EuroBlight

A potato late blight network for Europe

BRASOV - ROMANIA 13-15 MAY 2015

PPO SPECIAL REPORT NO. 17

Proceedings of the fifteenth EuroBlight Workshop

WAGENINGEN UR
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The James Hutton Institute

AARHUS UNIVERSITY

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Preface

EuroBlight Workshop Brasov, Romania 10-13 May 2015

A European network of scientists and other specialists working on potato early and late blight meet every 2nd year. 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 15th Workshop was hosted by the National Institute for Research and Development for Potato and Sugar Beet. The Workshop was attended by 103 persons from 22 countries, including Russia, Chile, Argentina, China, Israel and USA. Representatives from all countries presented recent research results regarding integrated control, decision support systems, resistance of varieties, late blight in organic potatoes and population biology of the late blight pathogen in potatoes. Since early blight is an increasing problem in Europe reports on this disease are also included.

The papers and posters presented at the Workshop and discussions in the subgroups are published in these Proceedings, PPO-Special Report no. 17. The current and previous Proceedings are also available on the EuroBlight website www.EuroBlight.net.

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PPO – SPECIAL REPORT NO 17 – 2015, 11-30

Epidemics and control of early & late blight, 2013 & 2014 in Europe

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INTRODUCTION

The EuroBlight late blight country profile was launched in 2007 to keep track of the development of late blight and its control in Europe in individual countries and over years. This paper reports the development and control of late blight in Europe, 2013 and 2014.

One important motivation for sharing data is that the single results in this way can be analysed in a pan-European context. When data are available over several years it will be possible to analyse the data over years and across countries. This is especially interesting now that all countries in Europe have to adapt to the new EU pesticide package implemented by 2014. Using the data we collect before and after 2014 might be used for impact assessment of this EU regulation. We will also use the data to stimulate to collaboration, harmonisation and coordination between institutions and different stakeholder groups.

At the workshop in Brasov special attention was drawn to the collaboration between global networks, and colleagues from North-America, South-America and Asia were invited to present their results and to participate in discussions how collaboration on a global scale can be strengthened. The parties' ultimate aim is to gain new knowledge about populations of *P. infestans,* how these populations evolve, how local strains are spread from one continent to another and how we most effectively can control *Phytophthora infestans* on the field level. The European monitoring initiative, which has been operating for two years now, has already given the parties a better understanding of the strains of *P. infestans* that are active in Europe. This information enables a more targeted use of crop protectors and helps growers to choose potato species with the right levels of resistance. A second area of concern in the workshop was the increasing problems with fungicide resistance related to the control of late blight and early blight. One answer from the EuroBlight consortium to this question was the recommendation of implementing IPM 2.0 – as expressed in the official statement from the workshop. This paper

reports the development and control of late blight in Europe, 2013 and 2014 and thereby describe the foundation for the further insight in the structure and behaviour of the European *P. infestans* (meta) population.

METHODS

A questionnaire about late blight and early blight development and control was answered by the EuroBlight country editors. The detailed questions can be found in previous proceedings. A new section will deal especially with Alternaria reflecting the initiation of the EuroBlight Alternaria group, headed by Hans Hausladen, TUM, Germany.

The reports per country published below are the abstracts of the country reports only slightly edited. The abstracts of the country reports are sorted according to regions in Europe. General trends and observations on disease development, fungicide use etc. are discussed in the section of summary information. 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 2013 and 2014 is shown for all European countries on maps in Figures 1-2. The same data are combined into marker plots per year in Figure 3.

Estonia

2013: The first late blight outbreaks were recorded during the first days of July in allotment gardens. Dry and hot weather in July kept disease at low levels and major late blight epidemics developed later, in the second half of August. The growers started fungicide applications in late June.

2014: After a cold and rainy June late blight was detected in early July in early and main crop. After a dry July the disease progressed faster in August after heavy rains at the end of July. The growers started fungicide applications in last days of June. Systemic fungicides (Ridomil Gold, Tattoo) were used in first sprays to protect new leaves.

Four to six spays have been used in both years, the most commonly used active ingredients have been mancozeb, fluazinam and mandipropamid. One-two treatments with cyazofamid were used to protect tubers against blight. The potato growers´ cooperative Talukartul - in cooperation with the Estonian Crop Research Institute - used the Negative prognosis and Fry models provided by fieldclimate.com for timing of fungicide application. Together they run a network of 13 automatic weather stations located in major potato growing areas.

Latvia

2013: The crop emerged in the last week of May. June was warm, hot and dry, and the first observations of blight were on the 25th of June. The first protective application of fungicide (systemic or translaminar + contact) was made just before the infection period. After a dry July the epidemic development happened in untreated fields after 9 August. The control of LB was good to moderate in 2013 in Latvia.

2014: Spring of 2014 was early. April was warm and dry. Beginning of May was cold and rainy, but during the second half of May the air temperature reached 29°C. The first warning of *Phytophthora infestans* in 2014 was received earlier than in previous years – 12 June. July was hot and dry and heavy attacks were only found after 11 August. Weather conditions were

favourable for the development of tuber blight. During the last years *Colletotrichum coccodes* has become a major problem for potato growing – more important than late blight.

Lithuania

2013: The potato crop was planted late and the crop emerged in early June. A dry June prevented the establishment of late blight until mid-July in allotment gardens and in commercial fields one week later. Farmers started routine fungicide applications since the beginning to the middle of July. In total, 4 to 6 fungicide applications were enough to avoid late blight in the crop. Also, shorter (6-7 days) intervals between fungicide applications were more favourable for late blight control compared with longer intervals (from 10 to 14 days) in 2014.

2014: The spring of 2014 was dry and warm and crop development was very fast until the middle of June when average air temperature started to decrease. The first late blight symptoms were found late June in small-scale fields, but only later in mid-July in commercial fields. High temperatures in July and in the first part of August prevented disease epidemics but due to very dry and warm conditions the foliage started senescing relatively early. Late blight continued to spread in the potato crop but overall had little influence on final tuber yield. The potato crop was sprayed 4 to 6 times.

Russia

2013: A severe late blight development was observed on potato fields of the Kaliningrad, Leningrad, Vologda, Moscow, Bryansk, Novgorod, and Pskov regions. A moderate disease development was registered in the Orel, Tambov, Voronezh, Nizhni Novgorod, Kirov, and Arkhangelsk regions. The development of the late blight infection on the other territories of the European part of Russia was rather weak. Infected seed tubers represented the main source of the primary infection. The most popular fungicides were Abiga-Pic, Shirlan, Tanos, Acrobat MZ, Infinito, and Ridomil Gold MZ. The total number of treatments varied from 2 to 10. Owners of allotment gardens did not use any fungicides. The use of DSSes (Plant-Plus, VNIIFBlight) was rather rare. The most popular potato cultivars were: Rosara (31.7%), Red Scarlett (18.2%), Nevsky (13.1%), and Udacha. The volume of foreign and domestic cultivars used was 50 and 40%, respectively; the rest of the seed potato is unknown.

2014: A severe late blight development was observed on potato fields of the Republic of Bashkortostan and Kaliningrad, Leningrad, Pskov, and Novgorod regions. A moderate disease development was registered in the Smolensk, Moscow, Kirov, and Arkhangelsk regions. The development of the late blight infection on the other territories of the European part of Russia was rather weak. The main sources of the primary infection were infected seed tubers and, in some cases, allotment gardens with soil contaminated with oospores. The most popular fungicides were Abiga-Pic, Shirlan, Tanos, Acrobat MZ, and Infinito. The total number of treatments varied from 2 to 11. Owners of allotment gardens did not use any fungicides. The use of DSSes (Plant-Plus, VNIIFBlight) was rather rare.

Poland

2013: After a dry and warm spring the first outbreaks were reported on 7 June in the Łódzkie region and on 8 June in the Dolnośląskie region. After a week attacks were found widespread in commercial fields in central and western-south regions. The weather conditions in June- August was unfavourable for late blight. In the northern and eastern regions of the Poland the first outbreaks of blight were noted on 19 June – widespread attacks only after 15 July. Generally, in

the season 2013, weather conditions did not favor the late blight development. The farmers applied 1-6 sprays in 2013, most often 1-2. The active ingredients used on the largest areas were fluopicolide/propamocarb-hydrochloride and fenamidone/propamocarb-hydrochloride, metalaxyl-M+mzb, mandipropamid and cymoxanil +mzb. Level of tuber infection was on average 1,3% for tested fields. The first symptoms of early blight caused by *Alternaria* were observed on 5th June in the central region of Poland (Łódzkie region). During all growing season, up to harvest the disease caused some problems on potato fields, particularly on the cultivar Markies. *Alternaria* spp was recorded in 89,3% of observed crops in Poland.

2014: During a survey on 160 potato farms (131.8 hectares), late blight was observed on 95.6% of fields inspected. Stem blight was noticed only on 34.5 % and early blight on 82.7% of the inspected fields. The date of crop emergence was relatively early, 10-20 May, despite the fact that April and May were dry. Two periods of early outbreaks of late blight were observed during the growing season across the country. The first one occurred unusually early in the Dolnośląskie and Lodzkie regions, on the 19 May. In other provinces first infections emerged in June, mostly between the 11th and 20th June. In general, farmers applied 2-3 sprays on a ware potato due to drought and low infection pressure of the pathogen. The active ingredients used on the largest areas were: propamocarb-HCl and its mixtures, phenylamide (metalaxyl), cymoxanil, and a contact product – chlorothalonil. The first and the only, very early outbreak of the early blight was observed on the 21 May in the western part of Poland, in Podlaskie region. Generally, the disease appeared in the second half of June (late compared to normal) due to long drought and very limited rainfall. EB was recorded in 82,7% of the inspected fields but the infection pressure was very low on the majority of them. The final severity of early blight in unprotected plots reached 15-20%.

Czech Republic

2013: Infection pressure for the whole season is considered as medium compared to normal. LB was found in the crops in the beginning July. After a very dry July the epidemic spread of the disease was recorded in late August and in September. Re-grown parts of plants were particularly susceptible. The late onset of the disease did not cause important yield reduction, but tubers of susceptible varieties were severely infected in fields not desiccated timely. Therefore, problems with tuber blight also arose in the storage.

2014: Late blight was found early and with severe attacks in early potatoes due to widespread and high amount of rain in May. June was dry with unfavourable conditions for late blight development and the first occurrences of late blight in the normal potato production region were only focally found towards the end of June and in highly susceptible varieties in the first decade of July. Favourable conditions for late blight and its epidemic spreading were from the second decade of August and continued during September, when highly above-normal precipitation was recorded. Simultaneously, in the second decade of August extreme and very rapid development of early blight (*Alternaria solani, Alternaria alternata*) was recorded, which has not been previously found to such a great extent. Early blight dominated in the crop and overlapped signs of late blight. In many crops early blight highly limited or completely ended growth, and yields and starch contents were reduced.

Switzerland

2013: Due to rain and low temperatures in April and May many potatoes could only be planted very late, i.e. end of May. First late blight attack was registered on 21 May in South Germany in

1 km distance to the Swiss border. One week later, seven additional late blight attacks were registered in different major potato growing regions. In June, weather conditions were favourable for late blight and the epidemic spread easily. Over the entire season 2013, late blight played a rather secondary role as potatoes suffered more from the wet and cold start of the season, the dry period in July, early blight and aphids. Due to the weather conditions, yields were not according to the expectations.

2014: Most of the potatoes were planted during the first week of April. First late blight attack was observed early, i.e. first part of May in covered potato and gardens. In the central and eastern part of Switzerland, late blight pressure was higher than in the other regions. From July until mid of August, weather conditions were very favourable for late blight and several periods with up to 6 continuous days with MISPs were registered for all weather stations. Hence, late blight could spread very fast in all potato growing regions and the epidemic pressure was high. Due to wet soil conditions, it was very difficult to apply fungicide treatments in time. Harvest conditions were also difficult, as weather conditions stayed unstable.

Finland

2013: First blight attacks were recorded during the first week of July in unprotected potatoes. These outbreaks were probably launched by heavy rain showers at the end of June. The weather during July was mostly very dry and warm and the few blight outbreaks did not develop into epidemics. In unprotected potatoes blight progress was very rapid during the first weeks of August. In protected crops very little blight was present in the season 2013 and no tuber blight problems were reported. Typically four to six fungicide applications were done for blight control.

2014: The season 2014 was mostly very unfavourable for late blight development due to dry and relatively cool weather type. First late blight symptoms were detected during the latter half of July, which is exceptionally late compared to years during the past two decades. No severe blight outbreaks were reported in protected crops in 2014 and tuber blight was not a problem. Typically four to six fungicide applications were done for blight control. An increasing number of farmers started to add azoxystrobin in tank mixture with late blight fungicides to improve early blight control.

Norway

2013: There were a few days with high blight risk according to the Nærstad model on VIPS in the end of May and some in June in the south east part of Norway, which is the region with most potatoes. Late blight was wide-spread by the beginning of July. A dry period in July reduced the disease development, and the disease was kept under control in sprayed fields. In average the potato area was treated 7.8 times with late blight fungicides.

2014: In 2014 the blight risk was low during the whole season. According to the Nærstad model on VIPS there were only one day with moderate risk in May, two days in June, one day in July, five in August and four in September. Blight was not wide spread before August, and most potato lots had less tuber blight than normal.

Sweden

2013: The spring was cold and dry, but planting was done at normal time. In south Sweden, early attacks of late blight was observed in some places. Hot and dry weather during the summer gave a low risk of late blight outbreaks and many farmers prolonged their spraying

intervals. However, blight was found in some fields in August, and locally there were problems with tuber blight. Most organic potato crops could grow to full maturity with very little blight.

2014: A warm and dry spring resulted in early planting in South and Mid Sweden. In the South, the combination of the early planting and very blight conducive weather gave problems with early and heavy attacks of blight – often with stem lesions low in the crop. Some fields in the Southeast were desiccated in mid-June because it was impossible to control the blight despite very heavy use of fungicides. It was suspected that oospores could be the origin of the attacks, and analyses of samples taken in one field supports this. Already during June widespread attacks were reported from South Sweden but short spray intervals in combination with a change to hot and dry weather got the situation under control.

Denmark

2013: April was cold but dry and planting was normal, 10-20 April. The crop emerged at normal time for Danish conditions, 15-20 May. Blight was found first time 19 June and after a week in several conventional fields. There were no clear indications of attacks from oospores and all recordings of blight were after BBCH 31. In general, the growing season was medium blight favourable and farmers had no big problems regarding control. No blight in Fungicide and DSS field trials in three regions of the country.

2014: After a mild winter potato plantings started already in late March and most potato fields were planted 2-3 weeks earlier than normal. Crop emergence was relatively early, 5-15 May. During crop emergence a combination of heavy rain and high infection pressure resulted in the highest amount ever recorded of fields with attacks from oospores - for Central-South of Jutland in fields with narrow crop rotation. A week after blight was observed (25 May), several conventional fields were recorded with infections in the same region – at growth stages below BBCH 30. Due to intensive fungicide use and a shift in the weather to more unfavourable blight weather, most attacks were stopped. July turned out to be the second warmest in more than 100 years. The mean temperature was 19,5 °C and this is 3,9 °C above the normal temperature. Late blight was kept under control in August and September and yields were generally at medium level.

France

2013: Potato planting was only possible from the end of April and it lasted until May. A very exceptional cold season followed after planting in May, and crop development was delayed for 2 or 3 weeks. The overall late blight pressure started late in the season and remained average to low in all parts of the country and during the complete growing season. In Brittany, the far western part of the country, late blight pressure was constant for early potato production but manageable; for more continental main crop production, the weather conditions were not conducive to late blight outbreaks.

2014: Planting was possible early April in most Northern areas and weather was conducive for establishing the first infections as early as end of March. For the rest of the season and for all areas located in the North-eastern part of the country, mild temperatures and continuous rain falls was very favourable to high yield but as well very conducive to late blight pressure on all potato crops until harvest. In Brittany, covered crops had severe early late blight outbreaks just as early crops in open fields.

Belgium

2013: After a 'normal' winter with enough frost, and an exceptional cold spring season, disease pressure at the start of the potato season was low. Although prolonged wetness in a cold month of May caused some infection risk, the first sprayings could be carried out in good circumstances. From the beginning of June, and during the summer months July and August - remarkably sunny and with little rainy days - the weather was unfavourable for late blight most of the time. Nonetheless, few days with infection risk in August resulted in disease developments in untreated crops and in fields where the timing of sprayings was poor. An average of 14 applications (interval 8.7 days) were used in a susceptible ware crop variety (Bintje).

2014: The winter had no frost or winter days at all and the following spring season was very sunny, warm and dry, leading to an early planting of the potato crops. At the end of March, late blight was already detected on a dump pile; by the end of April, inoculum sources with diseased plants were widespread. It was only a matter of time - and weather - for an epidemic to start in the susceptible, fast growing young crops. Two infectious periods - 27 & 28 May and 4 & 5 June - did the job, with an explosion of late blight attacks in most fields as a result. The severity ranged from sporadic lesions to half of the crop affected, with a high level of stem blight. Some dry, sunny weeks in June, together with short spraying intervals, stopped further expansion of the disease. Nevertheless, late blight control was a problem during the rest of the season. All in all, 18 sprayings (average interval 6.4 days) were used in a susceptible ware crop variety to control the disease.

The Netherlands

2013: A cold spring resulted in a delayed crop emergence. May and June were cold so potato crops developed slowly. Only a few early outbreaks were reported from the starch potato area (North-east past of the Netherlands). The first late blight infestation in a field was on 2 June and Oospores were said to be the source of this attack. The summer months were rather dry and sunny without serious blight problems.

2014: The winter without frost was followed by an early and warm spring and the potatoes were planted in time. Due to the warm conditions in April crop emergence was early and the crop development in May was very fast. After early reports of blight on dumps in April, the first infections in fields were found in the third week of May, initially in the eastern part of the country, later-on all over in the potato growing areas. Thanks to a long period of stable dry and sunny weather in June, growers were able to get the disease under control. Fungicides as Infinito, Revus, Proxanil also proved their skills under these conditions. After this severe and difficult start of the crop protection season no big problems were encountered later on.

Germany

2013: Crops were planted in good conditions but the crop emergence was late 15 May to 5 June. The first outbreak of late blight in potatoes was recorded by end of May in plastic covered potatoes. Attacks in different regions and ware potatoes were found in June. The weather conditions for the development of late blight was moderate in the North and low in the South. The number of fungicide treatments was lower than normal. All kind of products were used. Attacks of early blight (*Alternaria solani*) seem to be an increasing problem in most of the German potato growing areas.

2014: The weather condition and the disease development were diverse across the country in 2014. The Northern and Western part of Germany had an early and severe late blight epidemic. In the Eastern and Southern part a normal late blight development was observed. The crop emergence was normal (5 -20 May). The first outbreak of late blight was recorded early May (very early) in plastic covered potatoes. Attacks in conventional fields were found late May to early June. The further development of late blight was completely different. In the Northern parts 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 high in 2014. All kind of products were used; especially mixtures were used in the Northern part of Germany.

Scotland

2013: Late blight developed unusually late in the growing season. The first crop outbreak was in Tayside on 12 August. The number of outbreaks was low, only ten confirmed outbreaks reported on the Potato Council-funded blight outbreak maps for Scotland. The progression of crop outbreaks (7 in number) in Scotland was 0 in May, 0 in June, 0 in July, 6 in August and 1 in September There were no confirmed outbreaks on outgrade piles of potatoes but there were three outbreaks on volunteers (8 August, 4 and 14 September).

2014: This year was in sharp contrast to 2013. There were many outbreaks of late blight in Scotland. Unusually the first crop outbreak was on the 30 May in South Ayrshire. Ninety-nine confirmed outbreaks were reported until the 16th of September when the last sample was submitted. The progression of crop outbreaks (97 in number) in Scotland was 1.0% in May, 19.6% in June, 57.7% in July, 20.6% in August and 1.0% in September There was one confirmed outbreak on an outgrade pile of potatoes (24 June) and one outbreak on volunteers (28 July).

England & Wales

2013: Planting was delayed due to low temperatures in early April. Rainfall was close to average during the season in most areas, although eastern regions received below average rainfall in June and September. Higher than average temperatures in July meant that irrigation was a priority for many crops nationally. Most late blight outbreaks were reported in August and September in England and Wales. Fifty-three outbreaks of late blight were reported in 2013 as part of the Potato Council funded outbreak maps in England and Wales, with the earliest report from a volunteer on 6 July in Wales, nearly two months later than the first report of 2012. No outbreaks were reported in May or June, 7 in July, 21 in August and 25 in September.

2014: Planting progress was good in 2014, with temperatures above average from March to June. Favourable conditions for late blight development were reported in early May in the South West, however, favourable weather was reported nationally from late May onwards. Most late blight outbreaks were reported in June and July in England and Wales. One hundred and sixty-seven outbreaks of late blight were reported in 2014 as part of the Potato Council funded outbreak maps, with the earliest report from a discard pile on 9 May in Norfolk, nearly two months earlier than the first report of 2013. Twelve outbreaks were reported in May, 84 in June, 48 in July, 24 in August and 9 in September.

Northern Ireland

2013: Dry weather early in the season (late May-June) prevented primary infection development and the first field outbreak of late blight was not reported until 17 July (the latest 1st report since 1981 apart from 2010 which was 2 days later). Although weather suitable for the spread of blight occurred in July and August, there was little inoculum to spread and few outbreaks were reported. Blight was well controlled in commercial crops with growers as usual making use of a wide range of active ingredients.

2014: This year the weather was more conducive to late blight with mild night temperatures and more rainfall early in the season. The first field outbreak was reported relatively early on 9 June and subsequently blight was reported in all potato-growing areas. Blight was controlled well in most crops. A few cases of more severe foliar infection were reported, but there were few reports of tuber blight. Growers used a wide range of active ingredients.

Ireland

2013: The weather conditions during 2013 did not favour late blight development. Most potato crops were planted in good conditions, however as temperatures were below average crop emergence and development was slightly delayed. During the summer months (June, July and August) temperatures were above long term averages while rainfall was below. This prevented epidemics of late blight from getting established. When conditions did favour late blight as conditions prior to these generally favoured application of fungicides no major outbreaks of late blight were reported. In response to the weather conditions hampering late blight development the applications of fungicides were altered to reflect this. This included number of applications and choice of products. The good conditions ensured low levels of tuber blight.

2014: The weather conditions during 2014 were generally more favourable to late blight development than 2013. Both the rainfall and temperatures in spring were above average and this resulted in early outbreaks of late blight being reported. From mid-June through to early August dry and warm conditions dominated which slowed the progress of any epidemics which had developed. In early August extremely heavy rain was recorded throughout Ireland which initiated further epidemics. In most commercial crops the application of fungicides protected crops, however where timings or choice of product were poor late blight did develop. The frequency and choice of fungicide product changed throughout the season reflecting the variability of weather conditions. Although weather conditions during August favoured late blight development low levels of tuber blight were reported.

EARLY ATTACKS OF LATE BLIGHT

In North-West Europe, early attacks of late blight are often found on dump piles or in potatoes covered with plastic. In 2013 dry and cold weather in May and early June prevented primary infection development from those inoculum sources and the first outbreaks of late blight in conventional fields were only reported by Mid-June in the Netherlands and Denmark, in July in France, Belgium Norway, Sweden, Finland or even late July or August in the UK and Ireland. In the Baltic countries and the European part of Russia LB was recorded in conventional fields in mid to late July. In conclusion, the year 2013 was not a blight year in Europe, early attacks from oospores were reported for Estonia, Sweden, Lithuania and the Netherlands.

The 2014 season was in sharp contrast to 2013. Mild winter, good conditions for early planting resulted in early crop emergence, and very early attacks (compared to normal) were recorded in England, Wales, Netherlands, Denmark and Sweden. In Denmark and Sweden early epidemics were driven by oospores as inoculum source. Oospores were also recorded in the reports from Estonia, Lithuania and Poland (Fig 5).

In 2014 attacks were extremely late in Norway and Finland compared to the other two Nordic countries and compared to previous years (Fig. 2).

Comparing the date when attacks were recorded in 5 or more conventional fields for 2013 and 2014, in 16 out of 20 countries attacks were earlier in 2014 than in 2013 (Fig. 3). This difference was 1½ -2 months in the UK and some regions in France. Only in Norway, Finland, Poland and Czech Republic late blight was found later in 2014 than in 2013 in conventional fields.

In the Baltic countries first recordings of blight was 10-20 days earlier in 2014 than in 2013 – early July compared to late July the previous year.

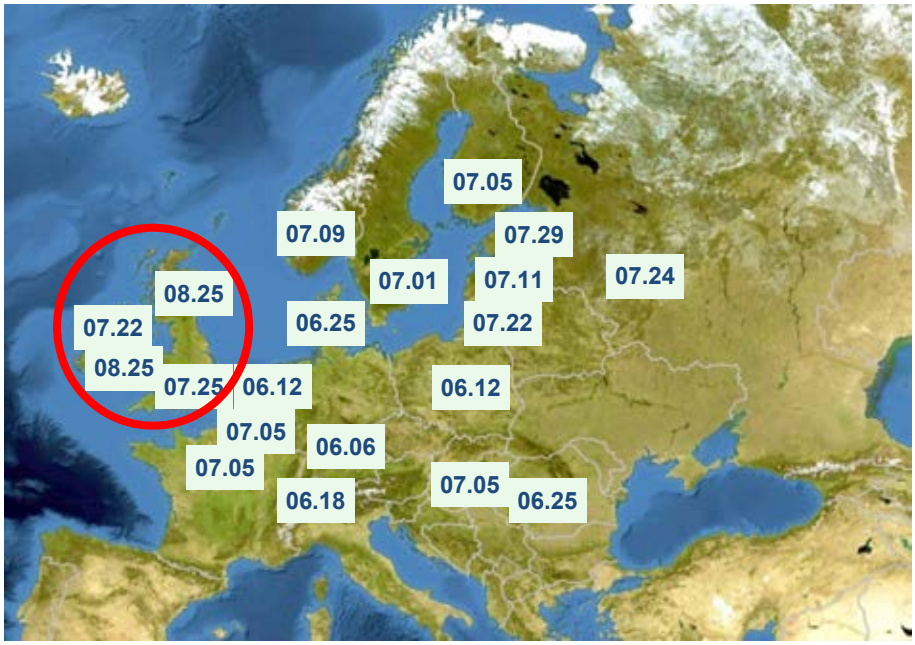

Figure 1. *Date of first observation of late blight in more than 5 conventional, normally planted potato fields, 2013*

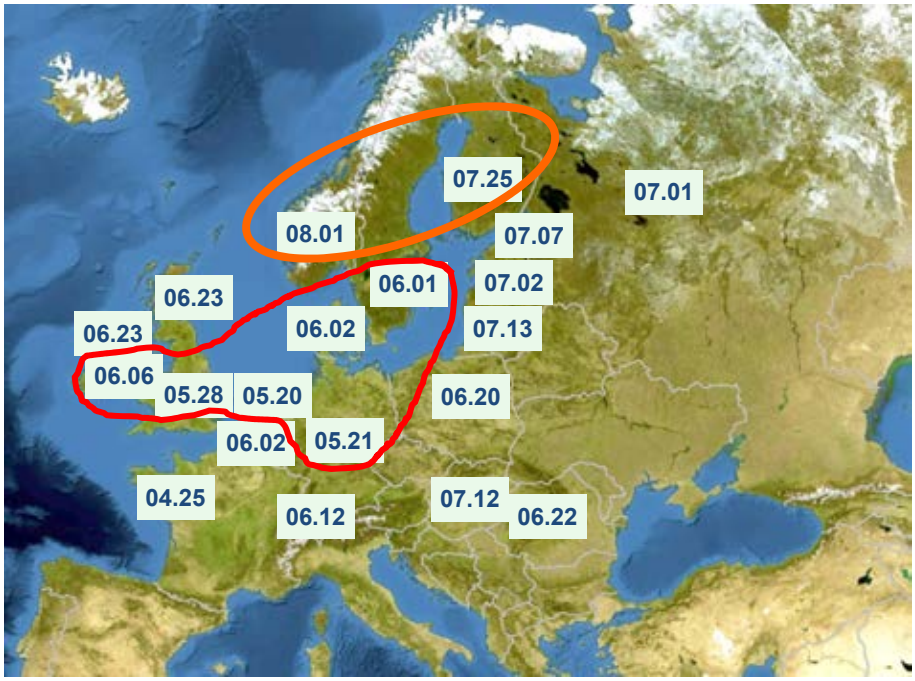

Figure 2. *Date when first infections were reported in more than 5 conventional, normally planted potato fields in 2014*

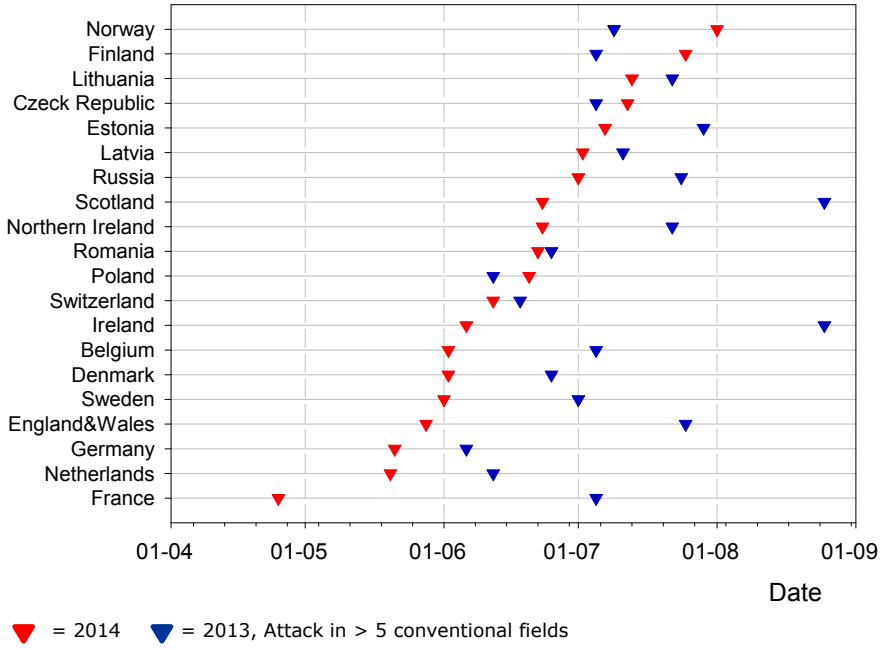

Figure 3. *Date when attacks were recorded in 5 or more conventional fields in 2013 (blue triangles) and in 2014 (red triangles)*

TUBER BLIGHT IN 2013 AND 2014

The level of tuber blight was reported as low in all countries in Europe, except for some regions and some type of potatoes in Russia, Latvia and Poland and Switzerland (Fig. 4).

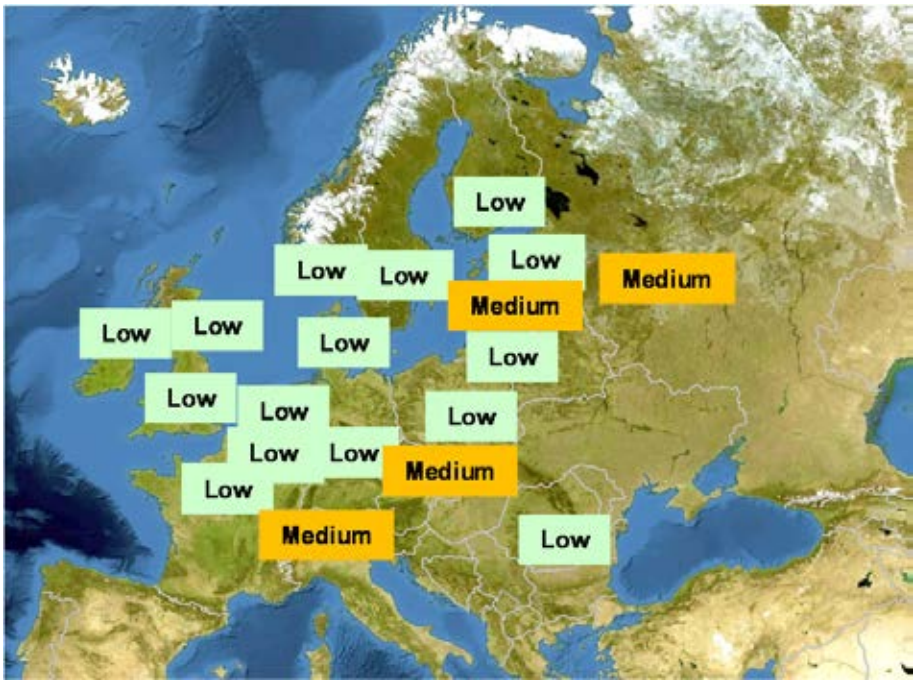

Figure 4. *The level of tuber blight attacks (low, medium or high) in 2014 compared to normal*

INDICATIONS OF OOSPORES

Infections from oospores were reported from Denmark, Sweden, Estonia, Lithuania and Poland in 2014 (Fig. 5).

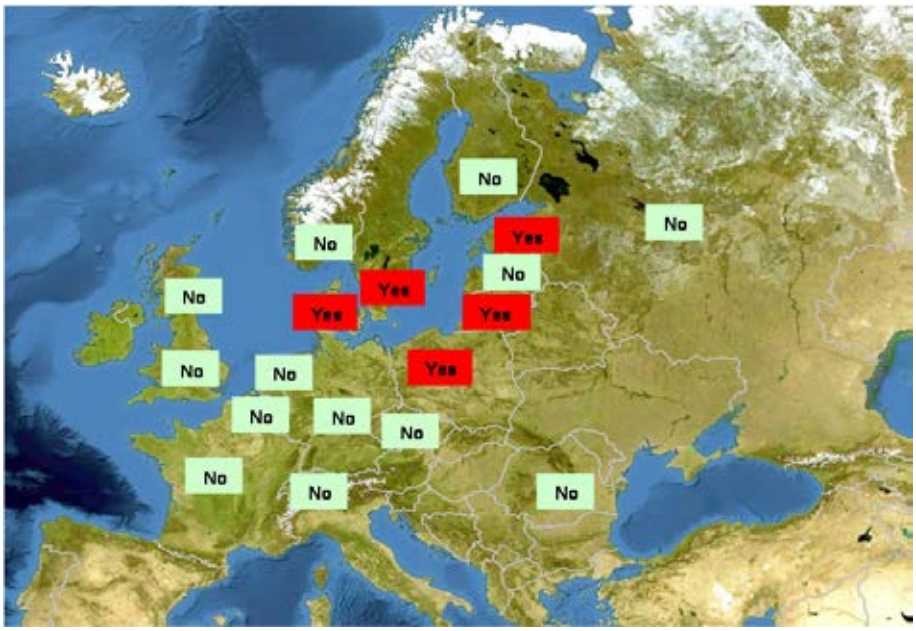

Figure 5. *Indications of oospores in Europe in 2014*

FUNGICIDES AND CONTROL STRATEGIES

In **Estonia**, systemic fungicides like Ridomil Gold and Tattoo were used in the first sprays to protect actively growing plants. Four to six spays were used in both years, the most commonly used active ingredients were mancozeb, fluazinam and mandipropamid, 1-2 treatments with cyazofamid were used at the end of the season to protect against tuber blight. In **Latvia**, the mean number of fungicide applications applied in ware potatoes 2013-14 was 3-8. The most frequently used active ingredients were: mancozeb, mefenoxam, propamocarb, dimethomorph, fluazinam, fluopicolide, mandipropamid, cyazofamid. In **Lithuania**, in average 4-6 fungicide applications is common practice. The first 1-2 applications are done with a contact fungicide, then two applications with systemic fungicides, and finally one or two applications with contact or translaminar fungicides. In **Russia,** 2013, according to the VNIIFBlight DSS, the calculated number of fungicide treatments was 3 (central regions), 2-3 (northwestern regions), 2-4 (southeastern regions), and ≤1 (eastern regions). In 2014 1-2 applications more recommended than in 2013, 2-5 applications per a season for all types of potato. In **Poland**, the farmers applied 1-6 sprays, most often 1-2. The active ingredients used on the largest areas were fluopicolide/propamocarb-hydrochloride and fenamidone/propamocarb-hydrochloride, metalaxyl -M+mzb, mandipropamid and cymoxanil+mzb. Farms producing potato for chips use fungicide applications more frequently than other potato-growing farms. The owners of allotment gardens

use no fungicides. In **Switzerland**, farmers control late blight at the beginning of the season with systemic fungicides, subsequently with protective or translaminar fungicides depending on the weather conditions and the late blight epidemic pressure. Farmers obtain such recommendations by using our DSS PhytoPRE, from their plant protection officer or from the newspaper. In organic potato production, copper products are often used to control late blight (max. 4 kg/ha^{1}year^{-1}). There is also a PhytoPRE version for organic potato production available, but it is rather seldom used. In general, farmers are aware of the possible infection sources and avoid waste piles and volunteer plants. In **Finland** the fungicide use against late blight was moderate, typically 4 to 6 sprays started during the first half of July. The use of mancozeb and fluazinam containing fungicides is steadily decreasing. The use of Revus, Ranman and Consento has increased especially during the rapid growth of potato. Infinito was registered at the end of 2014. In the **Czech Republic** conventional potato growers usually do 2 – 8 applications depending on maturity group and susceptibility of grown varieties. Early potatoes (1-2 applications), other ware potatoes (5–8 applications) and seed crops (4 – 5 applications). First applications mostly with Ridomil Gold MZ Pepite (mancozeb, metalaxyl M), Consento (fenamidone, propamocarb-hydrochloride), Dithane DG (mancozeb) and Galben M (mancozeb, benalaxyl). For further applications fungicides Curzate (cymoxanil, mancozeb), Revus (mandipropamid), Revus Top (mandipropamid, difenoconazole), Acrobat (dimethomorph) were used. Applications in the latter half of growing season were done with Infinito (fluopicolide, propamocarb-hydrochloride), Altima (fluazinam) and Ranman Top (cyazofamid). The highest efficacy was found in spraying schedules involving locally systemic fungicides with active ingredients cymoxanil, valifenalate, fenamidone, mandipropamid and fluopicolide and contact fungicides fluazinam and cyazofamid. In the Czech Republic 27 active ingredients in 64 fungicides are registered against late blight at present. Out of this number 28 fungicides contain mancozeb single or combined with an other active ingredient, the second most used active ingredient is cymoxanil, which is present in 17 fungicides. In **Norway**, 2013, the potatoes was treated 7.8 times with fungicides to control late blight, calculated as total amount of late blight fungicides sold divided by total potato area. In 2014 less blight fungicides were sold. Most potato farmers are members of the Norwegian extension service system and get their blight warnings through them from the VIPS system. However it is still common to apply fungicides on a weekly schedule, only slightly modified for the blight risk. In **Sweden,** contacts or translaminars are the main products, sometimes complimented with one or two treatments with a metalaxyl fungicide in the beginning of the spraying season. The number of sprays used in ware potatoes varies from south to north, with substantially more fungicide applications in the south. In 2014 the early attacks of late blight in the South gave a higher number of sprays compared to normal, but in other parts of the country the blight was controlled with normal or lower spraying intensity. In **Denmark,** Ridomil Gold is no longer allowed from 2014. Alternatively many farmers use a combination of Proxanil and Ranman/Revus. Due to the tax policy in Denmark, Dithane NT containing Mancozeb is not relevant to use anymore due to a relatively high price. More than half of the Danish farmers used Dithane as their first priority contact compound for many years. A change to alternatives seems to increase attacks of early blight. Therefore farmers now combine LB fungicides with fungicides especially targeted towards *Alternaria spp*. Good effects were experienced with strategies using Signum and Amistar. The Danish Blight Management decision support system is used by most farmers. Compared to a conventional strategy, this reduces the fungicide use by 10-30 % (as found in field trials). In **France**, 2013, due to a low to medium late blight pressure, growers achieved a fair control right after emergence with protectant products. Later on, because the disease pressure was diminishing, growers were able to return to normal and, occasionally, longer delay between

fungicide applications of simple protectant products. Very few translaminar and curative products have been used. The 2014 season was dramatically different since late blight pressure was high starting at emergence. Short intervals of 5 to 6 days, with protectant fungicides and products with efficient rain fastness were needed. Later in the season translaminar and curative activities of the fungicide applications were looked for in order to protect the crop. In **Belgium,** 2013, the average number of fungicide applications (susceptible variety, mainly cv. Bintje) was 14, which corresponds with an average interval of 8.7 days (from min. 6 to max. 13 days interval). In contrast 18 applications were needed in 2014, which corresponds with an average interval of 6.4 days (from min. 3 to max. 8 days interval). In the **Netherlands** the use of fluazinam solo is greatly diminished because of the problems with Green 33 in 2011. Most growers are using three or four different fungicides during the season, starting with Acrobat, Curzate, Valbon or Revus followed by Infinito, Banjo, Canvas and Ranman Top. In 2014 the use of fungicides was significantly higher than in 2013. Average last many years is appr. 14 sprays. In 2014 many growers spayed two times more. In **Germany**, a normal strategy is to start with a systemic or local systemic fungicide, then local systemic products (e.g. Revus, Infinito, Acrobat Plus, Valbon) and finally after flowering Ranman, Shirlan and fungicides containing Mancozeb. In **Scotland**, the main fungicide a.i.s used were fluazinam, cymoxanil + mancozeb, cyazofamid, cymoxanil and mandipropamid. In **England & Wales,** according to the UK pesticide usage survey report 250 published by DEFRA using 2012 figures (most recent published data), the average ware crop received 11 fungicide applications. This is an increase from 2010 where 10 applications on average were applied. Seed crops were treated with an average of 9 applications, the same as reported in 2010. Advisers are recommending that fungicides are applied at intervals no greater than 7 days apart. The three most frequently applied active ingredients were mancozeb/cymoxanil, fluazinam and cyazofamid for ware crops and cymoxanil, cyazofamid and fluazinam for seed crops. In **Northern Ireland,** the mean number of fungicide applications was about 10 in both years (ranging from 6 to 14). Growers made use of a wide range of fungicides. Fluazinam (Shirlan) was the most widely used active ingredient (used by 69% of seed growers). Growers also made significant use of Infinito (fluopicolide + propamocarb), Invader (dimethomorph + mancozeb), cymoxanil + mancozeb products, Dithane (mancozeb), Revus (mandipropamid), Merlin (propamocarb + chlorothalonil), Consento (fenamidone + propamocarb), Proxanil (propamocarb + cymoxanil) and Ranman (cyazofamid). Fubol Gold (metalaxyl-M + mancozeb) was used by a few growers. There were few blight outbreaks and these were well controlled (all <5% foliar infection). In **Ireland** the unfavorable blight weather influenced the choice of products. This included reduced numbers of applications and decreased usage of curative products. Where blight was seen the use of curative products ensured that no major epidemics were observed. More blight in 2014 increased frequencies of applications and the inclusion of curative fungicides in most applications early in the season. In response to dry spells growers did change fungicide products and frequency of applications. In some instances these were changed too quickly and epidemics were reported. The use of curative products, particularly cymoxanil was prevalent, especially early in the season and later following the heavy rain in August.

POPULATION CHARACTERISTICS

In **Estonia**, during the period 2010–2013, the A2 mating type frequency among the population of *P. infestans* was on average 40%. Both mating types were present in most of fields inspected (80%) where more than one isolate was tested. Metalaxyl-sensitive isolates prevailed (in average 66.3%) in the research period. The race structure was diverse and complex (the

average number of virulence factors per isolate was 6.4). Most pathotypes were unique, appearing only once. In **Lithuania**, a test of 93 *P. infestans* isolates, collected from all over Lithuania in 2010-2012 indicated a high and stable frequency of A2 mating type. On 45% of all studied potato fields, both mating types were recorded, suggesting sexual reproduction of *P. infestans* and possible oospore production in Lithuanian potato fields. Fourteen metalaxyl-resistant isolates were found among 71 isolates in the current study period, and sensitive isolates prevailed in all three years. Amongst 70 isolates studied, 38 avirulence pathotypes were found. The Lithuanian race structure was highly diverse and complex (the average number of virulence factors per isolate was 7.2). Most pathotypes were unique, appearing only once, and the four most common pathotypes comprised only 34% of the population (results of E. Runno-Paurson et al, 2015). In **Russia** the mating type, in the majority of the studied *P. infestans* isolates, collected from potato fields, were of the A1 type; the A2 type was reported only for single isolates. All isolates were identified as of complex races (5-11 virulence genes). In the Czech republic in 2013 the ratio of mating types A1:A2 was 40:60. In tests of resistance to fungicide active ingredients 33 % of isolates were found to be resistant to metalaxyl M. Resistance to dimethomorph was not detected. Resistance to propamocarb-hydrochloride was determined in 97 % of isolates in the central potato production region. In a similar test in 2014, the ratio of mating types A1:A2 was 27:73. In resistance tests, 20 % isolates were found to be resistant to metalaxyl M. Resistance to dimethomorph was not detected. 93% of tested isolates were resistant to propamocarb-hydrochloride. In **Sweden** the population of *P. infestans* shows a very high genotypic diversity with the biggest part of the variation found on the field or disease foci level. There are indications that the oospores as inoculum source are very important. In **Denmark** the *P. infestans* population is very diverse. Genotype Blue13 was found in 2011 and 2012 not in 2013 and at low level in 2014. None of the other named genotypes have been found yet in Denmark. In **Norway** the *P. infestans* population is diverse, with no dominant clones. SSR-fingerprinting of early attacks shows that genotypes clusters like families within fields. In **Finland**, no sampling was carried out in 2013 and 2014. In **France** the population has been monitored in collaboration with the EuroBlight network. With the easy-to-handle *P. infestans* collecting device, the Whatman FTA card, a thorough collection of samples has been possible with the help of professional partners, extension and technical institutes, breeders and advisors. The 2014 season has yielded some 200 samples, originating from most potato producing areas. The overall genotypic analysis confirms a balanced ratio of the 2 mating types A1/A2 and a predominance of the EU_13_A2 clone followed by the EU_6_A1 and EU_1_A1. The diverse clonal structure of the population tends to confirm that the asexual reproduction of *P. infestans* is still prevalent in the country. In **the Netherlands** the late blight population is a mix of different types. The Green 33 type hasn't been found last year. In Germany the genotype map show 25% EU-13-A2 and 75 % other genotypes. A Research project indicates that most of the *P. infestans* populations have a latent period between 48 to 72 hours. In **England & Wales** the predominant genotypes identified from field samples collected by advisers in 2013 were 13_A2 and 6_A1. A total of 53 isolates from 14 crops across **Northern Ireland** were obtained and characterised. Of these, 52 (98%) were A1 and all but one were 8_A1 (51), one was probably 6_A1. The only A2 isolate was 13_A2. Overall, 5% of isolates were phenylamide-resistant and this included the A2 isolate. In 2014 a total of 57 isolates from 29 crops across Northern Ireland were tested for mating type, of which 54 (95%) were A1 and 3 were A2. Of isolates tested for phenylamide sensitivity, 31% (of 61 isolates) proved to be resistant. Genotyping (using samples collected on FTA cards) is still in progress. To date this has shown that 8_A1 is the most frequent A1 genotype (43% of total), but 6_A1 and 12_A1 have also been detected. Genotype

13_A2 was present in 32% of samples. Results indicate that 13_A2 has been detected more frequently in FTA samples than among isolates.

USE OF DSSs

Several decision support systems for late blight forecasting and control are used in Europe but no major changes since the last reporting (Hansen *et al.* 2013) was noted.

ALTERNARIA 2013 & 2014

For long time *Alternaria* spp was a minor problem in North/West Europe. Some countries report that attacks of *Alternaria* spp is an increasing problem, and severe attacks were found also in countries beyond Central Europe in 2013 and 2014.

EB disease observation and EB disease progress

The date of first observation of early blight is shown for 2014 in Fig 6. The epidemic started mid of July (Germany) to end of August (Denmark). In 2013 the EB situation was similar to 2014.

In Table 1 the EB specific disease development in different countries is shown. Until the end of July 2014 the disease severity in the fields was lower than 20% in most European countries. Only in England, Wales and Germany the disease severity was between 20 and 50% at this time. In several countries the EB disease progressed in August and reached in Finland, Sweden, Germany and Czech Republic more than 50% EB disease.

EB: Identified Alternaria species

In most countries both Alternaria species *Alternaria solani* and *Altenaria alternata* were identified on infected potato leaves (Tab. 2). In June *Alternaria alternata* was the dominant species found in the central European countries. In Estonia only *Alternaria alternata* was identified in 2014 where else in England and Wales only *Alternaria solani* was found in 2014. Based on this data there is no correlation between the observed *Alternaria* species and the disease progression.

Fungicide usage and fungicide resistance

The following active ingredients were used in different countries to control EB: mancozeb, azoxystrobin (QoI), chlorothalonil, boscalid, pyraclostrobin (QoI) and difenoconazol. According to the regional registration also mixtures of these active ingredients are registered. QoI´s have a specific single-site mode of action and possess a high risk to the evolution of fungicide resistance due to point mutations. Loss of sensitivity to QoIs has been reported for *A. solani* in potato (Pasche *et al*. 2004) and for *A. alternata.* The monitoring data from 2014 indicate that in Germany, Belgium, Netherlands and Sweden the F129L mutation in *Alternaria solani* occurred. The G143A mutation in *Alternaria alternata* was identified in isolates from Germany, Netherlands and Sweden.

At the moment only limited DSS models are existing (PhytophthoraModel Weihenstephan in Germany, DACOM in Netherlands, Sweden and Poland) to optimise the control of EB.

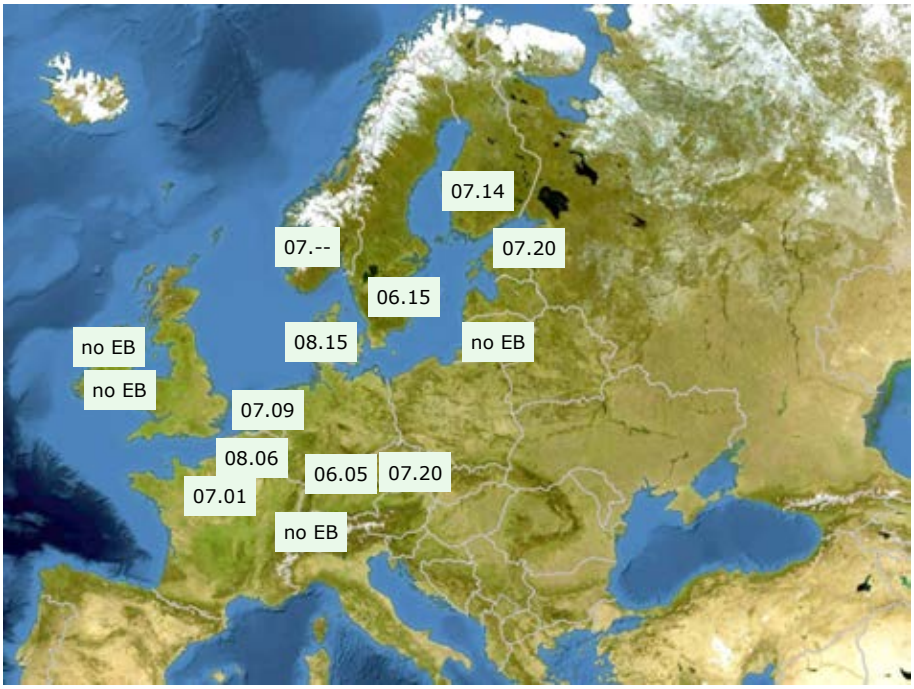

Figure 6. *First observation of early blight in 2014 in Europe*

Table 1. *EB specific disease severity in 2014 (2013) in different European countries*

	disease severity				
	May	June	July	August	September
Finland (2013)					
Finland (2014)					
Norway					
Sweden					
Denmark					
Estonia					
Lithuania					
Scotland					
Northern Ireland					
England and Wales					
Belgium					
Netherlands					
Germany					
Poland					
Switzerland					
Czech republic (2014)					
France					
		disease severity			
		< 20%			
		20-50%			
		> 50%			

Table 2. *Identified Alternaria species (Alternaria solani / Alternaria alternata) in different European countries*

	A. solani	Jun	Jul	Aug	Sep	remark
Finland	√		√	√	√	
Norway	√			√	√	
Sweden	√	√	√	√	√	
Denmark	√			√	√	
Estonia	not found					
Lithuania						
Scotland						
Northern Ireland						
Ireland						
England & Wales	√	no data				
Belgium	√		√	√	√	dominating species
Netherlands	√		√	√	√	
Germany	√		√	√	√	
Poland	√	no data				
Switzerland						
Czech republic						
France						

A. alternata	Jun	Jul	Aug	Sep	remark
√		√	√	√	
√			√	√	
√	√	√	√	√	with low frequency
not found					
√		√	√		
√	√	√	√	√	
√	√	√	√	√	
√	√	√	√	√	
√	no data				3 times more than A. solani

LITERATURE

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The potato blight population in Northern Ireland

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SUMMARY
Samples of late blight from 13 and 33 Northern Ireland potato crops in 2013 and 2014, respectively, were characterised for mating type, phenylamide resistance and SSR genotype. Fewer samples were obtained in 2013 than 2014 because dry weather in April and May 2013 limited the number of late blight outbreaks, whereas in 2014 the weather was conducive to infection. SSR analysis was carried out on single-lesion isolates of *Phytophthora infestans* in 2013, while in 2014 it was done on DNA samples from single lesions collected on FTA cards. In 2013, the incidence of both phenylamide resistance and the A2 mating type declined compared with 2012 (to under 5%), but in 2014, the incidence of phenylamide-resistant strains and of the 13_A2 genotype increased to over 30%. As in previous years, the population proved highly clonal: all A2 isolates belonged to the 13_A2 genotype; the predominant A1 genotype was 8_A1 in both years, but in 2014, 5_A1, 6_A1 and 12_A1 were also detected. The major A1 genotype in Northern Ireland since the mid-1990s has been 8_A1 (NI-1), whereas in Great Britain the newer 6_A1 has been the most frequent A1 genotype since 2007. The EuroBlight Late Blight Tool box was used to analyse and visualise 2013-2014 Northern Ireland *P. infestans* SSR data.

KEYWORDS
Phytophthora infestans, Northern Ireland, mating type, phenylamide resistance, SSR, population structure

INTRODUCTION

Late blight has challenged potato growers in Ireland since its arrival in 1845 and the subsequent Irish Potato Famine (1845-48). For much of the 20th century, global *Phytophthora infestans* populations were dominated by a single clonal A1 lineage, designated US-1. From the mid-1970s, migration events spread new strains of both mating types worldwide, displacing US-1 and triggering ongoing population changes. In Europe, some regions now have recombinant populations, while others have remained largely clonal.

In Northern Ireland, the A2 mating type was first identified in 1987 (Cooke *et al.,* 1995). By the mid-1990s, the Northern Ireland *P. infestans* population was highly clonal, with one major and

some minor A1 genotypes; the A2 mating type occurred at low frequency and no US-1 was detected (O'Sullivan *et al.,* 1995; Carlisle *et al*., 2001). In the period 1998-2002, the A2 mating type was not detected and there was a clonal A1 population with two major genotypes, designated NI-1 (equivalent to SSR genotype 8_A1) and NI-2 (5_A1) (Cooke *et al.,* 2006).

In the last 10 years, marked changes have occurred in *P. infestans* populations in Europe; new genotypes have been identified, most notably 'Blue 13' or 13_A2 (Cooke *et al.,* 2012a). These new types may have originated as sexual recombinants in mainland Europe and some have proved more aggressive and harder to control. In Northern Ireland, in 2005 the first A2 isolates since 1995 were detected and in 2007 the 13_A2 genotype was identified for the first time (Cooke *et al.*, 2009). Since 2008, *P. infestans* population studies in Northern Ireland have continued as part of all-Ireland projects led by Teagasc (Kildea *et al.,* 2010), currently MonPESC: **Mon**itoring **P**athogen **E**volution for **S**ustainable **C**ropping). Isolates have been characterised phenotypically (mating type, phenylamide resistance) and genotypically (SSR, RG57, mtDNA and *Pep* allozymes). A close association between markers has indicated a highly clonal population: only five genotypes were identified and within these, isolates had similar SSR patterns, shared a common mating type, RG57 fingerprint, *Pep* allozyme genotype, mtDNA haplotype and sometimes the same phenylamide resistance status (Cooke *et al.*, 2014). The major genotypes were 13_A2 (Blue 13), the only A2 detected (A2 genotypes present in Northern Ireland in the 1990s were not found) and 8_A1 (NI-1), the commonest genotype in the 1990s. The frequencies of these two genotypes fluctuated from year to year in inverse proportion to each other. Of three other A1 genotypes detected, two were present in the UK in the 1990s (5_A1/NI-2 and 12_A1); the third ('Pink 6', 6_A1), first identified in Great Britain in 2004 and in Northern Ireland in 2009 (Kildea *et al.*, 2013), has been the commonest A1 genotype in Great Britain since 2007 (Cooke *et al.,* 2012a). Results of *P. infestans* population studies up to 2012 in Northern Ireland have been reported in papers presented at previous EuroBlight Workshops and published in the Proceedings (e.g. Cooke *et al.*, 2012b; Cooke *et al.,* 2014). This paper reports results for 2013-14 and demonstrates the use of the new EuroBlight Late Blight Toolbox population tools on the Northern Ireland population.

MATERIALS & METHODS

Collection, isolation and storage of Phytophthora infestans isolates

Blighted potato leaf material was collected mainly from commercial seed crops by members of the Northern Ireland Department of Agriculture and Rural Development (DARD) Agri-food Inspection Branch. Once received, the blighted material was incubated and isolates established as previously described (Kildea *et al.,* 2010). In 2014, duplicate single lesions from each sample were also squashed onto FTA cards following the EuroBlight protocol (www.euroblight.net).

Mating type, phenylamide sensitivity and SSR determination

Mating type was determined as described by Cooke *et al*. (2006). The sensitivity of isolates to the phenylamide fungicide metalaxyl was determined using a floating leaf disk assay (Cooke *et al.,* 2006). For selected isolates, genotypes at the polymorphic allozyme locus, *Pep-1* (peptidase), were determined using cellulose acetate electrophoresis (Carlisle *et al*., 2001). In 2013, isolates were genotyped by SSR analysis at the James Hutton Institute (JHI) using a selection of the markers described by Lees *et al.* (2006) and Knapova & Gisi (2002) in

accordance with the protocol developed by EUCABLIGHT. In 2014, SSR analysis was carried out by JHI directly on the DNA collected on FTA cards.

Use of the EuroBlight Late Blight Toolbox
SSR characterisation data for the 2013 and 2014 Northern Ireland isolates were imported into the EuroBlight Late Blight Toolbox. The Toolbox was then used to visualise and analyse the data and compare the Northern Ireland population structure with populations in Great Britain and elsewhere in Europe (using Belarus and Sweden as examples). Bruvo distances were used for Principal component analysis (PCA); these are calculated genetic distances between individuals at microsatellite loci that can accommodate differences in ploidy (Bruvo *et al*., 2004).

RESULTS

Population characterisation 2013-2014
Dry weather early in the 2013 season (late May-June) prevented primary infection development and the first field outbreak of late blight was not reported until 17th July (the latest 1st report since 1981 apart from 19th July in 2010). Weather suitable for the spread of blight occurred in July and August, but there were few primary infection sources and not many outbreaks were reported. Blight samples were obtained from only 13 sites, of which 12 were commercial crops and one was an allotment; 54 isolates were established (up to five per site). In 2014, the weather was more conducive to late blight with mild night temperatures and more rainfall early in the season. The first field outbreak was identified relatively early on 9th June and subsequently blight was reported in all potato-growing areas. Blight samples were obtained from 33 sites, which comprised 27 commercial crops and six trial sites. Sixty *P. infestans* isolates were obtained from 30 sites (up to five per site), and 75 samples on FTA cards from 33 sites were SSR genotyped.

In 2013-2014, as in the whole period since 2005, the annual percentage of isolates containing phenylamide-resistant strains showed a similar trend to the annual proportion of A2 mating type isolates (Figures 1-2). This is indicative of hitch-hiking selection: growers in Northern Ireland now make little use of phenylamide fungicides and so the incidence of phenylamide-resistant strains is not related to their selection by phenylamide usage, but depends on the incidence of the invariably phenylamide-resistant 13_A2 genotype. This is further evidence for the clonality of the Northern Ireland *P. infestans* population.

In 2013, the incidence of both phenylamide resistance and the A2 mating type declined compared with 2012 (5% phenylamide resistance, 2% A2 mating type, Figures 1-2). In 2014, the incidence of phenylamide-resistant strains increased to 31%; although the incidence of A2 mating type isolates was only 7%, the incidence of the 13_A2 genotype in DNA samples collected on FTA cards was 33%. This indicated a problem which became particularly apparent in 2014 when SSR genotyping of the Northern Ireland population was done using DNA collected onto FTA cards for the first time rather than DNA from agar cultures of the pathogen. The 13_A2 genotype proved much more difficult to isolate into axenic culture than the 8_A1 genotype so that mating type determination on isolates in culture under-estimated the incidence of A2 types (whereas phenylamide resistance testing using spore suspensions generated from blight samples on leaves did not impose this bias). Of 11 sites where 13_A2 was detected, three sites were sampled onto FTA cards only (isolation not attempted), from three sites A2 mating type isolates

were obtained, but from five sites only A1 isolates were obtained although 13_A2 and A1 genotypes were detected in the DNA from FTA cards and phenylamide resistance testing showed the presence of resistant strains.

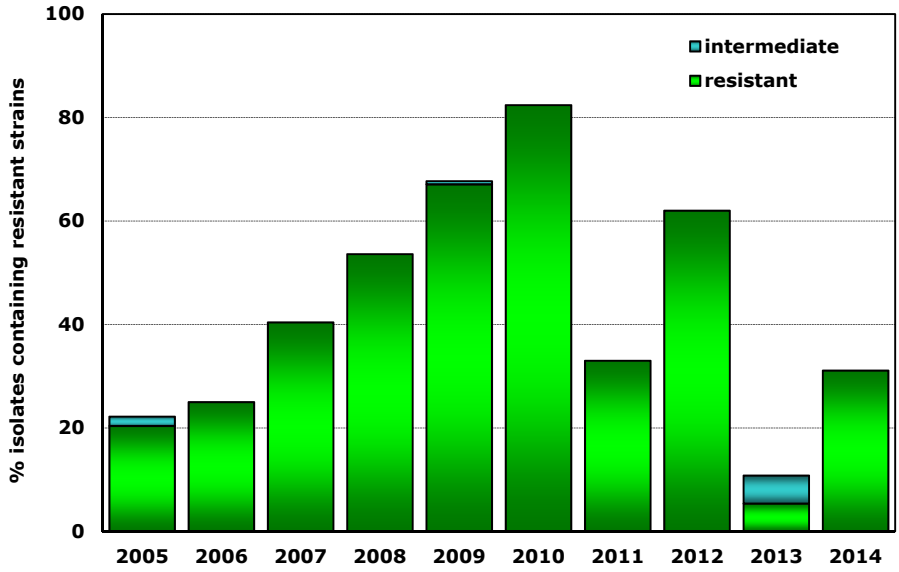

Figure 1. *The percentage of Northern Ireland Phytophthora infestans isolates containing phenylamide-resistant strains, 2005-2014*

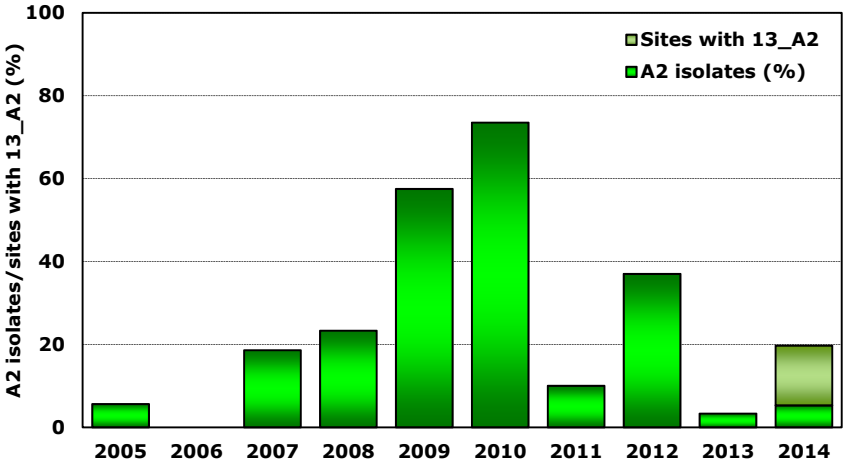

Figure 2. *The percentage of Northern Ireland Phytophthora infestans isolates of the A2 mating type, 2005-2014*

SSR genotype characterisation of 2013 and 2014 isolates revealed that all A2 mating type isolates belonged to the 13_A2 type and no other A2 genotypes were detected (Fig. 3). In 2013, of 53 isolates characterised by SSR, the only 13_A2 isolate was obtained from a tuber grown in an allotment in Belfast; this represented 2% of the detected genotypes. In 2014, as noted above, 13_A2 was identified at 11 sites (eight commercial crops and three trial sites) and constituted 33% of the detected genotypes (25 of 75 samples genotyped).

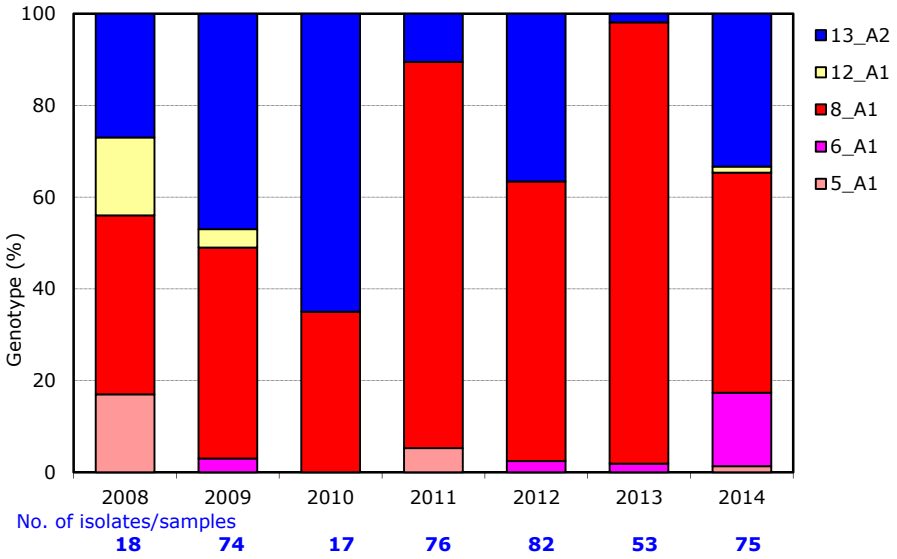

Figure 3. *Northern Ireland Phytophthora infestans genotypes 2008-2014*

Four A1 genotypes were identified by SSR in 2013-2014 (Fig. 3). In 2013, all but one A1 isolate belonged to the 8_A1 genotype (51 isolates, 96% of detected genotypes). One 2013 A1 isolate was not successfully characterised by SSR, but was phenylamide-sensitive with the *Pep* allozyme genotype *96/96*, typical of 6_A1 (Cooke *et al.,* 2014) and was tentatively assigned to that genotype (2%). In 2014, A1 types were identified in 50 of the 75 FTA samples analysed and these belonged to four genotypes, 8_A1 again predominated (48% of all samples genotyped), 6_A1 was present in 16% of samples (its highest detected occurrence in Northern Ireland), and 5_A1 and 12_A1 were each represented by a single sample.

Comparison of the *P. infestans* genotype occurrence in Northern Ireland with that in Great Britain between 2008 and 2014 (Cooke, D.E.L., personal communication) showed a clear difference between the two geographical regions throughout the period. While in both regions the only A2 genotype detected was 13_A2, in Great Britain the commonest A1 genotype detected in every year was 6_A1, while in Northern Ireland it was 8_A1. In years when 13_A2 was less frequent its place was taken by 6_A1 in Great Britain, but 8_A1 in Northern Ireland (Fig. 3).

Use of the EuroBlight Late Blight Toolbox

The EuroBlight Late Blight Toolbox genotype frequency maps for 2013 and 2014 clearly show the differences in genotype frequencies between Northern Ireland, Great Britain (England, Scotland and Wales) and mainland European countries, in both years (Fig. 4). Northern Ireland was the only location where the A1 genotype 8_A1 predominated.

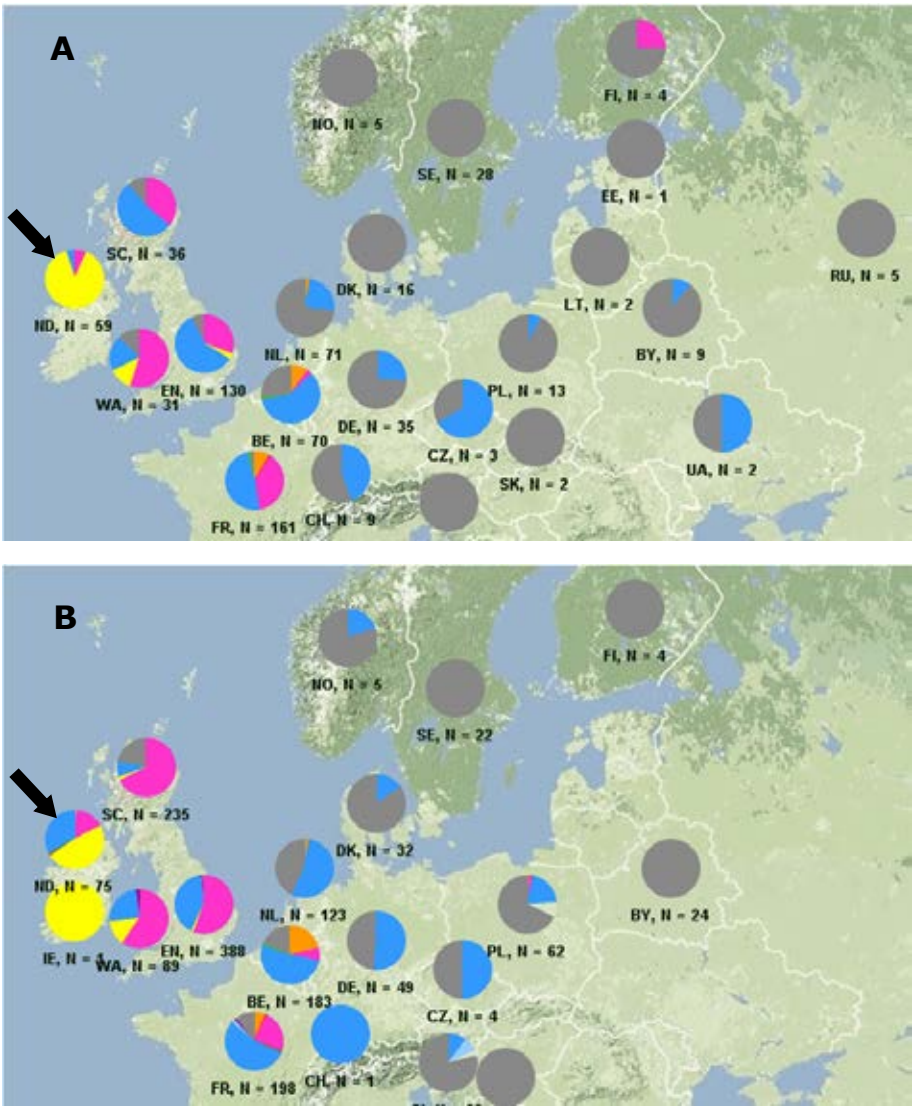

Figure 4. *Euroblight Potato Late Blight Toolbox genotype frequency maps for 2013 (A) and 2014 (B) showing Phytophthora infestans genotype frequencies in Northern Ireland (ND, arrowed) compared with those elsewhere in Northern Europe*

The number of analysed samples per multilocus genotype in Northern Ireland, England, Scotland and Wales was compared with Belarus and Sweden on the basis of data submitted to the EuroBlight Late Blight Toolbox (Table 1). This showed that in Belarus and Sweden the number of samples per multilocus genotype was 1.0 in both years (i.e. every analysed sample represented a unique genotype), whereas in Northern Ireland the corresponding figure was 3.9 and 3.6 in 2013 and 2014, respectively, and in Great Britain it was between 2.8 and 6.6. This result is in agreement with the *P. infestans* populations in Belarus and Sweden being recombinant in contrast to populations in the UK, which exhibited considerable clonality.

Table 1. *Comparison of the number of isolates/DNA samples (N) per multilocus genotype (MLG) in Belarus (BY), Sweden (SE), Northern Ireland (ND), England (EN), Scotland (SC) and Wales (WA) in 2013 and 2014*

Population	N	MLG	N/MLG
2013			
BY	9	9	1.0
SE	28	26	1.1
ND	59	15	3.9
EN	130	30	4.3
SC	36	6	6.0
WA	31	11	2.8
2014			
BY	24	24	1.0
SE	22	22	1.0
ND	75	21	3.6
EN	377	57	6.6
SC	235	55	4.3
WA	89	15	5.9

PCA of Bruvo distances from the 2013 and 2014 data from Northern Ireland, England, Scotland, Wales, Belarus and Sweden showed that the Northern Ireland data clustered together and this clustering was largely associated with genotype 8_A1 in 2013 (Fig. 5) and with 6_A1, 8_A1 and 13_A2 in 2014 (Fig. 6).

Multiple Spanning Network (MSN) trees for the same countries for 2013 and 2014 similarly indicated clustering of the Northern Ireland data with the circles representing *P. infestans* genotypes in Northern Ireland being large (i.e. representing clonal genotypes) compared with the small dots indicative of single isolate genotypes in Belarus and Sweden (Fig. 7-8).

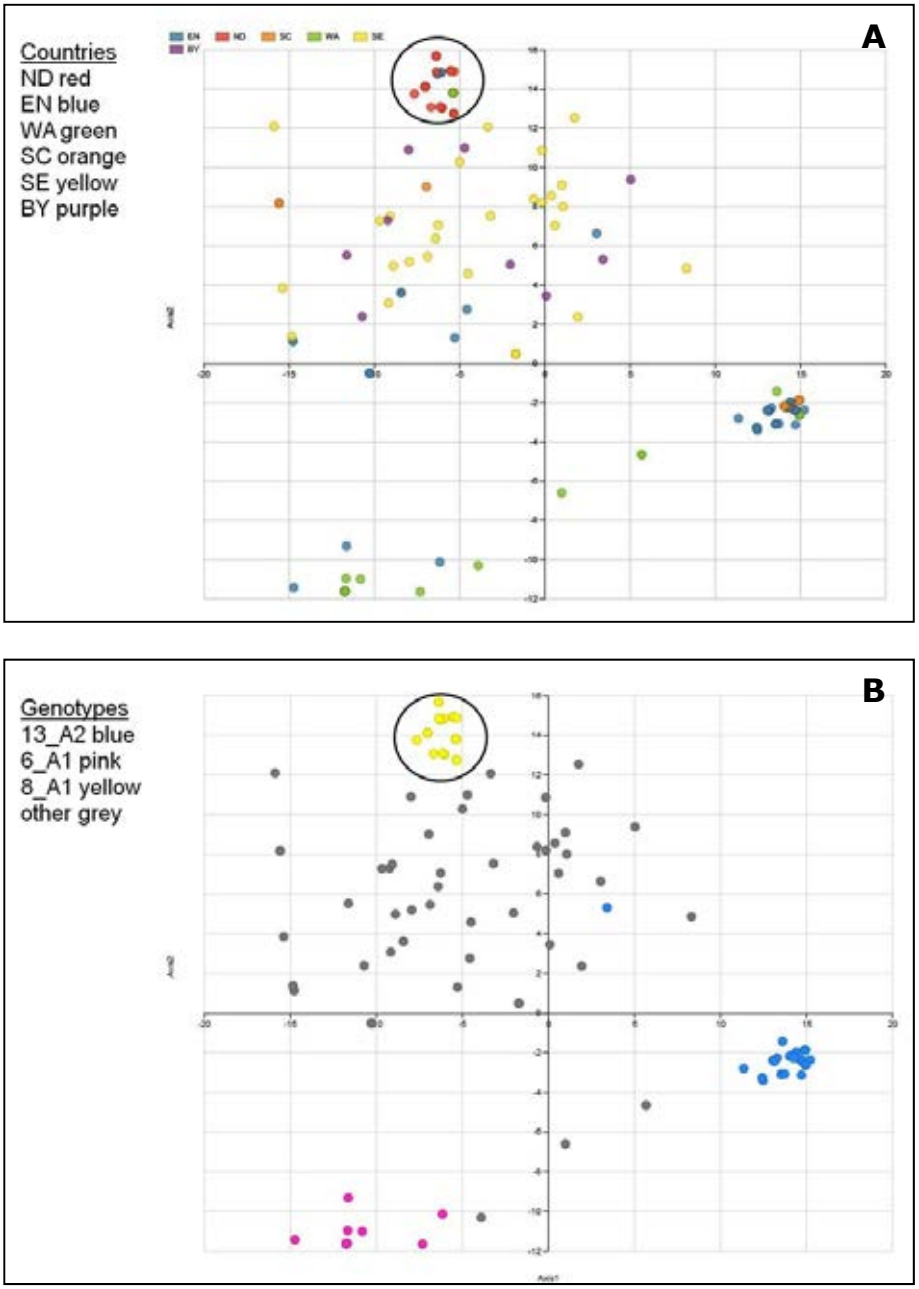

Figure 5. *Euroblight Potato Late Blight Toolbox Principal Component Analyses of Phytophthora infestans SSR data with Bruvo distances as input. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2013; data shown by Country (A) and by Genotype (B), 8_A1 cluster circled*

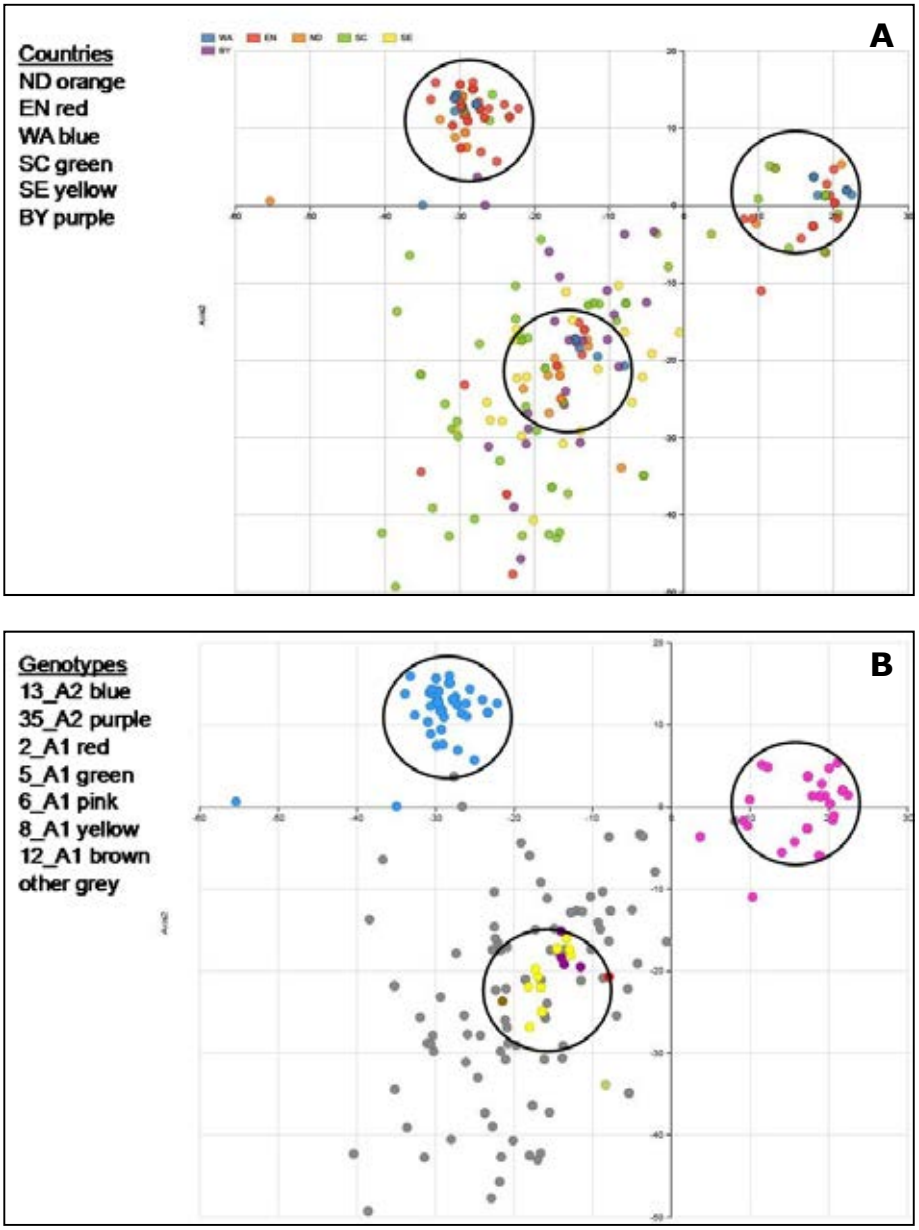

Figure 6. *Euroblight Potato Late Blight Toolbox Principal Component Analyses of Phytophthora infestans SSR data with Bruvo distances as input. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2014; data shown by Country (A) and by Genotype (B), 6_A1 (right), 8_A1(left below) and 13_A2(left above) clusters circled*

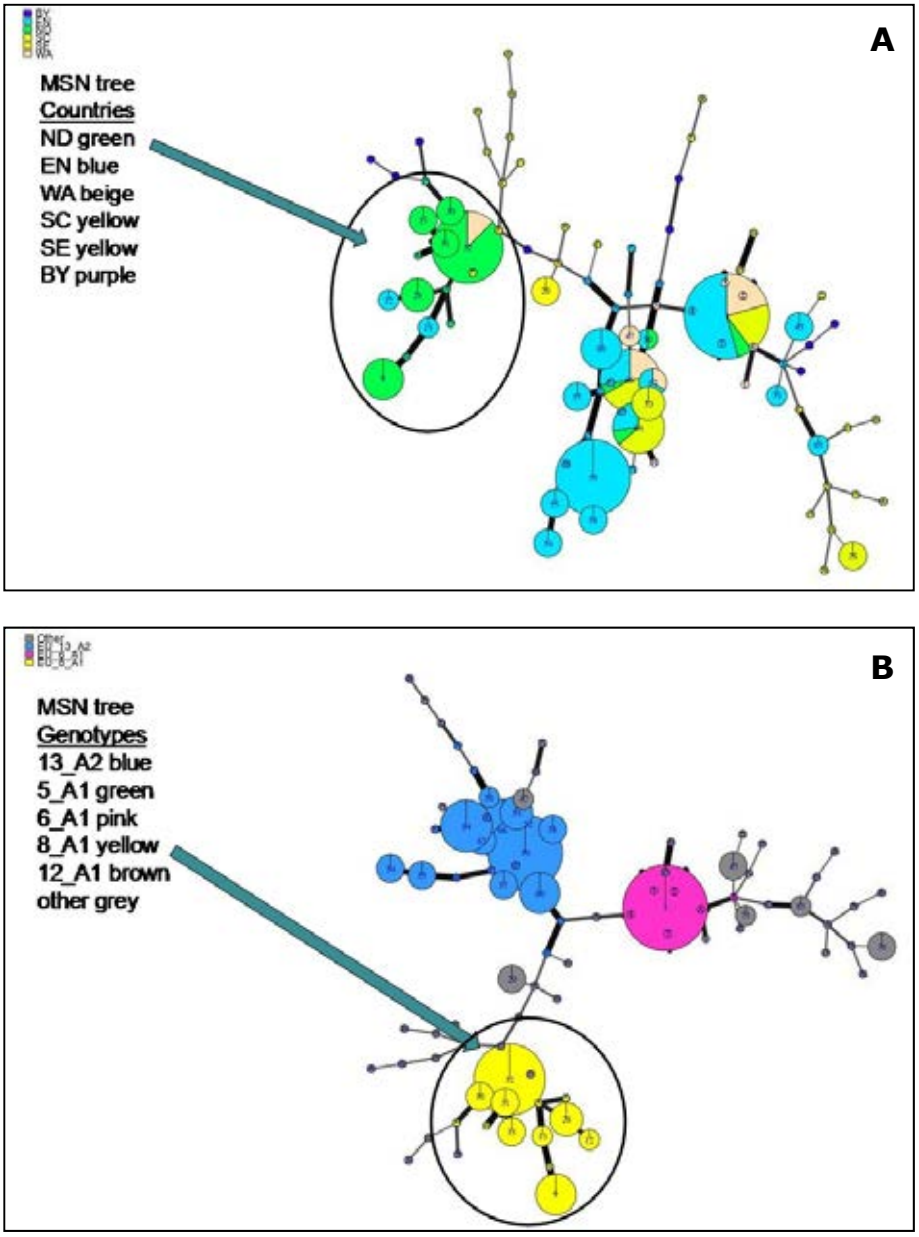

Figure 7. *Euroblight Potato Late Blight Toolbox Multiple Spanning Network Trees of Phytophthora infestans SSR data. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2013; data shown by Country (A) and by Genotype (B), 8_A1 cluster circled*

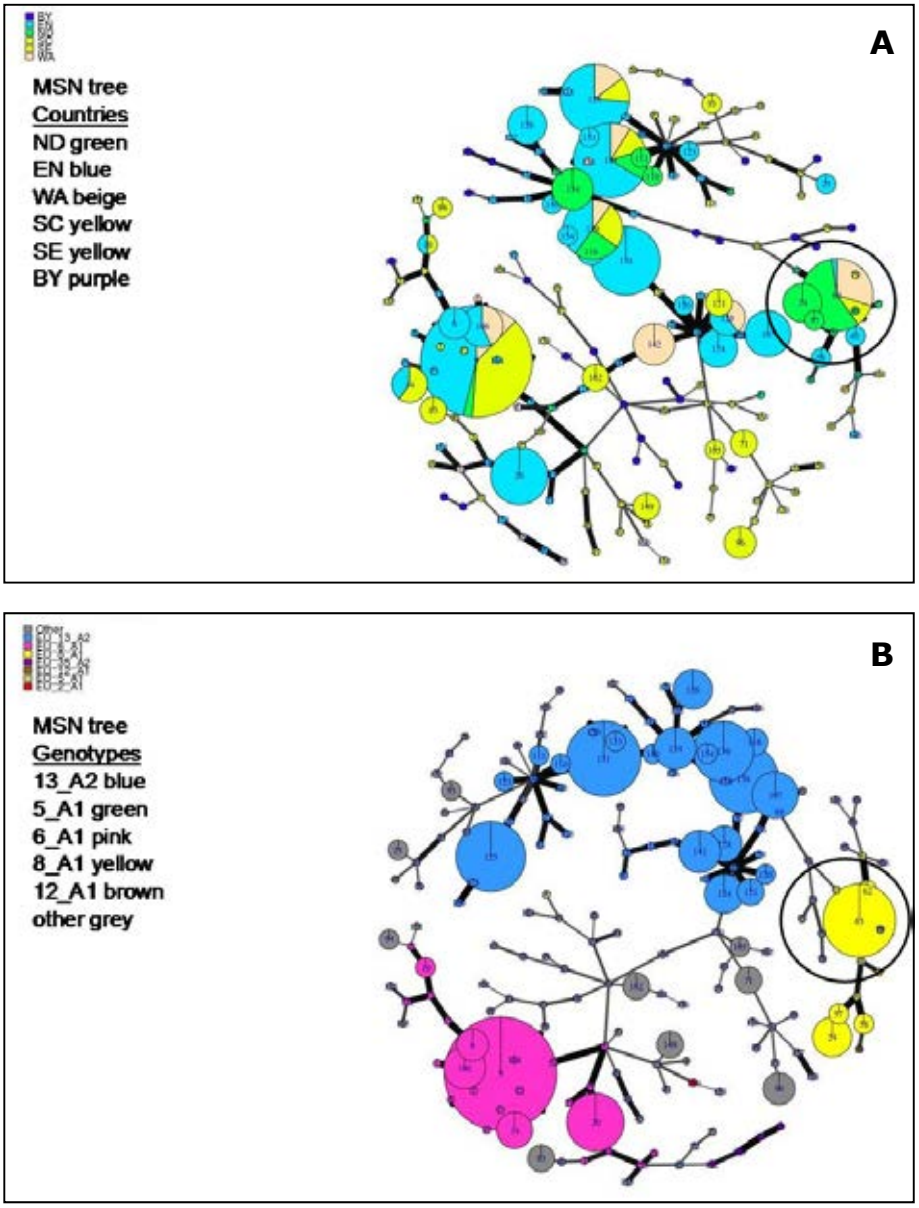

Figure 8. *Euroblight Potato Late Blight Toolbox Multiple Spanning Network Trees of Phytophthora infestans SSR data. Northern Ireland (ND) compared with England (EN), Scotland (SC) and Wales (WA), Belarus (BY and Sweden (SE) in 2014; data shown by Country (A) and by Genotype (B), 8_A1 cluster circled*

DISCUSSION

Studies of the Northern Ireland *P. infestans* population, begun in 1981 when phenylamide-resistant strains were identified (Cooke, 1981), were subsequently extended to investigate mating type (Cooke *et al.,* 1995). Since the mid-1990s, a range of approaches has been used to characterise the population and these have developed as new techniques became available. Results have shown that for the past 20 years, the population has remained highly clonal, but subject to ongoing change from year to year (e.g. Cooke *et al.,* 2014). The Northern Ireland population, as might be expected, contains a subset of the genotypes/clonal lineages present in other European *P. infestans* populations, but throughout the period studied these have occurred at frequencies different from those in the neighbouring population in Great Britain so that results from Great Britain cannot be extrapolated to Northern Ireland. In contrast, comparison of *P. infestans* population studies in Northern Ireland with those in the Republic of Ireland indicates that the pathogen behaves as a single population across the island of Ireland, albeit with some regional variation (e.g. Kildea *et al.*, 2010). As elsewhere in northern Europe, the appearance of the 13_A2 genotype, first identified in 2004-2005 in isolates from The Netherlands and Germany (Cooke *et al.*, 2012a), resulted in major upheavals in the *P. infestans* population in Ireland north and south. However, remarkably, whereas elsewhere in Europe the A1 genotypes of *P. infestans* have also changed, in the island of Ireland the predominant A1 genotype has been 8_A1 (equivalent to the multilocus RG57 genotypes IE-1 and NI-1; Griffin *et al.*, 2002; Cooke *et al.*, 2006) since the mid-1990s. Genotype 6_A1, which has increased in importance in Great Britain, particularly since 2011, although present in Northern Ireland, has remained relatively infrequent.

Understanding the pathogen population is a key component of control strategies, but a number of important questions remain. Among these are:

- Why do genotype frequencies in Northern Ireland and the Republic of Ireland differ from those in Great Britain?
- Why do genotype frequencies in clonal populations such as those in Northern Ireland fluctuate markedly from year to year?
- Why do some European countries now have recombinant *P. infestans* populations while others remain highly clonal?

Clonal populations are particularly subject to the influences of factors such as fungicide usage, cultivar and environment. The fungicides used for late blight control in Great Britain and Northern Ireland are similar and in both areas most cultivars grown are moderately or highly susceptible to blight, so these factors are unlikely to explain population differences. Environmental conditions may have a role; the Irish climate is cooler and wetter with mild winters, which may favour some genotypes such as 8_A1 while militating against others such as 6_A1, which is thought to be encouraged by warmer weather particularly in the spring. In a polycyclic disease such as late blight, repeated asexual reproduction can rapidly magnify the impact of even small selection pressures and also of stochastic effects. While factors such as latent period and sporulation capacity have major influences on genotype selection during the foliar epidemic phase of the disease, the impact of bottlenecks imposed by tuber infection, overwinter survival and success of primary outbreaks also needs to be taken into account and these may well have an important role in annual population changes.

Studies of *P. infestans* populations are valuable for formulation of integrated control strategies and for decision support systems, but without a greater understanding of the drivers of population changes, they remain largely reactive. Thus while they can describe the present or

immediately past populations, they have limited ability to predict the future. One area where future risks can to some extent be predicted is that of fungicide resistance. Monitoring for changes in the pathogen genome which could be associated with fungicide resistance e.g. to the carboxylic acid amide (CAA) fungicides (e.g. benthiavalicarb, dimethomorph, mandipropamid), is already being implemented across Ireland as part of the MonPESC project to provide an early warning of the development of fungicide resistance allowing loss of disease control and crop yield to be avoided (Kildea *et al.*, 2014). Rapid characterisation of the pathogen genotypes infecting specific crops can be achieved by SSR genotyping of DNA collected on FTA cards and this is now being used in the USA with a turn-around time of 24-48 h (Fry, 2015). Such an approach could be adopted in the UK and Ireland where currently population characterisation is done on an annual basis, but would require integration of extension and research programmes and additional funding.

In the longer term, while predicting some future changes in *P. infestans* populations may be possible, forecasting the emergence of new genotypes will be challenging. It remains unclear why some European and North American populations are clonal, despite the presence of both mating types, while other European populations have become recombinant. Ploidy differences may limit the success of sexual reproduction, but why in some in some geographic areas and not others?

ACKNOWLEDGEMENTS

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PPO – SPECIAL REPORT NO 17 – 2015, 45-50

Recent developments concerning the population biology and control strategies of *Phytophthora infestans* in the USA

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Late blight of potato and tomato has been sporadically important in the USA. Locally, there can be important epidemics annually, but there have been only two regional pandemics in the past several decades. In 1994/1995, the introduction of the US-8 clonal lineage caused major damage in most potato growing regions of the USA (Fry and Goodwin 1997; Johnson *et al.* 1997). In 2009, widespread distribution of the US-22 clonal lineage on tomato transplants led to a pandemic that was particularly hard on home owners and organic gardeners throughout northeastern USA (Fry *et al.* 2013).

The population of *P. infestans* in the USA continues to be dominated by relatively few clonal lineages (Hu *et al.* 2012; Fry *et al.* 2013). The most recent dominant strains are US-8, US-11, US-22, US-23 and US-24 (Fry *et al.* 2015) (Fig. 1). Individuals within a lineage are very similar to each other in most characteristics. However, there are important differences among lineages (Danies *et al.* 2013; Fry *et al.* 2015). For example, the most common lineages differ in terms of their response to mefenoxam, the most effective oomycete fungicide against sensitive strains (Fry *et al.* 1979). Mefenoxam is ineffective against resistant strains (Goodwin *et al.* 1996). From the mid-1990s to 2009, most clonal lineages in the USA were resistant to mefenoxam (Fry *et al.* 2015), so growers did not use mefenoxam during the mid-1990s to 2009 to manage late blight. We discovered in 2009 that the dominant lineage (US-22) was sensitive to mefenoxam and we've subsequently learned that some lineages dominant since 2009 have also been sensitive to mefenoxam (Hu *et al.* 2012; Saville *et al.* 2015). Thus knowledge of the lineage in a particular area provides crucially important information necessary to select the most effective management strategy.

Stimulated by the 2009 pandemic and supported by a USDA AFRI grant (from March 2011 through February 2016) a group of collaborators has combined to develop near real-time information on the populations of *P. infestans* in the USA. Microsatellite markers developed by Lees *et al.* (2006) were used to identify the clonal lineage of *P. infestans* in each sample that was submitted to a central laboratory for analysis. The specimens were sent mostly by collaborators via overnight courier and in the vast majority of cases the results were returned to the submitter within one or two days of receipt. The data are also reported on a national website (USAblight.org), which also contains a map illustrating the location (county) (Fig. 2). This

information was valuable to the submitter because each clonal lineage had reasonably consistent and unique fungicide resistance and host preference characteristics, which could help growers develop their management plans (Table 1) (Danies *et al.* 2013). During the AFRI project, use of the website increased dramatically each year, so that in 2014 alone, there were more than 25,000 visits to the website, which identified 349 active sites and more than 16,000 alerts were distributed to interested persons.

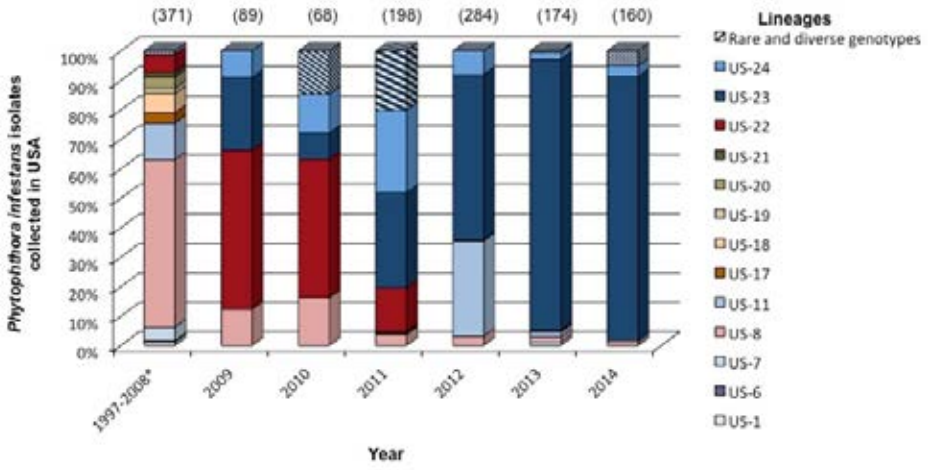

Figure 1. *Dominant clonal lineages detected in the USA from 1997 through 2014. * The data for 1997-2008 are from the Fry Lab; Hu et al 2012; and (Wangsomboondee et al. 2002); the data for 2009-2014 are from the Fry lab, the Ristaino lab and the USAblight consortium. The sample size for each year is indicated in parentheses at the top of each column. (This Figure and legend are from Fry et al. (2015)*

Table 1. *Phenotypic characteristics of the most common clonal lineages of Phytophthora infestans detected in the USA 2009-2014. (Data are from Childers et al 2015; Danies et al 2013, and Hu et al 2012)*

Lineage	Mating type	Host Preference	Mefenoxam sensitivity
US-8	A2	Potato	moderately resistant
US-11	A1	Potato and Tomato	resistant
US-22	A2	Potato and Tomato	sensitive
US-23	A1	Potato and Tomato	sensitive – moderately sensitive
US-24	A1	Potato	moderately sensitive

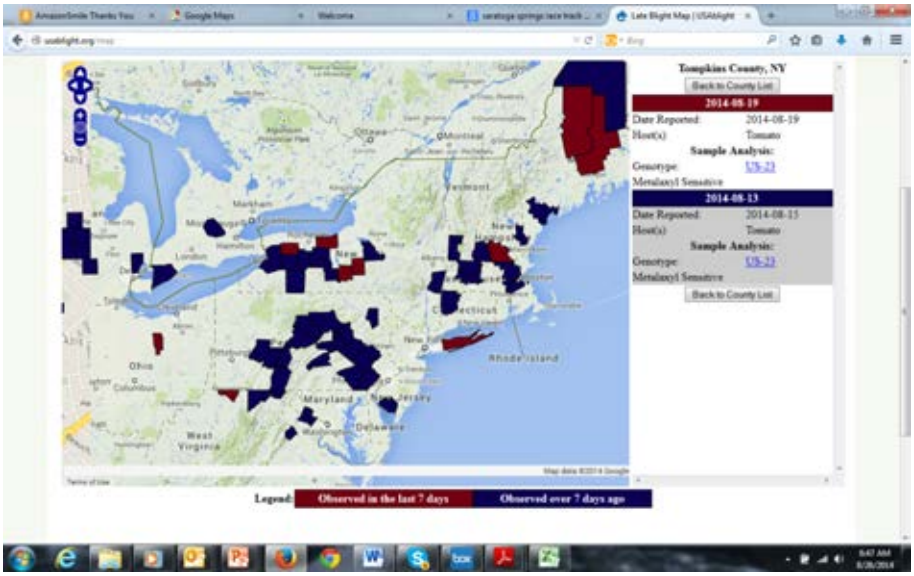

Figure 2. *Screen shot from USABlight.org/map from 19 August 2014. This map illustrates the reported occurrences of late blight during 2014. The most recent occurrences (in the previous 7 days) are indicated in maroon. This particular screen shot provides data for Tomkins County NY, and indicates that US-23 had been reported on 19 and 15 August 2014. The rapid identification of lineage and rapid reporting of location of late blight were crucial for precise informed management of late blight*

The USABlight site also has a link to a Decision Support System (DSS) for tomato and potato late blight management (Small *et al.* 2015a). The DSS is available on the web and integrates several models into a system that can be used to predict disease dynamics based on weather conditions, host-crop resistance, and management tactics. A grower identifies the location of her/his production unit of interest (field) and the system automatically obtains observed weather data from the nearest available weather station. The system also obtains forecast weather data from the National Weather Service - National Digital Forecast Database for that specific location. The DSS uses these weather data along with crop and management information to drive disease forecasting systems (Blitecast and Simcast). A validated mechanistic model of late blight generates location-specific management recommendations for fungicide application. An integrated alert system allows users to receive notification of upcoming critical thresholds via e-mail or text message. The DSS provides producers, consultants, researchers and educators with a tool to obtain management recommendations, evaluate disease management scenarios, or explore comparative epidemiology. In field and computer simulation experiments (Small *et al.* 2015b), DSS-guided schedules were influenced by prevailing weather and host resistance and resulted in schedules that reduced the amount of fungicide used by up to 50%. In situations with weather unfavorable to disease, the DSS recommended fewer fungicide applications with no loss of disease suppression. In situations of very favorable weather, the DSS recommended more fungicide applications but with improved disease suppression. On average the DSS improved the "efficiency" of each fungicide application. ("Efficiency" = the amount of disease suppression from each fungicide application.) The DSS provides an interactive system that helps users maximize the efficiency of their crop protection strategy by enabling well-informed decisions.

Knowledge of pathogen strain(s) in an area is very beneficial to late blight management. The simplicity of the population structure has been useful to the management of late blight in the USA (Fry *et al.* 2015). This is because the phenotype of most individuals within a lineage is relatively conserved. Characterizing the phenotype of an isolate can require weeks to months – particularly if one needs to work with the isolate in pure culture. However, determining the genotype of the pathogen from a sporulating lesion using simple sequence repeats [SSRs, or microsatellites] can be done in less than 24 hours. Thus, from knowledge of the phenotype of individuals in a lineage, we can typically predict the impact of certain management actions based on genotypic analysis of the pathogen in a sample. For example if tomato growers are aware of potato late blight in the area, and if they also know that the lineage causing potato late blight is US-8 or US-24, they can safely conclude that their tomato crop is not at great immediate risk – neither US-8 nor US-24 is particularly aggressive to tomatoes. In contrast, if the lineage on potato is US-23 they need to take immediate precautions because US-23 is very aggressive on tomatoes. Growers would also know that mefenoxam could be used to help protect their tomato crop because US-23 has been largely sensitive to mefenoxam in the USA. In contrast, if US-11 was on potato, then immediate action not involving mefenoxam would be necessary because US-11 has been consistently highly pathogenic to both tomato and potato and resistant to mefenoxam (Saville *et al.* 2015). Of course it is necessary to continually monitor the phenotypes of diverse strains to learn if strains with new epidemiologically important traits have appeared.

There are several reasons that it is important to continue monitoring populations in the USA. One reason is that new lineages can become dominant. New lineages have been introduced via migration (Goodwin and Drenth 1997) and via recombination (Gavino *et al.* 2000). While populations of *P. infestans* remain strongly clonal in the USA, two recombinant populations have been described since 1990. These populations have been ephemeral because after the initial detection, there has not been further production of recombinant individuals and most strains were not detected subsequently – probably because most of the recombinants were not as fit as the dominant genotypes. The first such recombinant population was detected in the Columbia basin of the Pacific Northwest in 1993 (Gavino *et al.* 2000). The authors postulated that the parents of this population were US-6 and US-7, and that one of the progeny was US-11 (Gavino *et al.* 2000), a lineage that has been very troublesome for more than 20 years. However, other progeny of this recombination event have not been detected for many years.

The second recombinant population has been reported recently from the northeastern part of the USA (Danies *et al.* 2014). The majority of isolates were detected in central/western New York State. These isolates were detected in 2010 and 2011, but not in 2012 or 2013. As in the Pacific Northwest in 1993, this population contained diverse individuals in a somewhat localized region and had great diversity for allele combinations based on analysis of allozymes, mating type, RFLPs, and microsatellites (Danies *et al.* 2014). The parents for this population were postulated to be US-22 (A2) and at least two other genotypes. Using a recent protocol that identifies at least 36 mitochondrial haplotypes, these individuals were all determined to have the same mitochondrial haplotype (H-20), the same haplotype as US-22 (Danies *et al.* 2014). As with the 1993 population, most individuals from this 2010/2011 population have not been detected since 2011 (Danies *et al.* 2014). However, these two reports of recombinant progeny in the USA demonstrate that sexual reproduction is possible in the USA and may happen again. Because recombinants will have different phenotypes from either parent, and because there's a chance

that one of them could become a dominant lineage, it's crucial to determine the phenotypic characteristics of any recombinant individual.

In summary, the population of *P. infestans* in the USA remains quite simple with a few clonal lineages dominating. Currently, US-23 is most dominant. Reports of the lineage in an area enables one to develop an efficient management strategy; this is because the phenotypes of the common lineages have been determined, so it is possible to predict phenotype from knowledge of lineage. A collaboration among investigators throughout the USA assures that this information is available via a public website. A web-based DSS enables further efficiencies by using local weather data and local weather forecasts to drive two late blight forecasters.

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PPO – SPECIAL REPORT NO 17 – 2015, 51-56

Recent developments concerning the population biology and control strategies of *Phytophthora infestans* in Asia and Africa

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INTRODUCTION

This is an overview of the population dynamics of *Phytophthora infestans* in sub-Saharan Africa and Asia. I attempt to give an short review of published historical efforts to study the population dynamics and also more recent developments, particularly related to standardization, data management and collaboration. Being more of a review, the paper does not follow the standard format with sections for methodology, results and discussion but is rather divided by themes.

POTATO IN AFRICA AND ASIA

Prior to looking at the *P. infestans* population in these regions, it is important to understand something of the dynamics of the pathogen's most widely grown host: potato.

Potato is now globally the fourth major crop in terms of total production and the third most important food crop (much of maize is used for feed). Nonetheless, while potato is widely grown, production is concentrated in certain areas, including Asia (Fig. 1). China and India are the first and second producers globally, and for this reason Asia is the region with the greatest potato production (Fig. 2). Potato production is not nearly as important in Africa but it is nonetheless widely grown and plays a very important role in poverty alleviation and food security on the continent (Low *et al.*, 2007).

BRIEF HISTORY OF *P. INFESTANS* POPULATION STUDIES IN ASIA AND AFRICA

There have been a flurry of papers on the population dynamics of *P. infestans* in China in the last few years. A Google Scholar search turns up 14 since 2011. However, only one attempts to make nationwide analysis (Li *et al.*, 2013); this may be due primarily to the fact that China is so big and potato production so extensive. An interesting characteristic of previously-mentioned paper is that it was the first report of the 13_A2 ('Blue_13') clonal lineage in China (discussed more below).

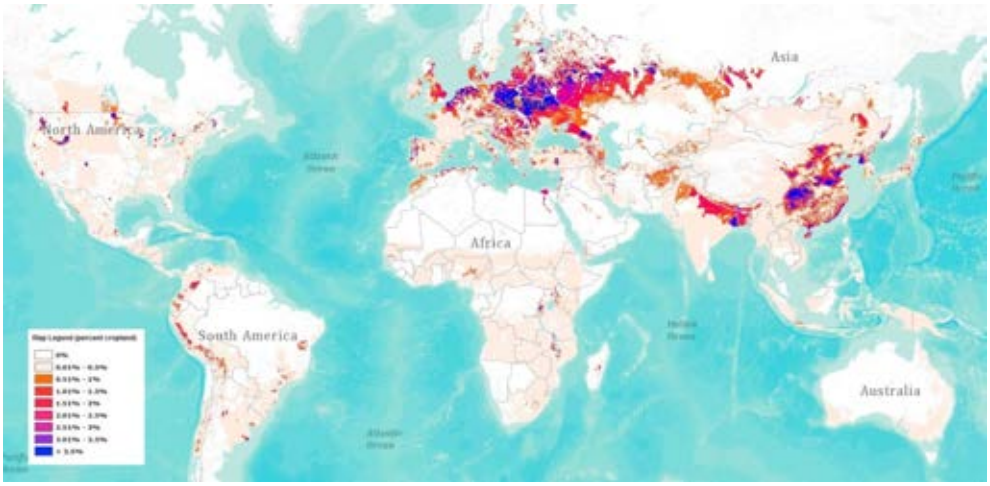

Figure 1. *Global potato production, source is RTBMaps (http://www.rtb.cgiar.org/RTBMaps/)*

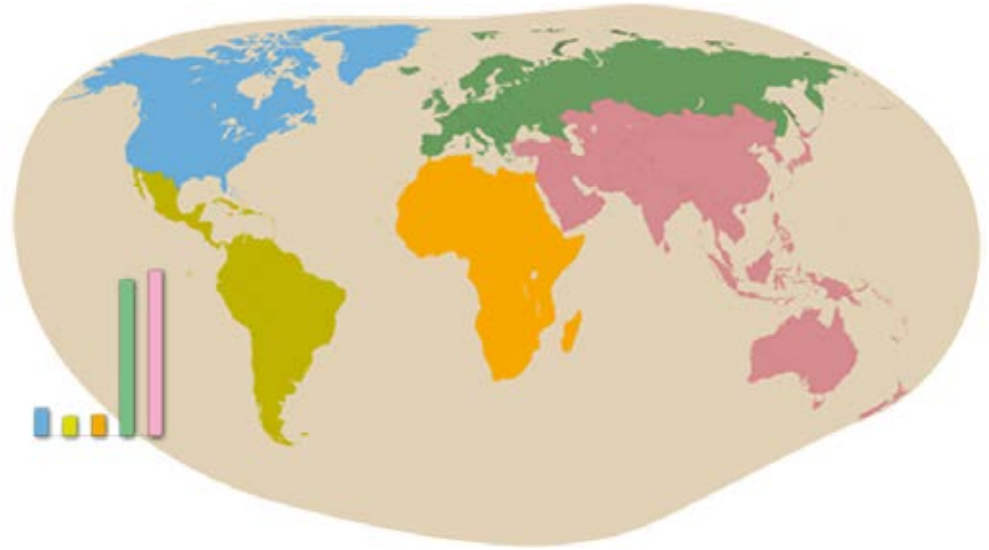

Figure 2. *Regional potato production. Source, FAO (http://www.fao.org/potato-2008/en/world/)*

Much less has been published about the pathogen population in other parts of Asia, with only a few articles looking at the population dynamics using markers that allow comparison across studies. Nonetheless, from these few it is possible to make some interesting observations. For example, while A2 phenotypes were detected relatively soon in China after their appearance in Europe (Zhang *et al.*, 1996), a study published in 2008 (Le *et al.*, 2008) from Vietnam indicated that the pathogen population in that country was still the "old" US-1.

Another important aspect of population studies in Asia is the growing evidence that the lineage A2_13 (blue_13), which has been problematic in Europe, is now relatively widespread in many parts of Asia as it has been reported in India (P. Chowdappa *et al.*, 2015), China (Li *et al.*, 2013) and in Nepal (D. Cooke, personal communication). Thus, it would appear that A2_13 is common through S. China, at least parts of the Himalayas and India. A recent set of studies from NW China indicates that this lineage is not present there (Tian *et al.*, 2015).

What little is known of the *P. infestans* population in E and SE Africa presents at least some enigmas. The earliest reports involving marker data indicated that at least some isolates collected in Rwanda were not US-1(Forbes *et al.*, 1998). However, since that time and until relatively recently, other reports indicated that the US-1 clonal lineage was dominant in eastern sub-Saharan Africa (Vega-Sanchez *et al.*, 2000; McLeod *et al.*, 2001). In contrast, it would appear that if the US-1 lineage was dominant at one time in Ethiopia, it has been displaced for more than a decade. Schiessendoppler & Molnar (2002) found no US-1 isolates in Ethiopia, but rather a population characterized by the mitochondrial DNA Ia haplotype.

In the last few years it appears that the situation in sub-Saharan Africa has been very dynamic. In collections done in 2007 in eight African countries (Burundi, Kenya, Rwanda, Tanzania, Uganda, Malawi and Mozambique and South Africa), only two fields in Kenya had a new clonal lineage, which the authors labeled KE-1 (Pule *et al.*, 2013); all the other isolates were US-1. Excluding the enigmatic reports about Rwanda noted above, and the one study from Ethiopia, this represented the first indication of a new clonal lineage in sub-Saharan Africa. The presence of a lineage in this region that was not US-1 was also made evident when isolates collected in 2009 were found to belong to an old European lineage known as 2_A1 (Were *et al.*, 2013). In that study, the 2_A1 lineage was found on more farms than was the US-1 lineage, indicating that if it was the same one found by Pule *et al*. (2013), than it appeared to have spread since 2007. Subsequently, Njoroge *et al*. (2015) demonstrated extensive spread of KE-1 (Fig. 3) and also that KE-1 and 2_A1 are identical.

COORDINATION, STANDARDIZATION AND DATA MANAGEMENT

To date there has been little effort to formally coordinate researchers in Africa around the theme of late blight, but there has been an effort to begin implementing data standardization and management. In general, due to a significant number of publications on the theme, and other publicity around Euroblight meetings and Web-based applications (www.euroblight.net), there is a growing understanding among researchers of the utility of using common markers which can allow comparison across studies. Consistent with this, some recent studies have been based on a set (or subsets) of simple sequence repeat (SSR) markers. This has allowed many data from Africa to be included in the Euroblight online database, which means that maps of the isolate locations are publically available.

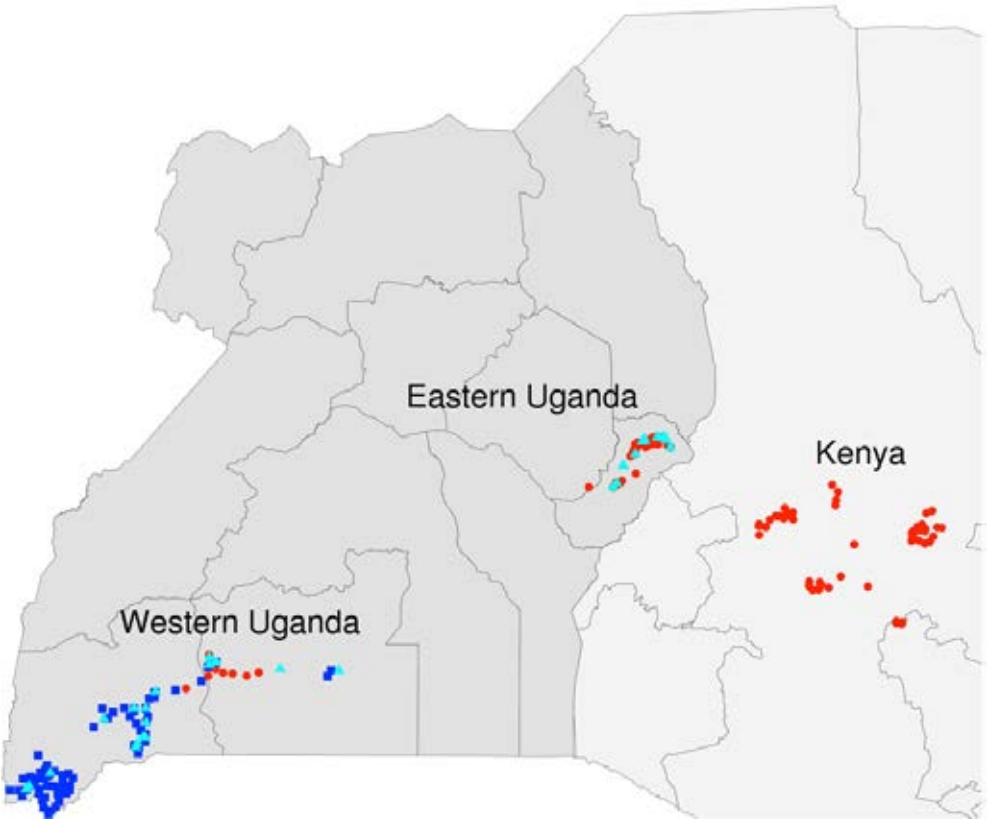

Figure 3. *The Phytophthora infestans population in Kenya and Uganda showing occurrence and distribution of US-1 and KE-1 lineages. Red circle = KE-1 from potato; blue square = US-1 from potato; cyan triangle = US-1 from tomato. (From Njoroge et al, 2015)*

Asia contrasts with Africa in that there has yet to be a standardization of marker approaches and data are not available in the Euroblight database, or any other common database. However, Asia also contrasts with Africa in that there have recently been several efforts to coordinate among researchers. Efforts for coordination in Asia began with a meeting of researchers in Nepal in late 2014. Twenty-five researchers attended this meeting, which was held in Dhulikhel just outside of Kathmandu, Nepal. The workshop was organized by the International Potato Center (CIP) and the National Potato Program of Nepal, and was sponsored by the Rural Development Administration of S. Korea and the CGIAR Research Program on Roots, Tubers and Bananas. Twelve countries were represented. The primary output of the workshop was the development of a roadmap to create a proposal for a region-wide network of collaboration on the subject of potato LB; the network was referred to as Asiablight.

Subsequent to the Nepal workshop, there was a meeting in Chongching, China, which focused on IPM of potato late blight. In that meeting, the idea of an Asia-wide network for late blight was further discussed and was highly appreciated by the participants.

This was followed by a special session of the 2015 Euroblight meeting in Brasov, Romania, in which regional late blight networks and global coordination were discussed. In that meeting Asiablight was discussed as well as USAblight and Latinblight (TizónLatino). More about the Brasov meeting is available on the Euroblight Website.

More recently the network got a more formal 'kick-off' at a meeting organized as a satellite to the World Potato Congress (WPC) in Yanqing, China, near Beijing (Fig. 4). About 40 people participated in the meeting, with about half from Chinese potato research groups and the rest from non-Chinese participants at the WPC. The meeting was designed to give participants a better understanding of how well Euroblight has worked, progress in Latinblight and a suggestion for initial activities in Asiablight. One important aspect of the meeting was the discussion of a project to get a course map for Asia of major genetic groups of *P. infestans*. The consensus was that this is a good activity for Asiablight because of it's feasibility and because it provides a clear opportunity for public and private sectors to work together.

Figure 4. *Asiablight satellite meeting at World Potato Congress held in Beijing in 2015*

COMMENTS

Efforts to 'globalize' the approach of Euroblight have gone on now for more than a decade. Implementing such networks in developing countries is difficult for a number of reasons, not the least of which is funding. One aspect that has led to sustainability in Euroblight is the private sector sees value in the network and thus supports. It would appear that a major challenge for making networks functional and sustainable in developing countries would be to find a model that provides a similar framework, i.e., one that allows both private and public sectors to benefit from the interaction.

One obvious shortcoming of the recent efforts to improve coordination among researchers in the developing world is the lack of emphasis on tomato. Tomato is an important crop in Latin America, Africa and Asia and undoubtedly plays an important role in the dynamics of the overall population of *P. infestans,* and potentially in disease management. Future efforts to develop these networks should include all important hosts of the pathogen.

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Assessment of genetic hotspots for *Phytophthora* resistance and their use as molecular markers in potato breeding

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SUMMARY
At present, only a few molecular markers are successfully used in potato breeding to select for quality and pathogen resistance (e.g. Song *et al.* 2008, Saderzeh *et al.*, 2006). Especially in resistance breeding the availability of reliable markers would help to accelerate the accumulation of different resistance loci in the potato genome (Schwarzfischer *et al.*, 2010). In our study we assessed published markers within six genetic hotspots for resistance against *Phytophthora infestans*. A variety trial with more than 150 cultivars and breeding clones was used to obtain resistance values (rAUDPC) for each cultivar. Results from genetic analyses showed that six markers produced distinct amplification products from resistance genes derived from wild *Solanum* species in all 150 clones. We then associated marker results with field resistance values. We observed that cultivars which were marker positive for S. *demissum* resistance genes R1 and R3b showed no higher resistance values than cultivars without *S. demissum* background. This result is consistent with observations, that resistance obtained from *S. demissum* R-genes have been overcome by *Phytophthora* pathotypes. However, all 13 clones in the trial set that showed positive marker results for the *S. bulbocastanum* gene *Rpi blb3* proved to be highly resistant against late blight. Interestingly, the majority of the clones, which were found marker positive for *Rpi-blb* genes, have neither *S*. *bulbocastanum* nor *S. stoloniferum* in their pedigree.

KEYWORDS
Potato resistance breeding, *Phytophthora infestans*, R-Genes, Molecular Markers

INTRODUCTION

In potato farming late blight caused by *Phytophthora infestans* poses one of the highest production risks. In conventional farming the problem is met by application of a range of potent pesticides. However, under organic farming conditions only copper based products are presently licensed on the market. Although these chemicals reduce late blight infection they rarely prevent infestation of susceptible cultivars completely. Consequentially, varieties grown under organic

farming conditions need to thrive under reduced pest management, increased mechanical stress and generally lower nitrogen availability than on conventionally managed fields.

To meet the demands of organic potato farmers the German Federal Ministry of Food and Agriculture has since 2012 promoted a joint project which is funded through the "Federal Organic Farming Scheme and other forms of sustainable agriculture" (BÖLN). The project brings together the German expertise in potato breeding: the Julius Kühn–Institut (JKI), the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), the Bayerische Landesanstalt für Landwirtschaft (LfL), and German potato breeding companies. The aim is to develop new suitable varieties for organic farming which show higher resistance to potato diseases and help reduce accumulation of copper on organically managed lands.

To tackle this task a variety of modern and historic cultivars from potato breeders and the IPK gene bank, respectively, are used to combine quality traits with the late blight resistance of JKI pre-breeding clones and other selected clones. Phenotypic results from a variety trial are used to choose suitable breeding partners. Breeding activities are carried out at the JKI and the LfL to produce a large number of potato seed. Where possible, the progeny is preselected using genetic markers for virus, nematode and late blight resistance.

A central aspect of the project is to select suitable clones in field trials under organic farming conditions. In a participative approach scientists and organic farmers have been choosing from more than 2,000 individuals per year to establish a late blight breeding pool for organic farming since the project started in 2012.

METHODS

Field trials

To assess late blight resistance a set of 150 cultivars and breeding clones was set up in a field trial. Material included LfL breeding clones, pre-breeding material from the JKI, historic cultivars from the IPK gene bank, cultivars from German potato breeders and selected varieties from other European potato breeders. Between 2012 and 2014 the trials were set up each year on three organically managed trial fields, two in Southern and one in Northern Germany as shown in Figure 1. The trials were arranged applying a randomized block design with 10 plants per plot and two replications per field. One trial was set up with fungicide protection to prevent late blight infection and determine the maturity of the cultivars. The five cultivars Anuschka, Ditta, Jelly, Lolita and Princess were used as reference. Phenotypic assessment of *Phytophthora* resistance was carried out under natural infection on the organically grown potatoes. During the epidemic late blight was documented using the percentage of disease-affected foliage (including dead leaves) once or twice a week. Resistance values were obtained calculating the relative area under disease progress curve (rAUDPC) and corrected for maturity using standard methods described in Truberg *et al.* (2009).

Marker assisted selection

To establish genetic markers for *Phytophthora* resistance breeding we analysed gene regions on six potato chromosomes (see Gebhardt *et al.*, 2006 and references within). Specific amplificates were obtained for markers shown in Table 1. Here we applied standard PCR procedures as described in the particular publications (Table 1) using Thermoprime Taq DNA Polymerase (Thermofisher). All amplificates were checked by sequencing through the LMU sequencing service (http://www.gi.bio.lmu.de/sequencing) and sequence analysis.

Figure 1. *Position of field trials. Yellow dots show the position of organically managed trials under natural infection.* ***Legend: UE*** *- Uelzen,* ***ND –*** *Neuburg/Donau,* ***FS*** *- Freising,* ***LL*** *– Landsberg/ Lech*

Table 1. *Overview over markers and gene regions assessed in this study*

Gene	Marker	Origin	Chromosome	Reference
Rpi-blb3	Blb3	*S. bulbocastanum*	4	Zhu *et al.*, 2012
Rpi-abpt	Abpt1	*Solanum* spec.	4	Kim *et al.*, 2012
R1	R1	*S. demissum*	5	Ballvora *et al.*, 2002
Rpi-blb1	Blb1	*S. bulbocastanum*	8	Fadina *et al.*, 2013
Rpi-sto1	Sto1	*S. stoloniferum*	8	Zhu *et al.*, 2012,
R3b	R3b	*S. demissum*	11	Rietman *et al.*, 2011

RESULTS AND DISCUSSION

Field trial

Between 2012 and 2014 six out of nine organically managed environments were used for evaluating late blight resistance of cultivars. Because of unfavourable weather conditions in 2013 and 2014 three trials were not infected by *P. infestans*. Estimation of resistance values showed between 83 % and 93 % identity with $R^2 = 0.67$ ($\alpha<0.99$) and $R^2 = 0.89$ ($\alpha<0.99$). Determination of maturity on the fungicide treated trial fields confirmed that material from all maturity indices were present. Late maturity and late blight resistance values could be shown to be positively correlated ($R^2 = 0.,12$; $\alpha<0.95$).

As shown in Figure 2 high resistance could be observed in all JKI pre-breeding clones as well as in a few selected cultivars i.e. Biogold, Vitabella, Bionica, Toluca, Carolus, Tübinger Circe, Saka 6, Axona, Kuras and Sarpo Mira. From the five cultivars Anuschka, Princess, Lolita, Ditta und Jelly which were used as internal standards, only Jelly displayed slightly better resistance values.

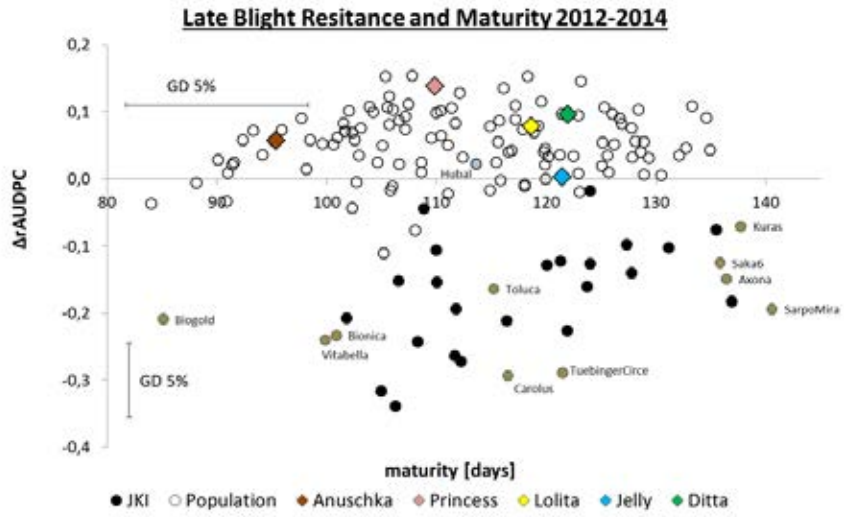

Figure 2. *Maturity corrected late blight resistance (ΔrAUDPC) vs. maturity obtained in field trials between 2012 and 2014 of JKI-prebreeding clones, populations, and standard varieties*

Marker-assisted selection

To find resistance donors in the breeding material which can be used by marker assisted selection (MAS), six potentially useful genome regions were analysed as shown in Figure 3. Distinct amplificates were obtained in all clones for markers Blb3, Abpt1, R1, Blb1, Sto1 and R3b.

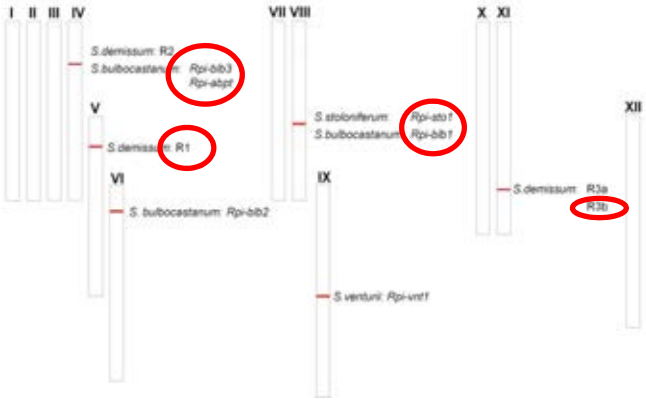

Figure 3. *Regions in the potato genome investigated as molecular markers. Rectangular bars depict chromoses, roman numerals indicates chromosome number. Markers with distinct sequence results are highlighted with red circles*

By correlating positive marker signals and high field resistance, cultivars carrying *S. demissum* derived markers showed no field resistance against *P. infestans* (see Fig. 4). Positive correlations were detected for markers derived from *S. bulbocastanum* and *S. stoloniferum* from two genome regions: Blb3 and Abpt1 (chromosome IV) and Blb1, Sto1 (chromosome VIII). Only one JKI pre-breeding clone was found marker positive for this gene region. As this clone also carries markers for other gene regions, no conclusions can be drawn.
Blb3 and ABPT were amplified from Biogold, Vitabella, Bionica, Hubal, Saka 6, Kuras and nine JKI pre-breeding clones. Interestingly, none of these marker positive JKI clones had *S. bulbocastanum* in their pedigree. Two of the Blb3/Abpt marker positive clones had a *S. stoloniferum* background. The remaining clones came from a mixed breeding background with wildtype genes from *S. andigena*, *S. phureja* and *S. demissum*. While there are no nucleotide differences between all obtained sequences from this marker, therefore *blb3* gene homologues seem to be present in some individuals of these wild species.

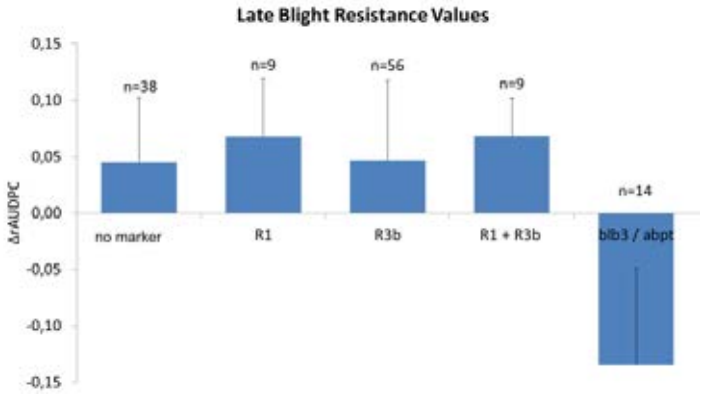

Figure 4. *Correlation between marker results and late blight resistance values. Results show that varieties carrying S. demissum derived genes R1 and R3b show no field resistance to present-day's population of P. infestans in these field trials. Breeding clones carrying BLB3 and ABPT, however, were highly resistant*

CONCLUSIONS

The field trials set up to evaluate late blight resistance showed that a representative number of German varieties and breeding clones revealed great differences in both, days to maturity and susceptibility against *P. infestans*. Many high-quality cultivars revealed a low degree of late blight resistance. However, pre-breeding material from the JKI and some selected varieties can be used as donors for late blight resistance in potato breeding. Gene regions found to carry resistance genes may be used as molecular markers. However, more markers need to be developed, analysed and evaluated within field trials. These markers need to target distinct wild type genes and produce single products from the potato genome. Markers found in this study which correlate with high field resistance will hopefully be evaluated in a future project using populations derived from breeding partners described in this study.

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Late Blight Prediction in Maine

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SUMMARY
Late blight is not controlled by predictions but is controlled by application of sound disease control principles. Late blight prevention in Maine starts with reducing initial inoculum to delay the onset of the epidemic. Elimination of dump piles, control of volunteer potatoes, and the use of seed treatments effective against seed-borne late blight are practices used to reduce initial inoculum. The potential for late blight to appear is predicted with the accumulation of severity values using the model NoBlight. As 70 severity values nears, late blight generally has reached detection levels. Tiers or risk areas are used to customize application materials in response to traditional inoculum sources. Partial to complete field destruction during the growing season has proven successful in some salvage situations.

KEY WORDS
Solanum tuberosum, late blight, prediction

INTRODUCTION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a devastating potato disease and has been around over 150 years with much written about it over that time. Late blight regularly causes loss in many potato production systems (Stark and Love, 2003). Late blight prediction in Maine consists of a combination of prevention, prediction, and spread prevention.

Late Blight Prevention

Late blight prevention in Maine focuses on reducing initial inoculum. *Phytophthora infestans* over seasons in infected tubers, cull piles, and in infected volunteer plants. Reduction of initial inoculum is the cheapest and most effective method of delaying the onset of disease epidemics and therefore reducing the impact of the epidemic. Reducing the initial inoculum effectively slows or delays the portion of the disease progress that is exponentially increasing. The exponential portion is the fastest rate of disease increase during a late blight epidemic and occurs where the disease proportion in the field increases by a million fold *e.g.* from 0.000001 to 1.0 percent disease incidence.

Elimination of dump piles is an effective means of reducing the initial inoculum. In Maine, late blight epidemics traced to dump piles are virtually unheard of, that is not the case everywhere

potatoes are grown. Dump piles include refuse plies from seed cutting operations as well as a disposal pile of unmarketable potatoes. Elimination of potato dump piles is part of a holistic approach to late blight control.

Volunteer potatoes, also called self-sown potatoes or ground keepers, are surviving over-seasoned potatoes from a previous potato crop. These too, can contribute to late blight epidemics, but again are more of an issue in areas that have less severe winters that Maine.

Potato seed treatments effective against seed-borne late blight are routinely used and have proven successful in Maine. The largest contributor to late blight epidemics in Maine is seed-borne inoculum. Seed infected with *P. infestans* establishes the pathogen in the host field. Epidemics initiated from seed-borne inoculum start early in the growing season and without warning. If seed-borne *P. infestans* is present in the field and moving up and sporulating on the stems, there is no control. These plants, and possibly the entire field, should be destroyed. The destruction will help protect other fields from inoculum spread; the field most likely would never have produced a marketable or storable crop. The most effective means of reducing seed-borne *P. infestans* is through reduced disease the previous season. Reduction of the amount of inoculum on the seed-potato crop has been the most successful approach to late blight control in Maine.

Late Blight Prediction

In Maine, the potential for late blight to appear is predicted with the accumulation of severity values using the model NoBlight (Johnson, 2006). The NoBlight model initiates accumulation of severity values starting at 50 percent plant emergence. Once 18 severity values have accumulated from emergence, the first spray is recommended. Subsequent applications are recommended based on additional severity value accumulation during the previous seven days (Johnson, 2006). NoBlight (Johnson, 2006) and Blitecast (Krause, Massie, and Hyre, 1975) both weigh relative humidity more heavily than rainfall in predicting the need for an application. Most late blight prediction models, including NoBlight, operate mainly in the logarithmic phase of the disease epidemic, which is after the exponential phase. Most late blight prediction schemes operate under the assumption that initial inoculum is present, but at very low levels. No prediction scheme will be successful with high levels of initial inoculum. A Smartphone app of NoBlight is currently being tested.

In years that late blight has been present, reporting of finds has occurred at or near a total accumulation of 70 severity values. Making the assumption that a 1 percent detection threshold has occurred, the epidemic has left the exponential phase and entered the logarithmic phase. More empirical than experimentally developed, using a 70 severity value threshold has been a good predictor of when late blight would be discovered in the region.

Growers can make informed decisions as the severity value accumulation nears this value. Should predicted weather keep the grower from being able to make the next application, it may prove beneficial to reduce the spray interval. A similar situation may also lead to a change in protection material.

An additional component of late blight prediction in Maine is the use of "tiers." Tiers are approximately 12 km divisions along the eastern edge of Maine. These demarcations, partially experimentally and partially empirically derived, are in response to traditional inoculum sources.

More frequent use of translaminar fungicides in the tiers closer to the traditional inoculum sources has proven very successful in delaying the onset of late blight epidemics in the region.

Late Blight Spread

The dilemma faced by grower is which field can be salvaged and which field should be destroyed. This technique has been highly successful in many situations and should always be a first consideration. Generally, the best value from this practice is on the first localized infections early in the season. If the disease is distributed at low levels in the field, crop removal is of limited value. If local inoculum is present and continually entering the field, that too, is a situation when crop removal is of limited value. As the disease is already present in the field, the goal is containment and limiting pathogen spread. Removing the hot spots in the field combined with chemical applications is far more successful than either practice alone.

Aside from the seed-borne late blight issue, once late blight is present in a field, most of the prediction models become less useful. In fact, late blight at a very close proximity should be treated similarly to late blight in the field.

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PPO – SPECIAL REPORT NO 17 – 2015, 67-68

DuPont™ Zorvec™ disease control: The first member of a novel class of oomycete fungicides

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Late blight caused by *Phytophthora infestans* remains one of the most important limiting factors in potato production, resulting in decreasing yields and affecting tuber quality. Applications of effective fungicides are an important part of an overall integrated pest management (IPM) control strategy for potato late blight. Characterizing and comparing the attributes and features of fungicides is critical to understanding best use in an effective late blight disease management program.

DuPont™ Zorvec™ is the global trade name for oxathiapiprolin (approved ISO common name), a novel fungicide recently discovered by DuPont and the first member of a new class of piperidinyl-thiazole-isoxazoline fungicides. It acts at a unique site of action in oomycete pathogens with no known cross-resistance to other fungicides. *In vitro* studies, scanning electron microscopy (SEM), and whole plant studies were conducted to characterize performance of oxathiapiprolin compared with current commercial fungicides used to control late blight. Studies have demonstrated: 1) high intrinsic activity against *P. infestans*, 2) an effect on multiple stages of pathogen development, 3) systemic movement within the host plant, 4) protection of new growth, and 5) quick rainfastness.
This combination of attributes allows oxathiapiprolin to provide consistent and reliable disease control, even under the most severe conditions. Oxathiapiprolin is highly effective for the control of *P. infestans* and other economically important oomycete pathogens at use rates much lower than current commercial fungicides. Its new mode of action makes oxathiapiprolin a valuable option for fungicide resistance management strategies, and its minimal impact on key beneficial organisms provides a strong fit within integrated pest management programs. A favorable toxicological and environmental profile, combined with low use rates, provides a new effective tool to potato growers.

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Impact of oils tank mixed with late blight fungicides on leaf blight control in three growing seasons

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SUMMARY

Two field trials were established in Ayrshire in each of the three years to compare the foliar blight control achieved by commonly used blight control products with and without the addition of the mineral oil Olie-H @ 3.1% of the spray volume. The first trial was to examine the effects at the rapid canopy expansion phase from rosette stage to full crop canopy. The second was targeted at canopy stable when full crop canopy had been achieved.

Olie H (and an alternative mineral oil, Newman Cropspray 11E, and the vegetable oil, Headland Fortune) consistently improved foliar blight control for the three fungicides Percos, Revus and Invader. In 12 out of the 15 comparisons involving these fungicides, the improvement in foliar blight control was statistically significant. However, the impact of oil added to fungicide on leaf blight was greatly influenced by the fungicide product used in the tank mix. In 2011 Olie-H had deleterious effects when tank mixed with Shirlan or Ranman TP. The formulation of Ranman TP was changed to Ranman Top by the manufacturer during the period of study. Foliar blight control with Olie H added to the mix was not detrimental for the replacement Top formulation. Seasonal variability in foliar blight control with added Olie H was evident in the results for Valbon + ZinZan, Ranman Top and to a lesser extent Infinito.

For 22 out of the 27 paired comparisons, adding oil to the blight fungicide did not significantly affect blight-free yield. For three comparisons the oil plus fungicide had significantly higher blight-free yields. For two comparisons yield was significantly reduced in the oil tank mix compared with the straight fungicide. Four out of these five tank mixes with a significant yield response to added oil had an associated statistically significant difference in foliar blight. The exception was the reduction in blight-free yield with the Valbon + ZinZan and Olie H mix.

Tuber blight incidences in the six trials ranged from very low to low and therefore prevented any conclusion regarding the impact of oil plus fungicide tank mixes on tuber blight control. However, there was no evidence that adding oil to blight fungicides had a substantial detrimental effect on control of this aspect of the disease.

Symptoms of phytotoxicity associated with oil use were only observed with one treatment, i.e. Shirlan + Olie H. A much more frequent and widespread effect was the beading of water droplets on the surface of leaflets treated with oil, resulting in delayed drying of leaf surfaces. Greater leaf blight severity due to this delayed drying is a possibility but this could not be specifically tested in the field trials. The predominantly positive response to added oil suggests that any such enhancement of leaf infection, if it occurred, was relatively small.

KEYWORDS
Foliar blight, *Phytophthora infestans*, mineral oil, vegetable oil

INTRODUCTION

The work described in this paper was part of a larger project "Effectiveness of mineral & vegetable oils in minimising the spread of non-persistent viruses in potato seed crops in GB". The aim of the overall project was to investigate the potential for mineral oils to be used as a control method for potyviruses (non-persistent viruses) in seed crops subject to the GB Certification system of growing crop inspection and control programmes for persistent viruses (Potato Leaf Roll Virus, PLRV) and potato late blight (*Phytophthora infestans*). One objective of the project was to evaluate the risk of oils tank mixed with fungicides to crop safety and fungicide efficacy. The part of the project reported in this paper is the effect of tank mixes of oils and common blight fungicides on levels of foliar and tuber blight, including the identification of any phytotoxic effects.

MATERIALS AND METHODS

Field trials were established at SRUC, Auchincruive Estate, Ayr to compare the blight control achieved by commonly used blight control products with and without the addition of Olie H @ 6.25 l/ha in a tank mix in 200 litres of water per hectare. In 2013 the oils Cropspray 11E and Fortune were also tested (Table 1). One trial was to examine the effects at the rapid canopy expansion phase and another was targeted at canopy stable. The distinction is necessary due to the differing nature of the target plant at these stages. Applications made at rapid canopy need to protect new leaf growth between subsequent applications. At stable canopy the fungicide product is required to protect existing leaf area. Also, applications of oil tank mixed with blight fungicides are generally only approved up to tuber initiation. A positive, neutral or undesirable effect on foliar blight or tuber blight activity associated with the introduction of mineral oil to the tank mix could occur. Fungicide products that are commonly used during these phases of growth were included in the trials with and without the addition of Olie-H to the tank mix (Table 2).

Table 1. *Details of oils*

Product	Dose (% of spray volume)	Ingredient	Dose (litres per ha)
Olie-H [1]	3.1%	96% mineral oil (petroleum oil)	6.25
Cropspray 11 E [2]	2.5%	99% paraffinic oil	5.0
Fortune[2]	0.5%	75.0 % Oilseed derived fatty acid esters + n-butyl	1.0

[1] Not yet approved in the UK

[2] May be applied up to tuber initiation

Table 2. *Details of fungicides.*

Fungicide	Active ingredient(s)	Formulation	Rate (kg or L/ha)
Percos	ametoctradin + dimethomorph	SC	0.8
Revus	mandipropamid	SC	0.6
Invader	dimethomorph + mancozeb	WG	2.4
Ranman Top	cyazofamid	SC	0.5
Infinito	fluopicolide + propamocarb	SC	1.6
Valbon (+ ZinZan)	benthiavalicarb + mancozeb	WG	1.6 (+ 0.075% v/v)
Ranman TP	cyazofamid	KL	0.2 + 0.15
Shirlan	fluazinam	SC	0.3
Quell Flo	mancozeb	SC	3.3

All trials were planted with the cv. King Edward, chosen for its susceptibility to potato blight; foliar blight resistance rating 3 and tuber blight resistance rating 4. Trials were inoculated with genotype 13_A2 (the stable canopy trials in 2012 and 2013 were naturally infected by this genotype). Small areas at both ends of all plots that were not treated with fungicide were inoculated to provide even disease pressure within each trial. The experimental design was a randomised complete block with four replicate blocks. Plots were 3.4 m (4 rows) x 7.5 m. Each trial was treated with test fungicide treatments at the appropriate growth stage. At other growth stages common blanket sprays were applied (Table 3). In the rapid canopy trials the one initial blanket spray was at the rosette stage of growth. All test treatments were applied in 200 l/ha of water at 3.5 bar using Lurmark F03-110 nozzles to provides a Medium – Fine spray quality. The sprayer was tractor-mounted.

Weekly assessments of foliar blight were made to document disease progress over time. The severity (percentage of leaf area destroyed by blight) was recorded. In addition visual observations were made of phytotoxicity symptoms and moisture retention within the canopy. Yield and tuber blight were recorded for the treatments. Tuber blight was assessed pre- and post-storage (rapid canopy 2011 and 2012; stable canopy 2011) or post-storage only (rapid canopy 2013; stable canopy 2012 and 2013). Tuber blight was assessed by external inspection

of two random samples of 50 tubers from each plot. The samples were taken at harvest and the assessment carried out after the tubers had been thoroughly washed.
Data from the blight control trials were subjected to analysis of variance using Genstat 15th Edition. AUDPC values were calculated after angular transformation of the foliar blight severity data for each plot.

Table 3. *Fungicide programme structure for the rapid canopy and stable canopy trials (named fungicides are blanket sprays)*

Rapid canopy 2011	**Rapid canopy 2012**	**Rapid canopy 2013**
Shirlan x 1	Curzate M x 1	Curzate M x 1
Test fungicides x 4	Test fungicides x 3	Test fungicides x 4
Shirlan x 4	Quell Flo x 5	Quell Flo x 5
Stable canopy 2011	**Stable canopy 2012**	**Stable canopy 2013**
Shirlan x 1	Curzate M x 1	Curzate M x 1
Consento x 3	Revus + C50 x 3	Consento x 3
Test fungicides x 6	Consento x 1	Test fungicides x 7
	Test fungicides x 7	

RESULTS

The AUDPC values for the 27 paired comparisons of fungicide alone versus fungicide plus oil were used to summarise the 3 years' results. Fungicide plus oil treatments were categorised as having either significantly greater efficacy, significantly worse efficacy or similar efficacy compared with fungicide alone. The same categorisation procedure was used to summarise the impact of oil mixed with fungicide on blight-free yield.

Over the 3 years, 14 out of the 27 tank mixes of oil plus fungicide tested resulted in significantly improved control of foliar blight. For 11 tank mixes the oil had no significant effect on foliar blight control by the fungicide partner. Only two combinations of oil plus fungicide significantly impaired blight control. These two tank mixes were Ranman TP (the Twinpack formulation that was withdrawn from the market during this study) + Olie H in the rapid canopy trial in 2011; and the Shirlan + Olie H tank mix in the stable canopy trial in the same year. The testing of these two tank mixes was therefore discontinued after the first year.

Some core combinations of oil plus fungicide were tested three times to check the repeatability of results in different growing seasons, i.e. Olie H with Infinito, Invader, Percos, Revus or Valbon + ZinZan. The effect of Olie H on the efficacy of individual fungicides was most consistent for Percos but also consistent for Invader and Revus (Table 4). In 2013, the final year of field experiments, tank mixes of an additional mineral oil, Cropspray 11 E, or a vegetable oil, Headland Fortune (Headland Diamond) with Invader, Percos and Revus were tested. The impact of these two additional oils on blight fungicide efficacy was consistent with that obtained with Olie H. When the three oils were compared tank mixed with Revus or Percos all three tank mixes were generally significantly more effective than straight fungicide. When Cropspray 11E and Fortune were compared tank mixed with Invader, Fortune significantly improved control of foliar blight compared with Invader alone whereas Cropspray 11E gave equivalent control to the

fungicide alone. However, because different oils were only compared in 2013 and only in the rapid canopy trial, conclusions regarding the relative efficacies of the different oils must be considered to be preliminary.

A similar summary of blight-free yield results over the 3 years demonstrated that for 22 out of the 27 paired comparisons, adding oil to the blight fungicide did not significantly affect blight-free yield (data not presented). Yield was only significantly reduced for two oil tank mixes compared with the straight fungicide. The first was Olie H plus Ranman TP in the rapid canopy trial in 2011. This reduction was related to the very poor control of foliar blight for the tank mix compared with Ranman TP alone. The second, Valbon + ZinZan + Olie H in the 2013 rapid canopy trial, was not associated with significantly poorer control of foliar blight. For three comparisons the oil tank mix had significantly higher blight-free yields. In all three cases the elevated yields were related to significantly better control of foliar blight. The three were Percos + Olie H and Revus + Olie H (both in rapid canopy 2012) and Revus + Fortune (rapid canopy 2013).

The incidences of tuber blight in the six trials were low (data not presented); so low in 2013 that the data were not analysed. In the four earlier trials the incidences of tuber blight did not differ significantly for 11 of the 14 paired comparisons. In the rapid canopy trial in 2012 the addition of Olie H to Ranman Top resulted in significantly less tuber blight. A similar result was obtained for Valbon + ZinZan + Olie H in the stable canopy trial in 2011. However, in the same trial tank mixing Olie H with Invader produced significantly more tuber blight. These limited results prevent a conclusion being reached regarding the impact of mixing oils with blight fungicides on tuber blight control. The impact of Olie H on tuber blight control by Ranman Top, Valbon + ZinZan and Invader can't be explained by the effect of the added oil treatment on foliar blight control by the respective treatments.

Symptoms of phytotoxic damage were only observed in the 2011 stable canopy trial for one treatment: Shirlan plus Olie H. However, a frequently seen effect attributable to mineral oil treatment was the beading of water droplets on the surface of leaflets.

Table 4. *Percentage improvement in foliar blight control contributed by tank mixing oil with fungicide*

Fungicide	Oil	% improvement[1]		
		2011	**2012**	**2013**
Percos	Olie H	**47.2**[2]	**44.6**	**36.4**
	Cropspray 11 E			**23.7**
	Fortune			**26.5**
Revus	Olie H	18.9	**47.9**	**47.9**
	Cropspray 11 E			**31.1**
	Fortune			**49.5**
Invader	Olie H	**42.4**	**29.0**	45.7
	Cropspray 11 E			12.2
	Fortune			**25.3**
Ranman TP	**Olie H**	**-188.1**		
Ranman Top	Olie H		**49.9**	-3.2
Infinito	Olie H	12.9	11.0	-42.1
Valbon + ZinZan	Olie H	6.1	**29.8**	-6.6

[1] Calculated from the relative AUDPC values for paired treatments of fungicide alone and fungicide plus oil. A negative value indicates poorer control by the tank mix of oil and fungicide.

[2] Values in bold are statistically significant ($P<0.05$)

Fungicides that were tested with one oil once only are excluded from the above table

DISCUSSION

Seasonal factors can influence the impact of using oil with blight fungicides. For example, in each of the three stable canopy trials Infinito was assessed with and without Olie H. Although the result obtained in each year was similar, i.e. oil did not significantly change the AUDPC, the percentage improvement, or decline, in foliar blight control was markedly different in 2013 compared with the two previous years. It can be hypothesised that spray timing relative to infection period will have a confounding effect.

The impact of oil on foliar blight control was influenced greatly by the fungicide product used in the tank mix. In 2011 Olie-H had deleterious effects when tank mixed with Shirlan or Ranman TP. The work with Shirlan was not continued in 2012 and the formulation of Ranman TP was changed by the manufacturer to Ranman Top. In contrast, the oils consistently improved blight control for the three fungicides Percos, Revus and Invader; in 12 out of the 15 comparisons including these fungicides the improvement was statistically significant. Much greater variability in control was evident in the results for Valbon + ZinZan, Ranman Top and to a certain extent Infinito.

The reason for the variability with some fungicides can't be determined from the dataset generated by this study. Additional experiments conducted under controlled environments are required to provide the explanation. In theory there should be greater variation in response to added oil from fungicide products with two active ingredients compared with one. This is because the two a.i.s are likely to have different characteristics and also the oil may differentially enhance the contribution of the two active ingredients. However, there is no evidence that this is the case. Although inconsistent control of foliar blight occurred for oil mixed with Infinito (fluopicolide + propamocarb) and Valbon (benthiavalicarb + mancozeb) + ZinZan, it was also evident for Ranman Top (cyazofamid). Foliar blight control was considerably more consistent for oil plus Invader (dimethomorph + mancozeb), Percos (ametoctradin + dimethomorph) and Revus (mandipropamid).

Very different results for foliar blight control were obtained for Olie H with Ranman TP in 2011 and Ranman Top in 2012. Differences between the blight epidemics in 2011 and 2012 will account for some of this difference but it is likely that much of the discrepancy was due to formulation differences between the two fungicide products. Both products provided 80 g of the fungicide cyazofamid per hectare.

Given the observed importance of the formulation of fungicide products to oil impact, it is important to acknowledge the limitations of the data set generated by this study. This study compared fungicide alone with fungicide plus oil. It can't be assumed that the results observed in these experiments will necessarily be replicated if oils are tank mixed with mixtures of blight fungicide plus for example aphicides. The formulation of, and adjuvant in, an aphicide are likely to differ from those of the blight fungicide. Previous work conducted by Scottish Agronomy Ltd found that tank mixing mineral oil with Biscaya (a neonicotinoid insecticide) should not be carried out due to incompatibility in formulation and the occurrence of phytotoxic symptoms.

The oils Cropspray 11E and Headland Fortune were only tested in one trial in one year with three fungicides. The results were similar to those achieved with Olie H, the oil tested most extensively. However, it should be noted that Cropspray 11E and Headland Fortune were tested in tank mix with the three fungicides that responded most consistently to mixture with Olie H.

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Strategies for the control of early blight (*Alternaria solani* & *A. alternata*) in Denmark

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SUMMARY
Field trials were performed in order to test the effect on early blight (*Alternaria solani* & *A. alternata*) of two sprayings compared with four and six sprayings at full and reduced dosages. Due to the dry July 2014 development in attack was not seen until the end of August with severe development in September. However, the attack was still sufficient to evaluate the different spray strategies. Since the development in attack came relatively late it was the strategies with sprayings late in the season that gave the best results for example in spray strategies with 4-6 sprayings which also had a high impact on yield. In average of two trials 2014 there was a tuber yield increase of 10%-19% after the different sprayings. In 11 trials 2010-2014 there was a tuber yield increase of 6.8% on average of the various treatments.

KEYWORDS
Potato early blight, *Alternaria solani, A. alternata,* control strategies, 2-6 sprayings, reduced and full dosages

INTRODUCTION

In recent years there have been many discussions on how the control of early blight should be targeted. Often the strategies have been supplementing the control of late blight (*Phytophthora infestans*) at the beginning of the season but now more focus is on extending the period during which the plants are protected to ensure a high yield potential. Field trials have been performed during the years (Bødker, 2014; Nielsen, 2013 and Nielsen 2014) and trial results from 2014 will be presented in this paper.

MATERIAL AND METHODS

Field trials with control of early blight were carried out in cooperation between Aarhus University and SEGES (Danish Advisory Service) at three locations (Flakkebjerg, Sunds and Billund). The potatoes were planted at the end of April 2014 and emerged at the end of May. The Alternaria trials were artificially inoculated at Flakkebjerg and Billund on 27th June with autoclaved barley

seeds inoculated with *A. solani* and *A. alternata* placed in the furrow between the plants. Each plot was scored as a whole for % disease severity (percentage coverage of all green leaves; EPPO guideline PP 1/2 (4), 2012).

DEVELOPMENT OF EARLY BLIGHT (*ALTERNARI SOLANI* & *A. ALTERNATA*)

Attacks of early blight are seen every year in the Danish potato fields, especially in the starch varieties with a long season. In many locations the first very small symptoms are seen from mid-July with disease development in August.

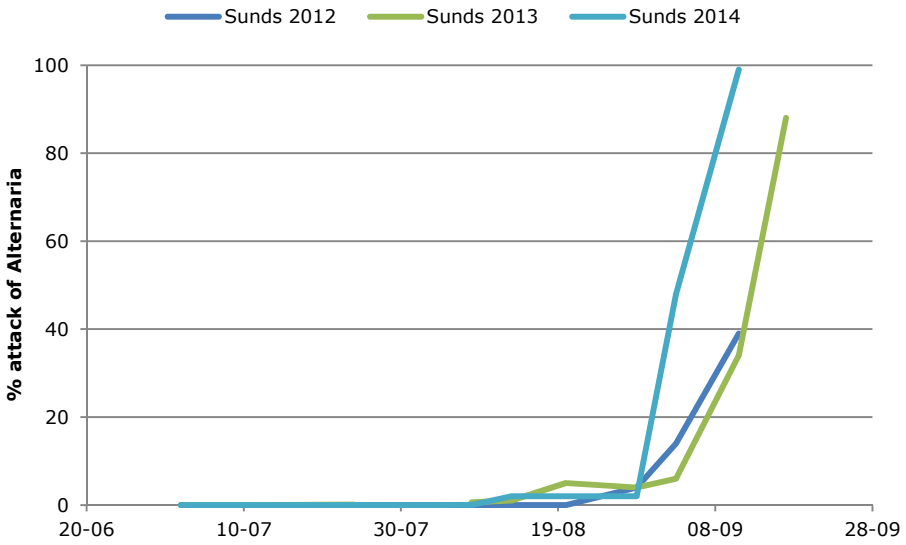

Figure 1. *Development of early blight (Alternaria solani & A. alternata) in untreated plots at Sunds (West Jutland) 2012-2014. Natural infestations. Variety Kuras*

Normally the epidemic development takes place in late August when severe attacks can be seen if the potato plants are not sprayed. Figure 1 shows the development of early blight in untreated field plots at Sunds in the western part of Denmark during the last three years in a typical potato growing area under natural infestations. The development in attack of early blight was almost similar in 2012-2014.

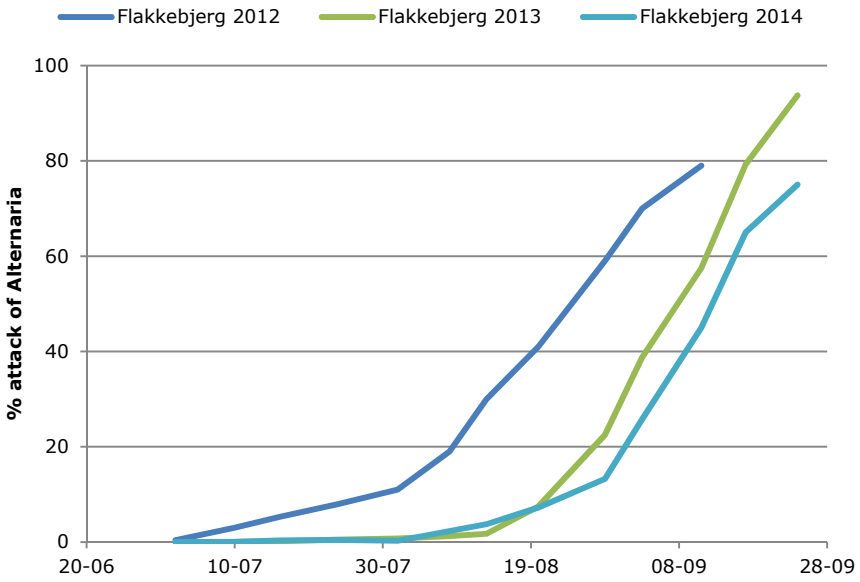

Figure 2. *Development of early blight (Alternaria solani & A. alternata) in untreated plots at Flakkebjerg 2012-2013. Artificial inoculation by inoculated barley seeds at the end of June. Variety Kuras*

At Research Station Flakkebjerg (100 km south-west of Copenhagen) the field plots are artificially inoculated and here the attacks of early blight are seen earlier in July (Fig. 2). The inoculation (as described under Materials and Methods) takes place at the end of June, and the first symptoms can be seen 7-10 days later.

The development in early blight at Flakkebjerg in 2014 was similar to the development in 2013 when the weather conditions in July also were dry but later than the development in 2012 (Fig. 2). Figure 3 shows the development in early blight at the three different locations.

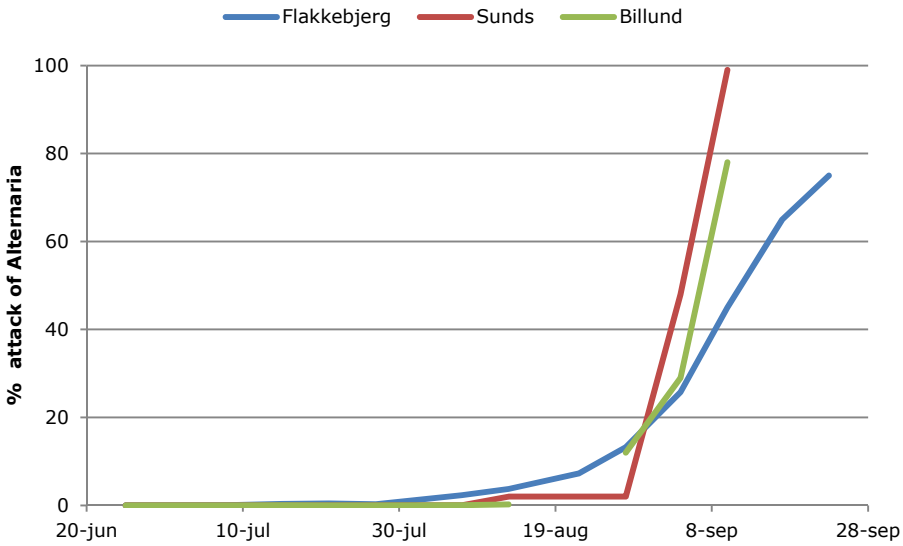

Figure 3. *Development of early blight (Alternaria solani & A. alternata) 2014 in untreated plots at Flakkebjerg, Sunds (West Jutland) and Billund (Central-Mid Jutland). Artificial inoculation at Flakkebjerg and Billund. Natural infestations at Sunds. Variety Kuras*

RESULTS

Field trials were performed in order to test the effect of two sprayings compared with four and six sprayings at full and reduced dosages. The start of the sprayings was at the very first symptoms at the beginning of July but early start before symptoms were observed was also tested. The trial set-up can be seen in Table 1.

Table 1. *Trial plan for testing different control strategies against early blight (Alternaria solani & A. alternata). Variety Kuras, 2014. Actual dates for the sprayings are indicated for the trial at Flakkebjerg. Set-up and the weekly spraying were almost the same in the trials at Billund and Sunds*

	17-jun	25-jun	01-jul	08-jul	15-jul	22-jul	29-jul	05-aug	14-aug	21-aug	28-aug	03-sep	09-sep
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Untreated*												
2			0,5 A		0,5 A								
3			0,3 A		0,3 A								
4			0,25 S		0,25 S		0,25 S						
5			0,25 S		0,25 S		0,25 S		0,25 S				
6			0,15S		0,15S		0,15S		0,15S				
7	Tridex	Tridex	0,15S		0,15S		0,15S		0,15S				
8			0,6 RT		0,6 RT		0,5 A		0,5 A				
9	0,6 RT		0,6 RT		0,5 A		0,5 A						
10			0,15S		0,15S		0,3 A		0,3 A				
11			0,15S		0,15S		0,3 A		0,3 A		0,15S		0,15S
12			0,075S		0,075S		0,15A		0,15A		0,075S		0,075S
13				0,15S		0,15S		0,3 A		0,3 A			

Tridex (2.0 kg/ha), RT: Revus Top (0.6 l/ha). 0.5A, 0.3A and 0.15A: Amistar 0.5 l/ha, 0.3 l/ha and 0.15 l/ha. 0.25S, 0.15S and 0.075S: Signum WG 0.25 kg/ha, 0.15 kg/ha and 0.075 kg/ha. All plots cover sprayed with Revus (0.6 l/ha) or Ranman Top (0.5 l/ha) at weekly intervals. Artificial inoculation at the end of June at Flakkebjerg and Billund, see Materials and Methods for details.

The results from the three trials can be seen in Figure 4 in which % leaf area attacked at the beginning of September is shown.

In 2014 the strategies in which most of the fungicide input was made in the first part of the season (e.g. 2 x Amistar) had the lowest effect (Fig. 4). However, it is interesting to note that the effect of the two early sprayings lasted approximately until the first week of September (6 weeks), but could not reduce the late attacks in September. Comparing the sprayings with Signum WG there was a clearly better control, using 4 sprayings than 3 sprayings and only small differences between 4 x 0,25 kg/ha and 4 x 0.15 kg/ha. Comparing the strategies using Revus Top, it seems that the first spraying at T1 (plot 9 in Table 1) was placed too early in relation to the actual disease development. Because of the relatively late start of the epidemic at the beginning of August there was in general a good effect of 6 sprayings. 2 x Signum WG 0.15 kg/ha + 2 x Amistar 0.3 l/ha + 2 x Signum WG 0.15 l/ha (60% dose level) gave a very high control level. Reducing this input to 30% dose level had an almost similar high effect. More details can be seen in Bødker (2014) and Nielsen (2014).

The results are similar to the results from 2013 when 2-4 treatments were compared. Best effect in 2013 was achieved with four treatments with either 4 x Signum WG or 2 x Revus Top + 2 x Amistar (Nielsen, 2013).

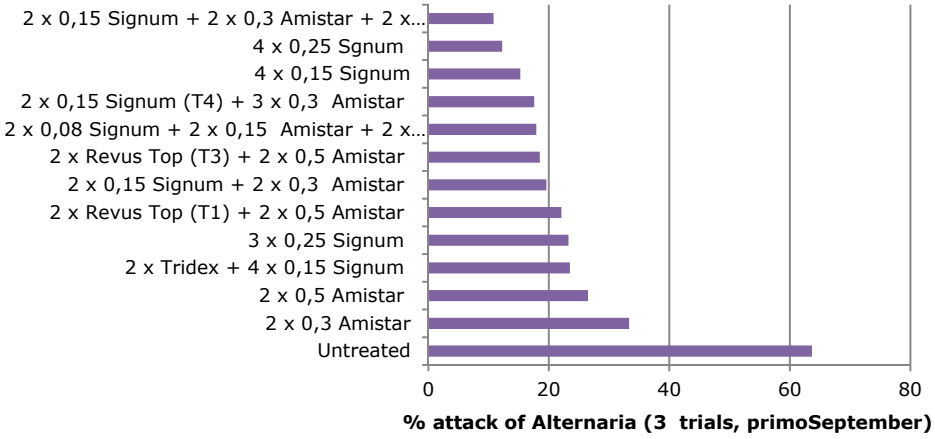

Figure 4. *Atack of early blight (Alternaria solani & A. alternata). Average of assessments at the beginning of September in 3 trials (Flakkebjerg, Sunds and Billund). The trial treatments have been arranged according to level of control. See Table 1 for details. Variety Kuras, 2014*

In average of two trials (Flakkebjerg and Sunds, the trial at Billund was not harvested) there was a tuber yield increase of 10%-19% after the various sprayings. The economy calculations in the different spray strategies showed that there was a high net yield increase relative to untreated from DKK 4,473 to DKK 7,380 Kr (15%-25% net yield increase). In 11 trials 2010 and 2012-2014 there was a tuber yield increase of 6.8% on average of the various treatments with a net yield increase of DKK 3.084-3.158 by using effective treatments (Bødker, 2014).

CONCLUSIONS

The trials with control of early blight (*Alternaria solani* & *A. alternata*) were performed in trials both with artificial inoculation and under natural infestations. Due to the dry July 2014 development in attack was not seen until the end of August with severe development in September. Although the start was relatively late in 2014 the level of attack was still sufficient to evaluate the different spray strategies. Since the development in attack came relatively late, it was the strategies with sprayings late in the season that gave the best results for example in spray strategies with 4-6 sprayings which also had a high impact on yield both in tubers and starch.

The results shows good effect of the late sprayings. What is needed now is more information on the start of the early blight treatments and the timing of the first spraying in order to optimize the length of the control period.

In average of two trials, 2014 there was a tuber yield increase of 10%-19% after the different sprayings (15%-25% net yield increase). In 11 trials 2010 and 2012-2014 there was a tuber yield increase of 6.8% on average of the various treatments with a net yield increase of DKK 3.084-3.158 by using effective treatments.

The results show that extending the control period with several sprayings had a high impact on the disease but it could also lead to an increased selection for fungicide resistance. It is crucial that anti resistance management is part of the spray strategy and includes fungicides with different modes of action.

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Study of the epidemiology of *Alternaria alternata* on potato

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SUMMARY

The involvement *of A. alternata* in early blight is discussed controversially. Brown spots developing on leaves of potato cultivar Markies are nevertheless often attributed to an *A. alternata* infestation. These symptoms appeared on Markies plants on two independent occasions in the greenhouse without any inoculation. In 75% of the necroses, neither *A. alternata* nor *A. solani* were present, which was confirmed by qPCR. Inoculation of Markies plants with *A. alternata* isolates derived from the same cultivar failed. Brown spots were also observed in field trials with Markies, but qPCR data did not indicate *A. alternata* as the causal agent of these necroses, either in the untreated control, in an artificial inoculation with *A. solani*, or in a double Ortiva® treatment. DNA amounts for this fungus were always low (< 2ng fungal DNA/µg total DNA) and *A. solani* predominant (up to 138 ng fungal DNA/µg total DNA). The Markies brown spots may have physiological causes.

KEYWORDS

Alternaria alternata, *Alternaria solani*, pathogenicity, disease progress, qPCR, potato cultivar Markies

INTRODUCTION

Early blight caused by *Alternaria* spp. is a problem in many potato growing areas of the world. In Germany, both *A. solani* and *A. alternata* can be found on early blight symptoms of potato leaves (Leiminger *et al.*, 2014). Although there is consensus about *A. solani* being a causal pathogen, the impact of *A. alternata* is discussed controversially. Stammler *et al.* (2014) doubt its role as a causal agent, and suggest it being rather a secondary invader which lives saprophytically on lesions and is therefore often isolated from leaf spots. In contrast, Droby *et al.* (1984) and Shtienberg (2014) state the pathogenicity of *A. alternata* towards potato leaves and the development of brown spots on the lower side of potato leaves after inoculation.

The potato cultivar Markies is a widely-used starch potato cultivar in Germany and there are many reports about the sudden appearance of a large number of small brown spots on its

leaves. Since *A. alternata* can almost always be detected on them, they are often attributed to an *A. alternata* infection, and Markies is considered to be highly susceptible to this fungus.
Markies was therefore used for inoculation experiments with *A. alternata* in the greenhouse. The disease progression of both *A. solani* and *A. alternata* was compared in a field trial with a naturally infected control, an artificial inoculation with *A. solani* and a fungicide treatment. To quantify the presence of both species in the crop, leaves were analyzed with a qPCR.

MATERIALS & METHODS

Artificial inoculation in greenhouse and field trials
Isolates of *A. solani* and *A. alternata* were grown on SN-Agar (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$ * $7H_2O$, 0.5 g KCl, 0.2 g Glucose, 0.2 g Saccharose, 0.6 ml 1n NaOH, pH 5.5, 20 g Agar-Agar, 1 l dist. H_2O) at 22°C and 12 h NUV-light for two weeks. For greenhouse experiments, eye cuttings of cultivar Markies were planted in Einheitserde Typ T, 12 cm pots and grown at ca. 20–22 °C in a greenhouse cabin. Spray inoculation was carried out with a spore solution (dist. H_2O + 0,05% Tween 20) containing 10^5 conidia/ml, applied till run off.
For field trial inoculation, sterilized shredded rye kernels were inoculated with spore solution, incubated for two weeks at 22°C and 12 h NUV-light, and scattered between the potato plants (cultivar Markies) on the hills.

Isolation from potato leaves
To verify the presence of *A. solani* and *A. alternata* on leaves showing symptoms, they were surface sterilized in 3% NaOCl for 1 min, washed in sterile distilled water, and pieces of leaf tissue showing necroses were cut out and placed on SN-Agar. After a 2–3 day incubation at 22°C and 12 h NUV-light, plates were examined for conidia formation.

Genomic DNA extraction
For DNA extraction, 10 leaves/plot and leaf level (middle and upper) were randomly collected, washed for 3 min in running tap water and ground in liquid nitrogen. DNA was extracted according to Fraaije *et al.* (1999) with modifications.

Real-time PCR
The primer pairs used for quantification of *A. solani* and *A. alternata* DNA were developed by Leiminger *et al.* (2014) and Schuhegger *et al.* (2006), respectively. The real-time PCR reaction mixture consisted of 10 µl Maxima SYBR Green qPCR Master Mix (2x) (Thermo Scientific), 0.3 µM each primer, 0.5 µl BSA (20 µg/µl) and 40 ng DNA in a 20 µl volume for both species. The 2-step PCR was performed in a Mx3005P QPCR system (Stratagene) with an initial step of 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing/elongation at 61°C for 1 min.

RESULTS

Greenhouse experiments (2013)
For the *A. alternata* inoculation experiments, 6-week-old eye cuttings of Markies should be used. However, shortly before the time of inoculation, around the beginning of flowering, the plants suddenly showed lesions on the middle and upper leaves that looked like an *Alternaria*

infestation (Fig. 1a,b). As the temperature in the greenhouse cabin in which they were grown was regulated by opening and closing the windows, a natural infection of the possibly highly susceptible cultivar by windspread inoculum couldn't be excluded.
Consequently, a second batch of Markies eye cuttings was grown in a closed cabin with air conditioning. But again, the upper and middle leaves of the nearly 6-week-old plants suddenly developed symptoms similar to early blight before they could be inoculated (Fig. 1c).

Figure 1a, b. *Symptoms on 6-week-old, non inoculated Markies plants in an open greenhouse cabin (11 June 2013)*
c. *Symptoms on 6-week-old, non-inoculated Markies plants in a closed greenhouse cabin with air conditioning (10 July 2013)*

As the involvement of relevant amounts of external inoculum could be excluded in the second batch, 40 leaf areas with symptoms were cut out, put on SN-agar and incubated at 22°C and 12 h NUV-light, to check if *Alternaria* spp. were actually present on them. However, only on 10 out of the 40 necroses *A. alternata* could be found, and *A. solani* on 5. Thus 75% of the symptoms were completely free of *Alternaria spp.*
A qPCR analysis of the leaf material confirmed these results: for *A. alternata* an extremely low DNA content of 1.48 pg DNA/µg total DNA was detected, and for *A. solani* a very low content of 88.58 pg DNA/µg total DNA.
It is discussed that wounds could provide an entrance for *A. alternata* and therefore enhance the infestation with the fungus. To test this hypothesis, the plants showing symptoms were inoculated with *A. alternata* isolates gained from the first batch of Markies plants. However, no

increase in leaf necrotisation could be observed; both inoculated and non-inoculated plants had 17% necrotic leaf area (NLA).

Field trials (2014)

To compare the disease progression of *A. alternata* and *A.solani*, a field trial with cultivar Markies was conducted.

This consisted of three treatments: control (natural infection, no fungicide treatment against early blight), artificial inoculation with *A. solani* (27 June, no early blight specific fungicide treatment) and fungicide treatment with Ortiva® (0.5 l/ha, 27 June + 31 July).

Visual assessment and rating of the necrotic leaf area was done at weekly intervals for the middle and upper leaf level. The real extent of leaf tissue colonization by both fungi was determined by qPCR for three timepoints.

The often reported sudden appearance of brown "A. *alternata*" spots on the leaves of Markies was observed in this trial (Fig. 2), independent of the treatment.

Figure 2a. *Brown spots on leaves of cultivar Markies in the field (5 July 2014, Ortiva® treatment 27 June 2014)*

b. *Brown spots on leaves of cultivar Markies in the field (11 Sept 2014, Ortiva® treatment 27 June and 31 July 2014)*

The *A. solani* qPCR results of the first date (Table 1, 31 July) show that both the artificial inoculation and the fungicide treatment were successful: the *A. solani* DNA content in the middle leaf level was highest in the inoculated plots (100.13 ng/µg total DNA) and very low in the fungicide treatment (0.5184 ng). 4.39 ng of *A. solani* DNA in the control indicates that the natural infection hasn't reached its epidemic phase yet, and was still at a low level. DNA contents of 0.0287, 0.0441 and 0.1174 ng in the upper leaves show that the fungus hasn't spread to the upper parts of the plants yet.

Table 1. *qPCR and visual necrotisation assessment data, field trial, cultivars Markies and Jelly, 2014 (DNA = ng fungal DNA/µg total DNA, % NLA = % necrotic leaf area, middle = middle leaf level, upper = upper leaf level, n.a. = not analyzed)*

	31.7.			14.8.			4.9.		
Cultivar, treatment, leaf level	A. a. DNA	A.s. DNA	% NLA	A. a. DNA	A.s. DNA	% NLA	A. a. DNA	A.s. DNA	% NLA
Markies, control, middle	1,1738	4,3900	14,5	0,7553	75,13	23,8	n.a.	n.a.	78,5
Markies, control, upper	0,0304	0,1174	1,4	0,1771	0,9265	6,3	1,1903	35,43	23
Markies, inoculated, middle	0,4681	100,13	23,3	1,0391	138,75	54	n.a.	n.a.	94
Markies, inoculated, upper	0,0071	0,0441	0,8	0,1570	0,6971	11,4	1,6003	57,13	30
Markies, fungicide, middle	1.9270	0,5184	10,1	1,1019	3,1798	16,6	n.a.	n.a.	49,5
Markies, fungicide, upper	0,0066	0,0287	0,8	0,1927	0,0525	8,7	0,9226	5,6118	25
Jelly, middle							0,2903	201,63	24,5
Jelly, upper							0,1682	114,38	12,5
Markies, greenhouse	0,0015	0,0886	17						

Two weeks later (Table 1, 14 August) the DNA amounts in the middle leaf level have increased in all treatments, but were still highest in the inoculation (138.75 ng/µg total DNA) and lowest in the fungicide treated plot (3.1798 ng/µg total DNA), indicating the continuing effect of the inoculation and the efficacy of the second Ortiva® treatment. On the upper leaves the colonization with *A. solani* was still low and on almost the same level in the control and the artificial inoculated plots (0.9265 ng/µg respectively 0,6971 ng/µg total DNA). This shows that both natural and artificial infection haven't progressed to the upper leaves yet. The second Ortiva® treatment resulted in the lowest DNA content in the fungicide treatment (0.0525 ng/µg total DNA).

A further two weeks later (Table 1, 4 Sept) the middle leaves couldn't be analyzed anymore. Due to their progressed necrotisation it wasn't possible to extract sufficient clean DNA. However, *A. solani* had spread to the upper leaves by this time. A slightly higher DNA amount of 57.13 ng/µg total DNA in the inoculated treatment compared to the control (35.43 ng/µg total DNA) indicated the continuing impact of the artificial inoculation. With 5.6118 ng/µg total DNA, the effect of the double Ortiva® treatment was still clearly visible in the fungicide plots.

The course of the leaf necrotisation progress (% NLA, Table 1) was similar to that of the *A. solani* DNA content. On 31 July for the middle leaves the highest value was found in the inoculated plots (23.3% necrotic leaf area), and the lowest in the fungicide treatment (10.1% NLA). However, one wouldn't expect a 10% leaf area necrotisation in a fungicide treated crop, compared to 14.5% in the untreated control. In addition, an *A. solani* DNA content of 0.5184 ng/µg total DNA was clearly indicating that the *A. solani* leaf infestation was negligible as a cause of this necrosis. The extent of necrotisation on the upper leaves was equally low in all three treatments (1.4, 0.8 and 0.8% NLA), but to high to be caused by the low *A. solani* colonization detected with the qPCR.

By 14 August (Table 1), an increase in necrotisation could be observed in all plots and leaf levels. In the middle leaves, the highest amount was still found in the inoculation treatment (54% NLA) and the lowest in the fungicide treatment (16.6% NLA). Similar to 31 July, the low colonization of the upper leaves and the high level of necrotisation, even in the fungicide treated variant, clearly showed that neither *A. solani* nor *A. alternata* was involved in the formation of these lesions.
By 4 September, necrotisation had reached 94% in the inoculation treatment and 49.5% in the fungicide treatment on the middle leaves. The upper leaves did not differentiate: with 23, 30 and 25% NLA the level was almost uniform. But, as seen previously, the DNA amount in the fungicide treatment was still low and, additionally, much lower than in the other two treatments. So again, *A. solani* can be excluded as a possible source of these necroses.

Regarding *A. alternata* disease progress (Table 1), the DNA amounts of this fungus found in every leaf level and every treatment were very low, never exceeding 2 ng/µg total DNA. With the exception of two dates (31 July, fungicide treatment, middle leaves and 14 August, fungicide treatment, upper leaves) they were distinctly lower than that of *A. solani*, which rose up to 138.75 ng/µg total DNA. Consequently, *A. alternata* can be excluded as the cause of the necroses found on the fungicide treated Markies leaves.
Comparing the results of cultivar Markies with an untreated, non-inoculated control of cultivar Jelly (Table 1, 4 September), located at the same field site, the degree of necrotisation in both the middle and upper leaf levels was much higher in all variants of Markies: 49.5 - 94% and 23–30% compared to 24.5 and 12.5% for middle and upper leaves in Jelly. The lower necrotisation in Jelly was associated with DNA contents of 201.63 and 114.38 ng/µg total DNA for *A. solani* and 0.2903 and 0.1682 ng/µg total DNA for *A. alternata* in the middle and upper leaves, respectively. The former amounts were much higher than the amounts connected with a similar necrotisation in Markies, and clearly reveal *A. solani* as the cause of the lesions in Jelly. The latter were lower, and maybe hint at a possible higher colonization due to the higher necrotisation in Markies.
The qPCR results of the greenhouse trials with cultivar Markies (Table 1, 0.0015 ng *A.a.* DNA/µg total DNA and 0.0886 ng *A.s.* DNA/µg total DNA), compared to field trial leaves with similar necrotisation (17% NLA), demonstrate that the lesions on the greenhouse plants were hardly colonized by either fungi, and thus must be caused by something else.

DISCUSSION

The pathogenicity of *A. alternata* towards potato is discussed controversially in the literature. In Germany, starch potato cultivar Markies is considered as highly susceptible, because brown spots can be regularly observed on its leaves and *A.alternata* can be isolated from them most of the time.
In greenhouse trials, different inoculation methods for *A.alternata* inoculation should be tested with Markies eye cuttings. However, the almost 6-week-old plants suddenly developed necrotic lesions resembling early blight symptoms, without any inoculation, on two independent occasions. The presence of *A.solani* and *A.alternata* in the necroses was tested mycologically and by qPCR. 75% of the symptoms were free of *Alternaria* spp. and qPCR showed that DNA amounts in the leaves were correspondingly low. Therefore both *Alternaria* spp. could be excluded as the cause of these symptoms. Furthermore, an artificial inoculation of Markies plants showing symptoms with *A. alternata* isolates derived from the same cultivar was not successful.

This is in accordance with Stammler *et al.* (2014), who found a low or no virulence at all for *A. alternata* in potato.

The progression of both *A. solani* and *A. alternata* under natural conditions was compared in a field trial, also with cultivar Markies. Visual assessment (% necrotic leaf area) and qPCR (ng fungal DNA/µg total DNA) data were collected for three dates in a non-inoculated, non fungicide (early blight) treated control, an artificial inoculation with *A. solani* and a double fungicide treatment with Ortiva®. The typical sudden appearance of brown spots on the leaves of Markies could be observed in all treatments, even in the fungicide treated. However, the *A. alternata* DNA content of the leaves never exceeded 2 ng DNA/µg total DNA and was, with two exceptions, always lower than the corresponding amount of *A. solani* DNA, which reached a maximum of 138 ng DNA/µg total DNA. Thus, an important impact of *A. alternata* in the formation of the necrotic lesions is unlikely. As the colonization of the leaves by both *A. alternata* and *A. solani* was marginal in the fungicide treatment (0.0066–1.9270 and 0.0287–5.6118 ng DNA/µg total DNA, respectively), *Alternaria* spp. could be excluded as the cause of the brown spots there.

The *A. solani* DNA amounts detected in the untreated control of the comparison cultivar Jelly were much higher than in Markies (201 and 114 ng DNA/µg total DNA compared to n.a. and 35 ng DNA/µg total DNA). In contrast, Jelly had a lower necrotisation level than Markies (24.5 and 12.5% NLA compared to 78.5 and 23% NLA). Together with the small amounts of *A. alternata* DNA, the lesions in Jelly could be clearly related to the *A. solani* infestation.

In summary, *A. alternata* can be excluded as the causal agent of the typical "Markies brown spots". They are possibly caused physiologically; in the greenhouse, they occurred with the beginning of flowering, i.e. the switch from the vegetative to the generative phase of the plant. Additionally, Markies reacts strongly to ozone exposure (data not shown); at low doses with development of necrotic lesions, at higher doses with defoliation.

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Evidence of strobilurine resistant isolates of *A. solani* and *A. alternata* in Germany

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SUMMARY
Early blight caused by *Alternaria solani* and *Alternaria alternata* is a highly destructive disease of potatoes. Control of early blight mainly relies on the use of preventive fungicide treatments. Because of their high efficacy, QoI fungicides are commonly used to control early blight. However, loss of sensitivity to QoIs has been reported for *A. solani* in potato (Pasche *et al.* 2001) and for *A. alternata* in different hosts (Vega & Dewdney, 2014).
Five hundred and five *A. solani* field isolates collected from 150 locations in Germany between 2005 and 2014 were screened for the presence of the F129L mutation in the cytochrome *b* gene; of these, 147 contained the F129L mutation. Sequence analysis revealed the occurrence of two structurally different *cytb* genes. F129L isolates have been found since 2009 onward. Sensitivity of *A. solani* isolates to azoxystrobin was determined in conidial germination assays. All *A. solani* isolates possessing the F129L mutation had reduced sensitivity to azoxystrobin. The results indicated an increase of the EC_{50}-value over the last three years.

In identical form two hundred and eight *A. alternata* field isolates were collected from 101 locations in Germany between 2005 and 2014. These isolates were screened for the presence of the G143A mutation in the cytochrome *b* gene; of these, 106 contained the G143A mutation.
Sensitivity of *A. alternata* isolates to azoxystrobin was determined in conidial germination assays. As well all *A. alternata* isolates possessing the G143A mutation had highly reduced sensitivity to azoxystrobin.

KEYWORDS
Alternaria solani, *Alternaria alternata*, cytochrome *b,* fungicide resistance, QoI

INTRODUCTION

Potato early blight occurs worldwide and is prevalent wherever potatoes are grown. Early blight of potato (EB), 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. Integrated pest management is mainly based on multiple fungicide applications. Prior to the registration of azoxystrobin (QoI) for potato in 2007 in Germany, EB control was mainly achieved by multiple and frequent application of mancozeb-containing fungicides. However, these fungicides were only moderately effective in the control of EB. Ortiva® (azoxystrobin) and Signum® (pyraclostrobin+boscalid) were registered in Germany as EB-specific fungicides in potatoes in 2007 and 2008, respectively. In contrast to mancozeb-containing fungicides, QoI fungicides turned out to be highly effective against EB. Strobilurins (QoIs) inhibit mitochondrial respiration in fungi by binding to the Qo site of the cytochrome *b* (*cytb*) complex, blocking electron transfer and inhibiting ATP synthesis. As QoI´s have a specific single-site mode of action they possess a high risk to the evolution of fungicide resistance due to point mutations.

The occurrence of fungicide resistance is a serious problem affecting disease management in agricultural ecosystems. The knowledge about the occurence of reduced-sensitive isolates is necessary for an integrated pest management.

The present study was carried out in order to determine whether a reduction in sensitivity to azoxystrobin has already occurred within German *A. solani* and *A. alternata* populations. The objectives of this research were to determine the prevalence of the F129L substitution among *A. solani* and the G143A substitution among *A. alternata* isolates collected from conventional potato fields throughout Germany between 2005 and 2014.

MATERIALS AND METHODS

Isolate sampling

Between 2005 and 2014 505 *A. solani* were collected from 150 locations and 208 *A. alternata* field isolates from 101 locations in Germany. All isolates originated from commercial potato crops naturally infected with EB. Infected leaflets were sampled gathered at random during EB disease epidemics between July and September of each year. Material was surface-sterilized in 5% NaOCl for 1 min and then washed in sterile, distilled water. One single conidium was transferred to synthetic low nutrient (SN) medium (1 g KH_2PO_4; 1 g KNO_3; 0.5 g $MgSO_4x7H_2O$; 0.5 g KCl; 0.2 g glucose; 0.2 g saccharose; 0.6 ml 1 n NaOH; 20 g agar; dissolved in 1,000 ml double distilled water). Only one isolate per diseased plant was collected. *Alternaria solani* or *Alternaria alternata* cultures were identified on the basis of morphological characteristics and spore size (Simmons, 2007).

Evaluation of the F129L substitution in A. solani and the G143A substitution in A. alternata

Genomic DNA of *A. solani* and *A. alternata* isolates was extracted from fungal mycelia cultivated on V8-medium under near ultraviolet light for 14 days at 21°C.

For genomic DNA extraction, *A. solani* and *A. alternata* isolates were cultivated on V8-medium under 12 h near ultraviolet light for 14 days at 21°C.

Mycelium and spores were carefully scraped off with a spatula and ground in liquid nitrogen. Genomic DNA extraction, PCR, probe hybridization and sequencing of a cytochrome *b* fragment for the detection of the F129L mutation was carried according to Leiminger *et al.* (2013).

In vitro QoI fungicide sensitivity assay

Fungicide sensitivity was determined using an *in vitro* plate assay, based on germination rate of conidia on fungicide-amended agar relative to non-amended media. Spores of *A. solani* and

A. alternata were produced by growing isolates on SN media at 20°C with an alternating 12 h photoperiod. Conidia were dislodged using a sterile glass rod and distilled H_2O. The conidial suspension of each isolate was spread onto the surface of fungicide-amended agar plates at different concentrations, as well as on fungicide free plates which served as control plates. After incubation of the plates, the germination of 100 conidia per plate was assessed.
Fungicide sensitivity was determined by comparing spore germination on water agar amended with azoxystrobin at different concentrations. Fungicide sensitivity assays were performed in the presence of 100 mg/l salicylhydroxamic acid (SHAM). SHAM was added to media to prevent the fungus from overcoming the toxicity of a QoI fungicide through an alternative oxidative pathway. Petri dishes with SHAM but without fungicides were used as controls. Fungicide sensitivity was measured as the concentration at which spore germination of *A. solani* was inhibited by 50% relative to the untreated control (EC_{50} value) and was determined for each isolate.

RESULTS

Detection of German A. solani field isolates carrying the F129L substitution
None of the *A. solani* isolates collected between 2005 and 2008 contained the F129L mutation. Two isolates, which contained the F129L mutation, were first observed in 2009. Both derived from the same field in Bavaria. In 2010, two further F129L isolates were sampled from two different locations, whilst in 2011 six different locations (all in Bavaria) showed F129L isolates. In 2011, the number of isolates carrying the F129L mutation increased strongly.
Thirty-nine out of 66 isolates (56%) contained the F129L mutation (see Table 1). In 2012 fifteen out of 88, 2013 69 of 183 and in 2014 20 out of 62 isolates contained the mutation. Within F129L isolates, the TTC to TTA nucleotide exchange was predominantly found. A CTC mutation was detected in only three isolates. The frequency of wild type isolates was 100% between 2005 and 2008, 95% in 2009, 80% in 2010 and 28% in 2011. Since 2013 the frequency of F129L mutants has been found as relatively stable between 32 and 37% (Table 1).

Table 1. *Details of Alternaria solani isolates collected from commercial potato crops in Germany, 2005 -2014*

year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Nr. of isolates	5	24	20	5	39	13	66	88	183	62
Nr of locations	3	22	13	3	22	9	12	14	25	27
Locations with F129L	0	0	0	0	1	2	7	6	20	18
Nr of F129 L mutants	0	0	0	0	2	2	39	15	69	20
Frequency % F129L					5,1	15,3	56,5	17,0	37,7	32,2

Detection of German A. alternata field isolates carrying the G143A mutation
From 2005 to 2014, 208 monoconidial isolates of *A. alternata* from 101 different locations were examined for the presence of the G143A mutation. For the detection of the G143A mutation 208 single-spore isolates from the years 2005 to 2014 and 101 different locations were

examined. In 2006 the first G143A mutant was found in *A. alternata* for Germany. In this year nine out of 43 isolates (21%) contained the G143A mutation. Since 2009 the frequency of G143A mutants has increased over 41%.
The molecular basis for the analysis of QoI resistance was carried out by using polymerase chain reaction of the cytochrome b gene. Within G143A isolates of *A. alternata*, only GGT the GCT nucleotide exchange has been detected so far.

Table 2. *Details of Alternaria alternata isolates collected from commercial potato crops in Germany, 2005 -2014*

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	in total
isolates evaluated	7	43	/	/	14	12	30	34	17	51	208
wildtyp isolates	7	34	/	/	5	0	7	20	7	22	102
G143A mutants	0	9	/	/	9	12	23	14	10	29	106
investigated locations	1	29	/	/	10	1	11	10	4	35	101
locations with G143A	0	9	/	/	5	1	8	5	0	18	46

In vitro assay of A. solani isolate sensitivity to QoI fungicides
Fungicide sensitivity was determined by assessing spore germination for a range of isolates on fungicide-amended and non-amended water agar in the presence of SHAM. EC_{50} values of *A. solani* baseline isolates collected in 2006 ranged from 0.058 to 0.1 µg ml^{-1} for azoxystrobin (Fig. 1). EC_{50} values for *A. solani* wildtype isolates, collected after registration of azoxystrobin between 2007 and 2014, were not significantly different from the mean EC_{50}-values of the baseline isolates.
F129L isolates collected 2009-2014 had significantly higher EC_{50} values compared to the baseline isolates. All isolates possessing the F129L mutation were less sensitive to azoxystrobin. Comparing mean EC_{50} values, isolates lacking F129L were approximately 4-fold more sensitive compared to F129L isolates collected from 2009 to 2012 (Fig. 1). Since 2013 the mean EC_{50} values of the F129L isolates have increased dramatically. The mean EC_{50} value of the F129L mutants collected in 2013 was 1.5 µg ml^{-1} for azoxystrobin, in 2014 10.2 µg ml^{-1}.

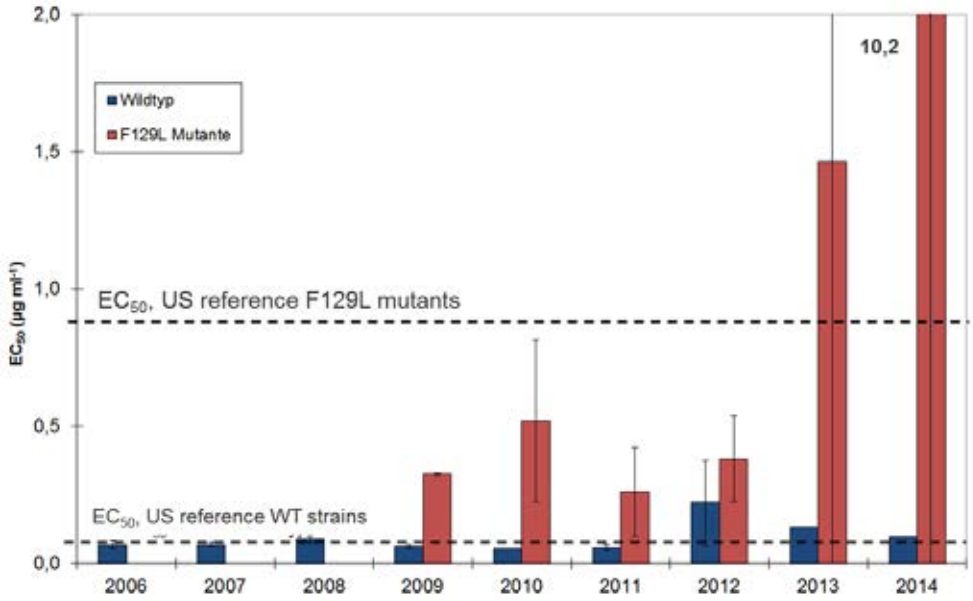

Figure 1. *In vitro azoxystrobin sensitivity assay of A. solani wild-type and F129L isolates collected between 2006 and 2014. Columns represent mean EC_{50} values, i.e. the effective fungicide concentration that inhibited spore germination by 50%. Bars represent standard deviations*

In vitro evaluation of isolate sensitivity of A. alternata to QoI fungicides

The germination rate of almost all *A. alternata* isolates evaluated in fungicide untreated control plates was over 90%. Compared to this the germination rate in the wild-type isolates was reduced to 8 – 65% by adding 0.1 µg ml^{-1} azoxystrobin. At a concentration of 1.0 µg ml^{-1} azoxystrobin the germination rate of wild-type isolates was less than 2% (Fig. 2).

By comparing wild-type and G143A isolates, G143A mutants showed a high germination rate at a concentration of 1.0 µg ml^{-1} azoxystrobin and even at 10.0 µg ml^{-1} azoxystrobin.

The mean EC_{50} values of the *A. alternata* mutant were at least 100 times higher than the mean EC_{50} values of the *A. alternata* wild-type isolates. Figure 3 indicates the fungicide sensitivity of *A. alternata* G143A mutants.

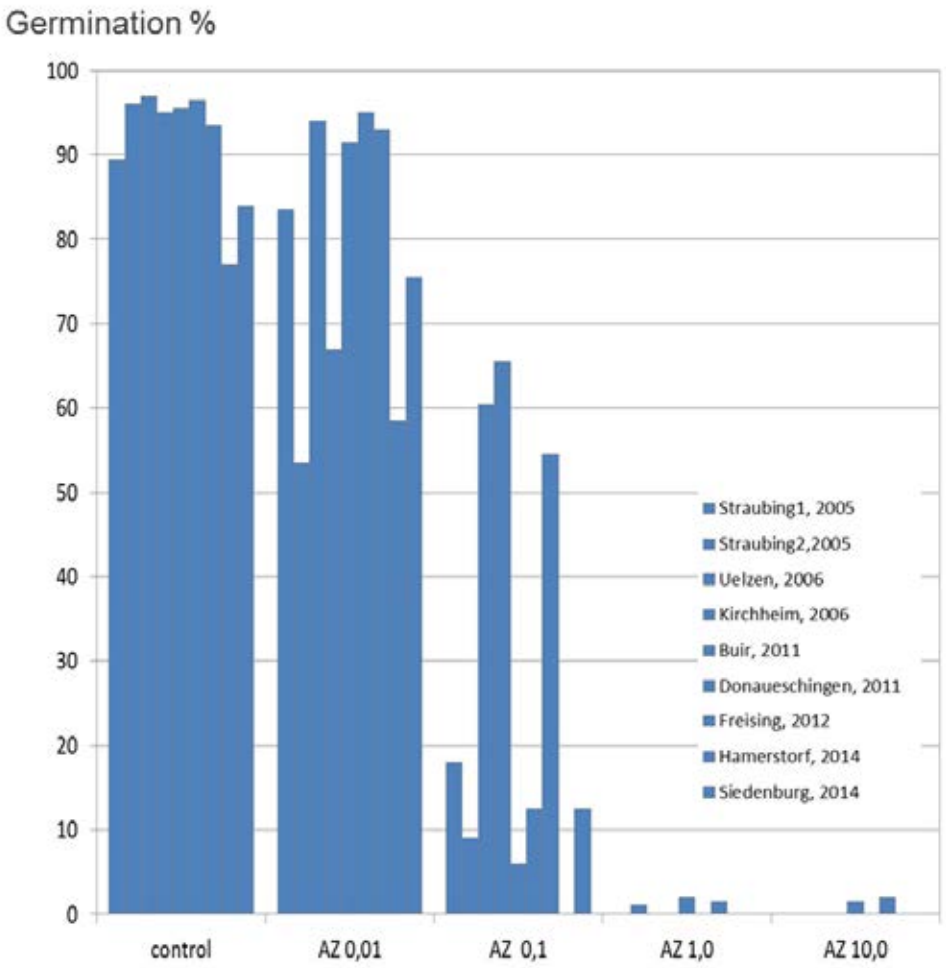

Figure 2. *Germination rate of A. alternata wild-type isolates from different locations by adding different fungicide-concentrations (AZ: azoxystrobin)*

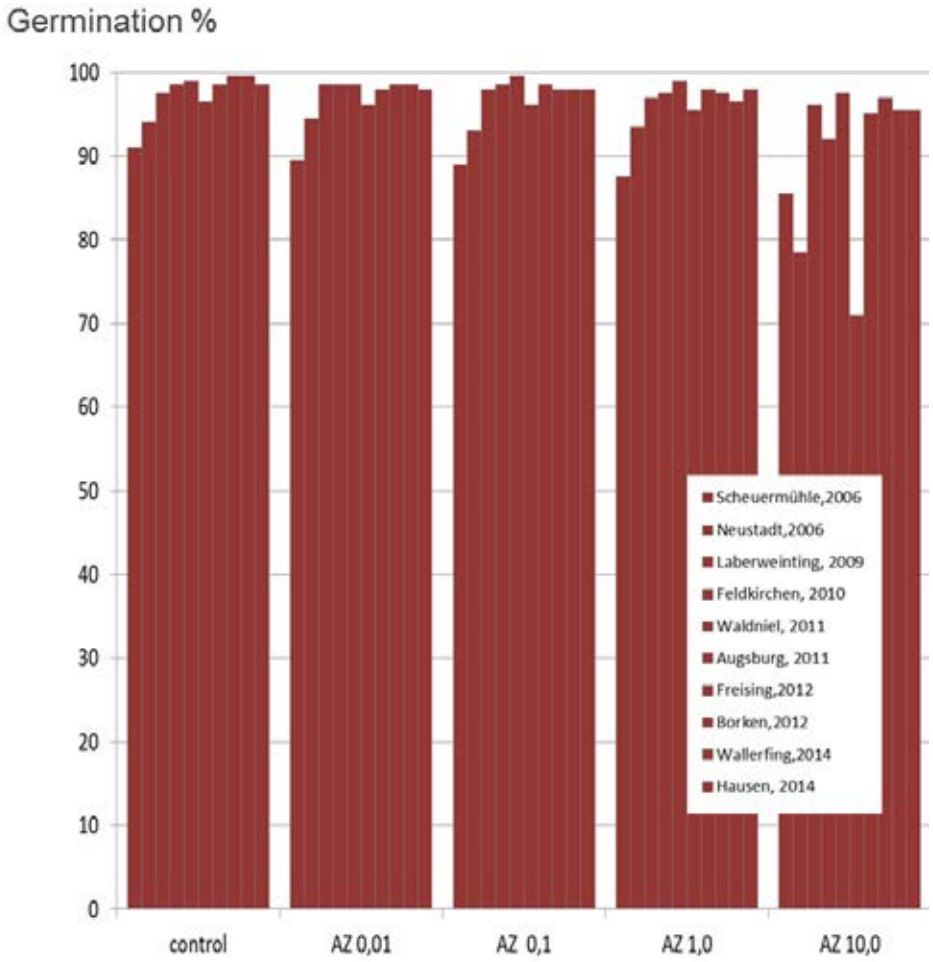

Figure 3. *Germination rate of A. alternata G143 isolates from different locations by adding different fungicide-concentrations (AZ: azoxystrobin)*

CONCLUSION

Resistance and reduced sensitivity to fungicides among fungal plant pathogens are significant problems concerning chemical pest management. QoI fungicides, which are widely used to protect crops, have been characterized as "high-risk" for the development of resistance. In Germany, QoIs were registered for the control of EB in potatoes in 2007 for the first time. First G143A isolates of *A. alternata* were found in 2006, before azoxystrobin was registered for application in potato. For the fungus *A. solani* first F129L isolates were found in 2009, two years after the first registration of azoxystrobin for potatoes in Germany. In the present study, isolates containing the F129L substitution displayed a shift in sensitivity to azoxystrobin in *in vitro* spore

germination assays. The restricted use of QoIs, in combination with management programs will be essential for the continued use of QoI fungicides for the control of EB.

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Fungicide strategies against early blight and presence of F129L in Sweden

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SUMMARY

Early blight on potato is an important disease in southeast Sweden and occurs in the middle part of Sweden even though it has not an intense epidemic phase there. The fungicide strategy against *Alternaria solani* dominates by strobilurins, sometimes in combination with boscalid. Potato cultivars with high host resistance would be preferable but there are no cultivars with high level of resistance available on the market. The susceptibility (host resistance) to *Alternaria* sp. is related to leaf position and senescence, which depend on plant developmental stage and maturity type where late maturing cultivars are more resistant. We have established methods for greenhouse testing of host resistance on intact plants and compared the lesion size to the disease scoring (AUDPC) in field trials and got good correlations (R= 0.96).

The common strategies against early blight were tested in field trials 2014. Two applications of Revus Top (mandipropamid and difenoconazol) followed by four applications of Signum (boscalid and pyraclostrobin) were applied with full and half doses in cultivars Kardal and Kuras. The timing of the first treatments was also tested but there was no or small effect on disease development between early or late first treatment. At the end of the season (September 2) there were still severe infections also with fungicide treatments and lowered doses intensified the infection. Full dose of the treatment strategy above was also tested against two applications of Amistar (azoxystrobin) and an untreated control in another field experiment. The untreated control and the Amistar treatment had similar disease development even though it was a small yield effect by Amistar. The Revus Top/Signum treatment was effective in inhibiting early blight until the end of August where the disease began to develop epidemically.

The occurrence of F129L was analysed in field samples from southern and middle parts of Sweden. In the southeast part (around Kristianstad) the genotype 2 (GII) dominated and the majority possessed the F129L substitution. In the middle part of Sweden only GII has been found and half of them possessed the F129L substitution in 2013 whereas none were found in 2014. Genotype 1 (GI) is rather scarce in Sweden but three isolates with the F129L (CTC) substitution have been found in the area surrounding Kristianstad (collected 2011 and 2013).

The effect of the F129L substitution on fungicide sensitivity was tested by Bayer CropScience, Monnheim (Dr. Andreas Mehl *et al.*). They found a reduced sensitivity to fenamidone but not to

trifloxystrobin in the isolates with F129L. However, there was no sign of reduced sensitivity to boscalid among 17 tested isolates. The sensitivity to azoxystrobin and pyraclostrobin is currently being tested at our lab.

The genetic structure of about 60 Swedish isolates of *A. solani* and 20 isolates of *A. alternata* (10 from Sweden and 10 from Tajikistan) were investigated with AFLP and microsatellite markers. The genetic variability was relatively high among isolates of *A. solani* and significant genetic differentiation was found among populations from different locations in southeast Sweden. Both GI and GII were found among the isolates but the F129L substitution was only detected in GII isolates.

KEYWORDS

Alternaria solani, early blight, fungicide sensitivity, genetic structure

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New fungicide and bactericide Zeroxxe®: *in vitro* assessment of fungicidal and bactericidal activity

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SUMMARY

Zeroxxe® is a new fungicide and bactericide product with a broad spectrum of activity against phytopathogenic fungi, oomycetes and bacteria. In Russia, Zeroxxe® is in the final stage of the four-year registration process as the world's first fungicide and bactericide containing nanosilver as an active component. An active substance of Zeroxxe® is silver nanoparticles (silver NPs, 3000 ppm) stabilized with environmentally safe biodegradable amphoteric surfactant. In vitro studies of Zeroxxe® revealed its high efficacy against a wide range of fungal pathogens of potato: *Rhizoctonia solani* (black scurf), *Phytophthora infestans* (late blight), *Colletotrichum coccodes* (black dot), *Helminthosporium solani* (silver scurf), *Alternaria solani* and *A. alternata* (early blight), *Fusarium solani* (dry rot), *Sclerotinia sclerotiorum* (sclerotinia rot). To assess the bactericidal effect of Zeroxxe® six types of pathogenic bacteria were tested: *Pectobacterium carotovorum* (soft rot of potatoes and vegetables), *Dickeya dianthicola* (black leg and soft rot of potato), *Agrobacterium tumefaciens* (bacterial cancer of fruit, ornamental plants and grapes), *Xanthomonas vesicatoria* (bacterial black spot of tomato), *Clavibacter michiganensis* (bacterial cancer of tomato and potato), *Xanthomonas campestris* (vascular bacteriosis of cabbage and rape). 30 minutes incubation of bacteria in the solution of Zeroxxe® (100 ppm of silver NPs) completely inhibits growth of all bacteria. In the case of 10 ppm solution 45-85% of all colonies were inhibited. The results prove the potential of Zeroxxe® as a fungicide and bactericide for pre-plant treatment of various seeds and tubers, treatment of vegetating plants, as well as for the treatment of tuber and root crops before storage.

KEYWORDS
Fungicides, bactericides, antibiotics, potato diseases, silver nanoparticles.

INTRODUCTION

Modern technologies of agriculture imply intensive use of plant protection products at all stages of production. Nearly all chemical plant protection products are more or less toxic for mammals; they (or products of their further transformation) accumulate in organs of treated plants, in the soil, get into water and air, which leads to undesirable environmental consequences. That is why special attention is paid to the development of plant protection products harmless for mammals and plants.

Silver influences a wide range of biological processes in microorganisms. Silver nanoparticles (NPs) can be subject to slow oxidative dissolution in close proximity to the cell membrane of bacteria and fungi, generating silver ions and thus causing their death. *Escherichia coli* was used to demonstrate the effect of silver on proteins of ribosomal complex as well as proteins participating in glycolysis and the cycle of tricarboxylic acids, which damages the synthesis of ATP and leads to quick cell death (Yamanaka *et al.*, 2005). Wide spectrum of action of silver ensures its use against a big number of different pathogenic organisms with no fear of appearance of new resistant strains.

In the XIX-XX centuries silver-based plant protection products were not widespread because of their high price, unlike copper-based pesticides. Nowadays copper pesticides are among the most popular in the world, despite their high application rate, which sometimes reaches 10 kilograms per hectare, and the negative impact on the environment. However, the advances in the synthesis and stabilization of silver NPs resulted in appearance of plant protection products with high efficiency at very low concentrations of active components, which makes their use economically justified and minimizes environmental risks related to their use.

Fungicidal effect of silver NPs was demonstrated in a number of researches. American researchers (Jo *et al.*, 2009) proved their high efficiency against germinating spores of *Bipolaris sorokiniana* (Sacc.) Shoem and *Magnaporthe grisea* (Hebert) Barr. The same research shows good prospects of application of NPs as a contact fungicide for protection of leaves of vegetating plants of ryegrass. Korean authors (Lamsal *et al.*, 2011) showed the effect of silver NPs on different agents of anthracnose (*Colletotrichum* sp.). The comparison of field and laboratory experiments showed that NPs are able to inhibit the development of *Colletotrichum* sp. in pepper plants in fields with the same efficiency as *in vitro*. In other research papers (Kim *et al.*, 2012, Kim *et al.*, 2009, Min *et al.*, 2009) high fungicidal effect of NPs against more than 20 phytopathogenic fungi was demonstrated, while the testing was conducted in different nutrient media.

The experience of different scientific groups showed that compositions based on colloidal silver stabilized with compounds of various chemical classes were efficient in most cases against phytopathogenic fungi and bacteria. That is why in 2012 the researchers of the Lomonosov Moscow State University and Grand Harvest Research International Ltd. have begun the development of the world's first fungicide and bactericide containing nanosilver as a main active component. This pesticide under trade name Zeroxxe® was tested in Russia to analyse its fungicidal and bactericidal activity in laboratory experiments and in field trials on many crops, its acute and chronic toxicity for animals, environmental impact, and many other parameters.

This article contains the results of the laboratory assessment of the fungicidal and bactericidal activity of Zeroxxe® against most serious fungal and bacterial phytopathogens.

MATERIALS AND METHODS

Samples of fungi *Colletotrichum coccodes* (Wallr.) S. Hughes, *Helminthosporium solani* Durieu & Mont., *Rhizoctonia solani* J.G.Kühn, *Fusarium solani* (Mart.) Sacc., *Alternaria alternata* (Fr.) Keissl. and *A. solani* Sorauer, oomycete *P. infestans* (Mont.) de Bary were isolated from infected potato tissue. Isolate of *Sclerotinia sclerotiorum* (Lib.) de Bary was isolated from the affected carrot. For the experiment one isolate of each species of fungi was taken.

Laboratory evaluation of fungicidal properties of Zeroxxe®

The resistance of isolates to fungicides was assessed on pea agar medium, supplemented with the corresponding fungicide at various concentrations (0.1, 1, 10, 100 ppm (=μg/ml) of silver NPs), and on the medium without fungicide (control). An agar piece containing fungi (5 mm in diameter) was placed into the centre of the Petri dish, which was then sealed with a paraffin film (Parafilm). Petri dishes were incubated at temperature of 23-25°C under natural light. The diameter of colonies was measured when the diameter of the fungi colony in the fungicide-free control sample achieved 0.7-0.75 of the diameter of the Petri dish. The radial growth on each concentration of fungicide was assessed in three replications (each isolate was put into 3 Petri dishes with the same fungicide concentration). On the basis of the averaged values of diameters of colonies the ratio of the sizes of colonies in the medium with fungicide and in the medium without fungicide were calculated. For each isolate the value of the effective concentration EC_{50} was determined, i.e. the concentration of fungicide required to slow down the speed of the radial growth of the colony twice with 50%.

Influence of Zeroxxe® on germination of zoosporangia P. infestans with zoospores (indirect germination)

Silver NPs stabilized with amphoteric surfactant (an active substance of Zeroxxe®) in concentration of 25 and 100 ppm were used in microbiological experiments in the form of aqueous dispersions. Fluazinam in concentration of 500 ppm was used as etalon fungicide, which corresponds to its recommended concentration in tank mixtures for treatment of vegetating plants. In our experiment germination of zoosporangia of four phytopathogenic isolates of *P. infestans* obtained from affected leaves of potato was analysed. The suspension of zoosporangia of *P. infestans* was obtained by means of washout from 8-days old pathogenic culture with distilled water. Then the suspension was mixed with the same amount of silver NPs or fluazinam solution in order to obtain aforementioned concentrations of active substances in culture media. The germinated (empty) zoosporangia were counted after 3 hours of cultivation at +10°C. In each sample 600 zoosporangia (6 samples, 100 pcs. on each) were accounted. The percentage of germinated zoosporangia was determined.

Estimation of bactericidal effect

For the estimation of bactericidal effect the bacteria were grown in agar nutrient medium during 24 hours at +28°C and then washed with sterile distilled water. Suspensions with concentration of 1000 CFU/ml were used for microbiological experiments. Bacterial suspension was mixed with Zeroxxe®-containing water solution (final concentration 10 and 100 ppm of silver NPs) and incubated for 30 min. Control samples were incubated with the similar volume of distilled water. After the incubation 50 μl of suspension were resuspended over the surface of agar nutrient medium. Colonies were counted after 48 and 72 h of incubation under +28°C and compared to the fungicide-free control sample.

RESULTS AND DISCUSSION

Laboratory evaluation of fungicidal properties of Zeroxxe®

The results show suppression of the radial growth of colonies of all tested species of fungi after addition of Zeroxxe® in the concentration of silver NPs more than 10 ppm (Table 1). High efficiency against *Rhizoctonia solani*, *Phytophthora infestans*, *Colletotrichum coccodes*, *Helminthosporium solani, Alternaria solani* and *Sclerotinia sclerotiorum* was noted. Fungicidal effect against *Alternaria alternata* and *Fusarium solani* was weaker. Differences of the efficiency of fungicides against strains *A. solani* and *A. alternata* correspond to our previous research: well known fungicides mancozeb, azoxystrobin, chlorothalonil were also less effective against *A. alternata* than against *A. solani* (Pobedinskaya *et al.*, 2012).

Table 1. *Influence of Zeroxxe® on radial growth of colonies of tested fungi*

Fungi/ oomycetes	Ratio of diameters of the colony on the media with different concentration of silver NPs and the fungicide-free control (in %)					EC_{50}*, mg/l
	0 (control)	0.1 ppm	1 ppm	10 ppm	100 ppm	
Phytophthora infestans	100	90**	55	33	0	3.1
Rhizoctonia solani	100	95	78	2	0	0.4
Fusarium solani	100	95	91	41	33	8.3
Colletotrichum coccodes	100	96	94	23	0	6.6
Helminthosporium solani	100	97	83	50	10	10
Alternaria alternata	100	93	107	52	41	28
Alternaria solani	100	92	92	35	22	7.7
Sclerotinia sclerotiorum	100	93	73	0	0	3.9

* - the concentration of a fungicide, causing a 50% delay in the colony growth rate as compared to fungicide-free control

** - the diameter of colonies was measured when the diameter of the fungi colony in the fungicide-free control sample achieved 0.7 to 0.75 diameter of a Petri dish.

Influence of Zeroxxe® on indirect germination of zoosporangia of P. infestans

According to the experimental results, Zeroxxe® certainly decreased germination of zoosporangia. In the control sample the average number of germinated (empty) zoosporangia in the visual field of the microscope at 150-fold magnification comprised 54-80 pcs, in the samples inoculated with 25 ppm of silver NPs – 0.2-12; at 100 ppm – 0.3-2.3 (Table 2). The maximum average number of germinated zoosporangia in the experiment with the etalon fungicide fluazinam reached 0.3. Thus, Zeroxxe® reduced germination of zoosporangia *P. infestans* at the level of fluazinam, though the concentrations of silver were lower.

Table 2. *Influence of Zeroxxe® on indirect germination of zoosporangia of P. infestans*

Variant	Average number of germinated zoosporangia in the visual field of the microscope at 150-fold magnification				
	Strain 1	Strain 2	Strain 3	Strain 4	Medium
Control (water)	69	80	60	54	65.8
Silver NPs 25 ppm	0.5	0.7	12	0.2	3.4
Silver NPs 100 ppm	0.5	0.3	2.3	0.3	1.4
Fluazinam 500 ppm	0.3	0	0	0	0.1
Least significant difference 0.95	5.3	5.0	5.4	5.2	5.2

Influence of Zeroxxe® on the growth of phytopathogenic bacteria

Incubation of bacteria in the solution of Zeroxxe® with silver NPs concentration of 100 ppm for 30 min completely inhibited the growth of colonies of the bacteria, while 10 ppm of silver NPs reduced the number of colonies by 45-85%. The number of colonies of *Dickeya dianthicola* and *Agrobacterium tumefaciens* significantly decreased even after the incubation with Zeroxxe® at silver NPs concentration of 1 ppm (Table 3).

Table 3. *Inhibition of growth of phytopathogenic bacteria after the incubation in Zeroxxe® solution for 30 min*

Species of bacterium	The number of colonies on the agar media after the incubation in Zeroxxe® solution with different concentrations of silver NPs, % of control		
	1 ppm	10 ppm	100 ppm
Pectobacterium carotovorum	87	45	0
Dickeya dianthicola	61	23	0
Agrobacterium tumefaciens	75	15	0
Xanthomonas vesicatoria	90	38	0
Xanthomonas campestris	97	55	0
Clavibacter michiganensis	92	38	0

Our experiments revealed high fungicidal and bactericidal activity of Zeroxxe®. The results of assessment of fungicidal effect of Zeroxxe® coincided in a number of parameters with the data obtained during assessment of fungicidal activity of silver NPs in other laboratories. Thus, in our research EC_{50} of silver NPs for the majority of the tested fungi was within the range 3.1-10 ppm; it reached its maximum for *A. alternata* and comprised 28 ppm. In the researches of other authors EC_{50} for non-stabilized silver NPs was as follows: for *Bipolaris* sp. 4.8-8.8 ppm, for *Magnaporthe grisea* 3.9-4.7 ppm, *A. alternata* (EC_{50}=38 ppm), *A. solani* (less than 10 ppm), *Fusarium* (9-55 ppm for different species), *Pithium* sp. (about 2 ppm), *Colletotrichum* (8-100 ppm for different species) (Jo *et al.*, 2009, Kim *et al.*, 2012, Lamsal *et al.*, 2011). For germinating sclerotia nanoparticles were more toxic than for mycelium: S*clerotinia sclerotiorum* (EC_{50}=1 ppm) and *Rhizoctonia solani* (less than 1 ppm). EC_{50} for silver NPs against

S. *sclerotiorum* (7 ppm) and *R. solani* (6 ppm) (Min *et al.*, 2009) exceeded EC_{50} revealed in our research (3.9 and 0.4 ppm respectively).

In general, the results on EC_{50} presented in the cited research papers are close to the ones determined for Zeroxxe® in our laboratory, but in most cases were higher. Evidently, the surface modification of nanoparticles increases the fungicidal effect.

It should be noted that as Zeroxxe® is almost completely harmless for animals and plants and not dangerous to the environment in recommended doses, it can be recommended for application in areas where the use of toxic pesticides is not permissible. For instance, it can be applied for treatment of potato (including ware and technical potato) tubers before placing for storage, during storage, and before planting, treatment of plants in glass houses and private kitchen-gardens.

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PPO – SPECIAL REPORT NO 17 – 2015, 109-118

Mancozeb: essential tool for sustainable protection of potato against late blight

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RUCCIA DANIELE, Product Regulatory Manager UPL

SUMMARY
Late blight (*Phytophthora infestans)* is still the most damaging fungal disease of potato, 2014 demonstrated this aspect again in many European countries, even if damages by early blight (*Alternaria solani/alternata*) are increasing. New active ingredients have recently been registered but most of them have a single-site mode of action and are prone to select resistant strains. Mancozeb has been registered for more than 60 years and thanks to its multi-site mode of action, it has consistently maintained its efficacy against both diseases. Looking at the evolution of *P. infestans* strains over the last 15 years, it has become critical to demonstrate how effective mancozeb is against the "new strains" of late blight: 13_A2, 6_A1, 33_A2. Studies conducted under laboratory and greenhouse conditions at the renowned University of Wageningen at the request of UPL Europe show high levels of efficacy on all strains confirming the practical results widely observed in commercial fields. Mancozeb continues to remain an essential tool in managing fungicide resistance on populations of *P. infestans* identified in the past and today.

KEYWORDS
Phytophthora infestans, fungicides, efficacy, new population

INTRODUCTION

In Europeans Countries, **late blight is the most important disease on potato, it is very frequent** and often very severe as we can see in the example of North of France in 2014 (Fig. 1 risks of sporulation given by the DSS Mileos): since last of April to the end of September, except for ten days in June, everyday was at the maximum level. The first symptoms appeared on potato dumps piles during planting and numerous fields were infected, even if the disease was controlled with a number of treatments.

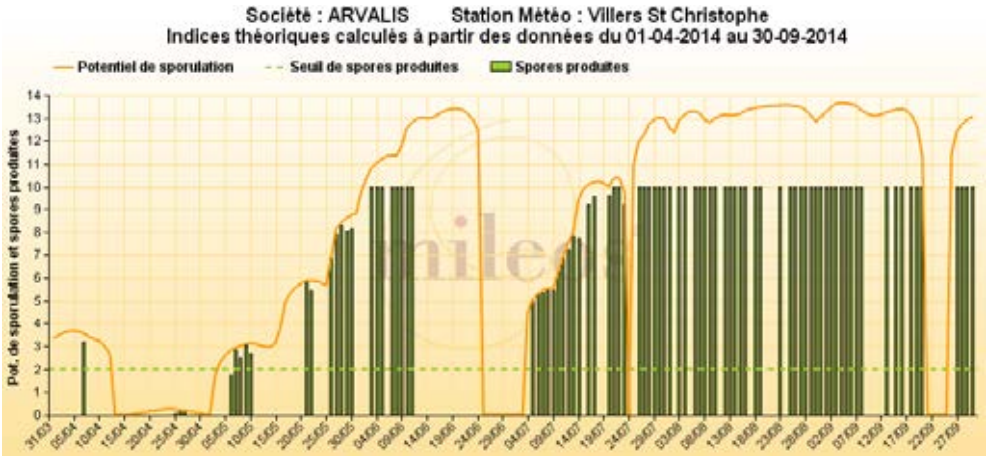


Figure 1. *Risks of sporulation given by the DSS Mileos*

If weather conditions are the essential elements of the development of the epidemic, it is imperative to take into account the **evolution of *Phytophthora infestans*.** For several years new strains have evolved. It should be noted the development of 13_A2 (Blue 13), detected in NL and DE in 2004, in UK and FR in 2005, in Ireland in 2007, and also present in many other EU countries since 2012. This strain is more aggressive at low temperatures (8°C) than the older ones; it is also resistant to Phenylamides.

6_A1 (Pink 6), was detected in NL in 2002, in UK and FR in 2004 and it seems very aggressive on the leaf at temperatures around 10°C. It was dominant in UK in 2011 and 2012, but has regressed during the last few years.

33_A2 (Green 33) was detected in NL in 2011, and is also present in BE and PL. Fluazinam is less effective on this strain. 33_A2 strains express a weak fitness, therefore regress when straight fluazinam is not used in a systematic way. Green 33 represented more than 20% of the strains in 2010 and 2011 in NL but only 6% in 2012.

Figure 2 shows the situation of the different strains in Europe in 2013 (Euroblight).

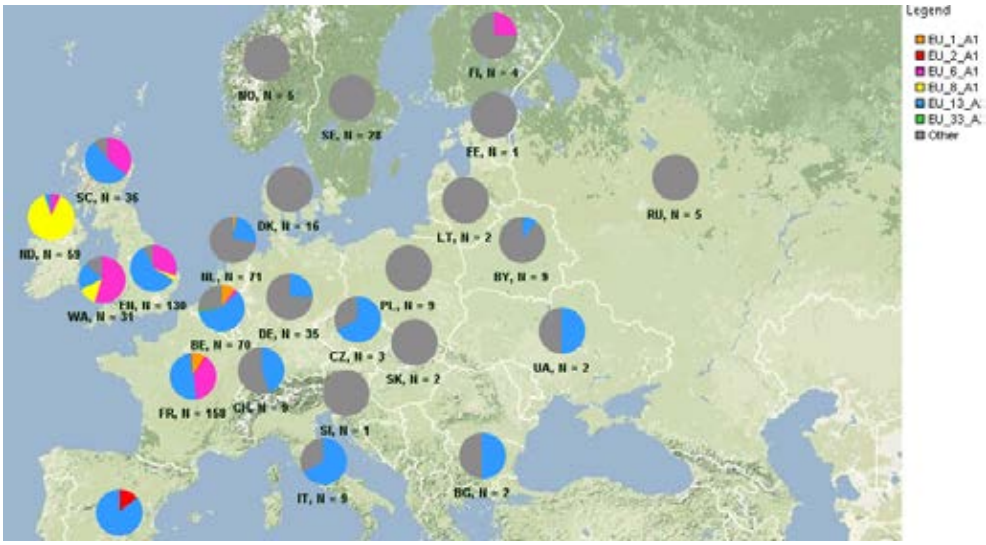


Figure 2. *P. infestans strains in Europe*

To fight against late blight growers have at their disposal **many fungicides,** the active ingredients are multiple but most of them have a single site mode of action against the fungus thus a high risk of resistance developing (Fig. 3). Only some ingredients have a multisite mode of action, e.g. mancozeb which has been registered for 60 years, with no known resistance and good efficacy against early and late blight.

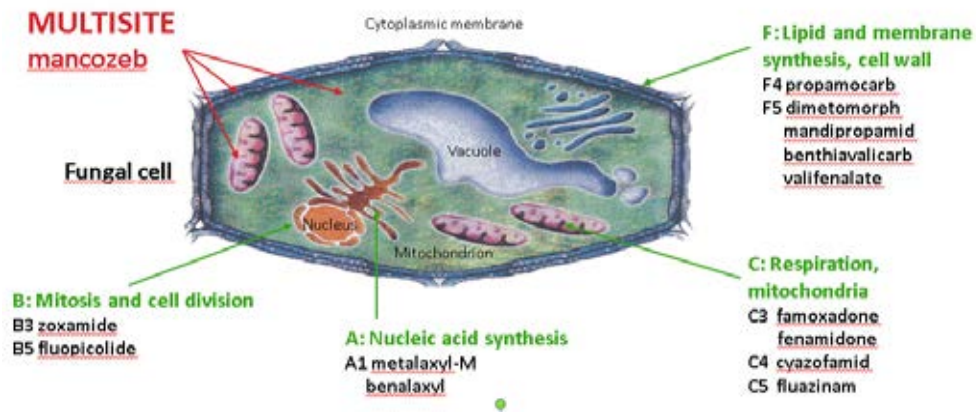


Figure 3. *Modes of action fungicides*

RESEARCH

Efficacy of fungicides to control different strains of potato late blight (Phytophthora infestans)
The trial was conducted by Applied Plant Research, part of Wageningen UR.

The efficacy of mancozeb (Penncozeb), to control potato late blight – *Phytophthora infestans* – was tested in the laboratory.

MATERIAL AND METHODS

Four different strains of *P. infestans* were used: Blue 13, Pink 6, Green 33 (coming from a field with a less susceptibility to fluazinam) and an isolate VK 98014 (old population).
Leaves (cv. Bintje) were punched to obtain leaf discs. Five potato leaf discs were laid on water agar. The fungicides were sprayed using a spray boom with three spray nozzles, placed 50 cm apart, 40 cm over the top of the leaf discs. Spray volume was 250 l/ha.
Spraying of the product (mancozeb 1500 g/ha) was carried out in a spraying cabin, 2 days before inoculation with potato late blight (preventive efficacy).
The isolates are cultivated on agar plates and potato slices. The inoculum density was set at approximately 10,000 sporangia per ml. Inoculation was carried out by spraying potato leaf discs over head with approximately 0.1 ml of inoculum. After inoculation the petri dishes containing leaf discs were incubated in a climate chamber.
Late blight disease severity was established 1 week after inoculation. Percentage necrotic foliage per leaf disc was determined.

RESULTS

Table 1. *Results of laboratory test*

Type of strain	Untreated % necrotic surface 7 days after inoculation	Mancozeb 1500g/ha % necrotic surface 7 days after inoculation (efficacy)
Blue 13	99,8	17,3 (82,7)
Green 33	96,8	2,5 (97,4)
Pink 6	95,5	5,0 (94,8)
VK 98074	91,2	2,5 (97,3)

DISCUSSION AND CONCLUSION

The efficacy of mancozeb is excellent on the strains Pink 6, Green 33, and the strain of the old population and very good on Blue 13.

The efficacy of mancozeb (Penncozeb) to control potato late blight – *Phytophthora infestans* – was tested in the greenhouse.

MATERIAL AND METHODS

The experiment was carried out with four replications. Each replication consisted of one potato plant. Analysis of variance on the late blight assessments was made based on the percentage necrotic foliage.

Four different strains of *P. infestans* were used: Blue 13, Pink 6, Green 33 (coming from a field with a less susceptibility to fluazinam) and an Orange genotype (old population).

The cultivated potato plants (cv. Bintje) were grown in pots. The pots with a content of 5 litres were filled with soil and the potato tubers were placed at a depth of 10 cm. From emergence until the experiment, the plants were placed in the greenhouse in Lelystad.

The potato plants were sprayed with the different fungicides in a spraying cabin developed by Applied Plant Research (PPO). The fungicides were sprayed using a spray boom with three spray nozzles, placed 50 cm apart, which is moving approximately 40 cm over the top of the potato plants. Spray volume was 250 l/ha.

The fungicides used were: mancozeb 1500 g/ha, mancozeb 1600 g/ha + metalaxyl-M 200 g/ha, mancozeb 1500 g/ha + fluazinam 200 g/ha, metalaxyl-M 200 g/ha and fluazinam 200 g/ha. The untreated control was sprayed with tap water.

The isolates were cultivated on agar plates and potato slices. An inoculum suspension was made by rinsing a one week old culture of *P. infestans* with tap water. The inoculum density was set at approximately 10,000 sporangia per ml. Inoculation of the potato plants was carried out by spraying the leaves over head with inoculum five days after spraying the fungicides. After inoculation the potato plants were incubated in a climate chamber for one week.

Late blight disease severity was assessed five times after inoculation. Percentage necrotic foliage was estimated. From the individual disease ratings a Standard Area Under Disease Progress Curve (stAUDPC) was calculated.

Analysis of variance on the late blight assessments was made based on the percentage necrotic foliage and sporulation

RESULTS

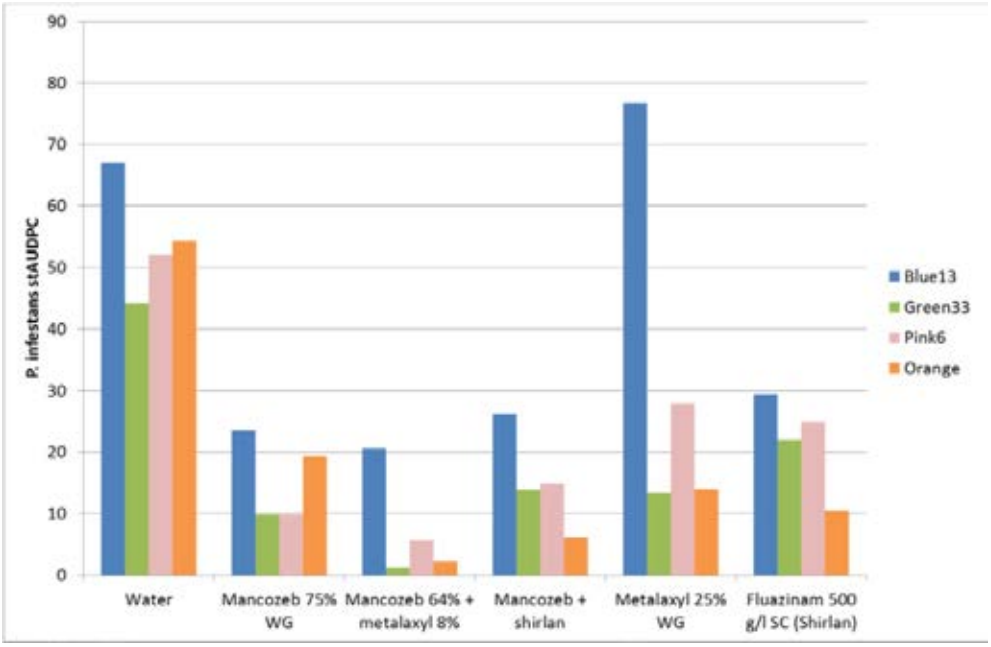
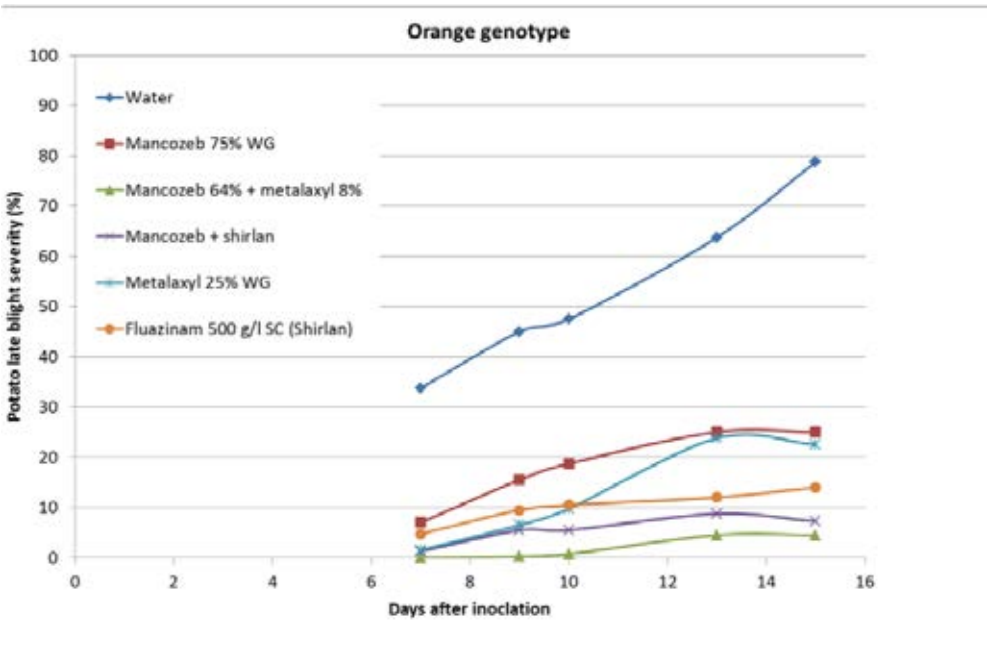
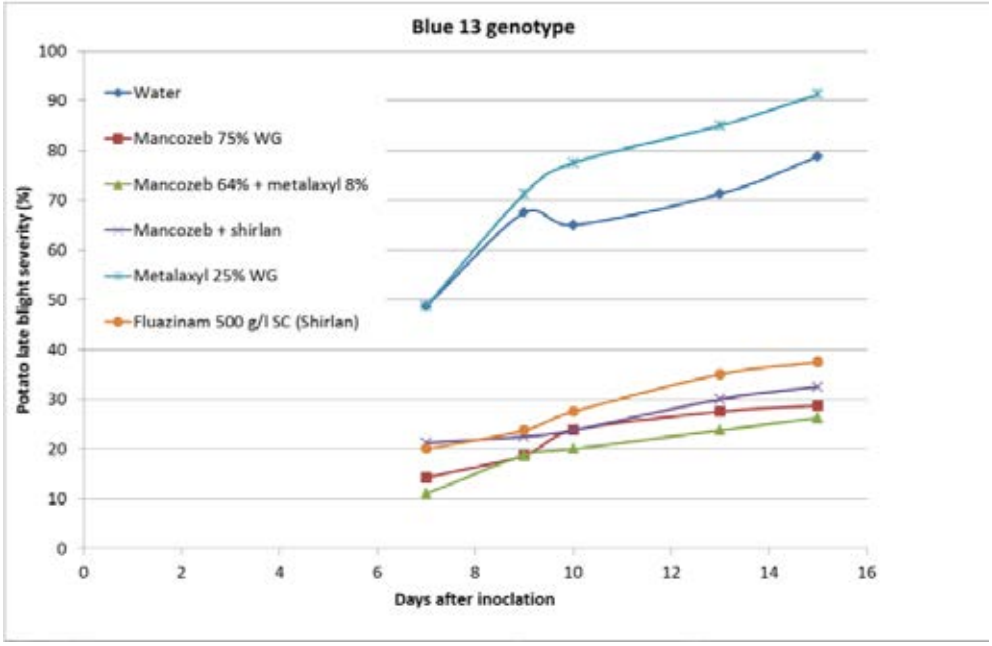
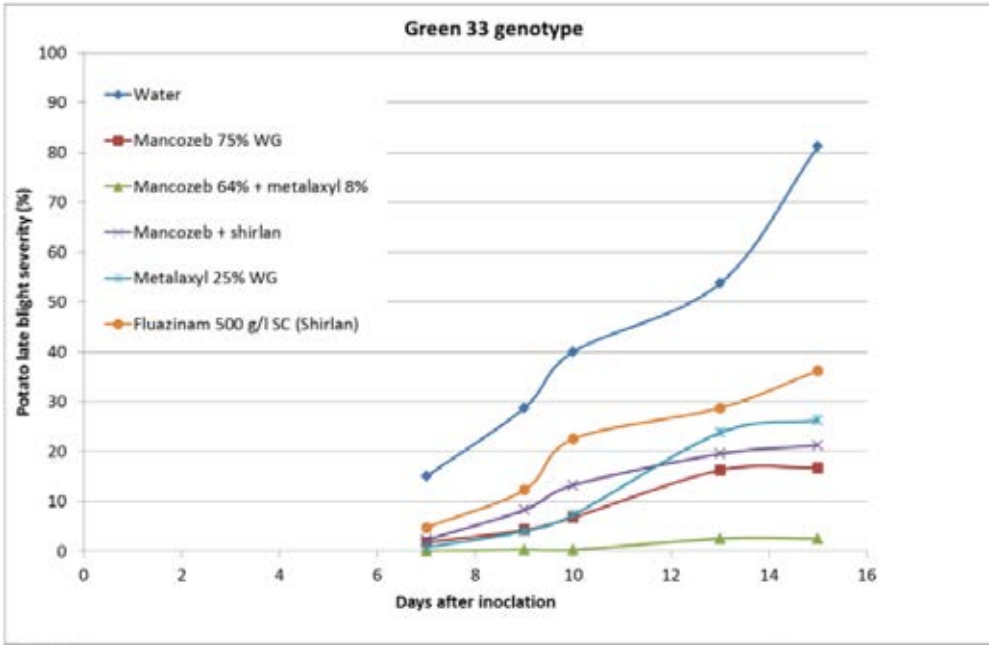


Figure 4. *Percentage necrotic foliage per treatment expressed as stAUDPC after inoculation with 4 different P. infestans isolates*




Blue 13 genotype
Water
Mancozeb 75% WG
Mancozeb 64% + metalaxyl 8%
Mancozeb + shirlan
Metalaxyl 25% WG
Fluazinam 500 g/l SC (Shirlan)
Potato late blight severity (%)
Days after inoclation
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16


Green 33 genotype
Water
Mancozeb 75% WG
Mancozeb 64% + metalaxyl 8%
Mancozeb + shirlan
Metalaxyl 25% WG
Fluazinam 500 g/l SC (Shirlan)
Potato late blight severity (%)
Days after inoclation
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16

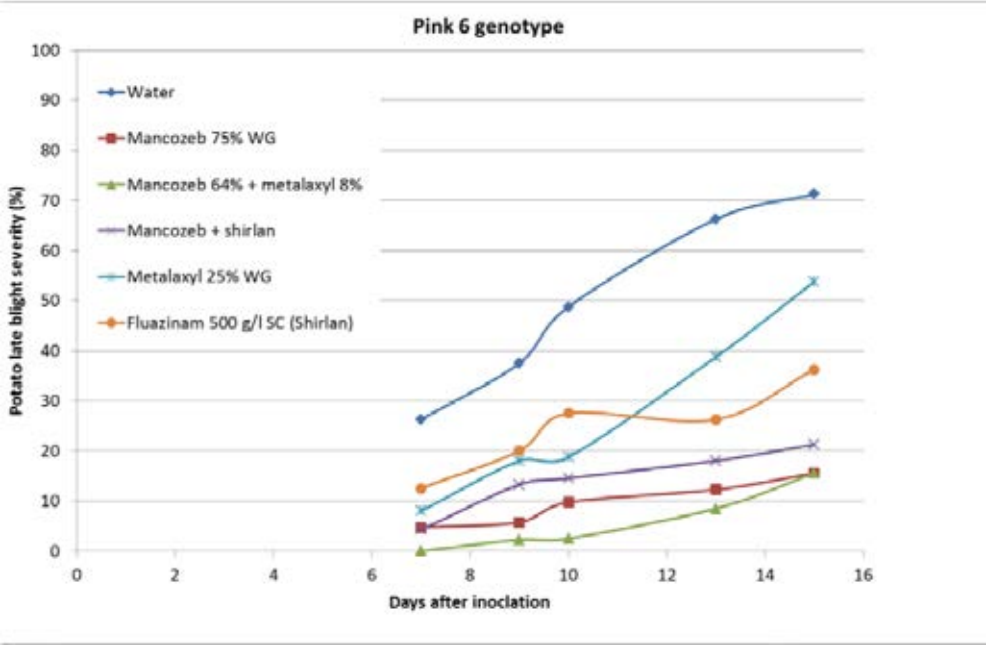


Figure 5. *Percentage necrotic foliage per treatment after inoculation with P. infestans isolates, representing clonal lineages*

DISCUSSION AND CONCLUSION

The infection level of untreated plants was 70-85% at the end of the experiment indicating that the artificial inoculation was successful for all four *P. infestans* isolates. All the fungicide treatments were able to reduce the percentage necrotic foliage compared to the untreated control, regardless of which *P. infestans* isolates were used except for metalaxyl-M when potato plants were inoculated with Blue 13

The absence of control of Blue 13 by metalaxyl-M confirms that this strain is metalaxyl-M resistant

The efficacy of mancozeb (B) to control Blue 13 isolate was significantly better than fluazinam (F) and metalaxyl-M (E)

Green 33 isolate was less effectively controlled by fluazinam (F) than mancozeb (B) and mancozeb + metalaxyl-M (C)

Pink 6 isolate was significantly better controlled by mancozeb (B), mancozeb + metalaxyl-M (C) and mancozeb + fluazinam (D) than fluazinam (F) or metalaxyl-M

The orange isolate was significantly better controlled with mancozeb + metalaxyl-M (C) than mancozeb (B) or metalaxyl-M

CONCLUSIONS FOR THE TWO EXPERIMENTS

Mancozeb is effective against all genotypes.

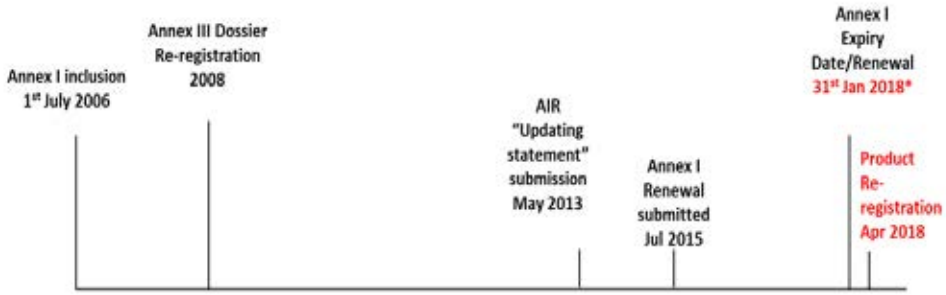
Mancozeb strengthens significantly the efficacy of fluazinam and metalaxyl-M, even on strains sensitive to these fungicides.

The stability of performance is ensured whatever the type of strains that is present in the field.
Mancozeb is an essential component of resistance management and therefore an important tool for developing efficient integrated management programs.

Next Step
UPL has commissioned Wageningen University to study the efficacy of mancozeb against *Alternaria*, especially against strains resistant to strobilurins.

REGULATORY STATUS

Mancozeb AIR 3 Timeline



Regulatory Hurdles
R63/H361d (possible harm to the unborn child) classification based on European Chemicals Agency (ECHA) evaluation on public literature in 2006 showing ED effects at unrealistic high concentrations.
Interim Criteria: Reproductive Category 2 + "toxic" effects on endocrine organs may be considered to have ED properties – as a result of its current classification.

ETU, a metabolite of mancozeb has been shown to affect the T3/T4 hormones in rats when dosed at very unrealistic high concentrations in public literature resulting in a R63/H361d classification.

DISCUSSION AND CONCLUSION

- Effects seen in the rat are not relevant to humans due to well known differences in physiology.
- When dosed at realistic exposure concentrations - New data shows that there is NO ADVERSE EFFECT.
- Proposed application to ECHA for removal of H361d classification.

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PPO – SPECIAL REPORT NO 17 – 2015, 119-120

Protocol for the artificial inoculation with *A. solani* in field trials (with infected kernels)

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SUMMARY
Protocol for the artificial inoculation with *A. solani* in field trials with infected kernels

KEYWORDS
Alternaria spp., artificial inoculation

INTRODUCTION

Early blight in potatoes is caused by the pathogen *Alternaria solani*.
The primary source of inoculum is fungal material which is overseasoning on plant debris within the soil. By adding additional infected material (kernels) the amount of primary inoculum is increased. This results in an earlier start of infestation and a faster disease progress during the vegetation.
This protocol can be used for field trials to check fungicide efficacy and host resistance and to compare the fitness of different *Alternaria solani* isolates.

MATERIAL UND METHODS

1. Let *A. solani* isolates grow on V8-medium at 22°C and 12h NUV-light for 14 days.
2. Put 150 g of cereal grains (wheat, barley, oat, rye) in an autoclavable bag.
3. Add 80 ml dest. H_2O.
4. Seal the bag with a plug and autoclave it two times (121°C, 20 min).
5. Take half of the content of one overgrown V8-petri dish, put it into the bag, and spread the inoculum by kneading the bag.
6. Incubate the inoculated bag at 22°C and 12h NUV-light for 4 weeks, turn it over and knead it again after 2 weeks.
7. Scatter 5g of inoculated grains/m^2 between the potato rows.

120

V8-medium:

Vegetable juice	200ml	
$CaCO_3$		2g
Agar		15g
dest. H_2O		800ml

PPO – SPECIAL REPORT NO 17 – 2015, 121-126

Virulence of *Alternaria* strains toward potato and tomato cultivars

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SUMMARY

Alternaria alternata is one of the causal agents of the early blight, a dangerous disease of potato and tomato, which is common for almost all regions, where these crops are grown. In this study the virulence and aggressiveness of *A. alternata* isolates, obtained from the leaves and tubers of potato and leaves and fruits of tomato, has been studied on 13 potato cultivars of different maturity groups and 5 large-fruited tomato cultivars. In the case of two isolates, obtained from tomato leaves and significantly differing in their virulence, the activity of subtilisin- and tripsin-like serine proteases has been also analyzed.

The performed study has revealed intraspecific differences in the virulence and aggressiveness of *A. alternata* toward the leaves of different potato and tomato cultivars. Some isolates successfully infected cultivars, highly resistant toward other isolates that probably evidences some potato and tomato cultivars have genes of specific resistance to *A. alternata.*

In addition, the revealed difference in the activity of serine proteases is observed on both interspecific and intraspecific levels and correlated with the virulence and aggressiveness of isolates. A high level of tripsin-like activity of secreted serine proteases is observed in highly pathogenic isolate. Thus, this parameter can be used as a marker for the study of the virulence and aggressiveness of *A. alternata* isolates.

KEYWORDS

Alternaria alternata, virulence, aggressiveness, serine protease activity

INTRODUCTION

Alternaria alternata is a common saprotrophic or parasitic fungus belonging to Ascomycetes. Like *A. solani*, *A. alternata* is a causal agent of the early blight of potato and tomato, a dangerous disease presenting in almost all cultivation areas of these crops.

Till recently it was considered that plants are infected with only large-spored *A. solani*, whereas small-spored *A. alternata* represents a secondary plant pathogen able to either infect plants together with *A. solani*, or to leave as a saprotroph on the necrotic lesions caused by other plant pathogens. However, today we have some data that *A. alternata* is able to infect plants independently, though the relation between the variability of the virulence and aggressiveness of different strains and their species-specific and organotropic specialization still remain unclear.

Hydrolytic enzymes secreting by necrotrophic plant pathogens provide the availability of macromolecular compounds as nutrients and, therefore, play an important role in the nutrition of such pathogens, including the early blight causal agents. This group of enzymes includes different proteases. Extracellular proteases, secreted by fungi, are able to macerate plant tissues and destroy cell wall components that allow a pathogen to overrun the natural resistance of a host plant. Thus, proteases not only act as digestive enzymes of fungi, but also participate in pathogenetic processes.

Some recent data allow us to suppose that serine exoproteases secreting by some plant pathogenic fungi can be considered as pathogenicity factors. For example, Dunaevskii *et al.* (2006) showed that plant pathogenic fungi, including *A. alternata*, secrete tripsin- and subtilisin-like proteases. Though the synthesis of proteases is a constitutive process, their repertoire in plant pathogens, including necrotrophic ones, should differ from that in saprotrophs. In this study we supposed that differences in the level of secretion of tripsin-like and subtilisin-like proteases occur at both interspecific and intraspecific levels and are able to serve as the markers of virulence and aggressiveness of isolates.

MATERIALS AND METHODS

Host plants

Thirteen Russian and Belorussian potato cultivars of different maturity groups were used in the study: first early (Zorachka and Lileya Belorusskaya), second early (Briz, Manifest, Romano, and Nevskiy), early maincrop (Volat, Lad, Skarb, and Yanka), and maincrop (Vektar Belorusskiy, Zhuravinka, and Ragneda). In addition, five commercial large-fruited tomato cultivars were used: Dubrava, Tomsk, La-la-fam, Verlioka, and Bych'e Serdtse.

Healthy meristemal plants were planted in enriched peat soil and kept in a greenhouse at 20-24°C and natural photoperiod. Watering was performed as required. No fertilizers were used during vegetation. To perform inoculation, the leaves of the 4-6 layers from the top were used. Leaves were placed in sterile Petri dishes onto object plates covered with wet filter paper.

Isolate sources

A. alternata and *A.solani* isolates were isolated from infected fresh leaves of potato and fresh leaves and fruits of tomato (Table 1). In addition, some isolates were collected from infected potato tubers within 10 days after the harvesting; isolation was performed only from conidiophores appeared on the live tissues of tubers. Infected samples were placed in wet chambers; isolation was carried out from a border of live and healthy tissue. The strain PPL 31 was kindly provided by the colleagues from the Laboratory of mycology and plant pathology of the All-Russian Research Institute of Plant Protection.

Table 1. *Description of Alternaria isolates used in the study*

Name	Species	Region of collection	Host plant, cultivar, organ	Year of collection
RPL 16	*A. alternata*	Ryazan region	Potato, cv. Ragneda, leaves	2012
RPL 21	*A. alternata*	Ryazan region	Potato, cv. Ragneda, leaves	2012
KPT 1	*A. alternata*	Kostroma region	Potato, cv. Udacha, tuber	2013
KPT 4	*A. alternata*	Bryansk region	Potato, cv. Bryanskaya Roza, tuber	2013
METL 5	*A. alternata*	Mariy-El Republic	Large-fruited tomato, leaves	2007
METL 12	*A. alternata*	Mariy-El Republic	Large-fruited tomato, leaves	2007
MTF 7	*A. alternata*	Moscow region	Large-fruited tomato, fruit	2013
PPL 31	*A. solani*	Primorye (Russian Far East)	Potato, leaves	2006
VTL 16	*A. solani*	Voronezh region	Large-fruited tomato, leaves	2014

Inoculum preparation and inoculation

To obtain inoculum (conidia), isolates were grown on potato-carrot agar up to the conidia formation (7-10 days). In the case of *A. alternata*, conidia were washed off from colonies by 10 ml of sterile water. In the case of *A. solani*, conidia were obtained by another way: aerial mycelium was removed after 7-10 days of incubation, and then Petri dishes were placed into refrigerator (+5°C) overnight and treated with UV radiation. After 3 days of incubation conidia were washed off from colonies by 10 ml of sterile water. The concentration of suspension used for inoculation was 100 conidia/μL. A drop (10 μL) of conidia suspension or sterile water (control) was placed on leaf surface, inoculated leaves were incubated for 7 days at room temperature, and then the level of infection was assessed. All experiments were performed in three replications.

Enzyme activity assay

The maintenance and cultivation of fungi was performed by submerged cultivation in liquid potato broth medium as described earlier (Valueva *et al.*, 2013). After 5, 10, and 18 days of cultivation mycelium was harvested on a weighed Whatman No. 41 filter paper, washed with a small volume of warm distilled water, heated overnight in an oven at +90°C, then cooled in a desiccator and weighed again. The longer drying did not result in a further weight loss. A crude culture filtrate obtained after the harvesting of mycelium was used for the enzyme activity assay.

The enzymatic activity of serine proteases was determined by the Erlanger's method (Erlanger *et al.*, 1961), using synthetic substrates BAPNA (Nα-benzoyl-DL-arginine-nitroanilide) and Z-AALpNA (N-carbobenzyloxy-L-Ala-L-Ala-L-Leu-pNa) in the assay of the tripsin-like and subtilisin-like activity, respectively. The initial substrate concentration was 0.5 mM. One unit of enzyme activity (U) was equal to the amount of enzyme hydrolyzing 1 nmol of the substrate per 1 min. Both BAPNA and Z-AALpNA substrates were purchased from Sigma-Aldrich (USA); all other commercially available reagents were of the highest grade. All experiments were performed in three replications.

RESULTS AND DISCUSSION

Virulence and aggressiveness of Alternaria isolates toward different potato and tomato cultivars

All isolates collected and isolated from tomato and potato leaves, tomato fruits, and potato tubers were able to infect potato and tomato leaves. Analysis of variance (ANOVA) show that

factors of cultivar, strain, and their combination influence the diameter of necrosis ($P<0.05$ for all variants). At the same time, we observe significant differences in the virulence of isolates toward the leaves of different cultivars of host plants. For example, all tested *A. alternata* isolates and *A. solani* strain used as a control strain were able to infect the following potato cultivars: Nevskiy, Lad, Volat, Briz, and Zorachka (Table 2). The maximum resistance was observed in the cvs. Lileya Belorusskaya and Yanka, which were infected with only one isolate, and also in the cv. Ragneda (infected with two isolates). Note that these three cultivars and also cvs. Romano and Zhuravinka were not infected by the control *A. solani* strain.

The study on tomato leaves showed that only cv. Dubrava was infected with all isolates studied; cvs. Bych'e Serdtse and Verlioka were infected with only one of three tested *A. alternata* strains and with the control *A. solani* strain (Table 3).

None of the studied isolates was virulent toward all studied potato cultivars. The highest virulence was observed in two isolates from potato tubers (KPT 1 and KPT 4) and one isolate from tomato leaves (METL 5), which were able to infect leaves of 10-11 cultivars. The lowest virulence was observed in the isolate obtained from tomato fruit (MTF 7), which infected only seven cultivars. The control *A. solani* isolate (PPL 31) did not show high virulence and infected the leaves of 8 out of 13 potato cultivars.

The same situation was observed concerning the virulence of the studied isolates toward different tomato cultivars. Only *A. solani* isolate was able to infect leaves of all tested tomato cultivars. Isolates of *A. alternata* collected from tomato fruits (MTF 7) and leaves (METL 5) infected leaves of four cultivars, whereas the METL 12 isolate infected only one tomato cultivar.

Table 2. *Pathogenicity of the tested isolates toward different potato cultivars*

Potato cultivar	Average diameter of necrotic lesions, mm								Number of virulent isolates
	Alternaria alternata							A. solani	
	Potato leaves		Potato tubers		Tomato fruits	Tomato leaves		Potato leaves	
	RPL 16	RPL 21	KPT 1	KPT 4	MTF 7	METL 5	METL 12	PPL 31	
Nevsky	18	20	15	10	15	10	3	10	8
Lad	4	4	25	24.5	3	5	15	8	8
Volat	3	3	6,5	10	3	10	3	10	8
Briz	10	10	30	5	25	15	20	20	8
Zorachka	3	3	8	15	3	4	3	7	8
Manifest	2	2	3	5	3	8	0	15	7
Vektar belor.	8	0	15	0	8	20	15	5	6
Zhuravinka	5	5	0	20	0	10	10	0	5
Romano	0	2	5	20	0	10	2	0	5
Skarb	0	5	20	6	0	4	0	27	5
Ragneda	0	0	0	20	0	20	0	0	2
Yanka	0	0	7	0	0	0	0	0	1
Lileya belor.	0	0	0	5	0	0	0	0	1
Total number of infected cultivars	8	9	1	11	7	11	8	8	
Average necrosis diameter, mm	4.1±1.7*	4.2±1.7	10.4±3.1	10.8±2.6	4.6±2.4	9±2.1	5.5±2.2	7.8±2.7	

* - a confidence interval for the significance level 0.05.

Table 3. *Pathogenicity of the tested isolates toward different tomato cultivars*

Tomato cultivar	Average diameter of necrotic lesions, mm				Number of virulent isolates
	Alternaria alternata			A.solani	
	Tomato fruits	Tomato leaves		Tomato leaves	
	MTF 7	METL 5	METL 12	VTL 16	
Dubrava	10	18	10	35	4
Tomsk	10	5	0	15	3
La-la-fam	5	5	0	5	3
Verlioka	5	0	0	5	2
Bych'e Serdtse	0	20	0	5	2
Total number of infected cultivars	4	4	1	5	
Average necrosis diameter, mm	6±2.1*	9.6±4.5	2±2	13±6.7	

* - a confidence interval for the significance level 0.05.

In this study only one component of aggressiveness was assessed: the diameter of necrotic lesions on infected leaves. According to the obtained results, isolates KPT 1 and KPT 4, obtained from potato tubers, and isolate METL 5, obtained from tomato leaf, were the most aggressive toward potato leaves of different cultivars: the average necrosis diameter was 8.9-10.8 mm. The same strains were also virulent toward the maximum number of potato cultivars (10-11 out of 13 cultivars; Table 2). METL 5 and KPT 4 isolates caused significant necrosis (20 mm) of leaves of cv. Ragneda, which was not infected with other isolates, including *A. solani*. The KPT 4 isolate was the only isolate successfully infected cv. Lileya Belorusskaya, whereas the KPT 1 isolate was the only isolate infected cv. Yanka.

Significant differences in the diameter of necrotic lesions were also observed for infected tomato cultivars (Table 3). The METL 12 strain was able to infect leaves of the cv. Dubrava, but did not infect other tomato cultivars. The METL 5 strain caused a strong infection of leaves of cvs. Dubrava and Bych'e Serdtse (18-20 mm), whereas the *A. solani* strain caused significant necroses on leaves of the cv. Dubrava (35 mm).

Serine protease secretion

In this study, we assessed the protease secretion by isolates, differing in their virulence toward potato and tomato; therefore, the studied isolates were cultivated on liquid medium based on thermostable proteins of potato. The study was carried out using two isolates, METL 12 and METL 5, collected from tomato leaves and significantly differing in their virulence and aggressiveness toward both potato and tomato leaves.

According to the obtained results, the dynamics of secretion of serine proteases was similar in both isolates: sharp increase in the activity from 5th to 10th days of growth and sharp decrease to the 18th day. In both isolates, the maximum activity of secreted proteases was observed at the 10th day of growth (Table 4).

The separate assessment of the activity of tripsin-like and subtilisin-like proteases resulted in an interesting observation. On the 10th day, the more aggressive METL 5 isolate demonstrated a higher activity of tripsin-like proteases than that of the less aggressive METL 12 isolate. At the same time, the activity of subtilisin-like protease was higher in less pathogenic METL 12 strain. These results correspond to the results of other studies. For example, Dunaevskii *et al.* (2006) showed that saprotrophic species *Trichoderma harcianum*, *Penicillium terlikowskii*, and

Penicillium chrysogenum are characterized by high activity of subtilisin-like proteases and do not produce tripsin-like proteases, whereas phytopathogenic species *Alternaria alternata*, *Botrytis cinerea*, and *Ulocladium botrytis* produce proteases of both types.

Table 4. *Dynamics of secretion of serine proteases by METL 5 and METL 12 isolates of Alternaria alternata*

Isolate	Average activity of serine proteases, U/g of dried mycelium*					
	Tripsin-like (BAPNA substrate)			Subtilisin-like (Z-AALpNa substrate)		
Day of growth	5	10	18	5	10	18
METL 12	8	162	4,2	22	14092	433
METL 5	0	298	2.9	46	1635	36

* U - the amount of enzyme that hydrolyzes 1 nmol of a substrate per 1 min.

Thus, the performed study demonstrated some intraspecific difference in the virulence and aggressiveness of *A. alternata* toward leaves of different potato and tomato cultivars. Some isolates were able to infect cultivars resistant to other isolates that makes it possible to suppose the presence of the corresponding specific resistance genes in potato and tomato cultivars.

Our study also showed that differences in the activity of serine proteases are observed not only at interspecific (Dunaevskii *et al.*, 2006) but at intraspecific levels and are connected with the virulence and aggressiveness of isolates. Though both isolates studied were isolated from alive tomato leaves, they probably differ in their trophic substrate: the METL 5 isolate, which is more pathogenic and has a high level of tripsin-like activity, is able to infect live tissues, whereas the less aggressive and virulent METL 12 isolate, characterized by a high level of subtilisin-like activity, grows on dead tissues and actively utilizes the substrate via the saprotrophic way. Therefore, a high level of the tripsin-like activity of extracellular serine proteases is observed in highly pathogenic isolates, and this parameter can be used as a marker for the study of the virulence and aggressiveness of *A. alternata* isolates.

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Efforts towards a harmonized early blight detection method, results of the first *Alternaria* ring test

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SUMMARY
Early blight is becoming an increasing problem in the potato cultivation. The aim of this research was to establish which fungi (*Alternaria* species) could be isolated from lesions in potato leaves collected in commercial fields and field experiments. By carrying out a ring test we wanted to establish whether different isolation procedures by the involved laboratories gave comparable results and thus to come to suggestions for a common EuroBlight isolation protocol for Alternaria spp.. Recovery rates of *Alternaria* spp. were high and seemed consistent for the three laboratories involved. Recommendations for an early blight isolation protocol are given.

KEYWORDS
Alternaria solani, Alternaria alternata, isolation, determination, Early Blight, potato, *Solanum tuberosum*

INTRODUCTION

Early blight is becoming an increasing problem in the potato cultivation. Within Europe there is no consensus on the causal agent of early blight. *Alternaria solani* and *A. alternata* are named the most often, although also other *Alternaria* spp. seems to be associated with early blight. Furthermore, often lesions on potato leaves are found from which no pathogen can be isolated. Several research groups collect infected potato leaves and all have their own method for isolation and determination of the fungi present in and on the potato leaves. The aim of this research was to establish which fungi (Alternaria species) could be isolated from lesions in potato leaves collected in commercial fields and field experiments. Furthermore we wanted to establish whether different isolation procedures by the involved laboratories gave comparable results and thus to come to suggestions for a common EuroBlight isolation protocol for *Alternaria* spp. Therefore potato leaf samples with lesions were taken and sent to the laboratories in München, Wijster and Wageningen.

MATERIAL AND METHODS

Potato leaves samples were taken from 13 locations and 5 countries (Table 1). Sub samples from the same location were made and sent to each of the three laboratories. The potato leaves were put between tissue paper which was put in an envelope and sent by mail. When available, information on spray schedule, cultivar and early blight severity was included.
Upon arrival each laboratory made the assessment according to their own protocol. From each sample at least 10 lesions were used to determine the causal agent.

Isolation and determination at TUM
Lesions from infected leaves were cut to a size of 0.5 to 1 cm. The pieces were surface sterilized in a 5% NaOCl solution for 1 minute followed by washing in sterile water. SN (slight nutritious) medium was used for production of spores. The samples were incubated at 20°C under UV-light with a 12/12h photoperiod during 3 to 6 days. Sporulation was checked under a light microscope and the *Alternaria* species found was established. The method can be found on the EuroBlight website http://euroblight.net/alternaria/protocols/

Isolation and determination at HLB
Lesions from infected leaves were cut. The pieces were not surface sterilized. The necrotic tissue was transferred to water agar containing 50 µg/ml streptomycin. The samples were incubated at 20°C with 16 h photoperiod during 3 to 8 days. No UV-light was used. Sporulation was checked under a light microscope and the *Alternaria* species found was established after three and seven days of incubation. A second assessment was made to check the first one.

Isolation and determination at Wageningen-UR
Lesions from infected leaves were cut to a size of 1 cm^2. The pieces were not surface sterilized. Water agar (1.5%) with ampicillin (200 mg/l) was used. Imprints of both sides of the lesions were made on the agar. Then the potato leaf cuttings with lesions were put in an upright position in the water agar. The samples were incubated at 15°C with 16 h photoperiod during two weeks. No UV-light was used. Sporulation was checked under a light microscope and the *Alternaria* species found was established. Both the imprint and the lesions were checked for sporulation. If no spores were formed the incubation was prolonged for 1 more week (usually this was not necessary). A little bit of agar with mycelium of *Alternaria* spp. was transferred to a new Petri dish with water agar to purify the sample. This was also a second check on the results. The method can be found on the EuroBlight website http://euroblight.net/alternaria/protocols/

Table 1. *Origin of samples from potato leaves with Early Blight symptoms sent to the three laboratories*

Nr.	country	sent by	field location	variety	fungicide treatment	disease severity	sampling date
1	Germany	TUM	Freising	Agila	untreated	?	15.07.2014
2	Germany	TUM	Freising	Maxilla	untreated	?	15.07.2014
3	Germany	TUM	Straßmoos	unknown	Mancozeb	moderate	25.07.2014
4	Belgium	PCA	Kruishoutem	Bintje	untreated	5%	08.08.2014
5	The Netherlands	HLB	Wijster	Festien	untreated	10%	11.08.2014
6	Sweden	SLU	Nymö	Kuras	untreated	?	31.07.2014
7	Sweden	SLU	Nymö	Kardal	untreated	?	07.08.2014
8	Sweden	SLU	Nymö	Kuras	untreated	?	19.08.2014
9	Germany	TUM	Niedersunzing	unknown	treated	moderate to high	24.08.2014
10	Belgium	PCA	Lozer	Bintje	untreated	0.5-1%	28.08.2014
11	The Netherlands	WUR	Westmaas	unknown	treated	?	01.09.2014
12	The Netherlands	WUR	Valthermond	Aveka	untreated	20%	03.09.2014
13	The Netherlands	WUR	Lelystad	Bintje	untreated	75%	02.09.2014

RESULTS

Alternaria solani was found in each sample by all laboratories, except for sample number 1 where the pathogen was missing at HLB and WUR (Table 2). *Alternaria alternata* was missing more often especially at Wageningen UR. Also when *A. alternata* was found it was less abundant than *A. solani.*

Table 2. *Alternaria identification from 13 locations at three laboratories*

Nr.	TUM	WUR	HLB	TUM	WUR	HLB
1	A.s1	missing	missing	A.a.	A.a.	A.a
2	A.s +	A.s.	A.s.	A.a.	missing	A.a.++
3	A.s ++	A.s.	A.s ++	A.a.	missing	A.a.+
4	A.s +	A.s +	A.s ++	A.a.	A.a.	A.a.++
5	A.s +	A.s.	A.s +	A.a.	A.a.	A.a.
6	A.s ++	A.s +	A.s ++	A.a.	A.a.	A.a.
7	A.s ++	A.s.	A.s ++	A.a.+	missing	A.a.+
8	A.s +	A.s +	A.s ++	A.a.	A.a.	A.a.
9	A.s +	A.s.	A.s	A.a.	missing	missing
10	A.s.	A.s.	A.s ++	missing	missing	A.a.
11	A.s +	A.s +	A.s ++	A.a.	A.a.	A.a.+
12	A.s +	A.s +	A.s ++	A.a.	A.a.	A.a.
13	A.s ++	A.s +	A.s ++	A.a.	A.a.	A.a.+

[1]: no sign means that the pathogen was found but was just present, + = moderately present, ++ abundantly present

DISCUSSION

In the first sample both HLB and Wageningen UR could not find *A. solani* whereas the pathogen was found by TUM. However this sample was taken at 17 July but arrived in the laboratories in The Netherlands more than one month later. Usually the samples arrived within one or two weeks after sampling. In the 12 other cases *A. solani* was found readily, although more abundantly when assessed by TUM and HLB compared to Wageningen UR. This could be method related, laboratory related or scale related. Nevertheless recovery rates were high and seemed consistent.

A. alternata was found in most of the samples as well, although less abundant than *A. solani.* The fungus was missing in samples 9 (HLB, WUR), 10 (TUM, WUR) and 2, 3 and 7 (WUR). The main difference in the procedure between TUM and HLB on the one hand and WUR on the other is the incubation temperature which is 20°C and 15°C respectively. Stammler (2014), showed that *A. solani* was recovered more easily when isolated at 16°C (72%), whereas *A. alternata* was more easily isolated at 22°C (86%). This could explain that *A. alternata* was not readily found by WUR.

Although it is known that UV-light can stimulate sporulation of *Alternaria solani*, in this experiment it was not a prerequisite.

CONCLUSIONS

The experiment was set-up to find the best method for determining *Alternaria* spp. on infected leaves. The following recommendations are given for isolation and determination.

- Sample leaves (app. 10 leaves), preferably from plots, which were not treated with Alternaria specific fungicides.
- In the case that leaves will be shipped, make sure that leaves are dried. Therefore leaves should be put between paper towels.
- cut out of little infection sites (1 cm^2), one lesion per leaf.
- surface sterilization is NOT obligatory, however it helps to reduce fungal/bacterial contamination (5% NaOCl, 1 min).
- Medium: no particular recommendations (water agar 1.5% with antibiotica or SNA).
- Incubation of petri dishes in climate room, temperature (15) 20°C, photoperiod (16/8h) is requested.
- UV-light is NOT obligatory but will support sporulation.
- spore formation can be visualized with a binocular within 3 to 7 days.

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Report of the Control Strategies Subgroup meeting on 13 May 2015: Discussion and agreements reached

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CHAIRMAN: HUUB SCHEPERS

Initially members of the *Alternaria* Subgroup attended a joint meeting with the Control Strategies Subgroup to allow aspects of *Alternaria* fungicide ratings to be fully discussed.

1. *ALTERNARIA* BLIGHT

1.1 Protocol for fungicide efficacy trials to provide ratings for Alternaria fungicides

There will be three field trials with *A. solani* in 2015; in Germany, Denmark and The Netherlands. Hans Hausladen presented the following updated outline of the protocol.

Treatments will override label restrictions in relation to number of applications
EPPO guidelines (EPPO 1/263(1)) will be adhered to
A susceptible variety will be used
The experimental design will be a randomised complete block
An untreated is to be included, either as plots or as spreader rows
Natural infection by *A. solani* is preferred but artificial inoculation is to be allowed provided the method used is the cereal grain one.
Misting of the trial is allowed but not preferred
The reference fungicide treatments are mancozeb @ 7-day intervals (reference for late blight fungicides with activity against early blight) and mancozeb @ 14-day intervals (reference for early blight specific fungicides)
The first application of the test fungicides is to be made at 6 to 8 weeks after crop emergence and definitely no later than the first appearance of symptoms
The severity (%) of early blight will be assessed every 7 days
The results of six trials with good results are required for a rating to be awarded to a test fungicide.
The first ratings are anticipated in 2017.
Fungicide products can have 7- and 14-day ratings
The ratings for the two mancozeb standards, and the ratings scale have not been finalised yet.

1.2 Changes to the early blight fungicide table

Prior to the Brasov workshop Belchim requested that the EuroBlight fungicide experts give an early blight rating for difenoconazole (Narita) based on the experts' experience with this product and also information provided by Belchim. Prior to the workshop a rating of +++ was agreed, with the table footnote that in some trials there were indications that the rating was +++(+). This rating was agreed at the subgroup meeting in Brasov.

Proposal: A footnote to the early blight table is required stating that the number of applications of test fungicides in the trials does not necessarily comply with the product label (Agreed).

Proposal: The dose rates of fungicide products should be added to the early blight table (Agreed).

Proposal: Comments on insensitivity risk for fungicides should be included in the early blight table (Not agreed). The consensus was that this needs to be considered further because the situation is not clear cut.

Proposal: Include information on the curative activity of fungicides against *Alternaria* (Not agreed). The subgroup's view was that it was too early to include information other than early blight leaf protection ratings.

2. CONTROL STRATEGIES SUBGROUP ALONE, LATE BLIGHT

2.1 Late blight fungicide table changes, as agreed at the 2013 workshop in Limassol

The A and B tables that existed previously have been combined.
The dates of first registration in Europe of the listed fungicide products have been added.
Fungicide products that are no longer marketed have been removed from the table.
A disclaimer covering fluazinam insensitivity in relation to genotype 33_A2 is now in place.
The table now has links to the early blight table and the FRAC website.

Proposal: The accuracy of some first registration dates should be checked (Agreed). The appropriate company should inform Huub Schepers of any necessary amendments.

Proposal: The link to the early blight table should be clearer (Agreed).

2.2 Ratings

The following ratings, based on trial results, were awarded and the table modified accordingly:

Fungicide	Leaf blight control ratings
benalaxyl-M + mancozeb	3.0
dimethomorph + fluazinam	3.7
mandipropamid + cymoxanil	4.4
(zoxamide + cymoxanil) + fluazinam	4.3

There were no changes to tuber blight control ratings or new ratings based on trials.

Qualitative ratings (0 to +++) were modified for dimethomorph + fluazinam after consideration by the EuroBlight fungicide experts of the information submitted by Adama.

If a company has information supporting revised qualitative ratings the agreed procedure is for the company to approach Huub Schepers.

Proposal: Phosphonates and host resistance elicitors should be considered for inclusion in the ratings table (Not agreed). It was stated that product registration is a prerequisite for inclusion. Companies with such registered products should approach EuroBlight to have products considered for trial evaluation.

Proposal: The decimal rating for a fungicide product needs to be confirmed (through an additional three EuroBlight trials) 7 years after the rating was conferred (Not agreed). Where there is suspicion of a discrepancy between a fungicide's rating and its current efficacy advisors need to report this to EuroBlight with supporting evidence (Agreed).

2.3 New initiatives and developments
Proposal: Information on the events of other blight networks, e.g. Latin Blight, USBlight and Asiablight, are to be placed on the EuroBlight website and in newsletters (Agreed).

Proposal: Links on the EuroBlight website to these other networks are required (Agreed).

Proposal: The experimental protocols on the EuroBlight website should be shared across the different blight networks (Agreed).

Proposal: FTA card samples should be submitted from Romania so that the country is included in the 2015 Monitoring of *P. infestans* in Europe programme (Agreed).

3. RECORD OF FUNGICIDE TABLES

The most up to date versions of the late blight and *Alternaria* fungicide tables should be accessed via the EuroBlight website. The fungicides tables in this paper are a record of the tables as at September 2015.

GENERAL COMMENTS ABOUT THE RATINGS TABLE FOR LATE BLIGHT FUNGICIDES

Ratings for leaf blight are based on results from EuroBlight field trials, and only compounds included in these trials are rated for leaf blight. The scale for leaf blight is a 2-5 scale (see technical report: Fungicide evaluation to rate efficacy to control leaf late blight for the EuroBlight table. Results 2006 – 2013).

Ratings for tuber blight are also based on results from EuroBlight field trials and only compounds included in these trials are rated for tuber blight. The scale for tuber blight is a 0-5 scale (see technical report: Fungicide evaluation to rate efficacy to control tuber blight for the EuroBlight table. Results 2009-2011). All other ratings are on a 0 to +++ scale.

There are few products with decimal ratings for tuber blight control compared with earlier subjective ratings. The 0 to +++ ratings can be obtained from the previous workshop proceedings.

The scores for individual products are not additive for mixtures of active ingredients. The dose rates in brackets are those used in the EuroBlight field trials to determine the leaf blight and tuber blight ratings. Ratings will be lower where fungicide insensitive strains are present.

The ratings given are for late blight fungicides currently registered in several EU countries and are for commercially available products containing one active ingredient, or two active ingredients as a co-formulated mixture, or tank mix on the product label. The ratings are NOT for the active ingredients themselves. 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 those for leaf and tuber blight control, are based on non-EuroBlight field experiments and experience of the performance of products when used in commercial conditions. Ratings for leaf blight and tuber blight control were each calculated from the results of a minimum of six EuroBlight field trials. Ratings, other than leaf and tuber blight control ones, are intended as a guide only and will be amended in future if new information becomes available.

DEFINITIONS (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.

NEW GROWTH
The ratings for the protection of the new growing point (new growth) indicate the protection of new foliage due to 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.

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
See section above on phenylamide resistance

Isolates of *P. infestans* have been found in The Netherlands resulting in lower field efficacy of fluazinam.

As stated earlier in this paper the fungicide insensitivity status of the European population of *Alternaria* species is currently not clear. Where resistant strains are present in high frequencies within populations the scores for the various attributes will be reduced.

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.

It is essential 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.

Late Blight Fungicide Table *The effectiveness of fungicide products and label mixtures for the control of P. infestans based on the highest dose rate registered in Europe (as at September 2015)*

	Effectiveness				Mode of Action			Rainfastness	Mobility in the plant	Year
Product [Dose rate (l or kg/ha)]	Leaf Blight[3]	New growth	Stem blight	Tuber blight[4]	Protectant	Curative	Anti-sporulant			
copper			+		+(+)	0	0	+	contact	1900
dithiocarbamates (2.0) [1]	2.0		+	0.0	++	0	0	+(+)	contact	1961
chlorothalonil			(+)		++	0	0	++(+)	contact	1964
cyazofamid (0.5)	3.8	++	+	3.8	+++	0	0	+++	contact	2001
fluazinam (0.4)	2.9		+		+++	0	0	++(+)	contact	1992
zoxamide+mancozeb (1.8)	2.8		+[5]		+++	0	0	++(+)	contact+contact	2001
amisulbrom+mancozeb (0.5+2.0)	4.5		+	3.7	++(+)	0	?	+++	contact+contact	2007
ametoctradin+mancozeb (2.5)	3.7	?[8]	?[8]		++(+)	0	0	+++	contact+contact	2011
famoxadone+cymoxanil			+(+)		++	++	+	++(+)	contact+translaminar	1996
mandipropamid (0.6)	4.0	++	+(+)		+++	+[6]	+(+)	+++	translaminar+contact	2005
mandipropamid+difenoconazole (0.6)	4.0	++	+(+)		+++	+[6]	+(+)	+++	translaminar+contact	2005
benthiavalicarb+mancozeb (2.0)	3.7		+(+)[5]		+++	+(+)	+	++(+)	translaminar+contact	2003
cymoxanil+mancozeb			+(+)		++	++	+	++	translaminar+contact	1976
cymoxanil+metiram			+(+)		++	++	+	++	translaminar+contact	1976
cymoxanil+copper			+(+)		++	++	+	++	translaminar+contact	1976
dimethomorph+mancozeb (2.4)	3.0		+(+)		++(+)	+	++	++(+)	translaminar+contact	1988
dimethomorph+fluazinam (1.0)	3.7	+	+	3.3	++(+)	+	++	++(+)	translaminar+contact	2012
fenamidone+mancozeb (1.5)	2.6		+(+)[5]		++(+)	0	+(+) [5]	++	translaminar+contact	1998
(zoxamide+cymoxanil) +fluazinam (0.45 + 0.4)	4.3								translaminar+contact	2013

Product [Dose rate (l or kg/ha)]	Effectiveness				Mode of Action			Rainfastness	Mobility in the plant	Year
	Leaf Blight[3]	New growth	Stem blight	Tuber blight[4]	Protectant	Curative	Anti-sporulant			
mandipropamid+cymoxanil (0.6)	4.4								translaminar+translaminar	2013
benalaxyl-M+mancozeb [2]	3.0	++	++		++(+)	++(+)	++(+)	+++	systemic+contact	1981
metalaxyl-M+mancozeb[2]		++	++		++(+)	++(+)	++(+)	+++	systemic+contact	1977
metalaxyl-M+fluazinam [2]		++	++		++(+)	++(+)	++(+)		systemic+contact	
(propamocarb+cymoxanil) + cyazofamid ((2.0)+0.5)				4.6					systemic+translaminar+ contact	2012
propamocarb+cymoxanil (2.0)					+(+)	++(+)[7]	++(+)		systemic+translaminar	2011
propamocarb+fenamidone (2.0)	2.5	+(+)	++		++(+)	++	++	+++	systemic+translaminar	1998
propamocarb+fluopicolide (1.6)	3.8	++	++	3.9	+++	++	++(+)	++(+)	systemic+translaminar	2006

Footnotes to Late Blight Fungicide Table

See caveats listed in the section entitled 'General comments about the ratings tables'

[1] Includes maneb, mancozeb, propineb and metiram. [2] See text for comments on phenylamide resistance. [3] Based on EuroBlight field trials in 2006-2012.

[4] Based on EuroBlight field trials 2009-2012 [5] Based on limited data. [6] In some trials there were indications that the rating was +(+).

[7] In some trials the curative activity was +++ [8] Observations from some trials indicated that both new growth and stem blight efficacy were ++

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

The scale for leaf blight is a 2 to 5 scale (2=least effective, 5= most effective).

The scale for tuber blight is 0 (no effect) to 5 (complete control).

Disclaimer : this is given in the text of this paper.

Early Blight Fungicide Table *Efficacy of fungicides for the control of early blight caused by Alternaria solani and Alternaria alternata (as at September 2015)*

Product	Efficacy
azoxystrobin	+++(+)
fluazinam	(+)
metiram/mancozeb[1]	++
propineb	++
chlorothalonil	+(+)
famoxadone+cymoxanil	++
fenamidone+mancozeb or propamocarb[2]	++
zoxamide+mancozeb	++(+)
pyraclostrobin + boscalid	+++(+)
difenoconazole+mandipropamid	+++
difenoconazole[3]	+++

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

[1]This rating applies to products containing mancozeb when used at the highest dose rates (>1500g/ha). This rating may not be appropriate where the rate of mancozeb used is lower, particularly where the second active substance is not effective against Alternaria. [2]In some trials there were indications that the rating was ++(+). [3]In some trials there were indications that the rating was +++(+). Ratings will be lower where fungicide insensitive strains are present.

Disclaimer: this is given in the text of this paper.

PPO – SPECIAL REPORT NO 17 – 2015, 139-140

State of the art and important research questions: Report from the EuroBlight Alternaria group

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1. OBJECTIVE

The discussion in the EuroBlight Alternaria group started with the discussion of objective of the subgroup. The group decided that the objective is to **increase the knowledge** of early blight. This is the basis for an IPM system to control EB and it provides information to support national development. The group agreed that there is more information necessary concerning:

- Monitoring of fungicide sensitivity
- Fungicide trials (→ rating of fungicides)
- Phenotypic characterization of the pathogens
- Decision Support System
- Host resistance

2. ACTIVITIES

The EuroBlight Alternaria group summarized the different protocols for isolation, spore production, artificial inoculation (greenhouse and field) and molecular detection of different Alternaria species. On the website there are 18 different "detailed lab protocols" listed dealing with:

- + qPCR
- + Artificial inoculation
- + Long-Term Storage
- + Growth and conidia production
- + Isolation of Alternaria species
- + Characterization of Cyt B mutations
  (F 129 L Mutation in *A. solani*, G143A Mutation in *A. alternata*)

The results of the diagnostic ring test 2014 were discussed. The group decided not to prolong this project.

3. PROTOCOL FOR FUNGICIDE TRIALS TO PROVIDE RATINGS FOR ALTERNARIA FUNGICIDES

Together with the fungicide rating group the protocol for fungicide trials to provide ratings for Alternaria fungicides was discussed. In comparison to the previous protocol the spray interval is now 7 or 14 days.

The main points are:

- Susceptible variety
- Weekly applications of Revus or Ranman Top to prevent late blight
- Two to five applications of Alternaria fungicides
- Test fungicides to commence before the start of the epidemic (approximately 7 to 8 weeks after emergence)
- Alternaria test fungicides to be applied at intervals of 7 or 14 days and at the highest label dose rate in Europe
- Two or more reference fungicides, i.e. mancozeb (1500 g a.i. per ha), Signum (0.25 kg/ha) and Ortiva (0.5 L/ha)

Fungicide rating trials according to the protocol will be conducted 2015 in Germany, the Netherlands and Denmark.

4. HOMEPAGE

All relevant publications (*Alternaria* on potato and tomato) as well as general remarks concerning "control of early blight" will be uploaded and updated on the EUROBLIGHT homepage.

5. NEW INITIATIVES

The members of the subgroup decided to initiate a cooperating project dealing with the widespread of the QoI fungicide resistance of *A. solani* in European potato growing areas. The project calls "Monitoring of sensitivity to fungicides (QoI) of *A. solani* isolates in Europe".
The project starts in 2015 with a limited number of *Alternaria solani* isolates and locations.

Posters

PPO – SPECIAL REPORT NO 17 – 2015, 143-144

Cultivation technology influences the occurrence of potato early blight (*Alternaria solani*) in an organic farming system

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Cultivation technology influences the occurrence of potato early blight (*Alternaria solani*) in an organic farming system

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INTRODUCTION

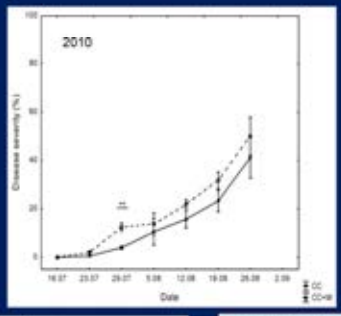
- Nowadays, organically produced products have become more popular than ever and interest in them is still growing fast.
- The early blight causal pathogen *Alternaria solani* has not been considered a great threat to potato in northern climate conditions in the past and has not been routinely sprayed against.
- In recent years, potato early blight has occurred with increasing frequency in European potato fields, including Northern area.
- The main aim of this research was to test how different organic cultivation technologies influence early blight development in disease-favourable conditions. A further aim was to test the suitability of a locally bred quite late blight resistant cultivar recommended for growing in an organic farming system.

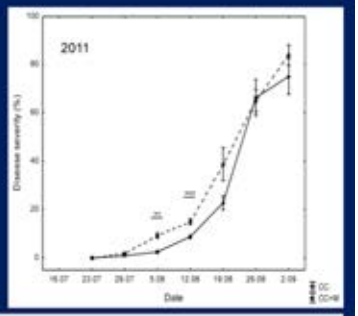
MATERIALS AND METHODS

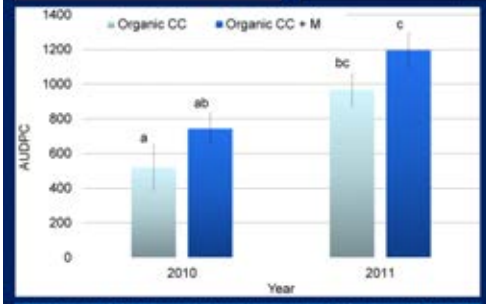
- Early blight (*Alternaria solani*) was evaluated in 2010 and 2011 on the plants of a potato cultivar 'Reet' in an organic farming experiment of the Department of Field Crops and Grassland Husbandry, Estonian University of Life Sciences located at Eerika (58°22' N, 26°40' E).
- The potato trial is part of a 5-year crop rotation experiment with red clover, winter wheat, peas, potato and barley following each other. The rotation experiment was started in 2008.
- 2 different treatments were used: CC (winter cover crop for green manure) and CC+M (CC+composted cattle manure, 40 t ha^{-1}).
- Early blight infection was assessed according to the 0–100% scale.
- The area under the disease progress curve (AUDPC) was calculated from the date of first occurrence of early blight until the last observation of the disease in the trial.

RESULTS

- Both growing seasons were very favourable for early blight development and evaluation.
- Winter cover crop (CC) plots had significantly slower progression of disease development than the CC + M in both 2010 and 2011 years.
- In both years, AUDPC values were very high; the mean AUDPC in 2010 was 632 and, in 2011, even higher – 1081.
- In 2010, the AUDPC value was 303 on CC plots and 990 on CC + M plots, that is three times higher.
- In 2011, the AUDPC value was 967 on CC plots 1195 on CC + M plots.







Mean area under the disease progression curve (AUDPC) values of foliar early blight on organic potato field trials in 2010-2011

CONCLUSIONS

- Potato early blight is a grave problem for potato growing in an organic farming system, and especially in years with higher than average temperatures.
- In both our study years, early blight infection was more severe in the plots with added cattle manure than in the cover crop plots.
- The development severity of early blight can be influenced by selecting appropriate growing technology, but more research on this is needed.
- In the changed climatic conditions, early blight can cause early defoliation of plants and crop death in susceptible cultivars, indicating the need for resistant potato cultivars.

ACKNOWLEDGEMENTS: The study was supported by the Estonian Foundation grants No 9432 and 9450; Target Financing SF1700057s09, project RESIST 3.2.0701.11-000 and Institutional research funding IUT36-2; this research was supported by European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, which is carried out by Foundation Archimedes.

PPO – SPECIAL REPORT NO 17 – 2015, 145-152

Combination of a pre-planting treatment of tubers with low-frequency pulse electric field and foliar treatments with Agat-25K microbial preparation to control the late blight of potato

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SUMMARY

The effect of a combined pre-planting treatment of seed tubers with low-frequency pulse electric field (LF-PEF) and the treatment of vegetating plants with the Agat-25K biostimulator, containing *Pseudomonas aureofaciens* strain H16 and its metabolites, on the total yield of potato and the level of leaf and tuber blight infection has been studied in comparison with two conventional chemical control schemes.

The studied combined treatment accelerates the growth and development of plants, delays the first manifestation of the leaf blight for three weeks, and significantly increases the total yield and yield of healthy tubers by 22 and 31.9%, respectively, as compared with the untreated control. The yield increase was similar to those observed for the chemical control schemes. The LF-PEF technology can be recommended either as the component of the integrated protection system, or as an alternative to the chemical protection of potato in the organic farming or in the cases, when the use of chemical pesticides is restricted.

KEYWORDS

Phytophthora infestans, potato, pulsed electric field*, Pseudomonas aureofaciens*, non-chemical control, organic farming

INTRODUCTION

Potato is one of the most important agricultural crops in the world. According to FAO (Food and Agriculture Organization), the global production of this crop in 2013 reached almost 376.5 mln. tons. The productivity and quality of this crop significantly depends on the level of protection against various potato diseases. Late blight, caused by *Phytophthora infestans* Mont. de Bary, is the most devastating disease in potato cultivation able to significantly reduce the yield. In Russia, average yield losses caused by *P. infestans* reach 4 mln. tons a year; in the case of epiphytoties, the total productivity can decrease almost twice (Filippov, 2012).

A common way to ensure a healthy and large potato yield is chemical control of diseases. In the case of Russia, the average number of seasonal fungicidal spraying makes 4-6 at small farms or 9-11 at large enterprises growing industrial potato. Potato growers from the most advanced potato-producing countries, such as the Netherlands, UK, Belgium, and France, usually apply about 12-19 fungicidal sprayings per a season (Hansen *et al.*, 2009). As a result, a significant pesticide contamination of the environment and agricultural products occurs.

Pesticide residues in vegetable food and soil pose a threat for both environment and human health. Potato belongs to agricultural crops, which contamination with pesticide residues is the most dangerous (Pesticides Residues Monitoring Report, 2006). Among nine fungicides, approved for the pre-planting and post-harvest treatment of tubers, four (Benorad, Kolfugo Super, Vitavax, and Vitavax 200) are prohibited for the use on a food potato because of medical constrictions. Among 27 fungicides applied during the plant vegetation to prevent the foliar blight infection, 14 contain mancoceb or other dithiocarbamates. When these fungicides get into soil and contact with water and oxygen, their acting substances degrade into ethylene thiourea; this compound causes carcinogenic and mutagenic effects and endocrine disorders in laboratory animals (Shykla and Arora, 2001).

The negative influence of fungicides on the human health and environment result in a necessity to search for any new environmentally friendly technologies able to improve crop protection and, at the same time, reduce the number of required chemical treatments. Such technologies can be also applied in the organic potato production, since the total rejection of any chemical protection significantly decreases the crop productivity (Hamouz *et al.*, 2005).

Among the most popular approaches providing the reduction of chemical treatments, one can mention the use of decision support systems and growing of resistant cultivars. In the case of the organic production, where systemic fungicides are not allowed, copper-based fungicides were used to control late blight; however, copper-containing compounds are also accumulated in soil, negatively influencing on the soil microbiota (Van-Zwieten *et al.*, 2004), so their application should be minimized. An alternative way of the non-chemical late blight control is the use of biogenic agents, such as microorganisms, plant extracts, chitosan, etc. (Stephan *et al.*, 2005; Kurzawinska and Mazur, 2008; Nechwatal and Zellner, 2014). However, none of the biopesticides alone provides a sufficient level of the late blight control as compared with the chemical fungicides (Finckh *et al.*, 2006).

Another group of "green" technologies represents a pre-planting treatment of tubers with heat or various physical fields to destroy pathogenic microorganisms persisting on tubers or to improve the development and resistance of developing plants (Forrer *et al.*, 2000; Kuznetsova, 2000; Cramariuc *et al.*, 2005; Pittman, 1972). The technology of the pre-planting treatment of seed tubers with low-frequency pulse electric field (LF-PEF) was developed at the Russian Research Institute of Precision Instruments and All-Russian Research Institute of Phytopathology and examined on several crops (Bel'kovets *et al.*, 2012). According to the earlier obtained data, the application of this technology alone increases potato yield by 15-20% as compared with the untreated control (Kuznetsova, 2000).

The purpose of this study was to evaluate the effect of a combined pre-planting treatment of seed tubers with LF-PEF and treatment of vegetating plants with the Agat-25K microbial preparation (*Pseudomonas aureofaciens* strain H16 and its metabolites) on the total yield and quality of potato and the level of leaf and tuber blight infection and to compare this non-chemical scheme with two conventional schemes of chemical protection.

MATERIALS AND METHODS

Electric field treatment

Three days before planting, potato tubers were treated for 24 h with modulated LF-PEF (20 kV/m and 16 kHz with modulating frequency 200 Hz).

Trial arrangement

Two-year field trials were arranged on the experimental potato field of the All-Russian Research Institute of Phytopathology (Moscow region). The area of each experimental plot was 40 m^2; the plots were randomly located on the field. Each variant was tested in four replications.

Land and field treatment

Potato (cv. Sante) was planted in May and harvested in September. The land treatment of the field included under-winter ploughing; spring ploughing; pre-planting furrow formation; application of organic fertilizers; and a pre-emergence treatment with a metribuzin-based Zenkor herbicide (2 L/hectare). During a vegetation season, the whole field was twice treated with a thiamethoxam-based Aktara insecticide (0.06 kg/hectare).

Experimental variants

In the first year, the following variants were tested:

1. Unprotected control.
2. Pre-planting treatment of seed tubers with pulse electric field.
3. 5× treatment of vegetating plants with a biostimulator Agat-25K (100 g/ha) containing *Pseudomonas aureofaciens* strain H16 and its metabolites.
4. Combination of a pre-planting treatment of seed tubers with pulse electric field and 5× spraying of vegetating plants with a biostimulator Agat-25K (100 g/hectare).
5. 5× treatment of vegetating plants with a chlorothalonil-based Bravo fungicide (3L/ha).

In the second year, the following variants were tested:

1. Unprotected control
2. 5× treatment of vegetating plants with a chlorothalonil-based Bravo fungicide (3L/ha)
3. Combination of the 3× treatment of vegetating plants with an Ordan fungicide (copper oxychloride + cymoxanil, 2.5 kg/ha) and 2× treatment with a mancozeb-based Penncozeb fungicide (1.6 kg/ha)
4. Combination of a pre-planting treatment of seed tubers with pulse electric field and 5× spraying of vegetating plants with a biostimulator Agat-25K (100 g/hectare).
5.

Disease development assessment

The level of the late blight development was assessed in accordance to the British Mycological Society scale (James *et al.*, 1972).

RESULTS AND DISCUSSION

In both trials the pre-planting treatment with pulse electric field accelerated the sprouting by 7-8 days as compared with the control.

The weather conditions of the first year of trials were very unfavorable for the late blight development, so it was impossible to evaluate the effect of the tested protection schemes on the disease development and tuber infection levels. At the same time, we evaluated the effect of biological (Agat-25K) and physical (LF-PEF) treatments and their combination on the yield

increase and compared these data with those obtained for the untreated control and treatment with chemical fungicide Bravo (treated control). The results of these trials are shown in Figure 1.

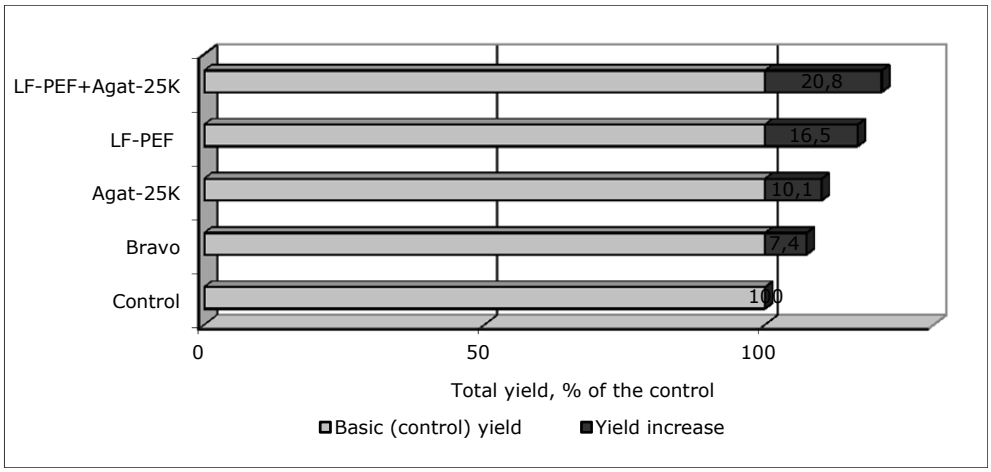

Figure 1. *Comparison of the total yield of potato obtained by the use of different schemes of protection under low infection press. Control, untreated plots; Bravo, 5× treatment with chemical fungicide (3L/ha), Agat-25K, 5× treatment with microbial preparation (100 g/ha); LF-PEF, pre-planting treatment with low-frequency pulse electric field; LF-PEF + Agat-25K, combined physical and biological treatment.* $LSD_{0.05} = 3.2$

According to the obtained data, all treatment schemes provided a positive effect on the total yield of potato as compared to the untreated control. There was no significant difference between the Bravo and Agat-25K variants, but in all other cases the difference was significant. The maximum effect (+20.8%) was obtained for the combination of the physical and biological treatments. Since the disease pressure was very low, the resulted effect can be explained by the stimulating effect of these types of treatments.

Next year the effect of the combined physical (LF-PEF) and biological (Agat-25K) treatment of potato was evaluated comparing to two different schemes of chemical protection, including the treatment of plants with the Bravo fungicide and the combined treatment with Ordan and Penncozeb fungicides. Due to dry weather, the first manifestations of the late blight appeared rather late, but rainy weather in the middle of summer provided very favorable conditions for the further disease development and tuber infection. Therefore, the leaf blight development rate, total yield and yield of healthy tubers were evaluated. The results of trials are shown in Figs. 2 and 3.

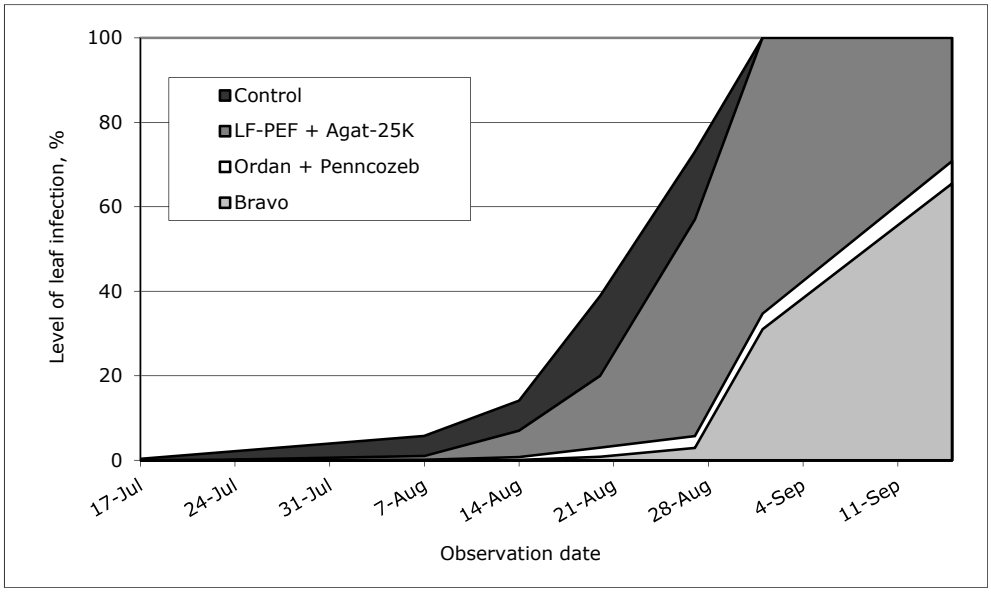

Figure 2. *Effect of different protective treatment on the development of potato leaf blight infection. Control, untreated plots; Bravo, 5× treatment with Bravo, 3 L/ha, Ordan + Penncozeb, 3× treatment with Ordan (2.5 kg/ha) plus 2× treatment with Penncozeb (1.6 kg/ha); LF-PEF + Agat-25K, pre-planting treatment with pulse electric field plus 5× treatment with Agat-25K (100 g/ha)*

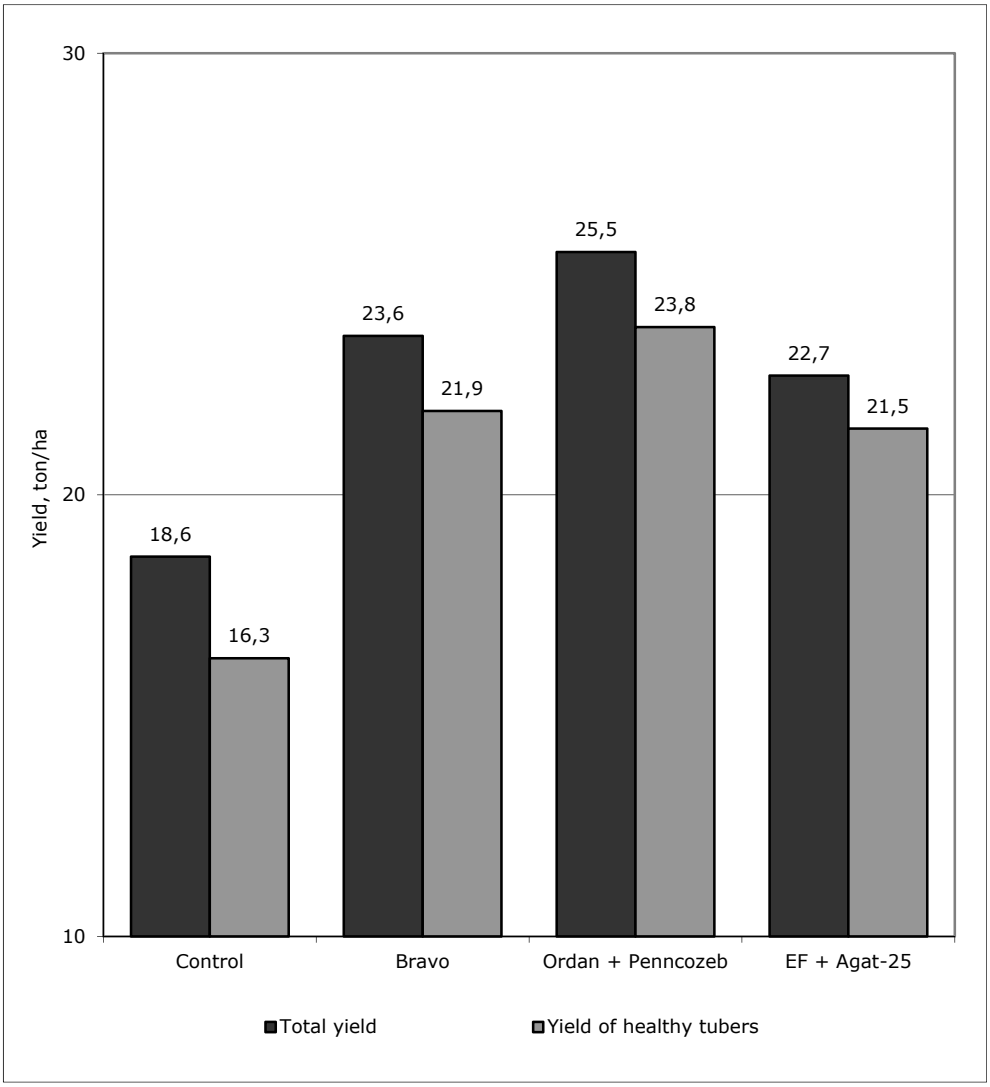

Figure 3. *Effect of various schemes of protective treatment of potato on the total yield and yield of healthy tubers under high infection press. Control, untreated plots; Bravo, 5× treatment with Bravo, 3 L/ha, Ordan + Penncozeb, 3× treatment with Ordan (2.5 kg/ha) plus 2× treatment with Penncozeb (1.6 kg/ha); LF-PEF + Agat-25K, pre-planting treatment with pulse electric field plus 5× treatment with Agat-25K (100 g/ha).* $LSD_{0.05}$ *= 4 (total yield) or 2.4 (yield of healthy tubers)*

In the case of untreated control, the first manifestations of the leaf blight infection were observed in the third decade of July, whereas in the case of the tested variants they appeared only three weeks later. To the harvesting time, the level of the leaf blight infection in the untreated control and LF-PEF + Agat-25K variants reached 100%, whereas in both chemical variants it was about 60%. Therefore, both chemical control variants provided a longer

vegetation period and, therefore, additional yield increase as compared with the environmentally friendly variant of protection.

In all protection schemes, the total yield was significantly higher than in the control (22-37.1%), but was not significant between the variants. In the case of chemical schemes of protection (Bravo and Ordan+Penncozeb), the total yield increase comparing to the control exceeded that of the ecologically friendly variant by 4.5 and 14.7%, respectively.

A rainy weather in the second half of the vegetation period caused a significant tuber infection level. In the case of the control, it was significantly higher (12.7%) than those in other variants (5-7%). The evaluation of the yield of healthy tubers showed a significant increase of this parameter for all protection schemes (92.8-94.7%) as compared with the control (87.6%). The difference between the tested protection schemes was insufficient.

Thus, the proposed ecologically friendly scheme of the late blight protection of potato provides a significant increase in the total yield of potato under both low and high infection pressure (20.8 and 22%, respectively), delays the first manifestation of the late blight infection by 3 weeks, and increases the yield of healthy tubers by 7.1%. The obtained data are similar to those obtained for two conventional chemical control schemes that makes it possible to recommend the joint application of the LF-PEF and microbial preparation Agat-25K as an alternative to the chemical late blight control in organic potato production or in situations, when the use of chemical fungicides is restricted; due to the stimulating effect of the pre-planting LF-PEF treatment, it can be also included into the integrated protection systems applied in conventional potato production.

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Displacement of *Phytophthora infestans* in East Africa

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Displacement of *Phytophthora infestans* in East Africa

Results from microsatellites, mtDNA, and effector screening

The authors would like to acknowledge support from the USAID project 'Development of late blight resistant potato biotech varieties' and the CGIAR Research Program on Roots, Tubers and Bananas

KE 1 dominates

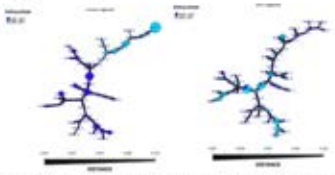
From appearing in just 2 fields in 2007, the clonal lineage KE-1 has displaced US-1 from potato in Kenya and much of Uganda based on collections made in 2011 and 2012.

Recent collections have revealed KE-1 even in Southwest Uganda.

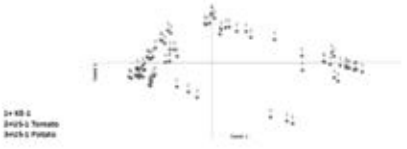
KE-1 has not displaced US-1 from tomato in Uganda.

Distribution of KE-1 (red) and US-1 from potato (blue) and tomato (cyan) in 2011 and 2012

Effector studies of IPIO indicate that RB may already be ineffective in East Africa.



Bruvo msn tree for potato isolates and US-1 from tomato and potato



PCA plot of microsatellite diversity from the 2011/2012 collection

Effector Diversity

Genotype	Ave-blb1			
	IPIO1	IPIO2	IPIO3	IPIO4
KE-1	a	b		
US-1 Potato				
US-1 Tomato				a

Class 1 (Ipio1 & Ipio2);
Class II (Ipio3);
Class III (Ipio4)

- Class I is **avirulent** and Class III is **virulent** on RB
- Isolates **lacking Class I** and **containing Class III** variant can overcome resistance provided by Rpi-blb1
- Class I ipio variants determine **avirulence** of *P. infestans* isolates on Rpi-blb1 plants
- US-1 potato isolates don't have class I variants
- KE-1 isolates have class I variants
- US-1 tomato isolates have class III variant
- Isolates in Uganda could break the resistance in Rpi-blb1

Kenya (P)	217	153	134	174	161	172	180	176	1a
	217	153	134	176	166	174	183	179	
					170				
Uganda	213	151	136	174	161	174	187	177	1b
Rwanda	217	153	136	174	166	174	180	177	
					170				
Kenyan	217	153	134	174	161	172	180	176	1a
Uganda (P)	217	153	134	176	166	174	183	179	
					170				
Mizzle (Wes	213	151	136	174	161	174	187	177	1b
Uganda (P)	217	153	136	174	166	174	180	177	
					170				

Some typical genotypes from Kenya and Uganda

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Do the Algerian *Phytophthora infestans* populations show genotypic structuration on potato and tomato?

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SUMMARY

Phytophthora infestans, the causal agent of late blight, is a serious threat to Algerian potato and tomato productions. This study was carried out to investigate host specialization on potato and tomato among Algerian and French *P. infestans* populations. A total of 74 Algerian isolates collected on potato and tomato from 2007 to 2014 were genotyped using 12 SSR markers, and compared with 43 French isolates mostly sampled in commercial tomato fields over the same period. Populations were clonal, with no evidence for sexual recombination and no geographic structuration, but significant differences were observed between the samples collected from potato and tomato. Potato Algerian isolates showed a low genotypic diversity; most of them belonged to the 13_A2 or to the 2_A1 clonal lineages. On the opposite, genotypic diversity of tomato isolates was high. Tomato isolates essentially fell into three genetic clusters: MLGs 23_A1, 2_A1, and a third group of unclassified MLGs belonging to both A1 and A2 mating types. Lineage 13-A2 was seldom found among the tomato isolates tested. Algerian and French 23_A1 isolates appeared to show preference for tomato, but were also pathogenic on potato. Under controlled conditions, on leaflets of a susceptible potato cultivar, they caused profuse sporulation, as do 6_A1 isolates, and small lesions similar in size to those produced by the 13_A2 isolates. Their virulence spectrum was very simple on potato and all were sensitive to metalaxyl. Overall, our data suggest host preference for either potato or tomato in some lineages (13_A2 on potato, 23_A1 on tomato), as well as the presence of ubiquitous lineages developing on both hosts (*e.g.* 2_A1). They also point to a possible role of tomato crops as 'refuges' of genetic diversity.

KEYWORDS

Late blight, *Solanum tuberosum*, *Solanum lycopersicum*, genotypic diversity, microsatellites, host specialization

INTRODUCTION

Phytophthora infestans, the causal agent of late blight, is a serious threat to Algerian potato and tomato productions, two economically important crops for this country. Common practice is to grow these two plants almost year round. For potato, two main crops are grown during a calendar year, one from January to May/June and the other from September to December/January. Moreover, commercial potato and tomato fields can be found in close proximity to each other or separately, according to the regions. These factors lead to the potential for late blight infections and aerial inoculum production at any time of the year. Except for studies with a reduced collection of isolates (Corbière *et al.*; 2010b), little is known about Algerian *P. infestans* populations on both plants. Therefore, we intended to further characterize Algerian isolates collected on potato and tomato from 2007 to 2014, and compared them with some French isolates sampled in commercial tomato fields over the same period.

We used neutral microsatellites to analyse *P. infestans* populations structure, in order to measure the distribution of genotype diversity on both hosts (potato and tomato) and gain insight into the pathogen reproductive system in Algeria, as sexual and asexual inoculum contribute differently to epidemic development. We also compared the phenotypic diversity of some of the main clonal lineages collected on potato and tomato for quantitative traits that are important in plant pathology, such as fungicide sensitivity, aggressiveness and virulence to potato. As knowledge regarding the host preference of *P. infestans* genotypes could provide information important for disease management strategies, the final objective of this study was to investigate host specialization on potato and tomato among Algerian and French populations.

MATERIALS AND METHODS

Isolates sampling

Isolates of *P. infestans* were collected from potato and tomato plants in six Algerian regions (Fig. 1). A total of 74 isolates was obtained in 2007 to 2014 (few in 2009-2010), generally either during May or in December and January, according to the growing season. Infected potato leaves and some stems from several cultivars were predominantly sampled in production fields, but also in trials around Algiers. Tomato leaves (n=10) with typical late blight symptoms were sampled from commercial fields or open-ended high tunnels.

In total, 43 French tomato isolates from leaves, stems or fruits were also collected during the same period, from commercial outdoor tomato crops in Southern France. Part of the isolates came from South-Western locations, around Marmande from four tomato fields of varieties Perfect Peel and Caladou, in July and September 2013 (n=18), and near Libourne from tomato trials in July 2014 (n=10). Other isolates were collected from South-Eastern France, near Avignon, in a greenhouse, in November 2007. In addition, several isolates were obtained from two gardens, one in Western France, near Rennes, on three tomato varieties (Pyros, Supersweet 100 F1 and Costoluto Genovese) at the end of August 2012 (n=13), and one in Eastern France, near Reims, in September 2014 (n=1).

Single-lesion isolates and pure axenic cultures were obtained as previously described (Corbière *et al.*, 2010b).

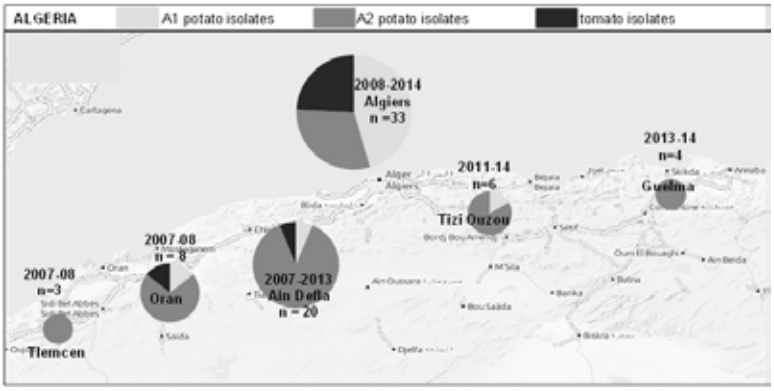


Figure 1. *Sampling of P. infestans isolates in Algeria. A survey was performed on potato and tomato plants from 2007 to 2014. Frequency of isolates from potato are presented in grey, according to their mating-type, A1 MT (clear grey) or A2 MT (dark grey) and frequency of isolates from tomato, in black*

Microsatellite genotyping and data analysis

DNA was extracted from lyophilised mycelium as previously described (Corbière *et al.*, 2010b). Twelve microsatellites loci were genotyped in each isolate : D13, G11, Pi04, Pi4B, Pi63, Pi70, *Pinf* SSR 2, 3, 4, 6, 8, 11. Amplification of the single sequence repeat (SSR) markers was carried out in multiplexed PCR assays, according to Li *et al.* (2013). PCR products were capillary electrophoresed on an automated ABI 3130 according to the manufacturer's instructions. SSR allele sizes were determined using GeneMapper version 3.5, and the microsatellite data were used to define multilocus genotypes (MLGs). Twelve reference isolates representing EU 1_A1, 2_A1, 6_A1, 23_A1 and 13_A2 were included in microsatellite analyses (Cooke *et al.*, 2012). In some isolates, loci with three alleles were observed (mainly for loci D13, G11, Pi 63, SSR 4, SSR 8). Such ploidy variation has been reported previously (Cooke *et al.*, 2012; Li *et al.*, 2013). Alleles were then scored using a binary representation of the presence or absence of specific alleles.

Two types of clustering methods were conducted to reveal genetic relationships among the *P. infestans* genotypes. Population genetic substructure was inferred using the STRUCTURE software (one representative isolate for each genotype) and the genetic distance between individual MLGs was calculated using the binary data. A minimum spanning network was then calculated using MINSPNET from the matrix and visualized using the Graphviz package.

Phenotypic characterization

Mating types of all isolates were determined by pairing each of them with known testers of *P. infestans*, either A1 and A2 mating-type, on pea agar medium.

Metalaxyl sensitivity, virulence and foliar aggressiveness tests were conducted with some isolates, as previously described (Corbière *et al.*, 2010b). As pathogenicity could be affected during axenic culture, isolates were inoculated onto detached leaflets of cultivar Bintje to prepare sporangia suspensions adjusted to 5×10^4 spores. mL^{-1} and chilled at 4°C for two hours before inoculation on plant material.

Sensitivity to metalaxyl was assessed on 20 French tomato isolates from Avignon and Marmande in a floating leaflet bioassay, with metalaxyl concentrations of 10 and 100 µg mL^{-1} alongside a pure water control.

Virulence tests of five tomato isolates from Avignon was carried out on detached leaflets of Black's differential set of 11 potato clones, according to the protocol detailed by Andrivon *et al.* (2011).

Aggressiveness was measured in a laboratory study, on detached leaflets of the susceptible cultivar Bintje. Four tomato isolates from Avignon (collected on November 2007) were compared in May 2008, to three 2_A1 isolates, eight 6_A1 isolates and eight 13_A2 isolates, sampled from potato during the same year. The A1 isolates were obtained from cultivar Bintje, in Western France (Ploudaniel) and the 13_A2 isolates from cultivar Agata, in central France. Incubation temperatures were 15°C night - 20°C light (with a 16 hours light period) and six technical repetitions per isolate were performed. Two components of aggressiveness, lesion area and number of sporangia per lesion, were scored six days post inoculation (dpi). Statistical analyses were carried out using the software R, version 3.1.0 (R Development Core Team, 2014) and the normality of variances was checked with the Shapiro-Wilk test. Differences in lesion area between clonal lineages were analysed with the Kruskal Wallis test, and mean values were compared with the Wilcoxon test, because normal distribution was not met to use parametric tests. Differences in spore production were analysed with ANOVA and mean values were compared with Tukey's HSD tests.

RESULTS

Genotypic structure of the populations

In total, 72 microsatellite alleles were detected over the 12 SSR loci, with two to fifteen alleles per locus. Allelic diversity per locus ranged from two or three (for loci Pi70, SSR2, SSR6), four or five (for loci Pi04, Pi4B, Pi63, SSR8, SSR11), seven (for locus SSR3) to ten or eleven (for loci G11, SSR4). Locus D13 had the highest observed number of alleles (fifteen). A large range of allele sizes was also noticed for SSR3 (from 173 to 272 bp), D13 (from 132 to 212 bp) and G11 (140 to 206 bp), whereas SSR2, Pi70 and SSR6 had the smallest range variation (2, 3 and 4 bp, respectively).

SSR genotyping of the 117 *P. infestans* isolates from potato and tomato resulted in 51 distinct multi locus genotypes. STRUCTURE analysis (Fig. 2) indicated the presence of three major clusters: cluster A with only tomato isolates (except one from Algerian potato), cluster B corresponding to the 13_A2 clonal lineage and cluster C with isolates from the 2_A1 clonal lineage. Further subdivision showed that cluster A consisted of three subclusters clusters, whereas cluster B (13_A2 MLG) cluster C (2_A1 MLG) remained as single groups. A sixth cluster was only composed of reference isolates belonging to the 6_A1 MLG, and 12 isolates were unclassified. This analysis showed that the *P. infestans* populations from potato and tomato were dominated by clonal lineages, but that isolates from tomato were much more diverse than their potato counterparts.

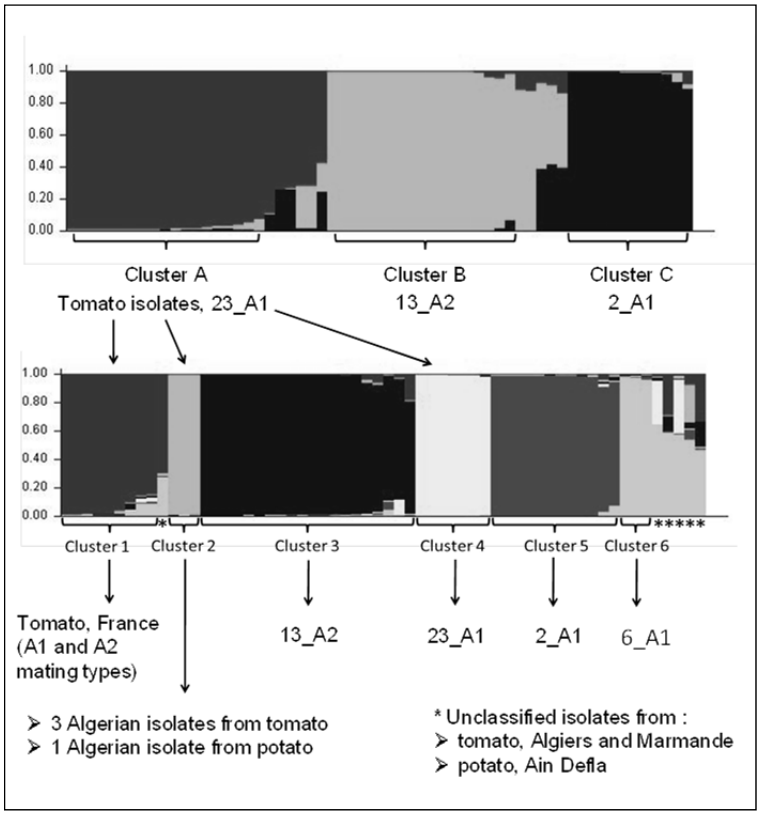


Figure 2. *STRUCTURE analysis of twelve microsatellite loci for P. infestans isolates collected from tomato and potato. Each grey color represents one population and each isolate is presented by a vertical bar.*

- Top : for K = 3, isolates were grouped into three main clusters : A with isolates sampled from tomato, B with 13_A2 MLG isolates and C with 2_A1 MLG isolates.

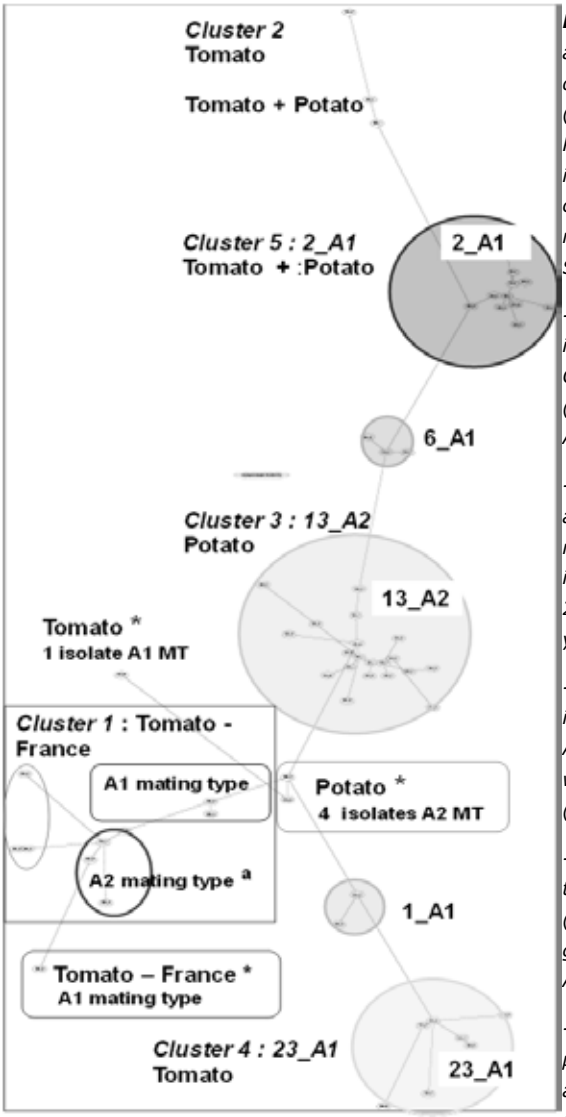
- Down : for K = 6, isolates were grouped into six distinct clusters. Cluster A was then divided in three clusters :
cluster 1 with French isolates from tomato,
cluster 2 with four Algerian isolates,
cluster 4 with 23_A1 MLG isolates.
Cluster 3 contained 13_A2 isolates and cluster 5, 2_A1 isolates.
Twelve isolates from tomato and potato were unclassified (*)

The network of all 117 isolates from the survey, plus 12 SSR reference isolates, split the isolates into five main clusters (Fig. 3). These clusters corresponded to the five clusters identified in the STRUCTURE analysis, among the sampled isolates. As no isolate from 1_A1 and 6_A1 MLGs were found in Algeria, nor on potato or on tomato, the sixth cluster in STRUCTURE only contained reference isolates.

Potato Algerian isolates collected between 2007 to 2014 did not show a high genotypic diversity. Most of them (n=35) belonged to the 13_A2 clonal lineage and they were sampled across all locations. 13_A2 seemed to be the dominant genotype on potato in Algeria. Nevertheless, within this clonal lineage, 20 MLGs were identified, which revealed sub-clonal variations. Most other Algerian isolates obtained from potato (n=20) belonged to the 2_A1 clonal lineage. They were mainly collected around Algiers, but were also found near Oran in 2007, Ain Defla in 2011 and Tizi-Ouzou in 2014. However, five isolates from Algerian potato plants remained unclassified. Four of them are A2 mating-type; they grouped together and are related to the 13_A2 clonal lineage. All were sampled in May 2012, on three cultivars (Sarpo Mira, Désirée and Timate) in three different locations of the Ain Defla region, one of the main potato production areas in Algeria. The last potato isolate grouped with tomato isolates from cluster 2. It was collected in ENSA trials at Algiers in January 2011, where the same genotype was found three months later on tomato.

All the tomato Algerian isolates analysed were A1 mating-type. Most of them were sampled around Algiers. Some (n=6) belonged to known MLGs : 2_A1 (cluster 5) or 23_A1 (cluster 4), but others (n=4) were unclassified. One isolate was unique, and the other three composed the cluster 2; they were obtained in two different years (2008 and 2011) and from three locations (Oran, Ain Defla and Algiers).

Tomato isolates from France were added in this study to further describe the genotypic diversity of *P. infestans* on this host. They were essentially fell into three clusters : MLGs 23_A1 (cluster 4) and 2_A1 (cluster 5), like the Algerian isolates from tomato, but also cluster 1 which is presently not clearly identified. In this study, we noticed that the 23_A1 clonal lineage was exclusively composed of tomato isolates; the French ones (n=7) were collected from Eastern and South-western France during 2007, 2013 and 2014. Surprisingly, cluster 1 contained both A1 and A2 mating type isolates, but they were distant from each other in the network. In this cluster, four A1 isolates were collected from Marmande in three fields in July and September 2013; the A2 mating type isolates were sampled in three fields in Marmande (2013) and also in a garden in Western France, in 2012. Several unclassified A1 mating type isolates (n=7), related to cluster 1, were also found in a single field in Marmande in July 2013. Finally, only one 13_A2 isolate was detected on tomato plants, although this clonal lineage is predominant on potato all over France.



[a] *A2 mating type isolates were also recovered from potatoes growing in a greenhouse and a volunteer in Brittany on 2013 - 2014 (Mabon et al., 2015).*

Figure 3. *Minimum Spanning Tree on the alleles at 12 SSR loci. indicating the genetic distance between multilocus genotypes (MLGs) and unclassified isolates of P. infestans. The 117 Algerian and French isolates sampled on tomato and potato were classified into five clusters and miscellaneous isolates (corresponding to STRUCTURE analysis, Figure 2):*

- Cluster 2 : four Algerian A1 mating type isolates (2 isolates collected from tomato at Oran and Aïn Defla in 2008 and 2 isolates (1 from tomato, 1 from potato) from ENSA, Algiers in 2011).

- Cluster 5 : 2_A1 MLG isolates from tomato and potato. On tomato : 5 isolates sampled near Algiers (2013 and 2014) and 1 French isolate from Marmande (2013). On potato : 20 Algerian isolates from all locations, each year.

- Cluster 3 : 13_A2 clonal lineage. Most isolates came from potato (35 from all Algerian regions, each year) and one isolate was only collected on tomato in France (Marmande, 2013).

- Cluster 1 : 27 French isolates from only tomato, but both A1 and A2 mating types (MT). They were sampled in fields and gardens, in all parts of France, except Avignon, during 2012 to 2014.

*- ***** unclassified isolates : from Algerian potato, 4 isolates (A2 MT, Ain Delfla, 2012) and from tomato : 1 Algerian isolate (A1 MT, Algiers, 2010) and 7 French isolates (A1 MT, Marmande, 2013).*

- Cluster 4 : 23_A1 MLG isolates. All of them were collected from tomato :

1 near Algiers (2013), 11 from Southern France, in Avignon (2007), Marmande (2013) and Libourne (2014).

Phenotypic traits of some French isolates collected on tomato

We observed a strong diversity in colony morphology of some isolates grown on pea agar (Fig. 4). The A1 and A2 mating type isolates from cluster 1 showed diverse mycelium aspects. All A2 isolates exhibited a distinct lumpy phenotype. The A1 mating type isolates (from cluster 1 and 23_A1 lineage) had a regular and even mycelium, whereas mycelial appearance of unclassified A1 isolates was airy.

Figure 4. *Colony morphology of French isolates collected from tomato. Isolates were grown on pea agar medium, at 15°C in darkness, during three weeks.*
Left : five A2 mating type isolates from cluster 1; their mycelium exhibited a distinct lumpy phenotype. Right : six A1 isolates sampled in Marmande in 2013; on right column : two 23_A1 MLG isolates; in middle column : two isolates from cluster 1 and on left column : two unclassified isolates

In the metalaxyl sensitivity assay, A1 mating type isolates (n=19) and one 13_A2 isolate (collected in Marmande in 2013) were compared. Only the 13_A2 isolate proved resistant to metalaxyl and a single one, a 2_A1 isolate (from Marmande) had an intermediate behaviour. All other 18 isolates were sensitive to metalaxyl : four A1 isolates from cluster 1, seven A1 miscellaneous isolates and seven 23_A1 isolates from Avignon (2007) and Marmande (2013).

We also examined the ability of five 23_A1 isolates to overcome foliar late blight resistance on eleven potato *R* genes differential plants, in a laboratory test. Virulence profiles of these isolates were simple. Four isolates only overcame three *R* genes, *R3*, *R4* and *R7*, while the last one had a more complex profile and overcame two additional *R* genes, *R1* and *R8*.

In the aggressiveness bioassay, 23_A1 isolates (n=4) were tested alongside isolates representative of three dominant MLGs in Algeria or in France, in potato crops : 13_A2 (n=8), 2_A1 (n=3) and 6_A1 (n=8). Differences in lesion areas were highly significant between MLGs (P<0,001) : 23_A1 and 13_A2 isolates showed smaller lesions than 2_A1 and 6_A1 isolates. As for lesion area, sporulation differed significantly between MLGs (P<0,01) : 13_A2 isolates presented the lowest spore production and 2_A1 isolates produced the highest number of spores, whereas 23_A1 and 6_A1 isolates were intermediate (Fig. 5).

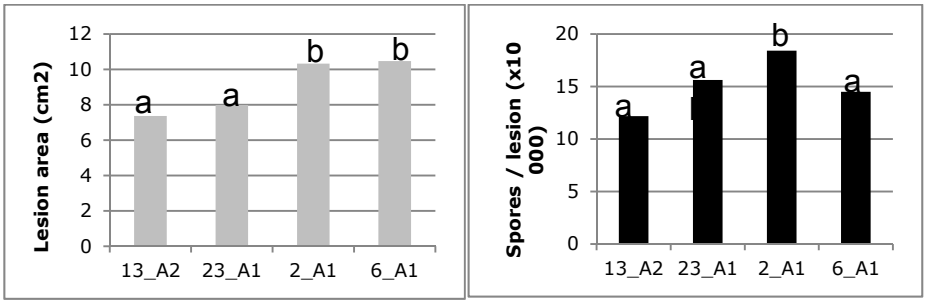


Figure 5. *Aggressiveness on cv. Bintje detached leaflets of 23 P. infestans isolates, grouped into four clonal lineages : 13_A2 (n=8), 23_A1 (n=4), 2_A1 (n=3), 6_A1 (n=8). Measurements made after six incubation days, at 15°C night / 20°C light (16 h light period).*
Left : mean lesion size (in grey) and right : mean number of sporangia per lesion (in black) within each clonal lineages. Different letters above the bars indicate significant difference between each lineage

DISCUSSION AND CONCLUSION

To understand the epidemiology of potato and tomato late blight, it is important to have a good knowledge of the genotypic diversity, structuration and relationships of *P. infestans* populations on its two main hosts. In this study, we characterized Algerian *P. infestans* isolates collected from potato or tomato at SSR loci and compared them with French isolates essentially sampled in commercial tomato fields at the same period (2007-2014). We revealed important aspects of the *P. infestans* populations in Algeria and an absence of geographic structuration, but differences between samples collected from potato and tomato. Throughout Algeria and France, we found that populations were clonal with no evidence for sexual recombination, even though both mating types have become distributed widely and found together in the same fields.

The low genotypic diversity of Algerian isolates obtained from potato (Corbière *et al.*, 2010b) was confirmed on a larger sampling. In the six Algerian regions sampled, the predominant population on potato was composed of the 13_A2 clonal lineage. Many studies have reported the current dominance of this lineage on potato since 2006, in Europe, especially in France, Great Britain and more recently outside Europe (Cooke *et al.*, 2012), but remarkably not in Tunisia where *P. infestans* populations were specific (Harbaoui *et al.*, 2014). On the opposite, we found that tomato was seldom infected with 13_A2, suggesting host preference of 13_A2 isolates for potato in Algerian and French commercial fields. However, the 13_A2 lineage has been found equally adapted to both hosts in some other countries, such as India (Chowdappa *et al.*, 2015). The 13_A2 lineage showed a sub-clonal population structure, with many minor variants which may have different fitness. The climatic, environmental or agronomic effects on the pathogenic traits of these sub-clonal variants have not clearly established, and may need to be considered in future research.
Constrasting with 13_A2, the 2_A1 lineage constituted another important lineage on Algerian potato crops but also on tomato. This observation may indicate that the 2_A1 lineage does not show any strong potato or tomato host preference. In France, the 2_A1 lineage, dominant before 2006 on potato (Mariette *et al.*, 2015), is also present on tomato, but is now infrequent on potato. In temperate regions such as the main French potato production areas, the low frequency of 2_A1 isolates on potato could be explained by a better inclusive fitness or particular

adaptative traits of 13_A2 new lineage isolates, compared with 2_A1 old lineage isolates, on this host. During epidemic stage, 13_A2 isolates may thus fast overcome potato crops which lead to the nonappearance of 2_A1 isolates and the displacement of the old lineages. We also noticed that many sub-clonal variants were found into the 13_A2 lineage (20 MLGs for 36 analysed isolates). This is in contrast with 2_A1 lineage where fewer minor variants were found (10 MLGs for 26 tested isolates), even though this lineage has been present for a long period in France and also probably in Algeria, and is detected on both potato and tomato.

STRUCTURE analysis revealed that most Algerian and French *P. infestans* isolates, sampled from tomato, were grouped together and belonged to one major cluster. These isolates were not found in Algerian and French potato fields; moreover the 6_A1, now dominant in French potato crops (http://euroblight.net/) was not detected on tomato. Further analysis showed that this major cluster could be further subdivided into three main subgroups (clusters 1, 2, 4) and some unclassified isolates, showing a higher level of genotypic diversity in populations collected on tomato compared to those from potato. This diversity is also reflected in the morphological aspect of axenic cultures on pea agar. This large genetic diversity also explains the high proportion of unclassified isolates from tomato in the minimum spanning network, compared to samples from potato. By comparison with reference isolates, we could only identify cluster 4 as 23_A1; the two other clusters and unclassified isolates were not referenced in the European data base (http://euroblight.net/).
Cluster 1 consisted of French isolates from A1 and A2 mating types, whereas cluster 2 only contained Algerian isolates from A1 mating type. The MLGs inside these clusters were not unique, but found in multiple locations or multiple years. For example, A2 mating type isolates, from cluster 1, were recovered from tomato in Western and Southern France, in 2012 and 2013 respectively. Interestingly, they were also detected in the early season of 2013 and 2014 from potato in Western France, on a volunteer and on early potatoes in a greenhouse which was cultivated with tomato during the previous year (Mabon *et al.*, 2015). Some isolates from cluster 1 could then also be recovered from potato in the early season. In the same way, in Algeria, an isolate from cluster 2 was found in a small potato trial where tomato crop was grown in a same location. It then appeared that isolates collected in commercial tomato fields showed preference for tomato, although they were also pathogenic on potato. Host preference of the isolates may be different in large area commercial fields and in gardens where potato and tomato were often grown close together or in tight successions during the crop season. Indeed, in British gardens, no evidence for host specialization was found on tomato and potato (Stroud *et al.*, 2015). Further studies are warranted to investigate the *P. infestans* host preference in the main Algerian and French commercial tomato areas, with and without potato fields in the same regions.

Several studies have characterized dominant clonal lineages and demonstrated the existence of variation among different clonal lineages in phenotypic traits, included phenylamide fungicide resistance, virulence profiles or aggressiveness. In Algeria and France, 23_A1 isolates were only found on tomato and in Great Britain on 2011-2012, this lineage was more frequent in garden tomato samples (20 and 14%) than in samples from commercial potato crops with a 0,23 to 0,56% frequency (Stroud *et al.*, 2015). However, it seems that the same MLG US-23, according to SSR profiles (Danies *et al.*, 2014), devastated potato crops in North America, since 2011. We then compared some phenotypic traits of Algerian and French dominant lineages on potato to those of some 23_A1 French tomato isolates, in order to investigate pathogenic characters of this 23_A1 MLG, on potato.

Differences in metalaxyl sensitivity was observed between MLGs : all A1 mating-type isolates from tomato, including 23_A1 isolates, were sensitive to metalaxyl, except one 2_A1 isolate (intermediate), whereas the 13_A2 isolate was resistant, as all 13_A2 Algerian and French isolates previously analysed (Corbière *et al.*, 2010a, 2010b). Nevertheless, absence of 23_A1 isolates on French potato could not be explained by this factor as very few phenylamide fungicides are now sprayed on this crop, in this country. Virulence spectrum of 23_A1 isolates differed strongly from those of other MLGs : they presented very simple profiles, whereas most 13_A2 isolates were virulent against nine to the eleven *R* genes of the differential set (Corbière *et al.*, 2010a, 2010b; Cooke *et al.*, 2012). Virulence patterns in 23_A1 isolates were also clearly distinct from those of potato A1 mating type isolates, which mostly overcame six to seven *R* genes (Corbière *at al.*, 2010a) and from those of Algerian tomato A1 isolates of cluster 2 (named ITC and AD), virulent against ten potato *R* genes (Corbière *et al.*, 2010b). Algerian and French 23_A1 isolates appeared to show preference for tomato, but they were also pathogenic on potato. On a susceptible potato cultivar, they caused profuse sporulation, as do 6_A1 isolates, and although limited necrosis were observed with 23_A1 isolates, they caused lesions similar in size to those produced by the 13_A2 isolates. In order to determine difference in host preference for isolates, cross-inoculations have now to be conducted on tomato and potato. Many factors (inoculum concentration, incubation temperature and humidity, plant age and growth period, …) have then to be accurately selected for valid conclusions (Danies *et al.*, 2013, Nowakowska *et al.*, 2014).
Interestingly, on potato, the 13_A2 isolates showed the smallest necrotic lesions and the lowest sporulation, whereas 2_A1 isolates had the highest lesion size and spore production. However 13_A2 MLG is now prevalent on potato in Western Europe and 2_A1 MLG previously dominant, is now sporadic. It then appears that aggressiveness measurements under controlled conditions are important criteria, but may not explain the total fitness of *P. infestans* isolates under different agro-ecosystems, during epidemics (*i.e.* thermal adaptation in relation with global warming) and during survival stages or may not reflect all the interactions between *P. infestans* isolates and the two hosts in field environment and under different climatic features.

In this study, high differences were found between the distribution of clonal lineages from tomato and potato commercial fields samples. In Algeria as in France, *P. infestans* genotypic diversity was higher on tomato than on potato, but unique genotypes were not frequent. Some lineages were mainly found on tomato and seemed to show a preference for tomato as a host, although some of them were also sampled on early potato, potato volunteer or potato grown close to tomato. Tomato crops may then act as a "refuge" for highly diverse genotypes, whereas commercial potato crops with low range of cultivars on large areas may select some lineages especially during epidemics.

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Effect of the in-furrow application of a Uniform fungicide on the late blight development on potato

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SUMMARY

In-furrow application of the Uniform fungicide (azoxystrobin + mefenoxam) during the planting of potato significantly reduces the late blight development in the course of a growing season. The effect has been observed in both laboratory and field trials arranged in 2012 and 2013 at the All-Russian Research Institute of Phytopathology.

The in-furrow Uniform application followed by the common schemes of the foliar treatment with Ridomil Gold MZ, Revus Top, and Shirlan in a tank mix with the Izabion biofertilizer delayed the first late blight manifestations by 40 days as compared to the untreated control and by 27-30 days as compared with the common protection scheme, which did not include the Uniform application. The resulting increase in the total and marketable yields made 30.5 t/ha and 42%, respectively, as compared with the untreated control, and 7 t/ha and 13%, respectively, as compared with the common protection scheme. The reduction of the number of foliar treatments by one in the Uniform-including scheme did not significantly influence the total and marketable yields and the start of the disease.

Thus, the addition of Uniform to the common potato protection schemes represents an efficient way to provide the late blight protection starting from the sprouting stage and to reduce the risk of the early infection of plants. In addition, it allows a user to delay the beginning of foliar spraying and to reduce their number without any losses in the plant protection efficiency; moreover, a significant increase in the total and marketable yields are provided.

KEYWORDS

potato, Uniform, azoxystrobin, mefenoxam, late blight, *Phytophthora infestans*, yield

INTRODUCTION

Potato is one of the most important crops in Russia, which, being inferior to China and India, takes the third place in the world potato production (FAOSTAT, 2012). However, the productivity of this crop in Russia still remains low as compared to other countries. One of the main reasons of insufficient crop productivity and poor quality of potato tubers is potato late blight (LB) caused by *Phytophthora infestans* (Mont.) de Bary (Anisimov *et al.*, 2009). In some regions of Russia,

the first symptoms of this disease are observed already at the crop emergence stage. The early disease appearance in the field, observed in recent years, resulted in the necessity of early fungicide treatments starting right from the sprout emergence (Filippov, 2012).

In the previous studies, performed at the All-Russian Research Institute of Phytopathology, it was shown that the in-furrow application of a Quadris fungicide (250 g/l of azoxystrobin) during the planting of potato delays the LB development up to 10-14 days as compared to untreated control (Kuznetsova *et al.*, 2009).

It is considered that, due to good water solubility (log Pow = 2.64), azoxystrobin has a good translaminar mobility in the case of a foliar spraying and shows a systemic relocation in the case of a soil treatment. The last effect is provided by the ability of azoxystrobin molecules to penetrate into plant tissues, where they are captured by roots and translocated acropetally to leaves and stems (Gisi, 2002).

In addition to antifungal properties, the majority of strobilurins positively influence on different aspects of the physiological state of plants, such as the improvement of plant viability under stress conditions, delaying of plant senescence (known as the «greening effect»), and improved drought resistance and water uptake.

In order to continue the study of the effect of a soil application of fungicides on the LB development during the vegetation season, a series of trials was conducted to evaluate a mixed Uniform fungicide containing two active ingredients, azoxystrobin (321.7 g/l) and mefenoxam (123.7 g/l).

MATERIALS AND METHODS

Both field and laboratory trials were carried out in 2012-2013 on the potato variety Red Scarlett; the effect of the fungicide application was assessed against both natural and artificial infection background. The area of each experimental plot was 40 m^2; the plots were randomly located on the field. Each variant was tested in four replications.

First-year trials

The purpose of the first-year trials was the study of the effect of the in-furrow Uniform application on the late blight severity during the vegetation period.

Trial arrangement

In 2012 the trial arrangement included the following variants:

1. In-furrow treatment with the Uniform fungicide (1.5 l/ha).
2. In-furrow treatment with the Quadris fungicide (3 l/ha) as a standard treatment.
3. Untreated control.

The soil application of Uniform and Quadris fungicides was performed using an experimental applicator RNF-1, specially intended for the in-furrow treatment of planting potato tubers; the application rate was 100 l/ha.

Description of the experiment

After the crop emergence, 30 potato leaves were collected every 7-10 days from each plot and placed into trays (0.3×0.4 m). The detached leaves were sprayed with a suspension of sporangia (15000 sporangia/ml), collected from an aggressive *P. infestans* strain, and incubated in the dark for 24 h at 20°C and 98% relative humidity. Then the leaves were placed into beakers with water under daylight. After 3-4 days of incubation, the number of necroses per 1 cm^2 of the leaf surface was calculated using a leaf area meter.

In the case of the natural infection background (field trials), we registered the date of the first disease manifestation and the further disease development on plants.

Second-year trials

The main purpose of the second-year trials was the evaluation of a successive use of fungicides applied in the following way: in-furrow application of the Uniform fungicide (1.5 L/ha) followed by the foliar treatment with Ridomil Gold MZ (2.5 kg/ha, mefenoxam+mancozeb), Revus Top (0.6 L/ha, mandipropamid+difenoconazole), and Shirlan (0.4 L/ha, fluazinam) in a tank mix with a biological fertilizer Isabion (2 L/ha) containing the complex of amino acids and peptides. In addition, it was important to examine, whether in-furrow Uniform application makes it possible to reduce the number of seasonal fungicidal treatments without any losses in the crop productivity and yield quality.

Trial arrangement

1. In-furrow application of the Uniform fungicide.
2. Foliar treatment with Ridomil Gold MZ (2×), Revus Top (2×), and Shirlan (1×).
3. Foliar treatment with Ridomil Gold MZ (1×), Revus Top (2×), and Shirlan (1×).
4. In-furrow application of the Uniform fungicide (1.5 l/ha) followed by the foliar treatment with Ridomil Gold MZ (2×), Revus Top (2×), and Shirlan (1×).
5. In-furrow application of the Uniform fungicide (1.5 l/ha) followed by the foliar treatment with Ridomil Gold MZ (1×), Revus Top (2×), and Shirlan (1×).
6. Untreated control.

The Isabion biofertilizer was applied twice a season in a tank mix with Ridomil Gold MZ and Revus Top.

Late blight development assessment

The LB severity assessment was performed according to the British Mycological Society scale (James, 1972). Based on the obtained data, the AUDPC curves were calculated for all variants tested.

After the harvesting, the total and marketable yields and the level of a tuber blight infection were additionally determined.

RESULTS AND DISCUSSIONS

First-year trials

According to the results of laboratory trials (artificial infection background), the use of the Uniform fungicide reduced the level of leaf infection with *P. infestans* as compared with the untreated control and Quadris application. Moreover, this effect was observed right after the sprout appearance and lasted until the haulm die-off.

The observed effectiveness of the artificial infection of leaves with *P. infestans* made 3-34% for the Uniform variant and 10-75% for the Quadris variant as compared to the control (100%; Fig. 1). Thus, in-furrow application of the Uniform and Quadris fungicides reduced the infection efficiency by 66-97 or 25-90%, respectively, as compared to the untreated control, and the protective effect of the Uniform preparation was higher and more prolonged than that of the Quadris fungicide.

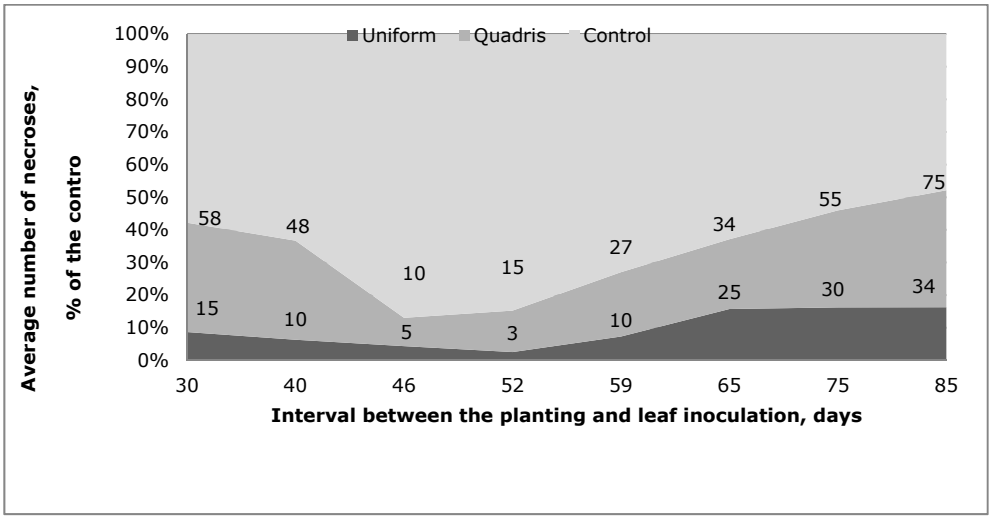

Figure 1. *Effect of the in-furrow application of Uniform and Quadris fungicides on the leaf blight infection level*

Weather conditions in 2012 were favorable for the severe late blight development. In such situation, in-furrow application of Uniform and Quadris fungicides delayed the manifestation of disease under field condition by 19 and 12 days, respectively; a certain suppressing effect was also observed for a later period (Fig. 2). The AUDPC value, describing the late blight development, was reduced by 1037 and 1035 in the Uniform and Quadris variants, respectively, as compared to the untreated control (Fig. 3).

Based on the obtained data, one can conclude that the application of the Uniform fungicide, containing two active compounds, azoxystrobin and well-soluble mefenoxam, provided the highest disease suppression that exceeds that of the azoxystrobin-based Quadris fungicide.

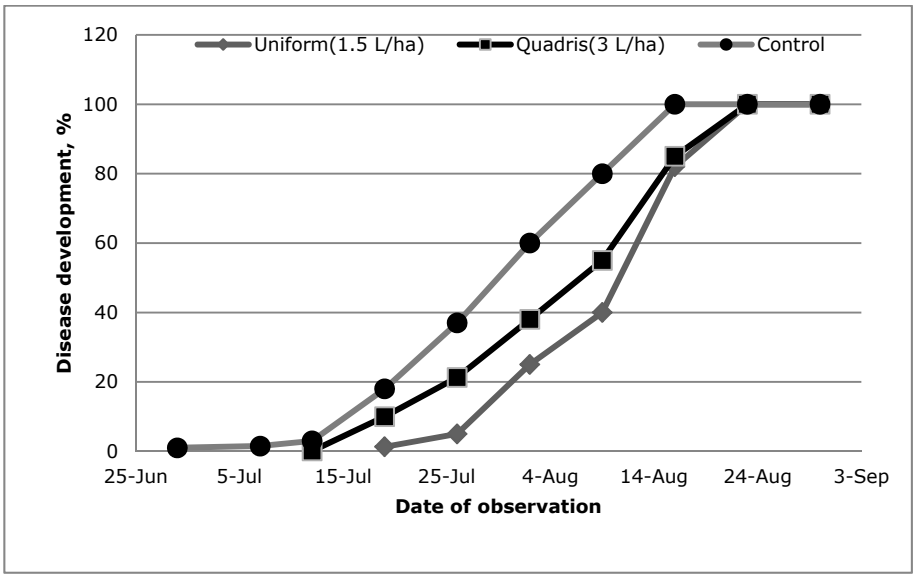

Figure 2. *Dynamics of the late blight development in different variants of protective treatment*

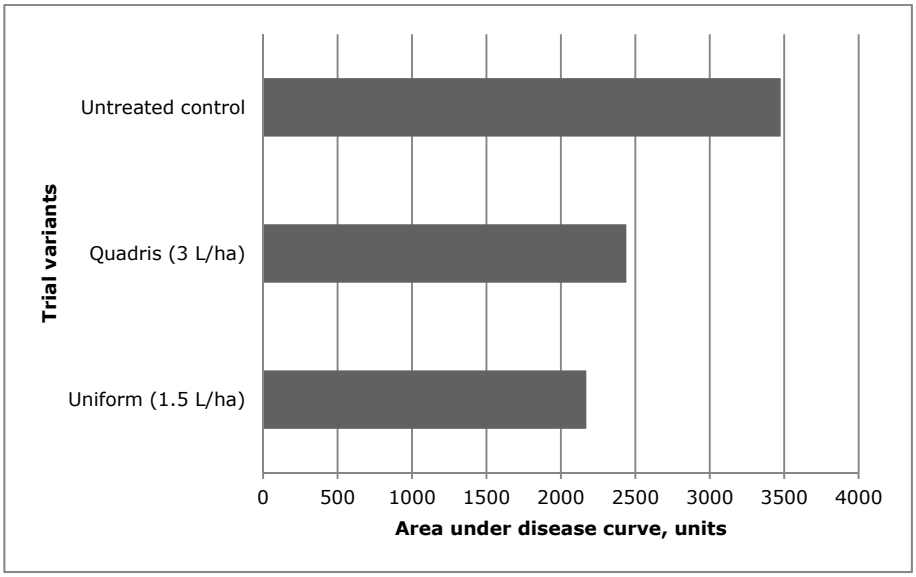

Figure 3. *Area under disease progressive curve (AUDPC) values obtained for different variants of protective treatment ($LSD_{0.05}$ = 135)*

Second-year trials

Like in 2012, the weather conditions in 2013 were favorable for the epiphytotic LB development. As a result, this disease had the most dramatic effect on both the total yield and the tuber quality. The first manifestation of the disease on the control plots was observed in the second decade of July, whereas the total haulm decay was observed already in the first decade of August. At the same time, Uniform application delayed the disease appearance by 14 days as compared to the untreated control and provided the further LB suppression (Fig. 4): the AUDPC value in the control and Uniform variants made 3421 and 2335, respectively (Fig. 5). Therefore, the Uniform application reduced the AUDPC value by 1086; the corresponding yield increase made 7.0 tons/ha (Fig. 6).

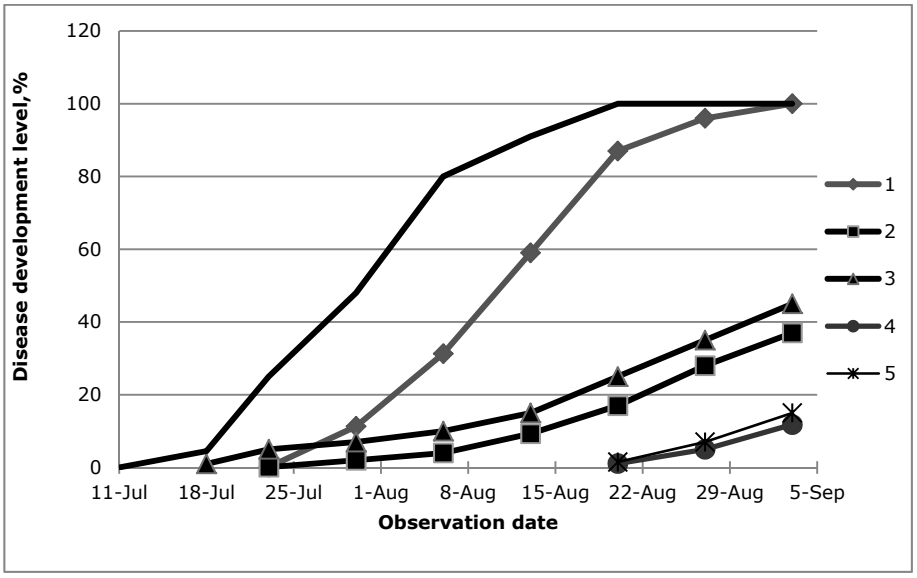

Figure 4. *Dynamics of the leaf blight development in the compared variants of treatment.* ***1****, Uniform;* ***2****, 2× Ridomil Gold MZ + 2× Revus Top + 1× Shirlan;* ***3****, 1× Ridomil Gold MZ + 2× Revus Top + 1× Shirlan;* ***4****, combination of 1 and 2;* ***5****, combination of 1 and 3;* ***6****, untreated control*

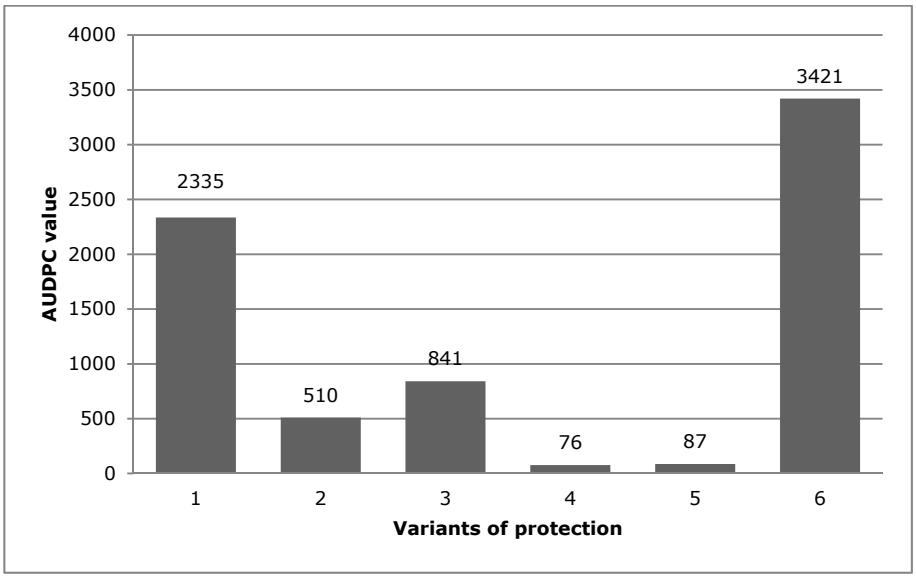

Figure 5. *AUDPC values for the compared variants of treatment.* ***1****, Uniform;* ***2****, 2× Ridomil Gold MZ + 2× Revus Top + 1× Shirlan;* ***3****, 1× Ridomil Gold MZ + 2× Revus Top + 1× Shirlan;* ***4****, combination of 1 and 2;* ***5****, combination of 1 and 3;* ***6****, untreated control (LSD* $_{0.05}$ *= 86)*

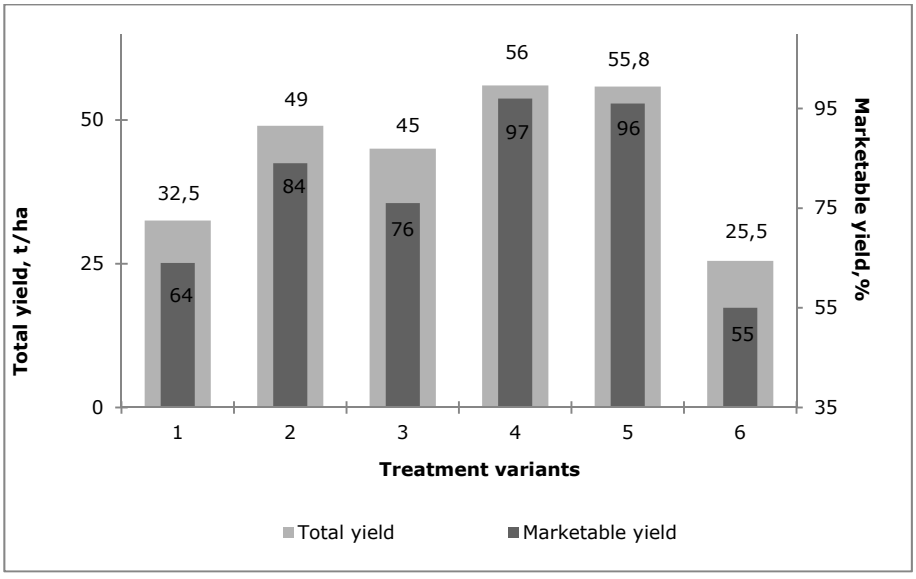

Figure 6. *The total (*$LSD_{0,05}$ *= 2.7) and marketable (*$LSD_{0,05}$ *= 3.2) yields obtained in the compared variants of protective treatment.* ***1****, Uniform;* ***2****, 2× Ridomil Gold MZ + 2× Revus Top + 1× Shirlan;* ***3****, 1× Ridomil Gold MZ + 2× Revus Top + 1× Shirlan;* ***4****, combination of 1 and 2;* ***5****, combination of 1 and 3;* ***6****, untreated control*

According to the obtained results, the sequential application of fungicides (in-furrow Uniform application followed by the protection of vegetating plants by the tank mix of common fungicides with the Isabion biofertilizer provided an efficient protection of potato against the late blight and high total and marketable yields. The start of disease was delayed by 40 and 28 days, and the AUDPC value was reduced by 3345 and 434 as compared with the unprotected control and the standard scheme of chemical treatment, respectively. As a result, the increase in the total and marketable yields comparing to the untreated control made 30.5 t/ha and 42%, respectively; comparing to the standard scheme of chemical treatment, this increase made 7 t/ha and 13%, respectively.

In the case of the variant 5 (skipped spraying during the sprouting stage), the disease also appeared 40 days later as compared to the untreated control (Fig. 4), and the AUDPC value was reduced by 3334 (Fig. 5). The increase in the total and marketable yields made 30.3 t/ha and 41%, respectively (Fig. 6) that was very close to the results obtained for the full treatment scheme (variant 4). Therefore, even in the case of the epiphytotic LB development, the in-furrow Uniform application made it possible to reduce the number of fungicide treatments during the vegetation period without any losses in the efficiency of plant protection, total yield, and the quality of harvested tubers.

In the variant 3 (reduced program of fungicide treatments without any Uniform application), the first disease manifestation was registered 7 days earlier than in the above variants. The further observations revealed more intensive disease development and increase in the AUDPC value by 331. As a result, the total and marketable yields were decreased by 4 t/ha and 8%, respectively, as compared with the variant 2 (common treatment scheme). These findings confirmed our earlier study showed that the delay or absence of the first spraying has a negative effect on the efficiency of the further protection (Filippov, 2012).

CONCLUSIONS

In-furrow application of the Uniform fungicide represents an efficient way to provide the late blight protection of potato starting from the sprouting stage and reduces the risk of the early infection of plants. In addition, such treatment allows a user to postpone the beginning of foliar sprayings and to reduce their number without any losses in the plant protection efficiency. The inclusion of the Uniform application into the common chemical protection scheme provides a significant increase in the total and marketable yields of potato tubers.

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Practical Experiences of Decision Support Systems in the Swedish Potato Field Trials for *Phytophthora infestans* 2011-2014

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Practical Experiences of Decision Support Systems in the Swedish Potato Field Trials for *Phytophthora infestans* 2011-2014

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Objective

To evaluate the use of decision support systems (DSS) for potato late blight (*Phytophthora infestans*) the two different DSS Dacom and VIPS have been evaluated in the Swedish potato late blight field trials during the last four years. Potato late blight is a severe and common disease in potato farming in Sweden. Thus, using DSS in order to spray at the right time and possibly to decrease the amount of chemicals at spraying can be a valuable tool in the work with integrated pest management (IPM).

Materials and Methods

Two different DSS were evaluated in the Swedish late blight field trials, predominantly in the susceptible variety Bintje, at three different locations in southern Sweden during 2011-2014 (Figure 1). The Dutch program Dacom was tested during 2011-2013 and the Norwegian web based system VIPS (Acronym for pest varnings, Bioforsk) during 2013 and 2014.

The two programs differ in usage and in what degree of freedom the user gets the information. Both programs require input of different weather data. Dacom also requires information about the crop, treatments and possibly additional weather information continuously from the user. Dacom gives the user a specific advice when to spray and with what kind of substance. VIPS on the other hand only gives information when there is an increased risk of infection.

The spraying information from both programs has been followed strictly as far as it has been possible due to weather conditions. For VIPS special rules for treatment, including reduced doses and permanent spraying intervals, were followed in order to be able to compare results from the different locations. Only in Sweden registered chemicals against potato late blight were used in the DSS treatments.

Table 1. Number of treatments in treatment programs decided by DSS in the Swedish late blight field trials during 2011-2014. Treatment programs with weekly sprayings included 11-12 sprayings. The trials were located in Kristianstad (L), Borgeby (M) and Lilla Böslid (N).

YEAR	DACOM; L	DACOM; M	DACOM; N	VIPS; L	VIPS; M	VIPS; N
2011	9	11	9	–	–	–
2012	8	10	9	–	–	–
2013	8	10	8	9	10	9
2014	–	–	–	12	8	9

Table 2. Performance (the lower number the better) of the DSS in the Swedish late blight field trials during 2011-2014. Totally 10-12 different treatment programs were accomplished per year. The DSS Dacom and VIPS were tested at the three different places Kristianstad (L), Borgeby (M) and Lilla Böslid (N). Results are based on harvest of tubers free from late blight infection.
* Totally 6 different treatment programs were tested in 2013 at Borgeby (M).

YEAR	DACOM; L	DACOM; M	DACOM; N	VIPS; L	VIPS; M	VIPS; N
2011	3	2	5	–	–	–
2012	7	2	1	–	–	–
2013	4	6	10	6	10	6
2014	–	–	–	2	3*	7

Figure 1. Locations (red dots) of the three potato late blight field trials in Sweden during 2011-2014. Kristianstad (NE Scania), Borgeby (SW Scania) and Lilla Böslid (S Halland). https://maps.google.com/maps

Results and Discussion

- The DSS tested have been found to be valuable tools in the control of potato late blight
- In the field trials the DSS have recommended less or the same number of treatments compared to weekly sprayings (Table 1)
- Controlling late blight using DSS have in average performed well compared to other treatment programs (Table 2)
- At some locals and years the warnings from the DSS have been disproportionate to the late blight infection pressure and attacks in the field
- Technical problems with weather data have sometimes hampered the use of the DSS

Conclusions

- Timing is more important than the number of sprayings
- DSS can decrease the number of sprayings compared to weekly sprayings
- DSS can give just as good results as weekly treatments with the same fungicides, but with less sprayings and possibly reduced spraying dose
- The costs of DSS can sometimes be compensated by more adapted and more efficient spraying
- The specific information from the DSS and work input from the user varies between different DSS
- The user can not only trust the information from the DSS, but also have to use its own experience and common sense
- The evaluation of DSS in the Swedish potato late blight field trials will continue in 2015, testing VIPS and the Danish Skimmelstyring

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Evaluation of seed tuber treatment products to control late blight in organic potato production

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SUMMARY

A selection of copper-free products for the control of late blight (*Phytophthora infestans*) infections in organic potato production was tested for their suitability as a tuber treatment in both laboratory and field trials. Both a storage and a growth test were performed under controlled conditions in the lab/ greenhouse using artificially inoculated tubers that were treated with the alternative products after inoculation. Several of these were able to reduce tuber infection after storage at 15°C in both frequency and severity, or to increase the number of healthy plants growing from the inoculated tubers. In organic production, tuber dressings with alternative, Cu-free preparations might help to reduce or retard late blight epidemics, to produce disease free seed tubers and to further reduce the copper input.

KEYWORDS

Phytophthora infestans, tuber blight, disease management, copper-free products

INTRODUCTION

Potato late blight epidemics caused by *Phytophthora infestans* often originate from (latently) infested seed potatoes remaining unnoticed during storage and brought to the fields at planting (Zellner *et al.*, 2011; Wharton *et al.*, 2012). Starting from an infected seed tuber, the pathogen can take several different ways to infect a potato plant and start an epidemic. When soils are sufficiently wet, sporulation on tubers might occur, leading to an infection of developing stems or neighbouring plants and tubers via the soil. Furthermore, the pathogen can grow on or inside the stem up to a certain height, and cause symptoms on aboveground parts of the stem. All these pathways will eventually lead to primary *Phytophthora* stem infections from which – via sporulation and secondary leaf infections – disease spots will develop that soon will affect the whole field. Such primary infections usually will occur relatively early in the season, earlier than secondary infections via airborne sporangia, as the primary inoculum originates directly from the tuber, and the pathogen can establish on the tuber and in the soil for a certain time before and during sprouting. In particular, this will happen when weather conditions are suitable for the pathogen and for the development of disease (i.e. moist soils and temperatures above 10° C).

Previous studies based on PCR analyses have shown that an average of 10% of the European seed tubers are (latently) infected with *P. infestans* (Zellner *et al.*, 2011) and as such, potentially carry the inoculum into the fields. Even if only 1% of these produce plants with diseased sprouts, this would eventually result in approx. 40 disease spots per hectare (assuming a density of 40,000 plants/ha) (Powelson *et al.* 2002).

Within the course of a project aiming at the reduction and avoidance of copper usage in organic farming, this study investigated different ways to reduce primary stem infections in organic potato production by seed tuber dressings with $Cu(OH)_2$ and other, copper-free products.

MATERIALS AND METHODS

Laboratory/ greenhouse tests were performed to test alternative substances for their ability to reduce tuber blight (brown rot) in artificially infested potato tubers. We have tested several commercial and non-commercial organic products and preparations in two different lab and greenhouse assays. In total, 15 Cu-free alternative products and $Cu(OH)_2$ were tested. (Table 1).

Tubers were infested by either a short full immersion in a sporangial suspension (5 sporangia/ µl) or by spraying with the suspension (ca. 0.5 ml/ tuber) and incubation at room temperature under high humidity for 24-48 h to initialise the infection. This treatment was meant to mimic natural tuber infestation at the end of the season with sporangia being washed onto the tubers from infected foliage. After that, dried tubers were treated by spray application or immersion with the various substances, as indicated in Table 1, at a rate of approx. 0.7 ml/ tuber. Tubers were then either stored or planted, as described below.

Two sets of laboratory tests with artificially infected tubers were performed: In a storage test inoculated tubers were treated with the products and stored in crates for 2-3 months at 15°C. Percent tuber necrosis (brown rot) and number of tubers with brown rot symptoms was recorded (equivalent to an autumn seed treatment). Up to 6 tests were performed with each product, each consisting of 10 or 30 tubers per treatment.

In a growth test in pots, inoculated tubers were treated with the products, immediately planted in pots after drying and grown for 2-3 months in a greenhouse (20-25°C). A chemical (conventional) control was included in these tests (Curzate, AI: cymoxanil und mancozeb). The number of healthy plants developing from the tubers was recorded (equivalent to a spring seed treatment). Up to 6 tests were performed, each consisting of 10 tubers per treatment.

Table 1. *Products and preparations used for tuber treatments in lab and greenhouse assays*

category	Active ingredient	Product status	Concentration	in test[1]
Control	Water	n/a	n/a	S/G
	Copper hydroxide	commercial	1.6%	S/G
	Curzate (conventional control)	commercial	0.025%	G
Microorganisms	*Aureobasidium pullulans*	commercial	1%	S/G
	Pythium oligandrum	commercial	7%	S
	Bacillus subtilis	commercial	1%	S/G
Plant extracts	Garlic product	commercial	1%	S
	Knotweed product	commercial	1%	S/G
	Liquorice extract	Test product	5%	G
	Horseradish/mustard product	commercial	20%	S/G
	Horsetail product	Test product	2%	G
	Powder from dried *Brassica juncea*	non-commercial	20%	S
	Clove oil	commercial	1%	S/G
	Citrus product	Test product	0.8%	G
Defined chem. substances	Benzoic acid	commercial	2%	G
	Na-Phosphonate (= Phosphite)	Test product	1%	S/G
	Chitosan	commercial	0.4%	S/G
Other	Hot water 44°C	n/a	n/a (for 1 minute)	S/G

[1] used in storage (S) or growth test (G).

RESULTS

Several alternative, copper-free products and substances for potential use in organic farming were applied to artificially infected tubers to evaluate their effect on the establishment of tuber infections and their ability to prevent/ reduce the amount of brown rot.

Storage test: Infection rates were generally relatively high, showing that an established infection can hardly be halted by any alternative plant protection product, i.e. in a curative sense. Still, some of the products/ preparations were able to clearly reduce the number of successfully infected tubers and the amount of brown rot developing on the tubers after 6-8 weeks (Table 2). Some caused a considerable reduction in tuber necrotisation (brown rot) after storage, in particular chitosan, knotweed, Na phosphonate and clove oil, with a disease reduction of around 20-30%. A commercial mustard product performed best, with an about 60% reduction compared to the control. These effects, however, were non-significant in most cases, due to the high variability of the data.

Growth test: Again, infection rates were generally relatively high, but the good performance of the chemical control (cymoxanil/mancozeb) and the poor growth in the controls proved the suitability of the assay for the analysis of tuber treatment products. In the growth test, all but one product had led to an on average increase in number of plants developing from the tubers as compared to the control. Tubers treated with chitosan, *Aureobasidium*, phosphonate and

Curzate performed best. The mustard product, however, caused significant plant death (Table 3). Again, these effects were non-significant, due to the high variability of the data and relatively low number of tubers that could not be increased because of the complexity of the test and space limitations.

Table 2. *Storage test: Mean incidence and severity of brown rot in artificially infested tubers after application of alternative seed tuber treatments. Each tests consisted of 10 or 30 tubers per treatment*

treatment/ product	no. of tests	mean percentage of brown rot per tuber (relative to control)	mean no. of successfully infected tubers (relative to control)
Control	6	100%	100%
Garlic product	5	128%	105%
Copper hydroxide	6	112%	105%
Pythium oligandrum	6	111%	92%
Aureobasidium pullulans	3	102%	105%
44°C water (heat treatment)	3	102%	74%
Bacillus subtilis	5	101%	90%
Brassica juncea powder	1	95%	103%
Chitosan	5	83%	82%
Knotweed product	3	81%	83%
Na phosphonate	6	75%	75%
Clove oil	6	68%	65%
Horseradish/ mustard product	4	41%	49%

Table 3. *Growth test: mean number of healthy plants growing from artificially infected seed tubers 4-6 weeks after inoculation and application of alternative seed tuber treatments. Each test consisted of 10 tubers per treatment*

treatment/ product	no. of tests	mean no. (SD) of healthy plants
control	6	2.8 (1.0)
horseradish/ mustard product	2	1.0 (1.4)
Citrus product	6	3.0 (2.1)
Benzoic acid	6	3.2 (2.0)
clove oil	6	3.2 (1.9)
Knotweed product	6	4.0 (1.3)
Liquorice extract	6	4.0 (1.9)
Copper hydroxide	6	4.0 (1.8)
Bacillus subtilis	6	4.0 (1.5)
44°C water (heat treatment)	6	4.2 (2.5)
Horsetail product	6	4.3 (2.5)
Chitosan	6	4.5 (1.6)
Aureobasidium pullulans	6	4.6 (1.1)
Na phosphonate	6	5.2 (1.9)
Curzate	4	6.3 (1.5)

DISCUSSION

Early primary stem infections originating from infected seed tubers are the most important starting point for early and massive late blight epidemics in both conventional and organic potato production (Powelson *et al.*, 2002; Johnson, 2010; Zellner *et al.* 2011; Wharton *et al.* 2012). In contrast to any secondary infections originating from airborne sporangial inoculum these occur earlier in the season, making them particularly significant for the further development of the disease on a field scale (Zellner *et al.*, 2011). In organic potato production, early primary infections are a key factor for development and economic significance of late blight disease, as they determine the onset of an epidemic and thus, have a strong influence on potato yield at harvest. Therefore, the time when an infection and an epidemic start is even more important for organic farmers. As systemic fungicides are not available, copper currently is the only fungicide able to control late blight in organic farming (in Germany and other countries).

As in conventional farming, seed treatments with copper or alternative products also can be a way to reduce late blight in organic potato production (Benker *et al.*, 2006; Wharton *et al.*, 2012), as such treatments might be able to reduce the incidence of primary *Phytophthora* infections. They are meant both to reduce the risk of infection of the developing sprouts and to affect sporulation on the infected tuber in the soil. This will also provide protection of neighbouring tubers within the potato hill.

Our lab tests have shown that several alternative substances have the potential to provide protection against primary stem blight when applied as a seed dressing. The tests proved that seed dressings can act against *Phytophthora* propagules sitting on or inside the tuber periderm as germinating spores, and as such can prevent the further colonisation of the tissue and the infection of neighbouring tubers. Although infection rates were still generally high, the test showed that some of the alternative products are effective in spite of the high infection pressure of the artificial inoculation (much higher than in natural situations), and are able to reduce tuber infection during storage and after planting. As no curative effect can be assumed, this effect was most probably due to an action against propagules that have not yet germinated or not yet invaded the tuber tissue deeply enough. The strong toxic effect of the horseradish product on the developing sprouts in the growth test, however, clearly showed that aggressive substances like this are unsuitable as a seed dressing. In moist soils, the dressings seem to have an additional effect surrounding the tuber that might on the one hand protect neighbouring tubers, but on the other hand might be too aggressive for the developing plants.

In addition to the laboratory assays described above, field tests were performed with copper and a selection of Cu-free seed treatment products in 2012 and 2014 to test these substances for their ability to reduce stem blight (*Bacillus*, chitosan, Na phosphonate). Application was done in autumn or in spring. Although artificially infected seed tubers were used (planted next to the treated tubers to increase infection pressure, see Keil *et al.*, 2010), incidence of primary infections from tubers was low in these trials, most likely due to dry weather conditions unsuitable for the development of this type of symptom during spring. Therefore, no data on the effect of the seed dressing on primary stem infections were available. However, when (secondary) leaf infection data in 2012 were considered, an effect of all treatments on the degree of leaf infections could be observed in one stand, and an effect of the spring copper treatment in the other (data not shown). This effect could not be unambiguously proven to be an effect of the tuber treatments on the incidence of primary stem blight, but might still be considered as a retardation effect, the mechanism for which remains unclear. The different treatments might have caused a reduction of sporulation on the tubers, causing a decrease of sporangia present in the soil (the initial soil- and tuberborne inoculum), and a later onset of massive leaf infections. In the 2014 field trials, the copper (spring) treatment had caused

a significant increase in sprouting from the tubers (less failing tubers), indicating that a tuber treatment does have an effect on the development of the disease, at least on the tubers themselves, protecting them from infection, subsequent decay and failure.

Together with other agricultural and technical measures (such as foliar treatments or leaf removal) seed tuber treatments with copper and/or alternative products can be part of a management strategy to reduce the extent of early infections from infected tubers in organic potato farming (Benker *et al.*, 2006; Wharton *et al.*, 2012). Such tuber infestations are thought to be among the major pathways of *Phytophthora* inoculum onto the field, serving as starting points for subsequent late blight epidemics. Seed dressing might thus be able to achieve a further reduction and/or retardation of a late blight epidemic and thus, might help to better control the disease and its impact on potato yield. Furthermore, this strategy might further help to produce disease free seed tubers and reduce the copper input into the fields.

ACKNOWLEDGEMENT

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PPO – SPECIAL REPORT NO 17 – 2015, 187-188

Genotypic and phenotypic characterization of *Phytophthora infestans* isolates sampled in Brittany in two consecutive years (2013-2014)

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Genotypic and phenotypic characterization of *Phytophthora infestans* isolates sampled in Brittany in two consecutive years (2013-2014)

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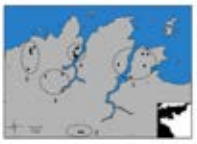
West European populations of *Phytophthora infestans* are characterized by frequent changes of major clonal lineages within a few years. In the aim to better control this pest, it is necessary to understand the mechanisms involved in these changes.
The objective of this study was to follow the evolution of *P. infestans* populations at a local scale for two consecutive years. For this purpose, we sampled potato fields, volunteers and covered crops in a small potato cropping area from northern Brittany (western France) in 2013 and 2014. This geographic area was chosen because of the presence of potato fields from April to August (early & ware potatoes), and a mild winter enabling the development of volunteers.
The 328 isolates collected during this period have been genotyped with 17 SSR markers and tested for foliar aggressiveness on three potato cultivars. This study provides elements allowing to better understand the epidemiology of *P. infestans* which are discussed in this poster.

1 Experimental protocol

❖ **Sampling of *P. infestans* isolates during two consecutive years**

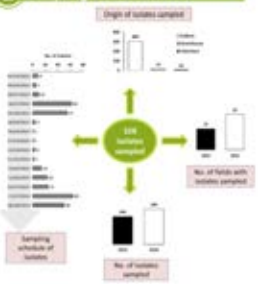


Geographic position of each potato field sampled during this study.
Fields sampled both in 2013 and 2014 (for culture and volunteers, respectively) are represented by black dots and fields sampled only in 2014 (for culture) are represented by white dots.
Fields were separated into five groups (1-5) based on their geographic positions.

❖ **Isolate characterization**

- Genotyping with 17 SSR markers:
  - ➢ **PiG8, PiG5, G11** (Knapova & Gisi, 2002)
  - ➢ **D13, Pi02, Pi04, Pi16, Pi33, Pi35, Pi63, Pi70** (Lees et al., 2006)
  - ➢ **PinfSSR2, 4, 6, 7, 8 & 11** (Li et al., 2010)
- Aggressiveness characterization on three potato cultivars (Bintje, Robijn & Milwa):

2 Sampling characteristics

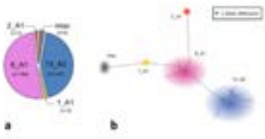


3 Genotypic characterization

❖ Among the 328 isolates, 60 multilocus genotypes (MLGs)

❖ The 60 MLGs split into five clonal lineages:

- Four already known (**1_A1, 2_A1, 6_A1 & 13_A2**)
- One not yet described, **termed as "misc"**, with a genotypic profile close to "tomato isolates" (**cf poster of Corbière *et al.***)



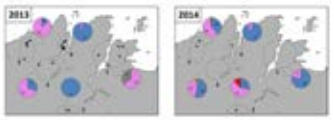
Genotyping of the 328 *P. infestans* isolates sampled in Brittany in 2013-14 with the clonal lineages frequencies (a) and the Minimum Spanning Tree based on the 17 SSR loci showing the evolutionary relationships among the multilocus genotypes (b).

4 Evolution of genotype frequencies

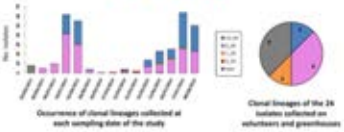
❖ Variation between nearby areas (***e.g.* 4 & 5**)

❖ Variation in a same area between 2013 and 2014 (***e.g.* 2**)

❖ Only 6_A1 & 13_A2 are recorded by the end of epidemics



Frequencies of clonal lineages in the five areas sampled in 2013 and 2014



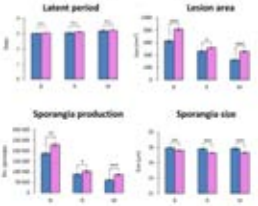
Occurrence of clonal lineages collected at each sampling date of the study

Clonal lineages of the 24 isolates collected on volunteers and greenhouses

5 Phenotypic characterization

❖ 6_A1 isolates sporulate more and cause larger lesions

❖ 13_A2 isolates produce bigger sporangia



Aggressiveness characteristics (means ± SE) of the 6_A1 and 13_A2 isolates measured on the three potato cultivars Bintje (B), Robijn (R) and Milwa (M). In total, 243 isolates were tested (144 6_A1 & 119 13_A2). Statistically significant effects, revealed with Student t tests, are indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001).

6 Discussion - Conclusion

❖ Two contrasted years for the late blight pressure, low in 2013 and high in 2014

❖ Brittany *P. infestans* populations are dominated by a few clonal lineages

- 95% of the 328 isolates sampled belong to 6_A1 or 13_A2;
- Large changes in genotype frequencies across space and time;
- No evidence of sexual reproduction, even with the presence of both mating types in some fields.

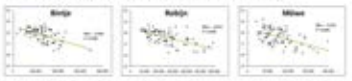
❖ Other clonal lineages sampled in the beginning of epidemics but not observed afterwards

- Lower fitness? Not adapted to higher temperatures?

❖ Lower aggressiveness of 13_A2 isolates compared to 6_A1... but this clonal lineage persists at very high frequencies

- Effect of the bigger size of 13_A2 sporangia?
  - ➢ **Higher infection rate of sporangia?**
  - ➢ **Higher survival rate of sporangia?**

❖ Trade-off between sporangia production and size



Relationship between sporangia production (no. sporangia) and sporangia size (µm) for 84 Brittany *P. infestans* isolates (6_A1 and 13_A2) sampled in 2014 tested on the potato cultivars Bintje, Robijn and Milwa. Pearson's rank correlations (ρ) and corresponding P-values, and the linear regression are indicated on the graphs.

7 Perspectives

❖ Evolution during epidemics of:

- **Genotypic diversity**
- **Aggressiveness levels**

❖ Influence of host variety on aggressiveness

- **Local adaptive patterns?**

❖ Focus on the trade-off between sporangia production and size

- **At the MLG scale**
- **Does it differ between geographic areas?**

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Influence of soil moisture to the occurrence of primary stem blight (*Phytophthora infestans*)

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Influence of soil moisture to the occurrence of primary stem blight (*Phytophthora infestans*)

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Introduction

The successful control of potato late blight (*Phytophthora infestans*) is mainly based on the right timing of the first fungicide treatments. The analysis of the Monitoring data (PhytophthoraModell Weihenstephan 1997 to 2010) indicates that the occurrence of late blight is influenced by the soil moisture. Bässler (2005) has shown that the soil type has an effect on the latent stem infection. The objective of this study was to check the influence of soil moisture to the occurrence of stem blight caused by *Phytophthora infestans*.

Materials and methods

Field trials were carried at the trial station near Munich. Artificial inoculated (*Phytophthora infestans*) tubers were planted between healthy tubers to determine the effect of irrigation and soil moisture on the incidence of primary stem blight infection (Fig. 1). The plots were irrigated in between the rows after planting (T1) and 28 days after crop emergence (T2). The stems of the healthy planted tuber were assessed.

Tab. 1: Field trial with inoculated tubers and additional irrigation at different timing

treatment	
1	not inoculated,
2	inoculated, no irrigation
3	inoculated, irrigation after planting (T1)
4	inoculated, irrigation 28 days after crop emergence (T2)
5	inoculated, irrigation after plant and 28 days after crop emergence (T1 + T2)

Fig. 1: Field trial with artificial inoculated tubers

Results

The crop emergence of the healthy tubers was not influenced by the artificial inoculated tubers placed 15 cm next to these tubers. The first symptoms of primary stem blight became evident 10 days after the irrigation at T2. In the plots without inoculated tubers no stem blight occurred. In the inoculated plots without additional irrigation after the crop emergence the stem blight frequency was less than 1%. Due to the irrigation after crop emergence the stem infestation was significantly increased.

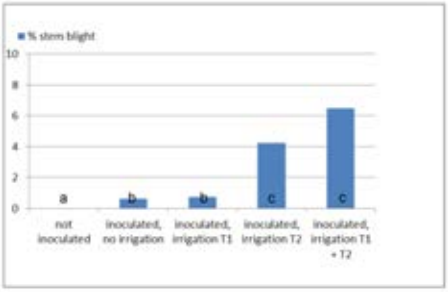

Fig. 2: Disease incidence of primary stem infection of *Phytophthora infestans*. Significant differences between treatments are represented by different letters (*P < 0.05, Tukey test*).

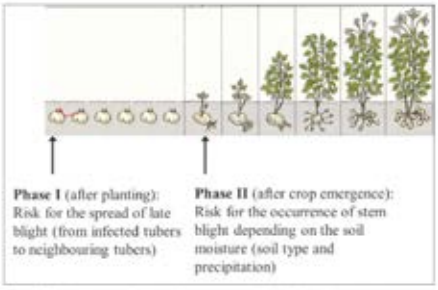

Fig. 3: Model "stem blight - risk assessment" is divided in two phases:
Phase I: Risk for the spread of late blight
Phase II: Risk for the occurrence of primary stem blight

Conclusions

The results indicate that the disease incidence of primary stem blight is influenced by soil moisture. In the phase I (after planting) *Phytophthora infestans* is able to grow in the sprout and/or release Zoospore in the soil which can infect neighbouring tubers (depending on the soil moisture). The phase II is relevant for the timing of the first fungicide treatment. High soil moisture after the crop emergence results in a higher risk for the occurrence of first stem blight.

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Late blight control and development in 2014 to NIRDPSB Brasov including technological elements

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Late blight control and development in 2014 to NIRDPSB Brasov including technological elements

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Introduction.

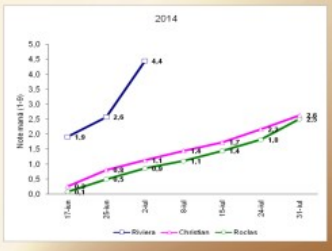
The research aim is to establish the density possibilities and late blight control in Riviera, Christian and Roclas varieties, grown without irrigation in the context of climate change. The main objective of the research is linked to highlight the complementary aspects that accompany chemical control of late blight. At present there is an impression, unjustified, that the treatments solve problems without due attention to cultural hygiene, agro – technical elements (density, weeds etc.).

Materials and methods

- Location of the field trial: NIRDPSB Brasov
- Size of plots: 25 m2
- Lay out of the plots of the field trial: randomized complete block design with 4 replicates. Planting was made in 31st March 2014. Cultivation and maintenance was in line with current good agricultural practice
- Potato cultivars: Riviera, Christian and Roclas
- First symptom of late blight observation: daily check for all plots after emergence till first symptom observed in one of the plots (2014, June 17th) Late blight assessment: plots are assessed for the extent of blight spots on the leaves. Each plot is assessed as a whole for percentage disease severity using a standard accepted severity key.
Yield assessment: two rows in the center of each plot were harvested mentioned the number and the weight of tubers with blight (blighted tubers assessments are usually based on a sample of 100 tubers per plot. When is necessary the tubers are cut to examine the flesh.)

No.	Day	TECH 1 Product	TECH 1 Product	Dose /ha	TECH 2 Product	TECH 2 Product	Dose /ha
1	17.06.	Dithane M-45	Mancozeb 80%	2,5 kg/ha	Ridomil Gold Mz 68 WG	Mefenoxam 45 + mancozeb 643%	2,5 kg/ha
2	25.06.	Bravo 500 SC	Clorotalonil 500 g/l	2,0 l/ha	Consent 450 SC	Fenamidon 75 g/l + mancozeb 375 g/l	2,0 l/ha
3	2.07.	Electis 75 WG	Mancozeb 68,5% + zoxamide 8,8%	1,8 kg/ha	Drago 76 WP	Cymoxanil 6% + mancozeb 70%	2,0 kg/ha
4	8.07	Bravo 500 SC	Clorotalonil 500 g/l	2,0 l/ha	Revus 250 SC	Mandipropamid 250 g/l	0,6 l/ha
5	15.07	Folpet 80 WG	Folpet 80%	2,0 kg/ha	Acrobat Mz 90/600 WP	Dimetomorf 9% + mancozeb 60%	2,0 kg/ha
6	24.07	Shirlan 500 SC	Fluazinam 500 g/l	0,4 l/ha	Bravo 500 SC	Clorotalonil 500 g/l	2,0 l/ha
7	31.07	Shirlan 500 SC	Fluazinam 500 g/l	0,4 l/ha	Shirlan 500 SC	Fluazinam 500 g/l	0,4 l/ha

Evolution of foliage destruction



Results and disscusions

Media effects of control technologies regarding late blight attack to different varieties (Brasov, 2014)

Variety	Late blight control technology	Late blight intensity 17 June*	25 June*	2 July*	8 July**	15 July**	24 July**	31 July**
Riviera	Tech 1	2,3 a	2,9 a	5,0 a	-	-	-	-
	Tech 2	1,5 b	2,2 b	3,9 b	-	-	-	-
Roclas	Tech 1	0,4 c	1,1 c	1,4 c	1,7 a	2,1 a	2,6 a	3,0 a
	Tech 2	0,1 d	0,5 e	0,8 d	1,2 b	1,4 c	1,7 c	2,3 b
Christian	Tech 1	0,1 d	0,9 d	1,3 c	1,6 a	1,8 b	2,3 b	2,9 a
	Tech 2	0,0 d	0,1 f	0,5 d	0,6 b	1,1 d	1,4 d	1,8 c
Mean	Tech 1	1,6	1,9	2,9	0,9	1,6	2,6	2,6
	Tech 2	0,9^{O}	1,2^{O}	2,0^{O}	0,5^{O}	0,9^{O}	1,7^{O}	1,6^{O}
DL 5% (Trat) =		*0,1	*0,1	*0,2	**0,1	**0,1	**0,1	**0,2
LSD 5% (Sol * Trat) =		*1,1	*0,2	*0,4	**0,2	**0,2	**0,2	**0,3

Control technology Tech 2 was more effective to the analyzed varieties throughout the period the attack intensity was lower. At the beginning of the observations, in 17 June the thickening on the row result in a significant intensity of attack only to Riviera variety. To Roclas and Christian varieties did not find significant differences of attack intensity during the observations.

Average interaction effects of late blight control technologies with the planting distances (Brasov, 2014)

Planting distance cm	Late blight control	Yield Riviera T/ha	Riviera Test Duncan	Roclas T/ha	Roclas Test Duncan	Christian T/ha	Christian Test Duncan	Mean T/ha	Mean Test Duncan
30	Tech 1	18,4	FF	24,8	BC	23,9	BC	22,4	B
	Tech 2	18,8	EF	26,8	A	26,2	B	24,6	A
25	Tech 1	16,8	F	23,1	CD	22,5	C	20,8	C
	Tech 2	20,6	DE	24,6	BC	24,4	B	23,2	AB
Average									
30 cm		18,6	C	26,8	A	25,1	AB	23,5	100 %
25 cm		18,7	C	23,9	B	23,4	B	22,0	93,6%
Tech 1		17,6	E	23,9	BC	23,2	C	21,6	100 %
Tech 2		19,7	D	26,7	A	25,3	AB	23,9	110,6%
Variety /experience		18,6	100%	25,3	136,0%	24,2	130,1%	22,7	CV = 7,2 %

DL 5% (Variety) = 8 t/ha (42,8%)
DL 5% (Distance) = 1,1 t/ha (4,5%)
DL 5% (Treatment) = 0,7t/ha (3,2%)
LSD 5% (Variety * Dist) = 2,6 t/ha
LSD 5% (Variety * Trat) = 1,7 t/ha
LSD 5% (Dist * Treat) = 1,4 t/ha
LSD 5% (Variety * Dist * Treat) = 2,4 t/ha

Average of total yield was 22.7% (CV = 7,2 %).
Average yield were 18.6 t/ha (100%) to Riviera, 25,3 t/ha (136,0%) to Roclas and 24,2 t/ha (130,1%) to Christian. Although Roclas and Christian yield exceeded Riviera with 36.0% and 30.1%, the differences were not statistically significant.
To the 25 cm distance plants density were more frequent and total production was reduced on average by 6.4%, densities effects manifested significant only to Roclas variety
By applying Tech 2 total production increased by 10.6% compared to the average production achieved after Tech 1.
Significantly better effects on average total production of Tech 2 occurred in all varieties studied and averaged over both densities.
Varieties responded differently to interaction between density and late blight treatment.

Conclusions:

- Riviera variety leaves little freedom protection, so the fight should begin as soon as the amount of inoculum in the medium is able to produce a general infection (after observing symptoms on leaves detached).
- The other two varieties, Christian and Roclas allow a reduction in the number of treatments.
- Reducing numbers of applications or reducing the amount of active substance used in treatment, or delaying the application of the first treatment, it can make a saving of one to three treatments at the beginning of the season.
- To Riviera and Christian varieties positive effects on production Tech 2 occurred significantly only when planting was done to turn higher density, at a distance of 25 cm. To Roclas variety yield differences are higher at the distance of 30 cm between Tech 2 and Tech 1.
- The biggest yield (28.8 t/ha) was achieved to Roclas variety using Tech 2 and a distance between rows 30 cm.

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Mancozeb, a key fungicide for the integrated control of the most frequent and detrimental potato diseases: late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*)

DUVAUCHELLE SERGE, HELLER JEAN-JACQUES

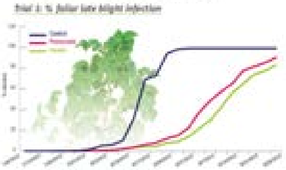
MANCOZEB, A KEY FUNGICIDE FOR THE INTEGRATED CONTROL OF THE MOST FREQUENT AND DETRIMENTAL POTATO DISEASES: LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) AND EARLY BLIGHT (*ALTERNARIA SOLANI*).

Duvauchelle Serge[1], Heller Jean-Jacques[2]

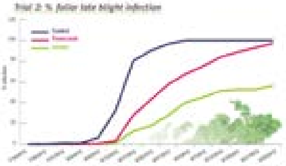
Introduction

Mancozeb still efficient after 52 years of using in EU

Trial 1: % foliar late blight infection



Trial 2: % foliar late blight infection



Efficacy against early blight

Percentage leaf area infected

Mancozeb is efficient on new strains of *Phytophthora infestans*

Treatments

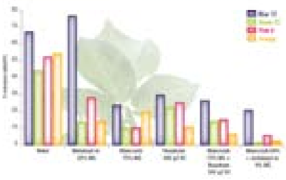
The fungicides used in the experiments and the applied dose rates

Inoculation with *P. infestans*

Disease observations

Results

Percentage necrotic foliage per treatment expressed as sAUDPC after inoculation with 4 different P. infestans isolates, representing large clonal lineages in Europe.



Discussion and conclusions

Mancozeb fungicide still widely used

Kind of grower and number of dithiocarbamate

Perception of the qualities of mancozeb by growers

CONCLUSIONS

Regulatory Overview

EU Mancozeb Task Force

Is Mancozeb safe?

Mancozeb AIR3 timeline

UPL Ltd
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Warrington
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Marker profiles of late blight resistance genes in complex interspecific potato hybrids

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SUMMARY

Stacking (pyramiding) several late blight (LB) resistance genes of diverse race specificity in one and the same potato plant provides for high, broad spectrum and durable resistance. Forty clones of standard potato varieties and interspecific hybrids comprising genetic material from two to seven wild *Solanum* species were evaluated for LB resistance and screened with SCAR markers for resistance genes *R1*, *R2/Rpi-blb3, R3a, R3b* and *RB/Rpi-blb1*. LB resistance of the hybrids was significantly related to the presence of the markers for resistance genes. However, the correlation was weaker than expected: apparently a considerable portion of LB resistance depended on the genes insufficiently researched in wild progenitors of interspecific hybrids. To verify the markers, we compared their sequences to those of the prototype genes from *S. demissum* and *S. stoloniferum*. In one case of incomplete match, indels and stop codons in the marker sequences can explain low LB resistance of the hybrid. In other cases, the observed polymorphisms in the marker sequences presume that the gene in question was probably introgressed from *Solanum* species other than *S. demissum* and *S. stoloniferum.*

KEYWORDS

Phytophthora infestans, wild *Solanum* species, potato hybrids, late blight, stacking resistance genes, DNA markers.

INTRODUCTION

Recently several very promising sources for breeding new potato cultivars with high, broad spectrum and durable LB resistance were developed by stacking several resistance genes (*R* genes) diverse race specificity in one and the same potato plant. Using genetic transformation, potato varieties were bred to carry three *R* genes (Jo *et al.*, 2014; Jones *et al.*, 2014), while traditional breeding methods produced hybrids comprising as much as five and seven *R* genes from several wild *Solanum* species (Rietman *et al.*, 2012; Kim *et al.*, 2012). In the latter case, new *R* genes introgressed by crosses are combined with polygenic LB resistance and other genes of agronomic significance (Gebhardt, 2013; Michelmore *et al.*, 2013).

Previously we screened clones of 17 interspecific potato hybrids and several standard varieties with SCAR markers of five *R* genes and demonstrated that LB resistance was associated with the presence of *R* gene markers and was vividly related to the number of these markers discerned in each particular interspecific hybrid (Khavkin *et al*., 2014). To make more manifest the link between LB resistance and the number of *R* genes per hybrid, we expanded the range of interspecific hybrids under study.

MATERIALS AND METHODS

Plant materials

We used the interspecific potato hybrid clones maintained at the N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia. These hybrids were selected for good tuber shape and yield, acceptable taste and resistance to LB, potato virus Y and golden nematode. All of the hybrids have in their pedigree from two to seven wild *Solanum* spp. Most hybrids displayed high foliage LB resistance in the field trials under natural infection conditions and high-to-moderate resistance in the laboratory assays. In the latter case, to evaluate LB resistance, plants were grown in glasshouse; detached leaves were infected with a highly virulent complex race of *Phytophthora infestans* (genes R1-R11; compatibility type A1) isolated in the Moscow region and scored against the susceptible cultivar Santé as a control (Rogozina *et al.*, 2014).

Molecular methods

Genomic DNA was isolated from potato leaves using the AxyPrepTM Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, United States). DNA samples were quantified at 260 nm with a NanoPhotometer P 300 (IMPLEN, Germany).

To screen the clonal collection of interspecific potato hybrids, we used six SCAR markers recognizing the CC and LRR regions of *R*-genes (Table 1, Fig. 1).

Table 1. *SCAR markers recognizing the R genes and their structural homologues*

R genes	SCAR markers	The prototype gene	Position on the prototype gene, bp	References
RB/Rpi-blb1/Rpi-sto1	RB-226	AY336128	3143-3368	Colton *et al.*, 2006
The same	Blb1-820	AY336128	2547-3143	Wang *et al.*, 2008
R1	R1-1205	AF447489	5126-6331	Sokolova *et al.*, 2011
R2/Rpi-blb3	R2-2500	FJ536325	1-2538	Kim *et al.*, 2012
R3a	R3a-1380	AY849382	1677-3056	Sokolova *et al.*, 2011
R3b	R3b-378	JF900492	94818-95195	Rietman *et al.*, 2012

PCR was run in a DNA Engine PTC 200 (Bio_Rad, United States). The PCR mix contained 10x PCR buffer, 2 mM $MgCl_2$, 100 ng of genomic DNA, 0.2 mM dNTP, 1 mM forward and 1 mM

reverse primers, and 1 U of *Taq* DNA polymerase (Fermentas, Germany), or 2.5 U of *Pfu* polymerase (Fermentas) in the case of the R2-2500 marker. PCR was run using the following programs: RB-226: one cycle of 7 min at 94°C; 35 cycles of 20 s at 94°C, 20 s at 50°C, 2 min at 72°C; one cycle of 5 min at 72°C; R1-1205: one cycle of 3 min at 94°C; 35 cycles of 35 s at 94°C, 30 s at 65°C, 2 min at 72°C; one cycle of 5 min at 72°C; Blb1-820: one cycle of 3 min at 94°C; 35 cycles of 35 s at 94°C, 35 s at 62°C, 2 min at 72°C; one cycle of 5 min at 72°C; R2-2500: one cycle of 30 sec at 98°C; 35 cycles of 10 s at 98°C, 10 s at 62°C, 2 min 30 sec at 72°C; one cycle of 10 min at 72°C; R3a-1380 and R3b-378: one cycle of 3 min at 94°C; 35 cycles of 45 s at 94°C, 45 s at 64°C, 1 min 40 sec at 72°C; one cycle of 5 min at 72°C; PCR products were separated by electrophoresis in 1% (w/v) agarose in 1x TAE buffer for 40 min at 6 V/cm and visualized under UV after staining with ethidium bromide.

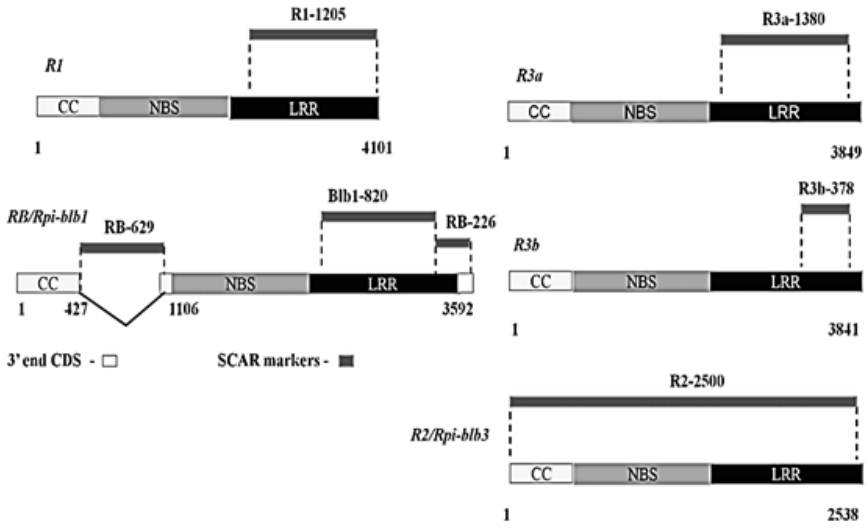

Figure 1. *SCAR markers of LB resistance genes. RB-629 marker was not used in this particular study*

Purified DNA fragments were cloned using pGEM-T Easy Vector System I (Promega, United States) and CloneJet PCR Cloning Kit, with vector pJet (both from Fermentas) in the case of R2-2500 marker. Plasmids were transferred into *Escherichia coli* XL1-blue chemically competent cells. For white-blue selection of transformants, cells were plated on an ampicillin-containing medium containing X-gal and IPTG. Plasmid DNA was isolated with AxyPrep Plasmid Miniprep Kit (Axygen Biosciences) according to the manufacturer's protocol. Cloned DNA fragments were sequenced using a 3130 Genetic analyzer (Applied Biosystems, United States) and Nanophor 05 (Institute of Analytical Instrumentation, St. Petersburg), and the chromatograms of nucleotide sequences were visually inspected using Chromas Lite 2.0 (www.technelysium.com.au/chromas_lite.html). All primers were synthesized by Syntol, Moscow (www.syntol.ru).

Bioinformatics methods

For multiple alignments of nucleotide sequences, we used SeqMan, Lasergene 7.0 (http://www.dnastar.com). Sequences of *R*-gene markers were assembled using a combination of the Martinez and Needleman-Wunsch algorithms (SeqMan, Lasergene 7.0). Predicted amino acid sequences were obtained with the Expasy Translate tool (http://web.expasy.org/translate). The data were processed using the Statistica 6 package (StatSoft, http://www.statsoft.com/).

RESULTS AND DISCUSSION

In this study, forty clones of standard potato varieties and hybrids comprising genetic material from two to seven wild *Solanum* species (Rogozina *et al.*, 2014) were evaluated for field and laboratory LB resistance and screened with SCAR markers for five LB resistance genes. The markers are described in Table 1 and Figure 1. The screening results are presented in Table 2 against the LB resistance indices.

Table 2. *Profiles of R-gene markers in potato interspecific hybrids and standard varieties (the presence/absence = 1/0 of the markers in particular genotypes) and LB resistance in laboratory trials with detached leaves*

№	Potato genotypes	*R*-gene markers*						Number of markers	LB resistance, points
		R1-1205	R2-2500	R3a-1380	R3b-378	RB-226	Blb1-820		
1	2372-60	1	0	**1**	**1**	0	0	3	6
2	97.13-9	0	0	**1**	**1**	0	0	2	3
3	97.1.17	0	0	0	0	0	0	0	4
4	2522-173	0	0	**1**	**1**	0	0	2	3
5	2584-7	0	0	0	**1**	0	0	1	4
6	2359-13	1	0	**1**	**1**	0	0	3	4
7	97.12-18	0	0	**1**	**1**	0	0	2	4
8	25-85-70	0	0	**1**	**1**	0	0	2	3
9	2585-80	1	0	1	**1**	0	0	3	6
10	2585-67	0	0	**1**	**1**	0	0	2	6
11	10/5-09	0	**1**	**1**	**1**	0	0	3	4
12	11/6-09	0	0	**1**	**1**	0	0	2	3
13	12/1-09	0	**1**	0	0	1	**1**	3	6
14	13/11-09	0	0	0	**1**	1	**1**	3	5
15	14/8-06	0	**1**	**1**	**1**	0	0	3	4
16	15/13-09	0	**1**	0	**1**	1	**1**	4	6
17	16/27-09	1	0	0	0	1	**1**	3	7
18	18/40-2000	1	0	0	0	0	0	1	4
19	134-3-2006	0	0	0	0	0	0	0	3
20	117-2	0	0	0	**1**	0	0	1	5
21	39-1-2005	0	0	0	**1**	1	**1**	3	6
22	25-1-2007	0	0	0	**1**	0	0	1	5
23	128-05-03	0	0	**1**	**1**	0	0	2	5
24	97-153-2	0	0	0	0	0	0	0	5

№	Potato genotypes	*R*-gene markers*						Number of markers	LB resistance, points
		R1-1205	R2-2500	R3a-1380	R3b-378	RB-226	Blb1-820		
25	99-4-1	1	0	0	**1**	0	0	2	nd
26	2(194-4т)	0	0	0	0	0	**1**	1	5
27	7(93-5-30)	0	0	0	**1**	0	0	1	4
28	53(34-5-003)	0	0	0	0	0	0	0	5
29	106 (171-3)	0	0	0	**1**	0	0	1	6
30	111 (38KBA)	0	0	**1**	**1**	0	**1**	3	6,5
31	113(50/1KBA)	0	0	0	0	1	0	1	6
32	118(118-5)	0	0	1	**1**	1	0	3	6
33	139	1	0	0	0	0	**1**	2	6
34	Alpha	0	0	0	0	0	0	0	3
35	Bintje	0	0	0	0	0	0	0	3
36	Eesterling	0	0	0	0	0	0	0	3
37	Escort	1	1	1	1	0	0	4	6
38	Gloria	0	0	1	1	0	0	2	4
39	Robijn	0	0	0	0	0	0	0	4
40	Sarpo Mira	0	0	1	**1**	0	0	2	7

*For prototype genes see Table 1. Cloned *R*-gene markers are highlighted in bold. nd – no data.

To verify the *R*-gene markers, we cloned several marker amplicons and compared their sequences to those of the prototype genes. The cloned markers corresponded to the prototype genes from *S. demissum* (*R1*, *R3a* and *R3b*) and *S. stoloniferum* (*RB/Rpi-blb1/Rpi-sto1* and tentatively *Rpi-blb3*).

Especially interesting are the cases of identity below 99-100%. In one case, in the hybrid 10/5-09, *R2* gene sequence was apparently damaged, and these changes can explain low LB resistance of the hybrid. In other cases, the observed polymorphisms in the marker sequences presume that the gene in question was probably introgressed from *Solanum* species other than *S. demissum* and *S. stoloniferum.*

LB resistance of the clones under study was significantly related to the presence of SCAR markers for the *R*-genes by the Spearman and ANOVA Kraskell-Wallace tests (Fig. 2). However, the correlation was weaker than expected. It became evident that a considerable portion of LB resistance depended on extra *R*-genes that we failed to recognize because of the limited set of markers. Such discrepancy was especially pronounced in the case of insufficiently researched wild progenitors of interspecific hybrids.

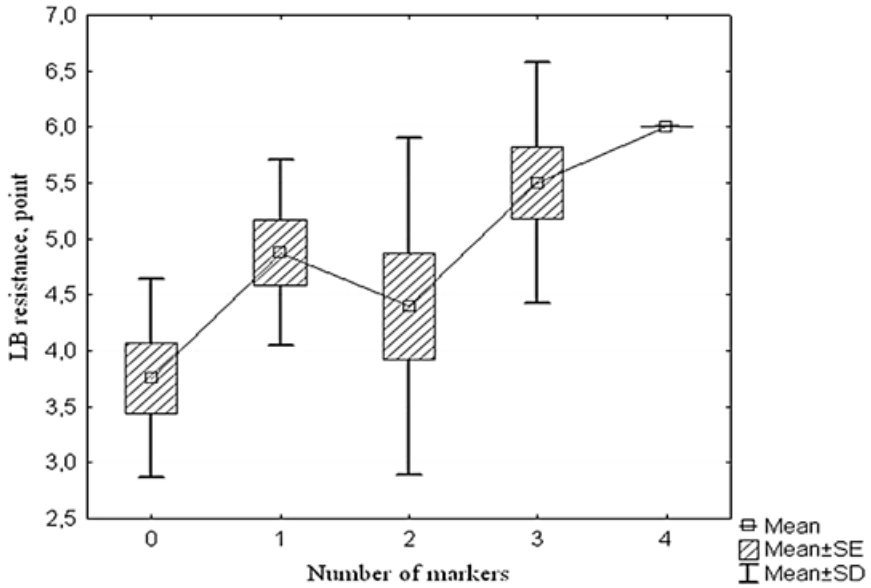

Figure 2. *ANOVA Kraskell-Wallace test relating late blight resistance to the number of SCAR markers of R genes per plant*

To illustrate such situation, let us turn to the highly resistant variety Sarpo Mira (Table 3). Here we find only two SCAR markers as compared to five *R* genes recognized by the method of effectoromics.

Table 3. *R genes in var. Sarpo Mira: two genes recognized by our markers and five by Avr genes*

SCAR markers	*R3a, R3b*
Avr genes (Rietman *et al.*, 2012)	*R3a, R3b, R4, Rpi_Smira1, Rpi_Smira2*

CONCLUSION

While the race-specific *R* genes are commonly held to be overcome (defeated) by new virulent races of *P. infestans,* our data support the hypothesis that these genes provide a discernible input to LB resistance (Gebhardt, 2013). Stacking *R* genes in interspecific hybrids obviously promotes their LB resistance.

ACKNOWLEDGEMENTS

The authors thank the Center for Collective Use of Equipment "Biotechnology" at the Institute of Agricultural Biotechnology for sequencing *Solanum* genome fragments. The study was supported by the ISTC - ARS-USDA (the project 3714p), by the Ministry of Education and Science, Russian

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PPO – SPECIAL REPORT NO 17 – 2015, 203-204

Observations regarding the origin of *Phytophthora infestans* inoculum in potato and tomato fields and distribution of the disease in Israel

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Observations regarding the origin of *Phytophthora infestans* inoculum in potato and tomato fields and distribution of the disease in Israel

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ABSTRACT

Late blight, caused by *Phytophthora infestans*, is one of the most important diseases of potatoes and tomatoes in Israel. Although the epidemiology and management of late blight have been studied in Israel for more than 50 years, it is still not clear what are the main sources of initial inoculum in both crops. The genotype of 41 *P. infestans* isolates sampled from potato and tomato crops in the 2013-14 autumn season, 2014 spring season and the 2014-15 autumn season was characterized. All isolates were categorized as belonging to the US-23 clonal linage, suggesting that infected potato seeds imported from European countries, did not serve as the source of initial inoculum in local production areas. The spatial and temporal onset of late blight symptoms in autumn 2014-15 may suggest that the disease was originated in a distinct area in the northwestern Negev from which it spread spatially country-wide.

HYPOTHESIS AND OBJECTIVES

It was hypothesized that if imported potato seeds serve as the source of initial inoculum to the production , then the *P. infestans* genotypes commonly observed in Israel would be comparable with those prevailing in the seed-exporting countries. Furthermore, it was hypothesized that inoculum originated from regions where potatoes and tomatoes are grown year-long infect the regions where these crops are grown with intermissions. The objectives of this study were: (i) to characterize the *P. infestans* genotypes prevailing in Israel, and (ii) to document the spatial and temporal onset of late blight symptoms around the country.

INTRODUCTION

Potatoes are grown in Israel in two main seasons: the autumn season (from September to February) and the spring season (from December to June). Seeds for the spring season are imported from European countries, mainly the Netherlands, Scotland, France and Germany. Seeds produced domestically in the spring season are used in the following spring plantings. Fresh tomatoes are grown year-long and processing tomatoes are grown from March to August (Figure 1).

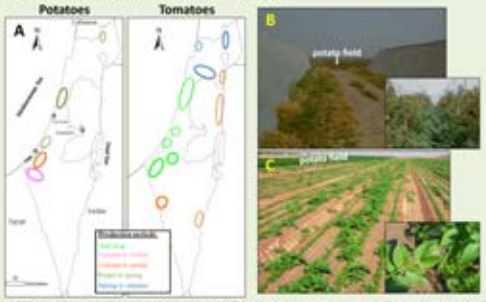

Figure 1. A. Production areas and cultivation periods of potatoes and tomatoes in Israel. The production area of potatoes is 15,000 ha and the production areas of fresh and processing tomatoes are 2,500 and 2,000 ha, respectively. Solid and dashed lines- primary and secondary production areas, respectively. In the northwestern Negev and the central sea-shore potatoes and tomatoes are grown side-by-side almost continuously. However, in other areas of the country these crops are not grown year-long and gaps of few months exist between two succeeding plantings. **B.** Neglected, late blight infected tomato net-houses, located adjacent to newly emerged potato field. *C.* Late blight infected potato volunteer plants growing within a newly emerged carrot field, located adjacent to another potato field.

Late blight, caused by *Phytophthora infestans*, is one of the most important diseases of potatoes and tomatoes in Israel. The disease is observed yearly, but the its prevalence and severity vary from year to year, from region to region and between fields (potatoes and processing tomatoes), net-houses and greenhouses (fresh tomatoes). Variation is governed by the availability of inoculum, the suitability of weather conditions to the pathogen and the management practices employed by the growers. Although the epidemiology and management of late blight have been studied in Israel for more than 50 years, it is still not clear what are the main sources of initial inoculum infecting both crops in the different plantings and production areas. The possible sources of initial inoculum are:

Possible source of initial inoculum	Potatoes		Tomatoes	
	Autumn season	Spring season	Fresh	Processing
Within the field / greenhouse				
Imported seeds	-[a]	+	-	-
Domestic seeds / transplants	-	-	-/+	-/+
Soil-borne oospores	-/+	-/+	-	-/+
From outside the field / greenhouse				
Near-by potato or tomato crops	++[b]	++	++	+
Volunteer potato plants	++[c]	+	+	-
Crops located in other regions	+	+	+	+

[a]The likelihood that the different possibilities serve as sources of initial inoculum:
"-" = irrelevant; "+/-" = possible, but not likely; "+" = possible; "++" = high probability
[b]See Figure 1B
[c]See Figure 1C

RESULTS

1. *P. infestans* genotypes prevailing in Israel

The genotype of 41 *P. infestans* isolates sampled from potato fields from the northeastern Negev were characterized. Of which, 9 were sampled in the 2013-14 autumn season, 20 isolates in the 2014 spring season (generously analyzed by David Cooke) and 5 in the 2014-15 autumn season. Seven isolated sampled from tomatoes growing in other areas of the country were characterized as well (Figure 2). **All** isolates were categorized as belonging to the US-23 clonal linage.

Figure 2. Location of the potato fields and tomato greenhouses and net-houses from which *P. infestans* isolates were sampled in autumn 2013-14, spring 2014 and autumn 2014-15.

2. The spatial and temporal onset of late blight in autumn 2014-15

The first late blight symptoms in autumn 2014-15 were observed in the northwestern Negev region in late October 2014. In early November the disease was observed in potato fields and tomato greenhouses in that area and within two months symptoms were observed in all production areas of the country. Spatially, there was a clear pattern in disease onset from the first site towards and south, south-east, east and north-east (Figure 3).

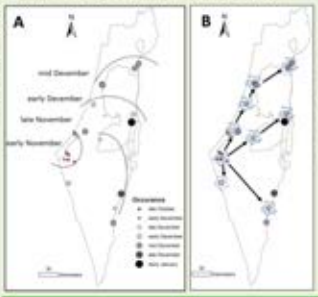

Figure 3. A. The spatial and temporal onset of late blight symptoms in potato fields and tomato greenhouses in autumn 2014-15. **B.** Hypothesized country wide spatial and temporal distribution of *P. infestans*. Black arrows represent primary (long distance) and blue arrows represent the secondary (short distance) spread of the disease.

CONCLUSSIONS

1. All isolates *P. infestans* isolates collected from potato and tomato crops were categorized as belonging to the US-23 clonal linage. As this clonal linage is rare in the European countries from potato seeds are imported, it seems unlikely that imported infected seeds served as the source of initial inoculum in Israel.
2. Based on data recorded in 2014-15 we hypothesized that late blight epidemics in that year originated in the northwestern Negev (where host crops are grown year-round) from which the pathogen sequentially spread to other production areas of the country.

PPO – SPECIAL REPORT NO 17 – 2015, 205-206

POTATO LATE BLIGHT; managing the risk with up-to-date and field specific information

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POTATO LATE BLIGHT

managing the risk with up-to-date and field specific information

A new web application gives the potato grower access to all the relevant, actual and field specific information at a glance. The main screen acts as a portal and brings together following information:

1
Personal login for registering fields, fungicide applications and use weather data from the nearest weather stations.

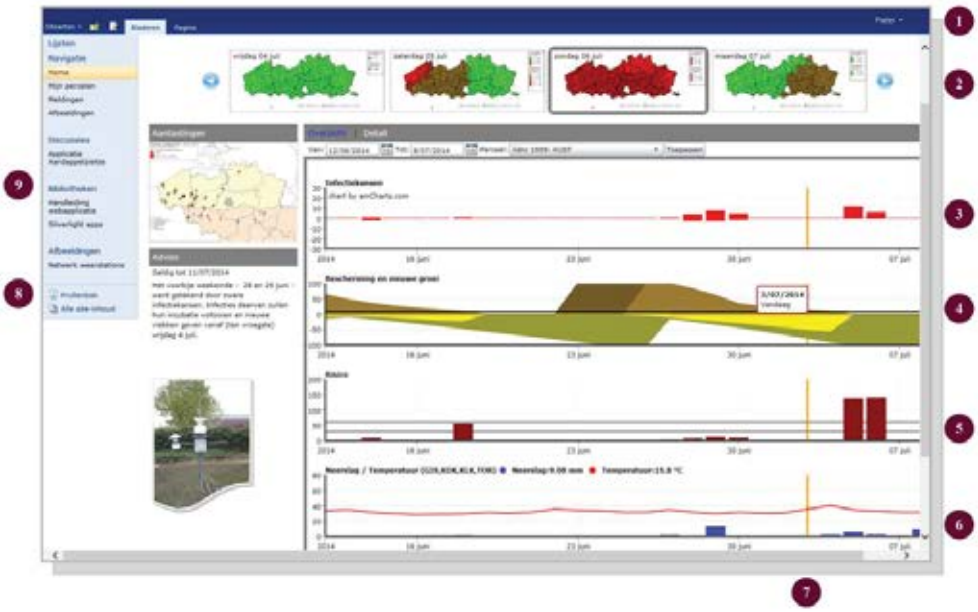
2
Film strip with the daily geographical spread of weather based infection risk in Flanders, on the basis of measurements in 48 automatic weather stations and detailed regional forecasts 96 hours ahead. Clicking on the thumbnail gives a detailed view of the map.

3
Consolidated daily values of infection opportunity, calculated both qualitatively and quantitatively (number of spores that successfully infect), for his location. The calculations of the disease model take into account: latent period, lesion growth, infectious period of lesions, spore formation and spore density, spore release and survival, germination and infection. Apparent cultivar susceptibility is dealt with by using different values for the components of resistance, e.g. infection efficiency, latent period, lesion size and growth rate, and spore density.

4
(a)Above the axis: the effect of fungicide applications. Based on the characteristics and dose rate of the fungicide used, the protection offered by the sprayings is calculated. Curative action (immediate post-infection period), protection of new growth and rain fastness of the fungicide are included in the calculation (based on hourly data).
(b)Below the axis: new growth, based on crop stage, and protection of new growth, depending on the type of fungicide.

5
The ultimate goal of this web application: the daily risk for my field, a combination of crop protection and infection opportunities. The calculated risk for the next few days, according to weather forecasts, allows the grower to take timely action and achieve an optimal timing of crop protection.

6
Additional information: rainfall and temperature measured in the nearest 4 weather stations, together with the forecast for your region (4 regions in Flanders are used for weather predictions)



7
All calculations are continually refreshed and use detailed hourly weather forecasts 96 hours ahead. The orange-coloured vertical line indicates the current date (=today).

8
A general description on the late blight situation in Flanders, updated twice a week, together with some advice on control measures. A more extensive report is available upon clicking on the textbox.

9
A daily updated late blight monitoring map of observed and reported inoculum sources and attacks of late blight, with indication of crop type (early, ware, dump pile, volunteers, ...), cultivar and severity. Clicking on the thumbnail gives a detailed view of the map.

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PPO – SPECIAL REPORT NO 17 – 2015, 207-208

Systemic acquired resistance to control potato late blight?

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WAGENINGEN UR
For quality of life

Systemic acquired resistance to control potato late blight?

A. Evenhuis, C.G. Topper & J.M. van der Wolf

Introduction

Potato late blight caused by *Phytophthora infestans* is usually controlled by application of fungicides on the foliage. One of the primary sources of *P. infestans* is latently infected tubers. Currently no application of fungicides on tubers to control potato late blight is available. By request of the Dutch Ministry of Economic Affairs the efficacy of systemic acquired resistance to control PLB was investigated. As a model the efficacy of salicylic acid was tested.

Method

Potato tubers (Bintje & Agria) were pre-sprouted for two weeks. Seed potatoes were then treated with products by spraying either 2 l/ton (chemical reference) or 5 l/ton (other) on the tubers. After 2 days the tubers were inoculated with *P. infestans*. One day later they were planted in pot soil in the greenhouse. The number of emerging stems was assessed. The experiment was carried out three years (2012-2014).

Figure 1: Treatment of tubers with active ingredients

Results and discussion

The emergence rate of potato stems in the greenhouse (Figure 2) is assumed to be a measure for the efficacy of the treatments to control the transition of *P. infestans* from tubers to stems (Figure 3). The chemical reference showed good control of *P. infestans* in the bio-assay.

Figure 2: Emergence of potato stems from *P. infestans* infected tubers

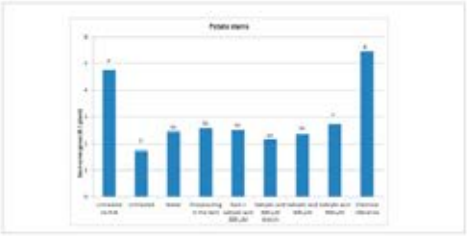

Figure 3: The effect of active ingredients on the emergence of potato stems from *P. infestans* infected tubers

Conclusions

- The chemical reference showed good control of *P. infestans* in the bio-assay.
- Systemic acquired resistance by applying salicylic acid showed some efficacy compared to the untreated control, however not compared to the water treatment or pre-sprouting in the dark.

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Ministerie van Economische Zaken

PPO – SPECIAL REPORT NO 17 – 2015, 209-210

Evaluation of foliar resistance to *Phytophthora infestans* in potato varieties in Belgium (2013-2014)

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Evaluation of foliar resistance to *Phytophthora infestans* in potato varieties in Belgium (2013-2014)

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Summary

Late blight caused by *Phytophthora infestans* is one of the most devastating diseases of potato. Under favorable environmental conditions and without fungicide protection, the foliage of susceptible varieties can be destroy in a few weeks. Its development is closely linked to climatic conditions: the mild and humid climate, frequently encountered in Western-Europe is usually responsible for epidemics. Farmers are interested to know information about resistance to late blight especially for the organic farming system. Indeed, the use of less susceptible varieties typically saves several fungicides applications on a season and reduce the environmental impact.

In 2013 and 2014, 36 varieties were tested for their susceptibility to late blight. This study was performed under natural conditions. The dynamic of the foliage destruction was measured and a score of 1 to 9 has been assigned to each of the varieties. 6 varieties have scored higher than 8,5. Results are also used to identify new genotypes in order to use it in our breeding program.

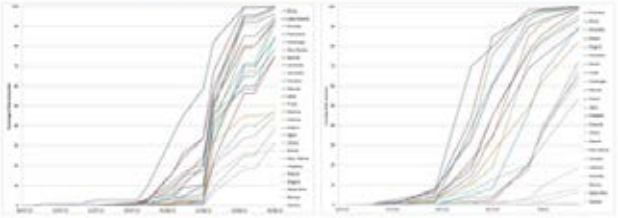
Materials and Methods

In 2013 and 2014, 36 varieties were tested for late blight resistance. Field trials were carried out at CRA-W station in Libramont (South-East of Belgium).
Trials were laid out according a randomized block design with four replicates. 24 plants of each varieties were grown in the field. No fungicide were applied.

To estimate the late blight resistance of potato in the field, the scoring of the foliage destruction was achived twice a week. Late blight infection was assessed according to the 0-100% scale, where 0 means the absence of any visible lesion and 100 means a 100% necrotic tissue.

The score were converted to the mean defoliation and used to calculate the rAUDPC (relative area under disease progress curve) for each variety. Reference varieties were Bintje, Agria, Gassore and Sarpo Mira.

Results of our observations were expressed in accordance with the 1-9 score scale (1: very sensitive and 9 resistant) (Table 1).



Graphe 1 and 2: Disease Progress curves for each variety from the field trials in 2013-2014.

Varieties	2013	2014
Premiere	-	1.0
Bintje	1.0	1.0
Charlotte	-	3.0
Lady Rosetta	3.0	-
Singra	-	3.0
Spunta	3.0	-
Nicola	-	3.0
Princess	4.0	-
Innovator	4.0	3.4
Linda	-	4.0
Franceline	4.0	-
Miss Bianka	4.4	-
Challenger	4.4	4.0
Désirée	5.0	5.0
Fontane	5.0	-
Annuik	-	5.0
Leonardo	5.1	-
Markies	5.2	-
Victoria	5.5	-
Amera	5.5	-
Agria	5.6	5.5
Triple	5.6	-
Lady	5.7	-
Bionica	6.1	-
Opdyke	-	6.6
Allians	6.8	6.4
Biogold	7.0	7.4
Vitabella	7.0	6.3
Miss Mallina	6.5	7.4
Gassore	7.4	6.8
Connect	-	8.6
Cephora	-	8.6
Alouette	-	8.6
Bionica	8.0	8.0
Carolus	8.0	8.0
Sarpo Mira	8.0	8.0

Table 1: Estimated value of potato cultivars for foliar resistance to *Phytophthora infestans* in natural conditions in Belgium (2013-2014).

Results

In 2013, the first late blight symptoms were recorded on 9 July on Bintje and Fontane. With dry conditions during July, infection rate remained very low until end of July with more intensive development in August with heavy rainfall.
In 2014, the first late blight symptoms were founded on 14 July. With wetter conditions during July and August, blight developed quite rapidly and most of foliage on susceptible varieties were totally destroyed on 5 August.
Based on two years observation data we identified 6 cultivars which are less sensitive or resistant: Sarpo Mira (Danespo, NL), Carolus (Agrico, NL), Bionica (Meijer, NL), Alouette (Agrico, NL), Cephora (Grocep,FR) and Connect (Den Hartigh, NL).

Eurobblight 2015 – Brasov Romania – 10-13 May 2015 - This work is supported by The Walloon Ministery of Agricultural

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Wallonie

PPO – SPECIAL REPORT NO 17 – 2015, 211-212

Toward marker assisted selection for late blight resistance in Sárpo potatoes

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Toward marker assisted selection for late blight resistance in Sárpo potatoes

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Introduction

Potato breeding is traditionally a slow and laborious process, typically taking 10-12 years from initial crossing to the production of a new cultivar (Bradshaw, 2009). The use of marker assisted selection (MAS) is one way in which breeders can attempt to shorten this process by screening progeny for markers known to be associated with desirable traits.

Background to late blight resistance

Breeding for resistance to *Phytophthora infestans* is important to reduce the frequent application of fungicides that provide the only other method of control. Organic growers are only allowed to use copper based preparations but the maximum allowable application of these is being reduced within the EU, while conventional growers are faced with increasing costs and a reduction in the number of active ingredients that are permitted.

It is logical to select parents that show a degree of durable resistance for inclusion in breeding programmes. One such cultivar is Sárpo Mira which, twelve years after official registration on the UK National List, still retains high levels of foliar and tuber resistance to all *P.infestans* genotypes to which it has been exposed (White & Shaw, 2009). This high level of resistance is now known to be conferred by at least five resistance genes (Rietman *et al.*, 2012), including the newly discovered *Rpi-Smira1* and *Rpi-Smira2*.

It has been proposed that *Rpi-Smira1* is located near the R3 gene cluster on chromosome X1, and genetic mapping of this area yielded eleven CAPS markers (Tomczynska *et al.*, 2014).

Figure 1. Sárpo Mira in background with the previously resistant cv. Lady Balfour in foreground. From SRT field trial Llanbedrgoch, North Wales, 2009. Durability of resistance in Sárpo Mira has also been reported by other groups throughout Europe.

Objective

To use molecular markers to screen a range of material from the Sárpo breeding programme and identify selectable alleles for resistance breeding.

Materials and Methods

DNA was extracted from eight varieties (Table 1) using a Quiagen DNEasy Plant Mini Kit and standardised to give a final concentration of 3 ng/ul. Extracted DNA was amplified in 20 ul PCR reactions for each marker contained 4 ul DNA, 0.2 ul forward primer and 0.2 ul reverse primer, 10 ul BiomixClear Taq mix and 5.6 ul PCR grade water. Primers used were those listed by Tomczynska *et al.* 2014 at a concentration of 20 pM. A touchdown PCR programme was run on a PTC-100 Programmable Thermal Controller (MJ Research Inc.)

PCR products were digested with restriction enzymes as described by Tomczynska *et al.* 2014. PCR and digestion products were resolved by 100v electrophoresis on 1.5% agarose gels stained with 10 ul Safeview (NBS Biologicals). Viewing under UV transillumination was carried out after 60 or 90 minutes, depending on the marker.

VARIETY	ABBREVIATION	BREEDER	RES. SCORE
Cara	C	IPM	5/7
Sárpo Mira	SM	Sárpo	7/9
Bionica	B	N. Vos	*
Sárpo Shona	SS	Sárpo	7/4
Clone #32	32	Sárpo	*
Maris Piper	MP	PBI	4/5
Axona	Ax	Sárpo	7/5
Athlete	At	Agrico	9/9

Table 1. Variety name, abbreviation as used in this work, breeder and published (source: British Potato Council Variety Database) late blight resistance score for foliage/tubers (higher scores are more resistant) for the eight potato varieties included in this study.
*** indicates no official data available.**

Results and Discussion

Polymorphism was detected with many of the markers associated with *Rpi-Smira1* in the other Sárpo material included in these experiments. The 45/X1 marker (Figure 2) was reported to have the strongest linkage to the proposed *Rpi-Smira1* resistance locus by Tomczynska *et al.* 2014. An allele of approx. 1100 bp was present in resistant lines and is visible in Sárpo Mira, Sárpo Shona, clone #32 and Axona.

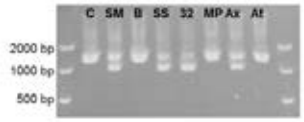

Figure 2. Agarose gel for marker 45/X1 with Cara, Sárpo Mira, Bionica, Sárpo Shona, clone #32, Maris Piper, Axona and Athlete. The unlabelled wells contain Easyladder1 for sizing the bands.

Although this work is preliminary, it indicates that five of the potential eight parents included in this study would be useful for future breeding. As well as marker 45/X1 (above) progeny segregating for four other markers can be selected to improve breeding efficiency. These have been suggested as flanking markers for the location of the main *Rpi-Smira1* resistance locus (Tomczynska *et al.*, 2014)

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Two orthologues of late blight resistance gene *R1* in wild *Solanum* species and derived potato varieties and hybrids

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SUMMARY
Sequences of the *R1* gene in *S. demissum* and *S. stoloniferum* differ by a 6-bp indel. This indel was used to construct SCAR markers R1-517 and R1-513 which reliably discerned *R1* orthologues from two species. The *demissum* marker R1-517 was present in *demissum*-derived potato varieties; however, we failed to find the *stoloniferum* marker R1-513 in potato varieties listing *S. stoloniferum* in their pedigrees.

KEYWORDS
Phytophthora infestans, Solanum demissum, Solanum stoloniferum, potato hybrids, late blight resistance, DNA markers, R1 gene.

INTRODUCTION

Recent arrival of highly aggressive strains of *Phytophthora infestans* (Cooke *et al.*, 2012; Fry *et al.*, 2015) has posed an urgent task both to search for new *Solanum* species manifesting high and durable late blight (LB) resistance and to look deeper into the stockpile of resistance genes in wild *Solanum* species already deployed in potato introgression breeding (Hein *et al.*, 2009; Rodewald and Trognitz, 2013; Sliwka and Zimnoch-Guzowska, 2013).

The *R1* gene for LB resistance cloned by Ballvora *et al.* (2002) from the potato genotype P6/210 was introgressed from *S. demissum*; the polymorphism of this gene was further characterized by Kuang *et al.* (2005) and Ballvora *et al.* (2007). Screening with the R1-1400 marker identified the *R1* gene in numerous *demissum*-derived potato varieties (Gebhardt *et al.*, 2004; Beketova *et al.*, 2006). *S. stoloniferum* has been also included into backcrossing programs for breeding LB resistant potato varieties (Bradshaw *et al.*, 2006). Gebhardt *et al.* (2004) found the R1-1400 marker in this species, and later Sokolova *et al.* (2011) reported the presence of the *R1* gene in many *S. stoloniferum* accessions using the marker R1-1205 (Fig. 1) modified from R1-1400.

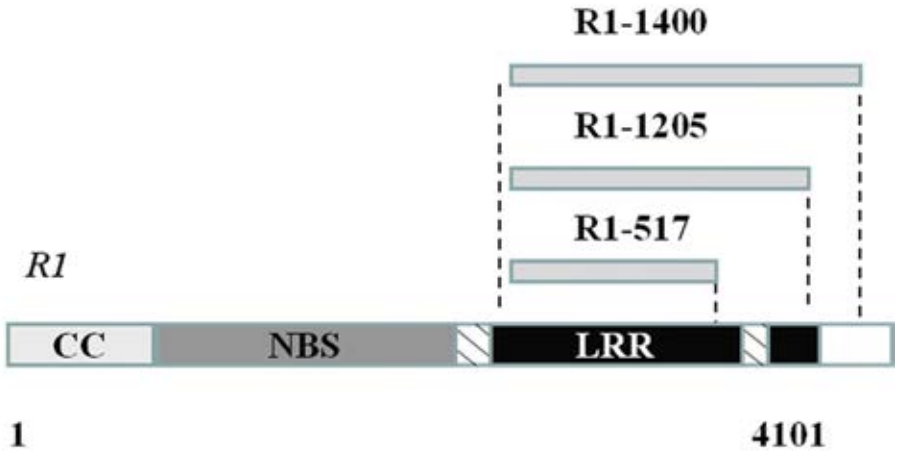

Figure 1. *The structure of R1-gene and the corresponding CC-NBS-LRR kinase and positions of the primers amplifying the markers R1-1400, R1-1205 and R1-517*

MATERIALS AND METHODS

Seeds or tubers of wild *Solanum* species were obtained from the collection of the N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia and USDA Potato Genebank, Potato Introduction Station, Sturgeon Bay, WI. Tubers of potato varieties and advanced interspecific hybrids come from VIR and also from the Institute of Phytopathology, Bol'shie Vyazemy, Russia, and the Institute of Potato Husbandry, Korenevo, Russia. When searching for *stoloniferum*-derived potato varieties, we turned to the cultivars manifesting extreme resistance to potato virus Y (PVY) and/or containing markers of the *Rysto* gene (Solomon-Blackburn and Barker 2001; Flis *et al.* 2005).The interspecific hybrids that comprised germplasms from two to seven wild *Solanum* species including *S. demissum, S. stoloniferum* and *S. papita* were described previously (Khavkin *et al.*, 2014). Standard protocols were employed for extracting DNA from young leaves, for PCR-amplification and cloning genome fragments and for phylogenetic analysis of nucleotide sequences.

RESULTS AND DISCUSSION

We cloned several fragments the *R1* gene corresponding to the marker R1-1205 from *S. demissum*, *S. hougasii*, *S. polytrichon* and *S. stoloniferum* (the sequences were deposited in the NCBI Genebank under accession numbers HM124852 - HM124854 and KP876031 - KP876037). By aligning all available *R1* fragments, we discerned three clusters in the dendrogram (Fig. 2). Cluster I combines various *R1* paralogues from *S. demissum* and *S. tuberosum* that differ from the prototype gene (NCBI accession AF447489) by several single nucleotide polymorphisms. Cluster II comprises the sequences from *S. demissum* identical to the prototype gene. Cluster III consists of *R1* orthologues from *S. hougasii*, *S. polytrichon* and *S. stoloniferum*; all these sequences differ from the prototype gene by a characteristic deletion (706-712 bp) which does not affect gene translation. This indel discerning the *R1* orthologues in two species was used to develop SCAR markers R1-517 and R1-513 (Fig. 3).

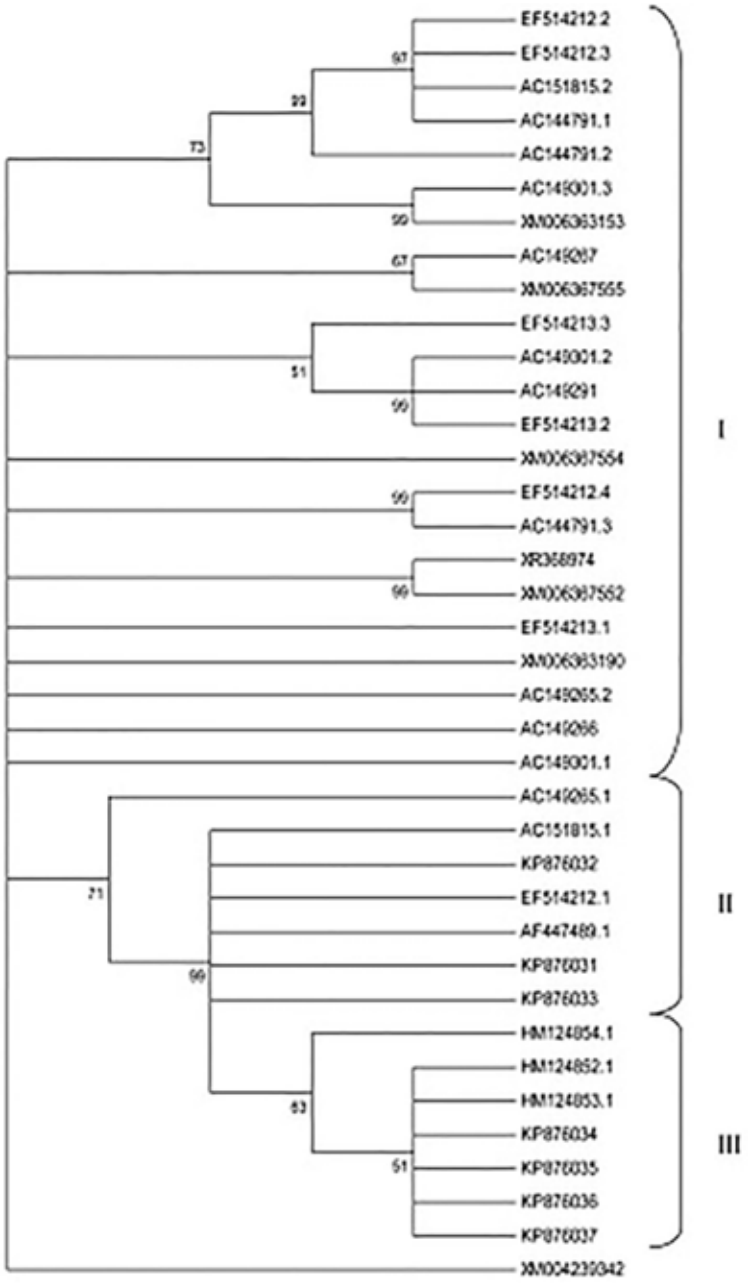

Figure 2. *Phylogenetic analysis of R1 homologues. The tree is built using the Neighbor Joining method; the bootstrap values were calculated for 1000 repeats. Listed are NCBI accession numbers. AF447489, the prototype gene (Ballvora et al., 2002); the tree is rooted against the XM004239342 homologue from S. lycopersicum*

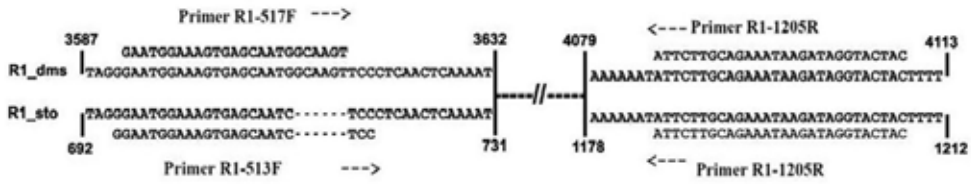

Figure 3. *Aligning R1 orthologues from S. demissum (AF447489) and S. stoloniferum (HM124853) to develop species-specific primers R1-517 and R1-513*

Screening large sets of *S. demissum* and *S. stoloniferum* accessions from the VIR collection proved that the *R1* gene was frequently found in both these species. The markers R1-517 and R1-513 were highly species-specific (Table 1).

Table 1. *Verification of markers R1-517 and R1-513*

Genotypes	SCAR markers		
(the number of accessions)	R1-1205	R1-517	R1-513
S. demissum (13)	11	13	0
S. stoloniferum (21)	6	0	6
S. polytrichon (9)	2	0	2

The presence of R1-1205/R1-517 markers in *S. demissum* was usually associated with LB resistance, whereas similar evidence for the markers R1-1205/R1-513 was insufficient in the case of *S. papita, S. polytrichon* and *S. stoloniferum* (Table 2). The *R1* fragments in *S. stoloniferum* and *S. polytrichon* were translatable; however, the functional activity of the *R1* gene in *S. stoloniferum* must be further attested by the methods of effectoromics (Vleeshouwers and Oliver, 2014). Characteristically, in wild *Solanum* accessions, we did not observe any consistent relationship between LB resistance and both extreme resistance to PVY and the presence of the Rysto marker. These data comply with the evidence on low incidence of combined resistance to both pathogenes in *S. demissum* and *S. stoloniferum* accessions stored in the USDA Potato Genebank (Bamberg *et al.*, 1994).

Table 2. *Screening wild Solanum species with the markers R1-1205, R1-517 and R1-513*

Series	Species	Accession	The presence/absence of markers (1/0)			Resistance to	
			R1-1205	R1-517	R1-513	late blight*	PVY (R/S)
Demissa	*S. demissum*	VIR 15174	0	1	0	MS	nd
		VIR 18521	0	1	0	R	nd
		VIR 23315	1	1	0	R	S
		PI 160220	1	1	0	R	S
		PI 160221	1	1	0	R	S
		PI 161176	1	1	0	R	R
		PI 161366	1	1	0	R	R, S
		PI 161729	1	1	0	MR, MS	R, S
		PI 175404	1	1	0	MR, MS	R, S
		PI 186552	1	1	0	S	R, S
		PI 218047	1	1	0	MR	S
		PI 275208	1	1	0	R	nd
		PI 498012	1	1	0	nd	nd
Longipedicellata	*S. stoloniferum*	VIR 3336	0	0	0	S	R**
		VIR 3360	0	0	0	MR	R**
		VIR 19196	0	0	0	R	R**
		VIR 20106	0	0	0	MR	**
		VIR 21618	0	0	0	MR	R**
		VIR 23561	0	0	0	S	S**
		VIR 23652	0	0	0	MR, MS	R**
		VIR 24263	0	0	0	MS	**
		VIR 24420	0	0	0	nd	**
		VIR 24972	0	0	0	S	**
		PI 195166	0	0	0	HR	R
		PI 195169	0	0	0	MR	**
		PI 255525	0	0	0	S	**
		PI 255533	1	0	1	S	nd
		PI 255534	1	0	1	R	**
		PI 275248	1	0	1	R	R
		PI 283109	0	0	0	nd	R**
		PI 498037	1	0	1	nd	**
		PI 545792	1	0	1	nd	nd
		PI 558477	1	0	1	nd	**
	S. papita	VIR 8816	1	0	0	R	S
		VIR 16888	1	0	0	nd	nd
		VIR 21547	0	0	0	MR	RS**
		VIR 16889	0	0	0	MS	**
	S. polytrichon	VIR 8815	1	0	1	MR	**
		VIR 16905	0	0	0	S	**
		VIR 18142	0	0	0	S	**
		VIR 19164	0	0	0	S	RS**
		VIR 23561	0	0	0	S	S**
		VIR 23563	1	0	1	S	nd
		VIR 24410	0	0	0	S	RS
		VIR 24462	0	0	0	S	**
		VIR 24463	0	0	0	S	**

*Late blight resistance, data from laboratory trials with detached leaves in the Institute of Phytopathology: R, resistant (points 8-9), MR, moderately resistant (points 6-7), MS, moderately susceptible (points 4-5), S, susceptible (points ≤3). **Presence of the Rysto marker. nd, no data.

LB resistance has been initially introgressed into cultivated potato from *S. demissum* and *S. stoloniferum* (Ross, 1986; Solomon-Blackburn and Barker, 2001); however, the input of particular *stoloniferum* genes to overall LB resistance has not been evaluated. As a step towards this goal, we used three *R1* markers to screen varieties and hybrids combining both *S. demissum* and *S. stoloniferum* germplasms or comprising *stoloniferum* germplasm alone. The data for some of these varieties are presented in Table 3.

Table 3. *Markers of the R1 gene in potato varieties comprising germplasm from S. demissum and S. stoloniferum as related to their LB and PVY resistance*

Potato varieties	Markers of the *R1* gene (1/0)			LB resistance*	Rysto marker (1/0)	Type of reaction to PVY***
	R1-1205	R1dms-517	R1sto-513			
Assia	0	0	0	L-H**	1	VH (ER)
Bobr	0	0	0	L-M**	1	VH (ER)
Effect	1	1	0	5/MS	0	VH (ER)
Fanal	0	0	0	H**	1	VH (ER)
Heidrun	1	1	0	M-H**	1	VH (ER)
Kolobok	1	1	0	3/S	1	VH
Resurs	0	0	0	4/MS	1	VH (ER)
Ronea	0	0	0	MS**	1	VH
Santé	0	0	0	4/MS	0	VH (ER)
Ute	0	0	0	H**	1	VH (ER)

*Laboratory trials with detached leaves in the Institute of Phytopathology: points and groups of LB resistance as in Table 2. **Resistance indices from www.europotato.org/. nd, no data. *** ER, extreme resistance; VH, very high resistance.

In some potato varieties combining germplasms from *S. demissum* and *S. stoloniferum* that manifested *stoloniferum*-derived extreme resistance to PVY (Effect, Heidrun) or contained the Rysto markers (Heidrun, Kolobok), we found both markers R1-1205 and R1-517 but never R1-513. Other varieties with extreme resistance to PVY and the Rysto markers (Assia, Bobr, Fanal, Resurs, Ronea, and Ute) were devoid of the *R1* gene markers. In potato genotypes listing in their pedigrees *S. stoloniferum* and *S. papita*, such as Chernskii, Corine, Talisman, Tuleevskii and Udalets, we did not find the markers R1-1205 and R1-513.

As a whole, LB resistance of potato varieties and hybrids, same as in wild *Solanum* accessions, was poorly related to extreme resistance to PVY and the Rysto markers; varieties Assia, Fanal, Heidrun and Ute were noteworthy exceptions. The absence of the *R1* gene in LB resistant potato genotypes comprising *stoloniferum* germplasm is probably best explained by a bottleneck in the founder *stoloniferum*-derived MPI breeding lines (Ross, 1986) that were successfully deployed to

introduce the *Rysto* gene to potato varieties: these lines were apparently devoid of the *R1* gene. There were several stumbling blocks for breeders, and probably a higher priority was given to PVY than to LB resistance (Solomon-Blackburn and Barker, 2001). Nevertheless, current biotechnologies for stacking agronomic genes by remote sexual and somatic hybridization and genetic engineering open new possibilities to elevate LB resistance by deploying the *stoloniferum R1* gene.

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The use of the VNIIFBlight model to analyse the severity of potato late blight over years and across regions

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SUMMARY
Two weather-based indices, number of risky periods during a potato-growing season and frequency of fungicide applications calculated by the VNIIFBlight decision support program, were compared with the expert evaluations of the late blight development level to monitor the severity of potato late blight. Both indices are calculated using the VNIIFBlight program for six years and seven different regions of Russia. The evaluation of these indices showed that the virtual frequency of fungicide applications provides more accurate results.

KEYWORDS
*Phytophthora infestans***,** risky periods of late blight, monitoring of the late blight

INTRODUCTION

The strategy of the potato late blight control should obviously depend on weather conditions. Potato-producing regions of Russia are located in several climatic zones, which significantly vary concerning the disease pressure level. Therefore, this difference between regions should be taken into account during the planning of protection measures.
The purpose of this study was to develop a tool for a spatial and temporal monitoring of the late blight (LB) development in potato-growing regions basing on meteorological information.

MATERIALS AND METHODS

We used three weather-based indices: 1) annual expert evaluation of the LB development on untreated potato plots, 2) number of risky periods during a potato-growing season, calculated by the VNIIFBlight program, and 3) frequency of fungicide applications calculated by the mentioned program.
The expert evaluation of the LB development on untreated potato plots was carried out by local specialists of the regional plant protection service. They determined three possible levels of the disease severity (low, medium, and high). Such estimation is usually rather rough and is based on either irregular inspections of potato plots, or the inquiry of local farmers. In some cases (for

example, in Moscow and Yaroslavl regions) the percentage of leaf area affected by the pathogen on test potato plots is estimated 3-4 times. Yield losses are then calculated using AUDPC values. The disease severity level is expressed as low (yield loss < 10%), medium (10-20%) and high (> 20%). In our case, we transformed this gradation into scores: 1 (low), 2 (medium, and 3 (high).

The LB risk periods for the whole season were calculated by the VNIIFBlight program using the following two equations:

$$y_1 = -32.47 + 0.75x_1 + 0.41x_2 + 0.41x_3 + 0.27x_4 + 0.74x_5 + 0.30x_6 - 0.07x_7 - 0.16x_8 + 0.06x_9 + 0.01x_{10} + 2.88x_{11} + 1.98x_{12} + 1.98x_{13} + 1.79x_{14} + 0.53x_{15} \quad (1)$$

$$y_2 = -31.34 + 0.63x_1 + 0.37x_2 + 0.42x_3 + 0.22x_4 + 0.65x_5 + 0.24x_6 - 0.06x_7 - 0.15x_8 - 0.13x_9 + 0.15x_{10} + 4.88x_{11} + 3.55x_{12} + 3.34x_{13} + 2.50x_{14} + 2.29x_{15} \quad (2)$$

where $x_{1,2,3,4,5}$ and $x_{6,7,8,9,10}$ are daily and night temperatures (^oC), respectively, and $x_{11,12,13,14,15}$ describe precipitations occurred in the 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days, respectively (yes/no).

The LB risk period occurred if y_1 was less than y_2 for five consecutive days. The period of calculation was 50 days and started short before the foliage closed in the row.

The third index represented the number of recommended fungicide applications calculated by the VNIIFBlight program (Filippov *et al.*, 2008). At this point, we considered that the average duration of a fungicide treatment effect is seven days. Therefore, we did not take into account weather data for seven days after each fungicide treatment even if these days were favourable for late blight development (Filippov *et al.*, 2008).

The described indices were determined for six consecutive years (2009-2014) in seven different regions of Russia: Kaliningrad, Leningrad, Vologda, Moscow, Tyumen, Voronezh and Kazan (Fig. 1).

Figure 1. *Russian regions included into the study*

RESULTS

The LB pressure levels in compared climatic regions significantly varied (Fig. 2). The seasons with severe attacks of late blight were more frequent in Kaliningrad, Leningrad and Vologda regions. In the Tyumen and Voronezh regions, most of the reported LB pressure levels were medium or low. In the case of the Kazan region, the LB development was low for the whole time of observations. The similar rating of the LB severity in the monitored regions was observed, when we used the number of risk periods or fungicide treatments as the indices of the disease severity (see table). For all three indices we obtained a good correlation between the expert estimation of the LB development and the VNIIFBlight calculation of the LB development risk based on either number of risk periods ($r = 0.84$), or the frequency of fungicide treatments ($r = 0.91$). In our opinion, the use of the last index is more preferable.

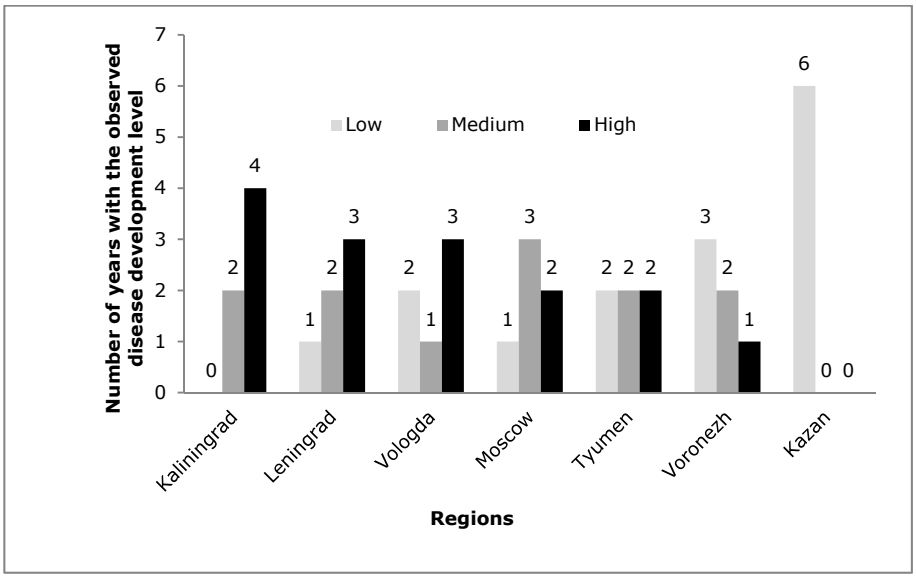

Figure 2. *Distribution of different levels of the potato late blight development in seven Russian regions across the period of 2009-2014 according to the expert evaluations*

Average values of two weather-based indices and expert evaluations of the late blight development assessed as tools for the monitoring of the late blight severity in different regions of Russia in 2009-2014

Regions	Number of risk periods[1]	Number of fungicide treatments[2]	Late blight development level[3]
Kaliningrad	16,5	4,3	2,7
Leningrad	18,7	3,6	2,3
Vologda	18,1	3,5	2,2
Moskow	10,7	2,8	2,2
Tyumen	9,8	2,5	2
Voronezh	4,3	1,5	1,7
Kazan	1,8	0,7	1

[1] number of risk periods per a season, calculated by the VNIIFBlight program;

[2] number of fungicide treatments recommended by the VNIIFBlight program;

[3] late blight severity level according to the expert estimations (3-score scale).

CONCLUSIONS

Basing on the performed analysis, we offer the following gradation of the LB severity based on the recommended number of fungicide treatments calculated by the VNIIFBlight program: low (0-1 treatment) medium (2-3 treatments), and high (≥4 treatments). The period of calculation should be 50 days starting short before the foliage closing in the row.

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PPO – SPECIAL REPORT NO 17 – 2015, 227-228

Variations in metalaxyl sensitivity of *Phytophthora infestans* isolates from Hungary

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Variations in metalaxyl sensitivity of *Phytophthora infestans* isolates from Hungary

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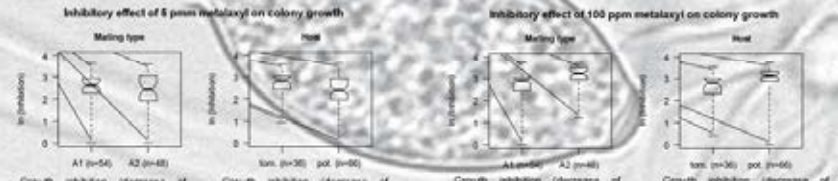
Potato late blight can cause serious damage under favourable weather conditions in wet, rainy years. Fungicide treatments are important part of the disease management. Systemic phenylamide fungicides were widely used, and are still in use for late blight control. However, resistance/tolerance to phenylamides is present in the pathogen population.

One hundred and two Hungarian *Phytophthora infestans* isolates were tested *in vitro* on pea broth agar in order to assess whether their response to metalaxyl differs according to their host, mating type and genotype. Isolates were collected from potato and tomato in 2001–2002.

Potato late blight symptoms.

Effect of metalaxyl on colony growth of various groups of isolates was different. Mann-Whitney test (also called the Wilcoxon rank sum test) revealed significant differences and so did Mood's median test. Growth medium containing 5 ppm metalaxyl had stronger inhibitory effect on isolates collected from tomato, than from potato. But 100 ppm metalaxyl, on the contrary, showed that isolates from tomato were less inhibited by the fungicide than isolates from potato. Mating type did not play a role as a grouping factor at 5 ppm (the difference was not significant), but A1 isolates were less inhibited by the fungicide at 100 ppm.



Growth inhibition (decrease of colony growth): 2.6 and 2.4 mm (U = 1429.5; P = 0.372).

Growth inhibition (decrease of colony growth): 2.8 and 2.4 mm (U = 831.5; P = 0.013).

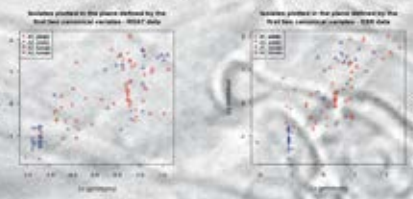
Growth inhibition (decrease of colony growth): 2.9 and 3.2 mm (U = 658; P << 0.001).

Growth inhibition (decrease of colony growth): 2.8 and 3.1 mm (U = 1674; P = 0.001).

Canonical correlation analysis was used to reveal the connection between metalaxyl resistance and two neutral DNA markers. RG57, a hybridisation probe used to be frequently chosen for identification of *Ph. infestans* genotypes (clonal lineages). Nowadays microsatellites (SSR), a useful marker in population genetic studies, are employed and we also involved 11 SSR loci of *Ph. infestans* in this study.

Hybridisation probe: isolates are characterised by their genetic fingerprint, but not with exact genotype data. For example 111-111011001100100101011.

Diploid microsatellite data reveal the true genotype: like the alleles of four isolates here at locus PI02 (162/162, 152/162, 152/162, 160/162).



Distribution of the isolates on the plot suggests a moderate correlation between response to metalaxyl and RG57-genotype (R_{CAN1} = 0.777). There are no well defined and separate groups according to mating type or host. The first pair of canonical variates represents 60.4% of the common variance.

Similarly to RG57, correlation between microsatellite genotypes and metalaxyl response was moderate (R_{CAN1} = 0.761). Individual SSR loci did not have strong correlation with metalaxyl response. The first pair of canonical variates represents 56.0% of the common variance.

There was no individual locus of the markers used being either dominant on the first axis (C_X , the genotypes) or directly linked to metalaxyl sensitivity. In addition, our isolates did not show aggregates on the plot according to their mating type or host plant. This suggests a highly diverse pathogen population without strong internal substructure, which may be the result of sexual propagation or intense migration.

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Virulence and aggressiveness of new *Phytophthora infestans* isolates collected in North-Western Russia as related to host plant resistance

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SUMMARY

In the recent decade, new aggressive lineages emerged in the West European populations of *Phytophthora infestans* which overcame many elite potato varieties known for high late blight resistance; these lineages have rapidly moved eastward displacing the previously known strains of the pathogen (Cooke *et al.*, 2012). To evaluate the changes in *P. infestans* populations which spread across the North-Western Russia, we ran in 2013-2014 pilot experiments with isolates obtained from leaves collected in the field plots of the Pushkin laboratories of the N.I. Vavilov Institute of Plant Industry (VIR) in the Leningrad region of Russia. Our study has supplemented, for the first time, the phytopathological characteristics of Pushkin isolates of *P. infestans* with the molecular evidence.

The evidence from phytopathological and molecular analyses of pure cultures of *P. infestans* consistently indicated the predominance of A2 mating type. We confirmed the previously observed tendency (Patrikeeva *et al.*, 2011) for complexity of the race profile in the Pushkin population and enhanced proportion of A2 genotypes, which is consistent with the general trend recently observed in the Western and Central Europe. The isolates with A2 mating type were sensitive to metalaxyl. Indices of aggressiveness of strains obtained from leaves of potato genotypes highly resistant to late blight were significantly lower than those of strains isolated from more susceptible varieties; the indices for aggressiveness did not expressly matched the profiles of virulence genes in the isolates. When genotyped with 12 microsatellite markers, most 2013 Pushkin isolates distinctly differed from those collected in the Western Europe and in the Leningrad region in 2008. In contrast, most 2014 Pushkin isolates resembled, by many discriminants, the Western-European genotypes of *P. infestans*.

KEYWORDS

Phytophthora infestans, potato late blight, SSR genotyping, A1/A2 mating types, aggressiveness

INTRODUCTION

Due to recent dramatic changes in populations of *Phytophthora infestans*, late blight is overcoming high resistance of many elite potato varieties. The most spectacular event has been the rise of an aggressive *13_A2* and *6_A1* strains of *P. infestans*, which is rapidly displacing other lineages of this pathogen in Great Britain, the Netherlands and other countries in the West Europe (Cooke *et al.*, 2012; Lees *et al.*, 2012; Li *et al.*, 2012) as well as in India (Chowdappa *et al.*, 2013) and China (Li *et al.*, 2013b).

MATERIALS AND METHODS

Phytopathological methods

In 2013, infected potato leaves were collected in the second decade of August, 2013 from ten potato genotypes that survived a severe late blight: var. Sarpo Mira, which manifested high level of late blight resistance in the European countries (isolate 131-13), and nine interspecific hybrid clones from the VIR collection. In 2014, infected leaves were sampled from 13 hybrid clones earlier, in the first decade of July. The ability of *P. infestans* isolates to damage various potato genotypes was evaluated in field and laboratory trials.

Leaves were stored between the slices of tubers of the corresponding potato varieties and in this way were transported to the Institute of Phytopathology, Bol'shie Vyazemy, Moscow Region. Samples of *P. infestans* were isolated by placing fragments of infected leaf tissue between ethanol- and flame- sterilized slices of tubers of the susceptible variety Bintje devoid of known R genes for late blight resistance. The sandwiches thus obtained were placed into sterile Petri dishes with moist filter paper disks on top and incubated for 3–4 days at 18–20°C in a growth chamber until the mycelia grew through tuber slices. Small samples of mycelia from tuber slices were transferred with a sterile needle to agarized oatmeal medium. Pure cultures were preserved at 5°C and maintained by transferring to fresh medium at 1–month intervals.

R-gene virulence phenotypes were characterized on detached leaves of potato differentials. The metalaxyl resistance test and the mating type pairing test were performed as described by Statsyuk *et al.* (2014) with some modifications. Mating type identification was based on the oospore development and the presence of CAPS marker (Judelson *et al.*, 1995).

SSR analysis

The microsatellite analysis of *P. infestans* isolates was conducted using the established set of primers for 12 SSR loci (Li *et al.*, 2013a). PCR was performed in a volume of 25 μl, 10 ng template DNA, 2.5 mM of each dNTP, 50X Encyclo polymerase mix (Evrogen, Russia), and a range of concentrations (Table 1) of the locus-specific primers synthesized by Syntol (Russia). Amplification reactions were run in a PTC-200/DNA Engine (MJ Research/Bio Rad, United States), with an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 58°C for 90 s, and 72°C for 60 s, and a final extension at 60°C for 30 min.

Table 1. *The SSR primers used in the study and the final concentration of each primer in multiplex PCR reaction*

SSR locus	Dye (Syntol)	Final con. (μM)
G11	tamra	0.3
Pi02/SSR3	tamra	0.3
SSR11	tamra	0.15
D13	fam	0.3
SSR8	fam	0.3
SSR4	fam	0.05
Pi04	r6g	0.05
Pi70	r6g	0.05
SSR6	r6g	0.05
Pi63	r6g	0.05
SSR2	rox	0.15
Pi4B	rox	0.3

Table 2. *Isolates of P. infestans collected in the Pushkin potato fields in 2013 and 2014*

Nos. on the dendrogram (Fig. 1)	Isolates	Virulence genes in isolates of *P. infestans*	Mating type assessment (oospore development /CAPS marker)	Resistance of *P. infestans* isolates to metalaxyl *
20	2 -13	12341011	A2/A2	S
21	4 -13	234810	A1/A1	S
22	111-13	12347891011	A1A2 /A2	S
23	7-13	124561011	A2/A2	S
24	106-13	12345781011	A1/A1	IR
25	103 -13	1234567891011	A1/A2	IR
26	132-13	12341011	A1A2 /A2	S
27	113-13	1234567891011	A1A2 /A2	IR
28	131-13	356781011	A1/A1	IR
29	87-13	1234567891011	A2/A2	IR
30	7-14	124561011	A1/A1	S
31	16-14	nd	A1/A1	S
32	18-14	nd	A1/A1	S
34	36 -14	12345671011	A2/A2	S
35	43-14	12367	A2/A2	S
36	53-14	125671011	N/A1	S
37	82-14	12345671011	A1/A1	S
38	109-14	12345671011	A1/A1	S
39	118-14	nd	A1/A1	S
40	119 -14	12345671011	A1/A1	S
41	132-14	12341011	A2/A1	S
42	133-14	nd	A1/A1	S
33	28 -14	1234567891011	A1/A2	S
-	standard	1234567891011	A1/nd	S

*S—sensitive; IR—intermediately resistant. nd - no data.

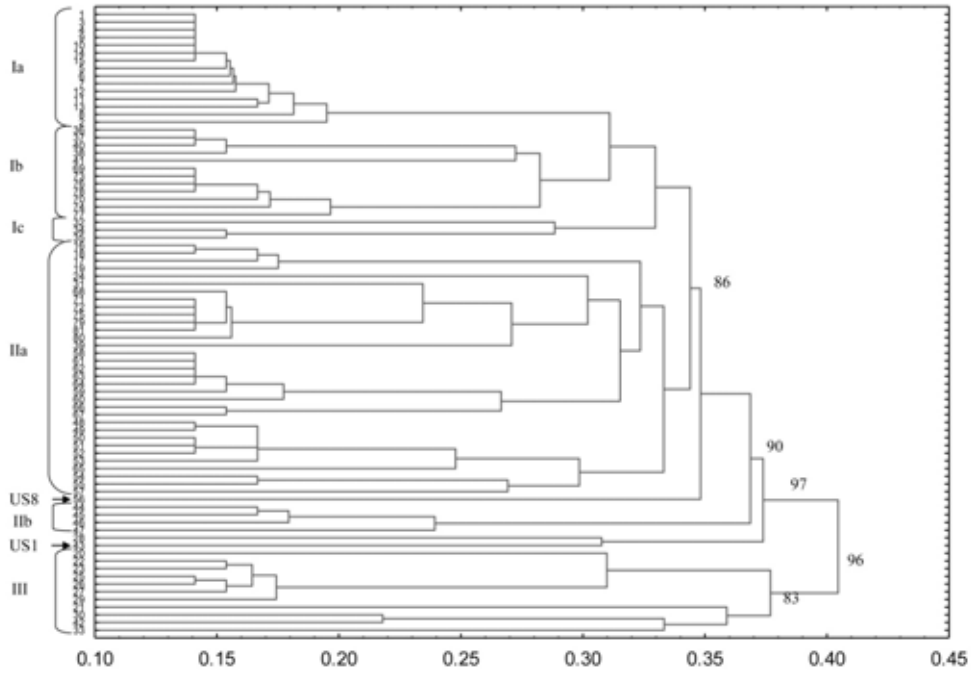

Figure 1. *Phylogenetic analysis of Pushkin P. infestans isolates listed in Table 1. The tree is built using the Neighbor Joining method; bootstrap values for 1000 repeats are shown when they exceed 0.70. P. infestans strains described by Li et al. (2013): 1 - 15 – 13_A2; 64 -10_A2; 16 – 19 – 6_A1; 44 - 46 – 2_A1; 47 – 4_A1; 48 – 53 – 8_A1; 54 – 55 – 5_A1; 58 – 64 – 1_A1; 65 – 10 A2; 66 - 67 – 23_A1. Reference strains: 43 – US1_A1; 56 – US8_A2; 57 – EC1_A1. 20 - 29 – 2013 Pushkin isolates. 30 – 42 – 2014 Pushkin isolates. 68 - 81 – isolates collected in the Leningrad region in 2008 (Statsyuk et al., 2014).*

SSR fragment lengths were determined with GeneScan-500LIZ standard (Applied Biosystems, United States) and Peak Scanner Software v 1.0, with genetic analyzers ABI Prism 3130x1 (Applied Biosystems) and Nanophore-05 (Institute of Analytical Instrumentation, Russia). To construct phylogenetic trees we used Neighbor Joining algorithm and software package PHYLIP Version 3.69 (Felsenstein, 1993).

RESULTS AND DISCUSSION

Virulence genes and aggressiveness of isolates

Among the isolates under study, we observed 23 complex virulence phenotypes possessing 5 to 11 virulence factors (Table 2). Four isolates with the A2 mating type (87-13, 103-13, 113-13 and 28-14) comprise all 11 virulence genes. In 2013, virulence genes 1, 2, 3, 4, 10, 11 were most frequent, and the gene 9 was found only in four isolates with the A2 mating type. In 2014, all genes, except 8 and 9, were represented with high frequency. The latter genes were

registered only in the A2 isolate 28-14. These data match fairly well the evidence obtained in 2008 under heavy infestation conditions (Patrikeeva *et al.*, 2011).

Evaluation of aggressiveness averaged by isolates and by tester varieties produced a wide range of indices (Table 3). Comparison of 2013 and 2014 data indicated higher values of aggressiveness in 2014, apparently because of earlier pathogen sampling.

Table 3. *Aggressiveness of P. infestans isolates on the tester varieties in 2013 and 2014*

A. Averaged by isolates

Isolates	Average aggressiveness
4-13	WA
106-13	MA
103-13	WA
132-13	MA
113-13	NA
131-13	WA
87-13	MA
7-14	MA
53-14	MA
28-14	HA
36-14	MA
43-14	WA
82-14	HA
109-14	MA
118-14	MA
119-14	MA
132-14	MA
133-14	MA
N161(standard)	MA

B. Averaged by tester varieties

Years	Santé	Alpha	Bintje	Escort	Eersteling	Robijn	Gloria	Sarpo Mira
2013	WA	WA	MA	WA	MA	WA	MA	NA
2014	MA	MA	HA	WA	HA	MA	HA	WA

NA - non-aggressive; WA - weakly aggressive; MA – moderately aggressive; HA - highly aggressive

SSR genotyping

In the Pushkin isolates collected in 2013 and 2014, we discerned 49 alleles in 12 SSR loci, 2 to 10 alleles per locus. We collated our data with the evidence for isolates collected in the Leningrad region in 2008 (Statsyuk *et al.*, 2014) and SSR allele sizes reported by Li *et al.* (2013a) for clone 13_A2, several A1 lines and reference genotypes US1-A1, EC1_A1 and US8-A2. All these data were used to build, by the Neighbor Joining method, the dendrogram presented on Fig. 1. This dendrogram comprises three major clusters and several subclusters. All 13_A.2 lines are found in the subcluster Ia; the subcluster Ib comprises some Pushkin 2014

isolates and some 2008 Leningrad isolates; the subcluster Ic combines three more 2014 Pushkin isolates. The subcluster IIa combines the A1 lines, the Leningrad 2008 isolates (also A1), A1 Pushkin isolates collected in 2013 and 2014, and quite unexpectedly, the reference strain 10_A2. The subcluster IIb brings together 2_A1 and 4_A1 lines. The isolate 131-13 from the leaves of the most resistant var. Sarpo Mira was found in a separate cluster. It appeared genetically similar to the strain US-1. In the cluster III, we find the rest of the Pushkin isolates collected in 2013 and 2014.

Thus, most 2013 Pushkin isolates (Cluster III) distinctly differ from those collected in the Western Europe and in the Leningrad region in 2008. In contrast, most 2014 Pushkin isolates were similar, by many discriminants, to the Western European genotypes of *P. infestans* (Table 4). Five 2008 isolates from the Leningrad region are the most difficult to interpret: with A1 mating type, they resemble 13_A2 line by many SSR alleles (Subcluster Ib).

Table 4 Fragment lengths (bp) of alleles resembling those found in the clone 13_A2

Isolates (Nos on the dendrogram)	Alleles of SSR loci											
	SSR11	D13	Pi4B	G11	Pi04	Pi63	Pi70	SSR2	Pi02/ SSR3	SSR4	SSR6	SSR8
36-14 (34)	341/341	154/156	205/213	138/154	166/170	270/279	192/192	173/175	266/266	285/295	240/242	260/266
43-14 (35)	341/341	154/156	205/213	138/154/168	166/170	270/279	192/192	173/175	266/266	285/295	240/242	260/266
LK-2/2.08 (69)	341/341	154/154	205/213	154/162	166/170	279/279	192/192	173/173	258/266	285/293	244/244	260/266
LK-3.08 (70)	341/341	154/154	205/213	154/162	166/170	279/279	192/192	173/173	258/266	285/291	244/244	260/266
LK-11/1.08 (73)	341/341	154/154	205/213	154/162	166/170	279/279	192/192	173/173	258/266	285/293	244/244	260/266
LK-13/1.08 (76)	341/341	154/154	205/213	154/162	166/170	279/279	192/192	173/173	258/266	285/293	244/244	258/264
LK-17/1.08 (78)	341/341	154/154	205/213	154/162	166/170	279/279	192/192	173/173	258/266	285/293	244/244	260/266

CONCLUSIONS

We have confirmed the previously observed tendency for complexity of the race profile in the Pushkin population of *P. infestans* and enhanced proportion of A2 genotypes (Patrikeeva *et al.*, 2011), which is consistent with the general trend observed in the Western and Central Europe up to 2013. The isolates with A2 mating type were sensitive to metalaxyl. The indices for aggressiveness did not expressly match the profiles of virulence genes in the isolates. We presume that in 2013, isolates of *P. infestans* were collected late in the season when only moderately resistant potato varieties stayed alive. In 2014, at the earlier stage of late blight progression, we also collected more aggressive isolates from susceptible potato genotypes.

ACKNOWLEDGEMENTS

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Development of LAMP-HRM for sensitive and specific detection of *Phytophthora infestans*

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Development of LAMP-HRM for sensitive and specific detection of *Phytophthora infestans*

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ABSTRACT

We developed the LAMP-HRM assay for detection of *P. infestans* in a RealTime PCR machine. Specific pathogen detection was achieved in the 18th minute of the reaction, which, in comparison with other diagnostic methods, shortens the analysis time. For instance, first late blight symptoms after inoculation of tomato plants with *P. infestans* are manifested only 4-5 dpi. We applied the downstream HRM analyses to estimate the variability of 96 pathogen isolates, collected from symptomatic tomatoes all over Poland, in the years 2009-2012.

INTRODUCTION

The oomycete *Phytophthora infestans* causing late blight, generates a significant global economic impact (losses) in production of potato and tomato alike [1,3]. In Poland, late blight reaches epidemic scale, and in many regions completely devastates the plantations of both crops [2]. Methods of integrated crop protection [2,3] may benefit from fast pathogen detection in the early stages of infection. This would help limit the expensive and undesirable chemical protection of crops until unequivocal pathogen identification, but requires a technique with high sensitivity and specificity [4,5,6].

One of the attractive methods for fast and reliable pathogen detection is LAMP (Loop-mediated Isothermal AMPlification). It is an isothermic PCR-based technique [7]. It can be run in standard qPCR machines, to observe the products' occurrence and increase in real time. Due to its high sensitivity and specificity, LAMP may compete with other (q)PCR methods [4,5,6]. Since its development in 2000 [7], LAMP has been successfully applied to detection of pathogens from various kingdoms (viruses, bacterias, fungi, and others).

In this project, we aimed at developing a LAMP assay for fast and reliable detection of *P. infestans*.

MATERIALS AND METHODS

We based our analyses on a collection of 96 isolates of *P. infestans*, originating from LB-symptotic tomato plants from all over Poland. These isolates showed high variability in their phenotypic characteristics (virulence on tomato testers and potato Black's standards, mating type, metalaxyl resistance) and molecular characteristics (mtDNA haplotype [8], genotype as per 12 SSR markers [9]).

As the negative controls, we used several other *Phytophthora* species (Unit of Diseases of Vegetable and Ornamental Plants, INH Skierniewice; L. Orlikowski group). All gDNA was isolated with the NucleoSpin Plant II kit (Mecherny-Nagel, USA). For analyses, we used the Isothermal MasterMix (OptiGen Ltd., Great Britain) in LAMP reactions with 6 primers, and the AccuMelt HRM SuperMix (Quanta Biosciences, USA) in the control reactions with 2 border primers. All analyses were run in a RealTime-PCR machine LightCycler II (Roche).

The pipeline with which we sought for the *P. infestans* detection loci is described in Table 1.

Table 1. Searching for the detection loci for *P. infestans*.

Step description	Effects
Literature searched for candidate loci.	10 potential detection loci.
Sequence searches, alignment for consensus sequences.	>1000 sequences analysed.
LAMP-cut-off of the consensus sequences.	6 potential sequences.
Testing the candidate sequences (2 primers; 6 primers) with gDNA of other Phytopthoras.	2 loci.
Variability analyses of the *P. infestans* collection.	

RESULTS AND DISCUSSION

By applying the presented research pipeline, we identified 6 candidate loci for LAMP-detection of various Phytophthoras (*P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. infestans*, *P. plurivora*). Based on the analyses of products of the 2-primer and 6-primer reactions (both, HRM and in-gel), two of these loci (ITS1, INF6) showed specific detection of *P. infestans* (Fig.1 A-D). The buildup of these two specific detection products started in LAMP reactions in the 18th minute (Fig. 1A). Melting analyses (HRM) with 2 border primers and with full LAMP 6 primers indicated differences in the respective products, even when such differences were not visible in gel (Fig. 1E).

The identified *P. infestans* specific loci (ITS1 and INF6) show different sensitivity of detection: This of ITS1 being significantly higher, exceeding 500 fg of gDNA (Fig 1A). Both, detection time and detection sensitivity surpassed these parameters when standard (q)PCR was used [6]. Yet, the is specific towards *P. infestans*.

Variability analyses of our collection of 96 *P. infestans* isolates were done with both specific loci, by coupling LAMP reaction with downstream HRM. We observed more variability in the products of ITS1 (Fig.2 A,B), which suggests polimorphism of the sequence undergoing detecion. This is in line with the high variability observed in general for this pathogen [2,3]. This results, however, requires an independent confirmation – be it with sequencing or capillary electrophoresis of the products.

The specificity of our assay needs to be further confirmed on a broader collection of Phytophthoras. Furthermore, the applicability of the pathogen detection needs to be checked on inoculated, asymptotic tomato plants.

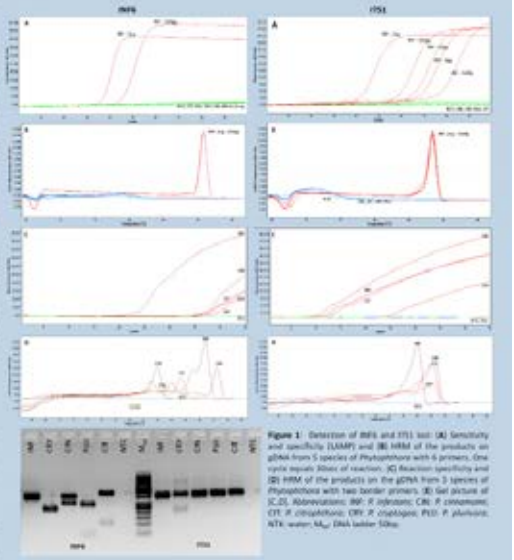

Figure 1 Detection of INF6 and ITS1 loci: (**A**) Sensitivity and specificity (LAMP) and (**B**) HRM of the products on gDNA from 5 species of *Phytophthora* with 6 primers. One cycle equals 30sec of reaction. (**C**) Reaction specificity and (**D**) HRM of the products on the gDNA from 3 species of *Phytophthora* with two border primers. (**E**) Gel picture of (C,D). Abbreviations: INF: *P. infestans*; CIN: *P. cinnamomi*; CIT *P. citrophthora*; CRY: *P. cryptogea*; PLU: *P. plurivora*; NTK: water; M_{100}: DNA ladder 100bp.

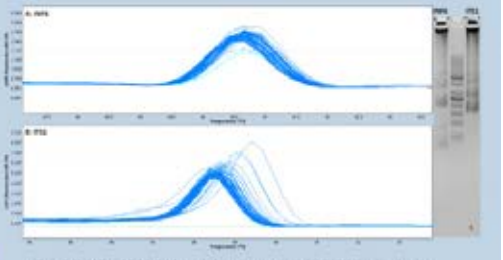

Figure 2: Variability of Polish *P. infestans* isolates, determined with LAMP-HRM (6 - primers) on the collection of 96 local isolates. Analyses were run for the (**A**) INF6 locus and (**ITS1**) locus. Exemplary products of LAMP reaction are shown in gel (**B**), against the 50 bp DNA ladder (middle lane).

CONCLUSIONS

1. LAMP coupled with HRM allows for specific and very sensitive detection of *P. infestans*. We suggest using the ITS1 locus, due to higher sensitivity of its detection.
2. The LAMP-HRM method can be used for analyses of *P. infestans* variability, thus adding to current array of phenotypic and molecular analytics tools.
3. Specificity of detection with our assays requires further confirmation with gDNA from other Phytophthoras (currently ongoing). Particular attention should be paid the ones closely related to *P. infestans* (clade 1 sensu 2012; 12 species).

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PPO – SPECIAL REPORT NO 17 – 2015, 239-240

The socio-economic value of mancozeb to the UK potato industry for the control of potato blight with wider implications in Europe

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The socio-economic value of mancozeb to the UK potato industry for the control of potato blight with wider implications in Europe

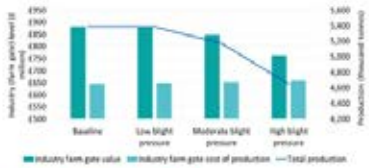
Aims

The project aimed to identify the value of mancozeb to the UK potato industry. Mancozeb is an important part of the resistance management strategy in the control of late blight (*Phytophthora infestans*), and is additionally used in the control of early blight (*Alternaria solani*). Interviews with growers and advisers examined the perceived short- and long-term effects in the event that mancozeb is removed from the market.

Short-term: mancozeb loss has financial implications for the UK

In high pressure disease years without mancozeb, interviewees expected an increased cost of production of **£484-1370/ha** would be required to maintain control of blight. In moderate (normal) years, extra predicted cost was **£75-444/ha**, compared to **£35/ha** in low pressure years. Yield losses of 0-4% (moderate pressure) and 4-14% (high pressure) were also expected.

Pesticide Usage Survey information was used to scale up farm level data to the national level. In moderate blight pressure years loss of mancozeb is estimated to cost the UK industry **£43 million** in reduced gross margin and reduce production by **215,000 tonnes.**



Long-term: resistance in 3 - 8 years

Current resistance strategies rely on multi-site actives such as mancozeb to help delay the time to first detected resistance (FDR). Predicted time to FDR for the main single site fungicide groups used in Europe varies from 3 – 8 years in the absence of mancozeb.

Chemical Group	Predicted FDR (years)	Resistance reported (*P.infestans*)
cyano acetamide-oxime	6.8	No
phenylamides	5.9	Yes
QiI fungicides	3.5	No
QoI fungicides	4.1	No
benzamides (pyridinylmethyl-benzamides)	5.5	No
benzamides (toluamides)	6.1	No
CAA-fungicides	4.2	No (laboratory mutants have been produced)
triazolo-pyrimidylamine	7.8	No

European implications

The next stage of the project involves determining the effects a loss of mancozeb would have across Europe. Experts across the continent will be invited to contribute their opinions and knowledge, to examine the risk of resistance in their respective countries.

Acknowledgements

Funding for this project has been provided by the EU Mancozeb Task Force

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F129L mutation found in Dutch *Alternaria solani* population

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WAGENINGEN UR
For quality of life

F129L mutation found in Dutch *Alternaria solani* population

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Relevance of the F129L mutation

Partial resistance to azoxystrobin was first found in the USA and is associated with the F129L mutation (Pasche & Gudmestad, 2008). In Europe strobilurines (QoIs) are frequently used to control early blight as well. A previous screening of German *Alternaria solani* population revealed the presence of the F129L mutation. In Germany a shift in sensitivity of *A. solani* to strobilurines was observed in in vivo tests (Leiminger et al., 2013).The objective was to assess whether the F129L mutation was also found in the Dutch *A. solani* population.

Alternaria solani isolate collection

In the Netherlands potato leaves with early blight lesions were collected in potato fields from 2001 to 2011. A total of 48 *A. solani* isolates were purified and stored in liquid nitrogen. The isolates were regrown at Wageningen UR and further investigations were carried out at the chair of Phytopathology (TUM).

Detection of the F129L mutation

To possess the amino acid substitution the cytochrome b gene was screened for all isolates. Therefore total genomic DNA was extracted. Depending on the genotype of the isolate a 214 bp (genotype I) respectively a 207 bp fragment (genotype II) was amplified with two different primer sets. Further investigations of the presence of the F129L mutation was performed by fragment sequencing.

Figure 1: Potato crop infected with early blight

Results

Of the 48 *A. solani* isolates, 47 were wild-type. One isolate, originating from a field in the South West of the Netherlands (2009), contained the F129L mutation. Two different genotypes were present among isolates derived from Dutch *A. solani* population (Figure 2). Genotype I was the most prevalent type among all isolates, whereas the F129L mutation occurred in type II isolates. Seven further isolates revealed genotype II, however were wild-type. So far no record of insensitivity to strobilurines of *A. solani* has been reported in the Netherlands.

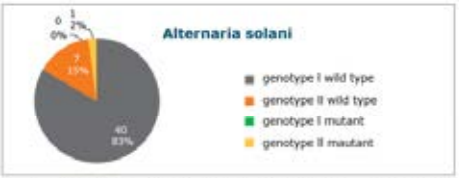

Figure 2: Genotype and F129L mutation in the Dutch A. solani population (n=48)

Discussion & conclusions

A small survey of *A. solani* in the Netherlands revealed the presence of the F129L mutant in one case.
In 2011 *A. alternata* isolates were detected in the Netherlands with a G143A mutation which makes them less sensitive to strobilurines (De Boerderij, 2012).
In order to reduce the selection pressure on *A. solani* populations and to prevent a lack of EB disease control, the use of QoI fungicides have to be restricted.
Furthermore monitoring the early blight population more extensively for the presence of the F129L and G143A mutation is recommended.
The consequence of the presence of the F129L and G143A mutations for the early blight control strategy in the Netherlands has to be investigated.

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Phytoalert: when less is more

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SUMMARY
Argentina is one of the main potato production countries in Latin America. Freshly harvested potatoes are available throughout the year, coming from four potato production areas and over different cropping seasons and weather conditions. The primary production area with the highest yields in the country is located in the southeast region of the province of Buenos Aires, where the weather conditions are very conducive to late blight development. Losses due to the absence of chemical control were on average 35.6 % of the total yield during the last 20 years. Fungicide treatment is the main strategy of control used to prevent the initial development of the disease
To increase the efficiency and sophistication of tools to control late blight, we developed Phytoalert, a decision support system which was implemented in high production fields of the southeast region of the Buenos Aires province. Phytoalert generates dynamic spray schedules that are matched alongside high-risk periods to avoid unnecessary fungicide applications, and provide an optimal timing for the remaining applications. The implementation of Phytoalert proved to have economical and environmental advantages which optimize the control of late blight with a reduction in the number of fungicide applications and in the amount of fungicide applied, thereby achieving lower production costs and environmental impact rates, without increasing the risks.

KEYWORDS
Phytoalert, DSS, Economical impact, Field use EIQ, late blight, fungicide

INTRODUCTION

Potato is an important staple crop in Argentina amounting to a production of around 2 million tonnes, according to latest statistics (Faostat, 2015). Based on production, Argentina is positioned in 32nd place globally and fourth in Latin America (Faostat, 2015). The market offers freshly harvested potatoes throughout the year, coming from the four main producing areas (early, semi-early, semi-late and late). Regarding to the varieties, Spunta dominates the fresh market and Innovator is the most cultivated by farmers supplying the processing industry mainly with French fries. The technology level of the potato farmers varies based on the size of the fields and the production goal. Potato production can be fully mechanized and achieve high technological levels with large and medium size farmers producing potatoes mainly for

processing industry. Although even smaller farmers producing potatoes for the fresh market still use intensive labour, especially during harvesting (Kessel *et al*, 2010).

Potato late blight caused by the oomycete *Phytophthora infestans* is the most serious disease affecting potato crop worldwide. The agriculture suffers staggering in yield and control measures of the pathogen of € 11 billion per year in yield (Haverkort, 2008). In the Southeast region of the Buenos Aires Province, an area with the highest yields in the country, the weather conditions are very conducive to late blight development. Losses to the absence of chemical control were on average 35.6 % of the total yield during the last two decades (Mantecón, 2009). A treatment based on fungicide sprays (with a weekly systematic program) is the main strategy control used to prevent the initial development of the disease (Mantecón 2000, 1998).

There is a growing desire in society to reduce the use of pesticides (Pretty and Hine; 2005, Beaumont 1993; Levitan *et al*, 1995; Levitan, 2000). The efforts are focused on incorporating some risk-reducing measures, standards and regulations into agricultural practices. At the local level, the Potato Research Group of INTA Balcarce developed a Protocol for integrating ware potato production, especially designed for the Southeast region of the province of Buenos Aires. This protocol proposes production alternatives in order to reduce the costs and the environmental impact, contributing to the sustainability of natural resources to develop a quality product that could be valued and accepted by consumers (Huarte *et al*, 2011; Rodriguez and Rodriguez, 2012).

To increase the efficiency and sophistication of tools to control the late blight, we developed a Decision Support System called Phytoalert. A collaboration between INTA, Wageningen University and McCain Argentina S.A. helped to the optimization on the practical implementation of Phytoalert in high production fields of the Southeast region of the Buenos Aires province (Lucca and Huarte, 2012). The DSS, integrates available information on weather conditions (meteorology and forecasted data), the disease cycle, the crop and previous spray applications to generate an optimal spray schedule. The spray applications are matched alongside high-risk periods to avoid unnecessary fungicide applications, provide an optimal timing for the remaining applications.

We assessed the economical and social - environmental impact of the implementation of Phytoalert, to control late blight during potato four crop seasons since 2010-11 until 2013-14. We compared a Control Calendar strategy based on fixed weekly fungicide spray schedule with a dynamic spray schedule suggested by Phytoalert during this period.

MATERIALS AND METHODS

We evaluated the impact that the implementation of Phytoalert would have on the costs, the fungicide sprays and the environment, in the framework of integrated production, compared with conventional potato production for which a Calendar Control is used based on a fixed weekly fungicide spray schedule to control late blight. This study was carried out in the southeast region of the province of Buenos Aires during four potato seasons (2010-11 to 2013-14) in high production fields of McCain Argentina S.A. with the variety Innovator.

Phytoalert is based on Simcast model (Fry *et al*, 1983). In order to take mainly preventive measures against late blight, we have included a weather forecast, to detect potential infection eventualities (critical periods). The system also includes information on the fungicides sprayed in the fields. The risk periods for late blight are detected based on Blight units and fungicide units.

For economical purposes we used a methodology to evaluate the cost of fungicide sprays in each production system. The methodology was based on fungicide prices (valued in US dollars for each season) and an updated cost of agricultural labour (application of fungicide) per season. To

calculate the cost of labour we used an agricultural labour unit expressed as UTA (which is the Spanish acronym for Unidad de Trabajo Agrícola) described by Garbers (2003).

To assess the total environmental impact of the fungicides applied in each season, we determined the Environmental Impact Quotient (EIQ) for Phytoalert and Control Calendar followed the methodology of Kovach *et al* (1992). For comparisons between the two different management strategies we used "Field Use EIQ" (http://www.nysipm.cornell.edu/EIQCalc/input.php), a value calculated for specific active ingredients and dosage rate, providing an indication of the potential environmental impact of specific pesticide formulations at the prescribed dosage. This tool considers a range of impacts to farm workers, consumers and the environment.

RESULTS AND DISCUSSION

During four potato crop seasons, implementing Phytoalert in high production fields of McCain Argentina S.A. proved to be an efficient control of late blight. No epidemics were developed in the fields where DSS advice had been followed. Phytoalert was sensitive enough to anticipate critical blight periods, performing mainly protectant sprays.

A summarized assessment of the average reduction on cost, number of sprays and on environmental impact measured by Field use EIQ index of Phytoalert and Control Calendar is shown in Figure 1.

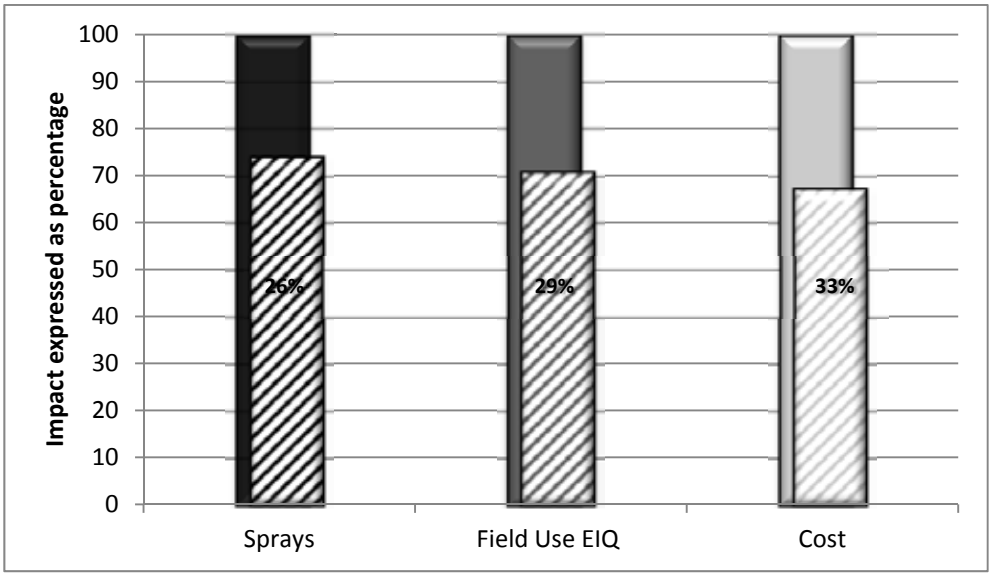

Figure 1. *Impact of the implementation of Phytoalert on the costs, the number of fungicide sprays and the environment (by Field use EIQ) compared with a Calendar Control strategy to control Late Blight. Percentage reduction are shown over the bars*

Phytoalert *Control Calendar*

The best disease control was achieved with Phytoalert associated with savings in fungicide sprayed during 3 out of the 4 seasons studied, which varied according to the pressure of the disease in each season. That reduction in fungicide sprays represented monetary savings of 33% on average during the seasons. Phytoalert, using a variety such as Innovator, did not require weekly fungicide applications and could reduce the number of sprays around 26%. Saving on sprays also implies a reduction in fungicide residues in the environment and lowering the risk of contamination to field workers and consumers. Comparing the "Field Use EIQ" using both late blight control strategies, Phytoalert showed reductions of up to 48 % over the whole Control Calendar programme and on average during the period studied of around 30%. The lower EIQ indices were mainly due to a reduction in fungicide input and sometimes based on the use of fungicide having a lower environmental impact. The main fungicides and doses used during these four seasons are shown on Figure 2.

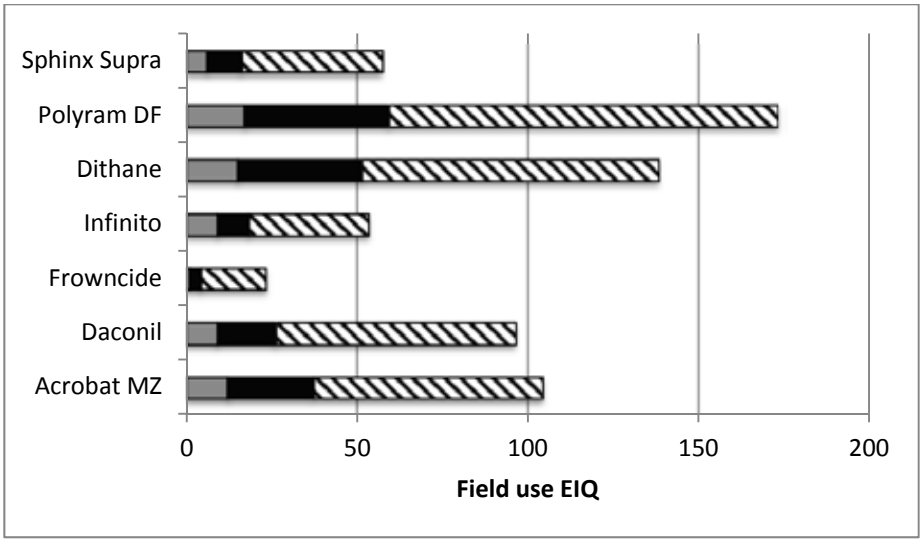

Figure 2. *Impact of Field Use EIQ components (consumers, workers and ecological) of the main fungicides and doses used during four seasons in the southeast of the province of Buenos Aires with Phytoalert and Control Calendar strategies.*
Field use EIQ components: Consumers *Workers* *Ecological*

According to Field use EIQ and based on environmental segments considered, the Ecological component show the highest relative weighting effects followed by Consumers and Workers components for the main fungicides used to control late blight during this period of study. Some commonly used products have a considerably larger theoretical environmental impact than many of the others. It could be possible to develop a dynamic spray schedule of fungicides with an appropriate product rotation to prevent resistance and having further a lower environmental impact. The incorporation of environmental risk indices in the table of fungicides given to the growers with the advice, will allow them to make better decisions in late blight control based not only on effectiveness and costs, but also on the side-effects of the fungicide on the environment.

The selection of fungicide with lower hazard rates could further reduce the EIQ index, but this will depend on the prioritization of economic or environmental interests within the production system, and also the availability of these fungicides on the market during critical periods for late blight.

CONCLUSIONS

The implementation of Phytoalert proved to have economical and environmental advantages. Phytoalert allowed us to take strategic decisions about late blight management with a reduction in the number of fungicide applications and in the amount of fungicide applied, thereby achieving lower production costs and environmental impact rates, without increasing the risks. The incorporation of environmental index into the fungicide table given to the growers with the advice will allow them to make better decision to control late blight based not only on effectiveness and costs, but also on the side-effects of fungicides on the environment. The EIQ index could further be reduced if the growers select fungicides with lower hazard rates, but that decision will depend on the prioritization of economic or environmental interests within the production system, and also on the availability of these fungicides on the market during critical periods for late blight.

The use of Phytoalert in the framework of an IPM system can optimize the control of late blight in the climatic conditions of the southeast of the province of Buenos Aires and is a useful tool that could be applied in other potato regions of Argentina as Tucumán and Mendoza. Even so, a validation should be carried out to make adjustments of the system if needed to minimize risks of potato growers.

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Tizon Latino: A Latin American network for the study of Solanaceae Blight Diseases

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Tizon Latino: A Latin American network for the study of Solanaceae Blight Diseases

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INTRODUCTION

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the most important disease of the Solanaceae family of plants worldwide, and is responsible for severe yield losses since it was first described in 1845, when it caused the renowned "Irish potato famine", causing the death and migration of millions of people. During the 1990's, new late blight epidemics of potato occurred once again throughout the world due to changes in the pathogen and migration of new genotypes. These new genotypes were more aggressive, resistant to some fungicides, and able to overcome cultivar resistance in commercial varieties.

For many years, much work and great efforts have been done to develop effective management strategies to reduce disease losses. Today, the most effective strategy is integrated management using cultivars with high levels of resistance to the disease and decision support systems that allows variable fungicide doses. Despite our collective best efforts, late blight continues to be a serious disease, and researchers around the world have focused on studies of the pathogen, disease epidemiology and ecology, genetic improvement of the host and integrated pest management.

Because the scientific community understands that cooperation is a key factor to solve a problem, collaborative networks have been established worldwide. Therefore, Euroblight, the European network, has 22 member countries which have standardized protocols and research objectives related to late blight and recently, for early blight (*Alternaria* spp). In addition, USABlight and AsiaBlight have been formed with similar objectives. TizonLatino was formed in September 2014 at the Latin America Potato Association meeting (ALAP) in Bogota, Colombia. It was born from the need to work together on a disease and hosts originating in Latin America, which causes serious losses and affects the food security of the region. Today, ten Latin American countries are part of this network.

OBJETIVES AND FOCUS WORK

The main objective of TizonLatino is to share knowledge and protocols on blight diseases and their causal agents, with the ultimate aim of advancing sustainable disease management.

The network will also be a platform where partners can work together to find financial support conduct research on Solanaceae blights and to strengthen close cooperation with existing networks in Europe, Asia and North America.

However, the Latin American network must find its differential areas of work with respect to other networks. We know that the most important differences are:

- The diversity of hosts in Latin America includes a large diversity of solanaceous species.
- The need to expand extension and collaborative research to facilitate the incorporation of scientific advances by farmers, especially the large number of small farmers, in their particular realities.

Therefore, the TizonLatino approach will be:

- Monitoring and characterization of pathogens.
- Study the effects of late blight on potato landraces due to climate change and its impact on diversity and food security.
- Search for durable resistance and breeding populations.
- Develop integrated pest management strategies using decision support systems, fungicides and resistant cultivars.
- Extension and technology transfer focusing on the development and implementation of management strategies, based on vulnerability and food security in Latin America and for adaptation to climate change.

EXPECTED RESULTS

Tizon-Latino will generate the following products:

- Standardized procedures for the measurement and data analysis to manage blight diseases:
  - Improve monitoring of pathogen populations in the areas where the new technologies will be established.
- A portal web with updated data on:
  - Pathogen diversity and distribution.
  - Susceptibility levels of different solanaceous plants.
  - Sources of resistance to incorporate in breeding programs.
  - Evaluation of fungicide efficacy.
  - Decision support system implementation.
- Scientists, technicians and extensionists trained in protocols and techniques related to technology transfer:
  - Improvement of the technical support to farmers.

Additional information can be found in the Tizon Latino website: https://tizonlatino.wordpress.com.