Shirten	4	9.9	437	
9 ligitus Simeno	3	5.4	461	
1.35 kg/to Dectric	- 5	2.0	460	
1.8 kg/ha Clears	- 1	2.4	484	
LID!	17.8	48	.11	

40 11	5.0 5.0 2.0	138 396
"		100
	2.0	.196
1421		
38	2.4	411
+	8.5	405
29	2.0	425
	2.1	410
12.8	. 192	39.
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#### Materials and methods

in 2001-2003 the effect of Ranman plus -Additive for Ranman- was evaluated on contract for the Norwegian Food Safety Authority in field trials with a number of other fungicides in Norwegian trials and trials are trials and trials are trials and trials and trials are trials and trials are trials and trials and trials are trials and trials and trials are trials and trials and trials are trials. The spraying interval varied from 7-18 days depending on the weather conditions. Conventional spraying technique and 400 liter of water per ha has been used.

In the trials from 2008 the effect of Ranman was tested together with two different doserates of the adjuvant Renol and compared with Ranman plus -Additive for Ranman-. Fig. 1 shows the trial plan and chosen dose rates. Both trials have been conducted in farmers fields.



#### Results

Tables 1-3 show the results from the trials in 2001-2003. In all trials Ranman - -Additive for Ranman- has been able to control late blight on the same level as other tested fungicides with no significant difference.

Fig. 1 shows results from 2008 with Ranman together with either -Additive for Ranman- or Renot in two different dose rates. The first late blight infections came late in 2008 at both locations. Anyhow late blight developed epidemically during a few weeks with 100 % infection in untreated piots at Jamen at desiccation time.

At the other location untreated plots were desiccated at 50% late blight attack.
At both locations all fungicide treatments gave good control of late blight. There was no significant difference in control of late blight between

late bight between treatments with Ranman plus -Additive for Ranman- and Ranman plus Renoi. There was no significant difference in control of late blight between the two dole rates of Renoi.

Protetters	XMX	Tiend	
-	EMMINY.	taliers	Mg/br
Coverer	42	2.5	299
0.15 Uhu Ravnan - 0.11 Uhu Additve	- 3	1.2	400
6.75 l/ha Rawnen = 6.75 l/ha Additive	1	2.4	406
IL3 She Shirten	3	5.6	179
1.5 i /ke Tylee		3.7	395
I.O L/ha Tytpe	1	3.4	404
150	12	-	32

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### Curatieve and eradicant activity of Proxanil

JOHAN TESTERS AND JOHAN DESNOUCK

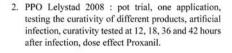
Belchim Crop Protection nv, Neringstraat 15, 1840 Londerzeel, Belgium info@belchim.com

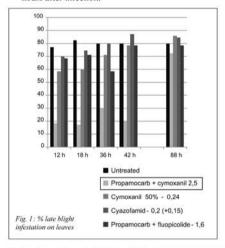
#### Curative and eradicant activity of Proxanil

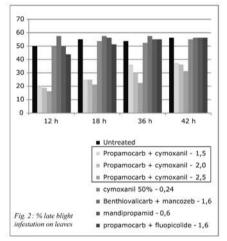
Jos Testers and Johan Desnouck, Belchim Crop Protection nv, Neringstraat 15, 1840 Londerzeel, Belgium, info@belchim.com

Proxanil is a new fungicide for the control of Potato Late Blight (*Phytophthora infestans*) and has been introduced in 2008. Proxanil is an SC formulation containing 400 g/l of propamocarb and 50 g/l of cymoxanil. The recommended dose rate is 2 to 2.5 l/ha.

 PPO Lelystad 2008: pot trial, one application, testing the curativity of different products, artificial infection, curativity tested at 12, 18, 36 and 42 hours after infection. The eradicant activity was tested 88 hours after infection.







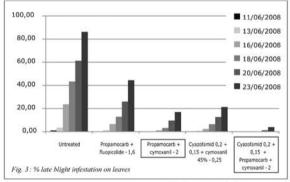
 Staphyt (France) 2008: Field trial, two applications followed weekly by fluazinam, testing the curativity and eradicant activity of different products, artificial infection (5/06), curativity tested at 48 hours after infection.

#### Conclusions

<u>Curative activity</u>: In comparison with cymoxanil alone (a compound known to have good curativity), the curative activity of the formulation cymoxanil + propamocarb is stronger, even though the limited curativity of propamocarb. This shows a

synergy of the formulation propamocarb and cymoxanil. Proxanil has the best curative activity in the presented trials.

<u>Eradicant activity:</u> None of the tested products has satisfying eradicant activity. Proxanil shows the best stop effect but it is not strong enough to stop the visual blight spots immediately. The combination with eyazofamid shows a good result. It demonstrates the importance of a good preventive product in the schedule to stop visual late blight.



Definitions curative and eradicant

From: Ninth Workshop of a European Network for Development of an Integrated Control Strategy of Potato Late Blight Tallinn, Estonia, 19-23 Oct 2005 Report of the funcicide sub-group.

Curative activity - the fungicide is active against P. infestans during the immediate post infection period but before symptoms become visible, i.e. during the latent period.

Evadicant activity (Anti-sporulant activity) - P.
infestans lesions are affected by the fungicide
by decreasing sporangiophore formation and/or
decreasing the viability of the sporangia formed.



Jos Testers and Johan Desnouck, Beichim Crop Protection nv. Neringstraat 15, 1840 Londerzeel, Beigium, info@beichim.com

Proxanil is a new fungicide for the control of Potato Late Blight (Phytophthora infestans) and has been introduced in 2008. Proxanil is an SC formulation containing 400 g/l of propamocarb and 50 g/l of cymoxanil. The recommended dose rate is 2 to 2,5 l/ha.

#### Trial results

1. PPO Lelystad 2008 : pot trial. one application, testing the curativity of different products, artificial infection, curativity tested at 12, 18, 36 and 42 hours after infection. The eradicant activity was tested 88 hours after infection.

Conclusions Curative activity: In comparison with cymoxanil alone (a compound known to have good curativity), the

curative activity of the formulation cymoxanil + propamocarb is stronger, even though the limited curativity of propamocarb. This shows a synergy of the formulation propamocarb and cymoxanil. Proxanil has the best curative activity in the presented trials.

Fradicant activity:

blight.

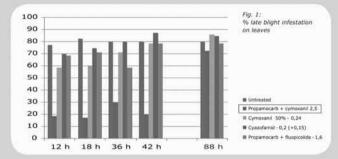
None of the tested products has satisfying eradicant activity. Proxanil shows the best stop effect but it is not strong enough to stop the visual blight spots immediately. The combination with cyazofamid shows a good result. It demonstrates the importance of a good preventive product in the schedule to stop visual late



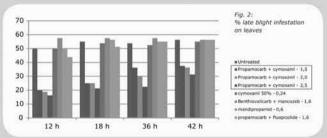
#### Definitions curative and eradicant

From: North Workshop of a European Network for Development of an Integrated Control Strategy of Potato Late Blight Tallinn, Estonia, 19-23 Oct 2005 Report of the fungicide sub-group.

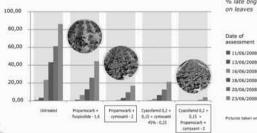
- Curative activity the fungicide is active against P. infestans during the immediate post infection period but before symptoms become visible, i.e. during the latent period.
- Eradicant activity (Anti-sporulant activity)
   P. Infestans lesions are affected by the fungicide by decreasing sporangiophore formation and/or decreasing the viability of



2. PPO Lelystad 2008 : pot trial, one application, testing the curativity of different products, artificial infection, curativity tested at 12, 18, 36 and 42 hours after infection, dose effect Proxanil.



3. Staphyt (France) 2008: Field trial, two applications followed weekly by fluazinam, testing the curativity and eradicant activity of different products, artificial infection (5/06), curativity tested at 48 hours after infection.



% late blight infestation

Pictures taken on 23/06/08

BELCHIM

PROXANIL®, product of Agriphar S.A., contains 50 g/l cy

### Can U.K. populations of P. infestans mate?

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#### Can U.K. populations of P. infestans mate?

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D.E.L. Cooke, SCRI, Invergowrie, Dundee, DD2 5DA, U.K.



#### Abstract:

Crops of potato were grown inside 3 polythene tunnels and infected with different pairs of A1 and A2 isolates of P. infestans. A fourth tunnel was inoculated with all 6 isolates. Oospores were formed within the foliage and crop debris was allowed to rot into the soil. New seed was planted in the same tunnels after four months. Lower stem and leaf lesions were observed soon after emergence. Both mating types were detected in all tunnels. Lesion samples were fingerprinted with SSR markers. Parental genotypes were not present and the evidence suggests that parents had recombined to yield new hybrid genotypes. Many of the "progeny" isolates showed tri-allele genotypes, typical of single-oospore progeny of P. infestans.

Key words: cospores; recombination; microsatellite markers

#### Interpretation and conclusions:

The genotypes detected in all four tunnels were non-perental. While there was evidence of recombination of parental alleles at many loci, evidence of non-Mendelian inheritance was also found. New alleles at several loci not detected in parental isolates may be artifacts or may be generated during sexual recombination. Tri-alletic isolates could have been trisomic or even triploid but mintures of genotypes cannot at present be ruled out. Single-cospore progeny with three affeles at one or more loci are known from in-vitro genetic analyses (Carter et at., 1999). Three more cropping cycles are planned to follow the evolution of genotypes in each tunnet.

#### Introduction

Due to the recent increase in frequency of the A2 mating type in U.K. both mating types are detected in some commercial fields. We need to find out if sexual mating and recombination is taking place and if new, fit genetypes are generated. We have selected common strains from the 2006 population and generated. Yet have selected common straints your the 2000 population and incoulated comps with compatible pairs of these within plastic greenhouses (tunests). Our plan is to continue to plant new cops in the tunnels each Spring and Autumn and to sample from the resulting lefected plants to find out if the genes detected in the parental strains are recombled in the new bight

#### Methods and Material:

Four turnels, 20m x 6.5m (see below), were each planted with aternating beds of cv. Maris Piper and cv. Bistje in March, 2007. Compatible strains of P. infestins were inoculated on 24 April. Common A2 strain Blue 13 was used in Tunnel 1. The corp was harvested in July and was destroyed. Fresh seed was planted on 7 October and blight was first detected and sampled on 31 Cetabor. Speak Jesins painted with the Cetabor. Speak Jesins painted was first detected and sampled on 31 Cetabor. Speak Jesins painted, work 10 Maris 200 October. Single-lesion samples were transferred to FTA cards (Whatman) prior to determining SSR genotype (Cooke et al.)



#### Acknowledgements:

We wish to thank the British Potato Council for funding and staff at Hentaes Research Centre and SCRI for their assistance

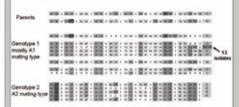
Microsatellite (SSR) genotypes of parents used to inoculate the tunnels are shown below. Alleles at 11 loci were assessed.

	_		-	**  #	-	-		-	-	-	-	**	-	**		-	*	mi	-			-	-	-	-	-
Farmer 6	15	200	1	765	1	=:	5	Ξ	п	I		π	ï	78	:	Ħ	ä	ŝ	4	#	200	1		=	84	۳
Turne )	3	22	1	:10	1	= 1	8:	8	4	1		ø	:	:8	:	ä	ä	:	ä	4	40	1	4	Ξ	100	:
Speed 3	100	-	Ė	-10	±	23		*	¥	±	Ħ	ш	ż	:01	:	Ħ	=	ż	2	=	200	Œ.	뇀	×	100	*

Blight occurred spontaneously on the second crop planted in each tunnel in November 2007.



Single-lesion isolates from each primary lesion were fingerprinted. Genotypes found in Tunnel 1 are shown below. Although two basic genotypes were found, variation was detected at several loci.



Similarly, two basic genotypes, one A1 and the other A2, were found in Tunnel 2. In Tunnel 3, one basic genotype was found and there was evidence of the presence of unique alleles from parents of Tunnel 1. The fourth tunnel had been infected with all 6 parental isolates. The new crop there became infected more slowly than those in the other three tunnels. Unique alleles from parents of genotypes 1\_A1, 13\_A2 (Blue 13) and 8\_2aA1 were detected.

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### Risk estimation to predict tuber blight

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## Risk estimation to predict tuber blight

#### P.J. van Bekkum<sup>1</sup>, A. Evenhuis<sup>1,2</sup> & G.J.T. Kessel<sup>1</sup>

#### Introduction

Tuber infections can result in high yield losses at harvest and / or during storage, infected bubers also form a source of inoculum for following cropping seasons. Therefore, fungicides are applied intensively to prevent leaf and subsequently siber blight.

To prevent tuber infection and to minimize the fungicide input, prediction of tuber blight infection risks can help to identify critical periods for tuber infection so that preventive measures can be adapted to specifically negate this risk.

The following (key) factors are important:

- · Tubers must be present
- Inoculum must be present in the foliage or the soil.
- Weather / soil conditions must be conducive for infection



Fotato crop severely infected with Phytophthora infections,

#### Risk estimation

A risk estimation can be made based on the relative conduciveness of the (combined) key factors for tuber infection (Figure 1).

A more detailed risk estimation could also include additional risk factors such as soil conditions (conducive for tuber infection), soil type, compaction of top soil layer and "humeling" furflux of sportangia into the ridge) and therefore tuber bight levels, infection risk is also influenced by the level of bight resistance of the cultivar and the virulence of the pathogen population.



Phytigalithore spores which can be washed aff to the soil and subsequently infect tubers.



intected tubers resulting from a period with high inoculum pressure and heavy rainfall

- weather		Tubers <sup>3</sup>			
		141	+	++	
Inoculum <sup>2</sup>		11-	-	100	
	+		123	133	
	++	-	10.0		

+ weather		Tubers					
		133	1040	++			
Inoculum			100	100			
	+	201	10/-	100			
	++	22		-			

++ weather		Tubers		
			+	++
Inoculum		lite.	100	192
	+			+
	++	lle.	++	++

No or very low risk	
Low - medium risk	
Medium - very high risk	

Figure 1. Whather, incoulum and presence of labors are key factors to estimate tubor adection risks. When all three factors are favourable tuber infection risks will be high.

- | Weather; --- No rain; + = >0 4 mm rain; ++ = > 4 mm rain
- <sup>3</sup> Installant No Dilght in cross: \* sparradic feature in the crops: \*\* frequent lesions in the crop
- \* Tubers: tuber initialization or earlier; + = early tuber filling; ++ = late tuber filling

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