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A potato late blight network for Europe

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Colofon

Mancozeb Taskforce

Preface

EuroBlight Workshop Aarhus, Denmark 14-17 May 2017

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 16th Workshop was hosted by Aarhus University, Department of Agroecology, Denmark.
The Workshop brought together 110 participants from Europe, South America, USA, Africa, Israel and China to share research results and identify current challenges and opportunities.
Representatives from all countries presented the late and early blight epidemics in 2015 and 2016 and 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. 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, PAGV-Special Report no. 18. The current and previous Proceedings are also available on the EuroBlight website www.EuroBlight.net.

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Table of contents

8

Papers

Epidemics and control of early & late blight, 2015 & 2016 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, 2015 and 2016.

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 Aarhus 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 effective can control *Phytophthora infestans* on the field level. The European monitoring initiative 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 fungicides and helps growers to choose potato varieties with the right levels of resistance. A

second area of concern is the increasing problems with fungicide resistance related to the control of late blight and especially early blight.

This paper reports the development and control of late and early blight in Europe, 2015 and 2016 and thereby describes 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.

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.

Estonia

2015: The first late blight outbreaks were recorded at the end of July. Heavy rainfall and warm nights at the beginning of August created very favorable conditions for the development of potato late blight. The potato growers started applying fungicides in the middle of July, for the first sprays systemic fungicides were used. At the beginning of August rainproof fungicides like Infinito, Ranman and Revus were used for the control of potato late blight.

2016: Heavy rainfall and high humidity during the summer of 2016 favored the development of potato late blight. First outbreaks were recorded in the first half of July. Already at the end of July, the untreated potato fields were severely infected by late blight. The potato growers started with the fungicide treatments at the end of June. The growers sprayed on average 3-6 times with fungicides during the season. Preferred active ingredients for the control were fluazinam, mandipropamid and mancozeb.

Latvia

2015: Spring of 2015 was earlier than other years. April and May were warm and wet. Crop emergence was completed by the end of May. Dry weather conditions in June delayed the crop growth and development, the first warning of the development of *Alternaria solani* was received on the 20 June when the temperature and humidity conditions were favourable for the development of the disease. The first warning of the development *P. infestans* of was received in end of June. The first protective fungicide application (systemic + contact) was made just before the infectious period. Beginning of July was warm, some days the air temperature reached 30°C, then entered the cool and wet weather till beginning of August. Temperature and humidity conditions remained favourable for the development of both diseases. Following August was very dry and hot (air temperature above 35°C).

2016: Spring of 2016 was warm and wet. End of May and beginning of June entered the heat (31°C) and drought. Due to colder and wetter weather from middle of June, the first warning of the development of *P. infestans* and *A. solani* was received on the 10 June. Because of frequent rain and favourable temperatures for crop growth, development of early and late blight started on the 11 June. The first protective fungicide application (systemic + contact) was made just before the infectious period. July was hot and wet. Temperature and humidity conditions

remained extremely favourable for the development of both diseases all of July and August. At August rainfall reach 128,5 mm support development tuber infection.

Lithuania

2015: The potato crop was planted in the beginning of May. The crop fully emerged nearly a month later – at the beginning of June. Summer period was very unusual compared with previous seasons. If average air temperature in June and July was only by 0.6 degree lower compared with the long term mean than amount of rain was significantly lower. By average, amount of rain in June was by 57.8% and in July – 23.7% lower compared with the long term mean. In August, average air temperature was 19.7 degree and it is by 3.0 degree higher compared with long term mean. Over this month, 19 days were with the average air temperature higher than 25.0 degree and 7 days of them were with the average air temperature higher than 30.0 degree. Amount of rain was even more significantly lower than in June and July. In August, were only 2 rainy days with the total 5.6 mm, while the long term mean of the entire month is 74.2 mm. Drop of the amount of rain was by 92.5%. Application of fungicides started in the first week of July. Fungicides were applied with 7-11 days intervals. In total, 5 applications were performed. The last application was performed on 10 August. Due to dry and hot July – August period late blight did not appear in the crop. The crop remained green until the middle of August. Then potato foliage started to wilt and by the end of the month reached full maturity.

2016: The potato crop was planted at the usual timing in Lithuania – end of April or beginning of May. In May, average air temperature was by 2.7 degree higher compared with a long-term mean; therefore potato crop fully emerged around three weeks after planting. Amount of precipitation over this month was relatively lower and consisted 52.5% of the normal. Summer period differed in comparison with perennial means. Average air temperature in June, July and August was higher by 1.8, 0.8 and 0.3 degree, respectively. At the same time amount of precipitation differed over entire period. In June it was only by 4.5 mm lower than normal. July and August were exceptional with unusually high amount of precipitation. In July and August it was by 52.4 and 35.4 mm higher compare to perennial means, respectively. Almost every second day over these two months was rainy. Warm and rainy weather conditions were very suitable for spreading of late blight in potato crops. Application of fungicides started since the beginning of July with 6-10 days intervals. First late blight symptoms were found on 12 July, so 2 applications were already applied before symptoms appeared. In total, 6 applications were needed to control late blight. The last application was performed by the middle of August.

Russia

2015: A severe late blight development (yield losses >20%) was observed on potato fields of the Kaliningrad, Leningrad, Vologda, Tver, Moscow, Murmansk, Kirov, Novgorod, and Pskov regions. A moderate disease development (yield losses 10-20%) was registered in the Kaluga, Ryazan, Smolensk, Bryansk, and Arkhangelsk regions. The development of the late blight infection on the other territories of the European part of Russia was rather weak (yield losses <10%). Infected seed tubers represented the main source of the primary infection. The most popular fungicides were Abiga-Pic, Shirlan, Tanos, Acrobat MZ, Infinito, Revus Top, Kurzat, Sectin Phenomen, 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 DSSs (Plant-Plus, VNIIFBlight, Agrodozor) was rather rare. The most popular potato cultivars were: Red Scarlett (24%), Gala (8%), Udacha (7%), Rosara (6%), Zhukovskiy ranniy (6%), Nevsky (5%), and

Impala (4%). The volume of foreign and domestic cultivars used by large agricultural companies was ~80 and 20%, respectively.

2016: A severe late blight development (yield losses >20%) was observed on potato fields of the Kaliningrad, Leningrad, Tver, Moscow, Murmansk, Smolensk, Novgorod, Pskov, Kaluga, Bryansk, Yaroslavl, and Kostroma regions. A moderate disease development (yield losses 10-20%) was registered in the Vologda, Ryazan, Ivanovo, Tula, Orel, Kursk, and Nizhni Novgorod regions. The development of the late blight infection on the other territories of the European part of Russia was rather weak (yield losses <10%). Infected seed tubers represented the main source of the primary infection. The most popular fungicides were Shirlan, Tanos, Acrobat MZ, Penncoceb, Infinito, Revus Top, Kurzat, Sectin Phenomen, 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 DSSs (Plant-Plus, VNIIFBlight, Agrodozor) was rather rare. The most popular potato cultivars were: Red Scarlett (14%), Gala (10%), Udacha (8%), Rosara (6%), Nevsky (5%), Impala (4%), and Zhukovskiy ranniy (4%). The volume of foreign and domestic cultivars used by large agricultural companies was ~90 and 10%, respectively.

Poland
2015: Most of May the weather was cool and dry. The conditions promoting the disease occurred during first ten days of June. The first symptoms of the disease were reported from the provinces Kujawsko-Pomorskie and Dolnośląskie (10 June). After about 10 days the disease has spread on the fields of central provinces (Łódzkie, Mazowieckie), the center of Pomerania (Pomorskie) and the north-eastern part of the country (Warmińsko-Mazurskie, Mazowieckie). The conditions at the beginning of July were not favorable to the development of the disease. The second peak of the spread of late blight occurred in the second half of July. The disease occurred in the following provinces: Mazowieckie, Podlaskie and Świętokrzyskie. Meteorological conditions in 2015 were not favorable to the development of the disease. Potato farmers performed from 1 to 5 control treatments, but most frequently 1-2 treatments were applied. The most commonly used active ingredients were fenamidone + propamocarb-hydrochloride, fluopicolide + propamocarb-hydrochloride, metalaxyl-M + mancozeb, cymoxanil + mancozeb and dimethomorph + mancozeb. The level of infection of tubers by late blight was low.

2016: The conditions in May and the first twenty days of June were not favorable to early infections of potato late blight. The earliest occurrence of the disease was reported in the southern part of the country (30 May, Dolnośląskie). About 10-15 days later, the symptoms were also observed in the central part of the country (provinces: Łódzkie, Mazowieckie and Kujawsko-pomorskie). In Pomerania, the disease appeared during last ten days of June and during first ten days of July. Most favorable conditions for the development of the disease (high rainfall) occurred in the provinces: Zachodniopomorskie and Pomorskie. In the central part of the country, further development of the disease was hampered by the high temperatures and lack of rainfall. Farmers growing potatoes in the growing season usually performed 1 to 5 treatments. The number of treatments on plantations for processing potatoes for chips and French fries, ranged from 10 to 13. Most often were used the following active ingredients: fenamidone + propamocarb-hydrochloride, fluopicolide + propamocarb-hydrochloride, metalaxyl-M + mancozeb, cymoxanil + mancozeb and dimethomorph + mancozeb. The level of infection of tubers was low.

Serbia

2016: an unusually mild winter followed by an early and warm spring with a lot of rainy days was favourable to late blight development. Due to the favorable conditions in spring, potato plants were planted a few weeks earlier than usual and the crop development was very fast. High humidity during March, April and May resulted in early outbreaks of late blight being reported throughout the potato growing regions, already in early June. Dry weather in late June and intensive use of fungicides resulted in control of the increasing late blight infection. During July the disease remained at a low level and a major late blight epidemic developed later, in the second half of August. The year had very favourable conditions for potato growing, but infection risks were present throughout the season until harvest and yields were generally at a medium level.

Switzerland

2015: Until end of March 2015, weather conditions were rather mild and dry. At the beginning of May ongoing heavy rainfall with mild temperatures started and up to six consecutive days with main infection and sporulation periods (MISPs) were registered for almost all weather stations in the DSS PhytoPRE. The amount of rain during these first days of May was as high as the common rain amount for the whole month. Therefore potatoes suffered from flooding and erosion. On 11 May, the first late blight attack was observed in the western part of Switzerland in a covered potato field. Other primary late blight infections were registered the following four days in the Swiss plateau. Until the end of June, weather conditions were very favourable for late blight and the epidemic could spread fast in all potato growing regions. Late blight pressure was particularly high in the central part of Switzerland. From July until mid of August, it was hot (temperatures > 30°C) and dry with occasional thunderstorms. Hence, late blight pressure was strongly reduced, lesions dried out and the epidemic could not spread anymore - in contrast early blight increased. Due to the changeable weather from mid of August onwards, formation of miniature stolons and new sprouts were observed.

2016: The potato season 2016 was very difficult due to the prevailing weather conditions. From mid of May until the end of June weather conditions were very favourable for late blight. Two first late blight attacks were registered on 18 May in the eastern part and the western central plateau of Switzerland. Within ten days from the end of May until the beginning of June for most of the weather stations 7 or more main infection and sporulation periods (MISPs) were registered in the DSS PhytoPRE. During this period, no fungicide applications were possible due to the wet soil conditions. Therefore, late blight could spread very fast in all potato growing regions. Some potato fields, especially in the central part of Switzerland, were completely destroyed during these days. Within a few days in June, number of registered late blight attacks increased from 10 to 100. Despite some dry weather periods in July, late blight pressure remained high during the whole potato growing season. High yield losses were observed due to these difficult circumstances.

Finland

2015: Very normal blight season: first outbreaks during the first week of July, epidemic development in untreated crops at the end of July – beginning of August. End of season not very conducive for blight development. Four to six fungicide applications, no severe blight outbreaks in fungicide treated fields.

2016: First blight outbreaks somewhat earlier than usual, epidemic development in untreated crops during the latter half of July. August was very rainy and conducive for blight development. Four to eight fungicide applications, locally severe blight outbreaks also in treated crops. Problems to apply fungicides at correct intervals due to continuous rain and wet field soils.

Norway
2015: There were only a couple of hours in May with high blight risk in the main potato growing areas according to the Nærstad model in VIPS (south east). June had from 2-10 days with infection risk, and the first real period with high risk was late June. By mid-July, late blight was widespread, and both July and August had long periods with conditions favourable for blight. Overall 2015 was an average year for late blight in Norway.

2016: The season was relatively wet, and there were more days with warnings according to the Nærstad model in both June, July and August, compared to the previous years. The conditions were more favourable for blight in the south east (close to the coast) compared to the eastern parts in the inland, especially in July and August. It was a good year for potato producers, with frequent rain but not very high amounts in most areas. The producers managed to keep late blight under control despite these conditions.

Sweden
2015: The blight epidemics started around mid to late July in south and mid Sweden, which is normal. Low temperatures restricted the attacks, which stayed at low to moderate levels during the season. Use of fungicides resulted in full control, and organic potato stayed mostly free of blight.

2016: The blight epidemics started around mid to late July in south and mid Sweden, which is normal. With the exception of a wet period in late June after which infected fields were reported, the season was very dry resulting in limited problems with blight. Normal fungicide use gave a very good disease control.

Denmark
2015: First observation of blight was recorded on 9 June in the south of Jutland on young plants (BBCH 30) probably infected by oospores. Inoculum sources in Mid-South Jutland were identified as oospores, dumps, volunteer plants and infected tubers. The milder winters increase the risk of attacks from dumps and volunteer plants in Denmark. Infections from oospores in the soil is causing early attacks of late blight in Denmark especially in starch potatoes with narrow crop rotations. Most growers do spray on a weekly schedule and vary the dosage according to the need as calculated by the Danish DSS. Slightly more blighted tubers in ware potatoes than previous years. Wet conditions during harvest of starch potatoes. Due to a change in tax regulation on pesticides, compounds with mancozeb are not attractive for the growers to use anymore. This has caused a shift in the use of compounds to Revus and Ranman and growers are facing the challenge to optimise an integrated control of both late blight and early blight.

2016: Dry and warm May resulted in early crop emergence. First observation of blight was recorded on 2 June in Central Jutland on young plants of the starch variety Eurogrande (BBCH 25) probably infected by oospores. Similar to 2015 early attacks were reported for starch potatoes with narrow crop rotations in the central-south of Jutland and inoculum sources is a mixture of oospores, volunteers and infected tubers. The summer was warmer than normal and

with more rain than normal across June, July and August followed by a dry spell in September. The weather based risk for late blight was high during the whole season. Active blight was found in many fields and Proxanil was heavily used and with good effect this year. Due to blight risk, infected fields and rapid new growth it was necessary to decrease to 5 day spray intervals in June and July in some regions (compared to 7 day intervals). Increase in the potato area from 40.000 ha in 2013, to 46.000 ha in 2016, mainly increase in starch potatoes (26.000 ha in 2016). Yield is mean level for all types of potato, but potatoes with narrow crop rotations do senesce earlier and have relatively lower yields than other fields, mainly due to more problems with soil-borne diseases. Two varieties for organic potatoes were tested with good blight resistance – Anouk and Alouette.

France
2015: After a winter with mild temperatures and high rainfall, potato planting was possible at the end of March and at the second part of April after a fifteen days rainy period. A very dry and hot period followed after emergence in May up to the middle of August. The late blight pressure was very low and started very late in August with a medium level. No significant late blight outbreaks were observed in the fields in the country, except in covered and early planted potatoes in Brittany (mostly in May).

2016: After a winter with very mild temperatures and medium rainfall, potato planting was possible from the end of March to the middle of May (until June in some flooded areas). A very exceptional cold and wet period followed after planting in May and June (except in Brittany), and the crop development was delayed for 2 or 3 weeks. The late blight pressure started late in May or early in June and was extremely high up to early of July. After the middle of July, the late blight pressure decreased to medium to low level, according to the areas. The first late blight outbreaks were observed from the end of May on a cull pile to the beginning or the middle of June in fields according to the regions.

Belgium
2015: Due to a mild winter with only a few frost periods, not that much leftover tubers were affected. By the end of April, the first diseased plants were found on different cull piles. Although the number of such inoculum sources continued to rise in the following weeks, the risk of spreading of late blight was low, due to the prevailing dry and sunny weather. The month of June was even drier, with lots of sunshine and very little rainfall, and very unfavourable for the disease. On top of that, a heatwave in the first half of July completely stopped the development of late blight. It was not until the end of August, with the resuming of more substantial rainfall, that late blight attacks were again observed in the fields, requiring sufficient protection against tuber infection.

2016: After – once again – a mild and wet winter, the first diseased potato plants were reported on 20 April, on different cull piles. Cold temperatures hampered the development of late blight during the month of April. From the second half of May however, the increasing number of inoculum sources in combination with very favourable weather for late blight, led to a high disease pressure in the period of emergence of the ware potato crop. High rainfall towards the end of May did not help very much, and made a good crop protection mandatory from emergence forward. This protection was very much hampered or even made impossible by the abundant rainfall during most of June, which also caused flooding and crop losses. The combination of continuous infection weather, active lesions and inoculum sources, a strong crop

growth and sometimes inaccessible fields, caused an enormous spread of the disease. Spray when and wherever feasible, was the only possible advice to give. From July on, disease pressure subsided somewhat, although the numerous stem lesions remained a concern and defined the choice of fungicides. Yet, the mostly warm and dry summer weather from then on, eventually brought the late blight situation back to normal. A late heatwave towards the end of August turned out to be the herald for an exceptionally warm and dry September, with low risk of tuber infection. The harvest of the potatoes was delayed until the second half of October.

The Netherlands

2015: After a winter without a real frost period the potato season was not really early. After a wet decade at the end of March, most potatoes were planted during the second half of April. Temperatures during spring were average, although May was a little bit colder than normal. After emergence of the crop a dry period started and lasted till half of July. Many field were irrigated. The second half of August and the first weeks of September were very wet. Harvest of the ware potatoes was rather late and started at the end of September. The first reports (South-West and North-West) of late blight on dumps and volunteers were already in May. But in the field the disease was hardly found until half of August. Until the end of the season there were no real blight problems in 2015.

2016: Potatoes were planted during a long period of time across the country. In the Southern part of the country most growers were able to plant at a normal time (April) but to the north most fields were planted in May. The last three weeks of June were very wet. In some regions the total amount of precipitation in June exceeded the 250 mm. Very favourable weather for late blight during a period of a fast growing crop! After first reports in May of blight on dumps, infestations in the field were found all over the country after the first weeks of June. In July the disease pressure decreased due to the weather. After changeable weather during the first two decades of August there was a remarkable sunny and hot period at the end of that month and in September.

Germany

2015: Crops were planted in good conditions and the crop emergence was normal between 10 May and 25 May. The first outbreak of late blight in potatoes was recorded by mid of May in plastic covered potatoes. Attacks in different regions and ware potatoes were found beginning of June. The weather conditions for the development of late blight was completely different. The Northern part of Germany had a severe late blight epidemic. On the other hand there was an extremely hot and dry summer in the Southern part of Germany. This resulted that even in untreated control plots no late blight progression was detectable. Overall the number of fungicide treatments was normal. All kinds of products were used. Attacks of early blight (*A. solani*) seem to be an increasing problem in the Northern part of Germany.

2016: The weather condition and the disease development were diverse across the country in 2016. The first late blight outbreak was reported mid of May in the early potato production areas (covered crop). Attacks in conventional fields were found 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. The use of fungicides was high in the Northern part and normal in the South. All kinds of products were used; especially mixtures were used in the Northern part of Germany.

Scotland

2015: The first late blight crop outbreak was in postcode AB30 on 8 July. There were only 19 confirmed outbreaks reported on the AHDB Potatoes-funded blight outbreak maps for Scotland. The progression of crop outbreaks (14 in number) in Scotland was 0% in May, 0% in June, 42.9% in July, 50% in August and 7.1% in September There were two confirmed outbreaks on outgrade piles of potatoes (25 July, 11 August) and three outbreaks on volunteers (31 July [2 cases], 7 August). The last sample was submitted on 24 September.

2016: The number of outbreaks of late blight in Scotland was intermediate. The first outbreak was an outgrade pile on 16 June in postcode IV8. The first crop outbreak was in postcode PH13 on 11 July. Forty-five confirmed outbreaks were reported on the AHDB Potatoes-funded blight outbreak maps, up until 23 September 2016 when the last sample was submitted. The progression of crop outbreaks (36 in number) in Scotland was 0% in May, 0% in June, 38.9% in July, 44.4% in August and 16.7% in September. There were two confirmed outbreaks on outgrade piles of potatoes (16 June & 22 August) and seven outbreaks on volunteers (22 July, 13 September [3 cases], 23 September and 28 September [2 cases]).

England & Wales

2015: Thirty-seven outbreaks of late blight were reported as part of the AHDB Potatoes funded Flight against Blight outbreak maps. Epidemic onset was late, with the earliest outbreak in England and Wales reported on 1 July near Portsmouth on the South Coast of England. Six outbreaks were reported in July, 22 in August, 9 in September and 1 in October. The majority of these were in commercial crops, however, 5 outbreaks were on volunteers, 1 in August, 3 in September and 1 in October. One outbreak was from an outgrade pile in August. Fungicide programmes were well underway by the time the epidemic started so control was generally good across England and Wales. According to the UK pesticide usage survey report 263 using 2014 figures, 98.4% of ware crops were treated with fungicides with an average of 12 applications per crop. The most frequently applied active ingredients to ware crops were mancozeb + cymoxanil, fluazinam, cyazofamid, mandipropamid and cymoxanil. For seed crops, all those surveyed were treated with fungicide and received an average of 9 fungicide applications. The most frequently applied active ingredients were cymoxanil, cyazofamid, fluazinam, cymoxanil + mancozeb and mandipropamid.

2016: One hundred and thirty-eight outbreaks of late blight were reported as part of the AHDB Potatoes funded Flight against Blight outbreak maps. Epidemic onset was late June with the earliest outbreak in England and Wales reported on the 26 May in the South West of England. Two outbreaks were reported in May, 50 in June, 60 in July, 14 in August and 2 in September. The majority of these were in commercial crops, however, 5 outbreaks were on volunteers (1 in June, 3 in July and 1 in August). One outbreak was from an outgrade pile in June. According to the UK pesticide usage survey report 263 using 2014 figures, 98.4% of ware crops were treated with fungicides with an average of 12 applications per crop. The most frequently applied active ingredients to ware crops were mancozeb + cymoxanil, fluazinam, cyazofamid, mandipropamid and cymoxanil. For seed crops, all those surveyed were treated with fungicide and received an average of 9 fungicide applications. The most frequently applied active ingredients were cymoxanil, cyazofamid, fluazinam, cymoxanil + mancozeb and mandipropamid.

Ireland

2015: The season was a low late blight season with only a small number of reported outbreaks. This was mostly due to weather conditions unfavourable to the development of late blight during the summer months – dry and warm early in the summer followed by wet but relatively cold conditions following. Where outbreaks of late blight did occur the application of preventative and curative fungicide chemistry ensured these outbreaks were kept under check. Low incidences of tuber blight were reported. The Irish *P. infestans* population continues to be dominated by three clonal linages EU13_A2, EU8_A1 and EU_6A1.

2016: Outbreaks of late blight were reported by the end of June, with weather conditions in late May and June favourable to the development and spread of late blight. However the spread of these outbreaks was kept in check through the use of both preventative and curative fungicides. Even though weather conditions continued to favour development of late blight no significant outbreaks were reported. This is mostly due to the prophylactic application of fungicides, in most cases at seven day intervals. Low levels of tuber blight were reported. Again as in previous season the Irish population continues to be dominated by three major clonal linages, although the proportions of each do change between seasons.

EARLY ATTACKS OF LATE BLIGHT

In 2015 weather conditions in the beginning of the growing season were not favourable for late blight development except in Germany, France and Switzerland where the early attacks resulted relatively quickly in infected conventional fields. In some other countries first attacks were found early (Belgium, Netherlands, Sweden) but because of unfavourable weather conditions for late blight it lasted until July or even August before conventional fields were infected. In conclusion, the year 2015 was not an overall blight year in Europe (Figure 1 and 2).

In the 2016 season the favourable weather conditions for late blight development caused early attacks in many countries. In most countries followed quickly by infections in conventional fields (Figure 3 and 4).

Comparing the date when attacks were recorded in 5 or more conventional fields for 2015 and 2016, in 12 out of 17 countries attacks were earlier in 2016 than in 2015 (Figure 5).

Figure 1. *Date of first observation of late blight in more than 5 conventional, normally planted potato fields, 2015*
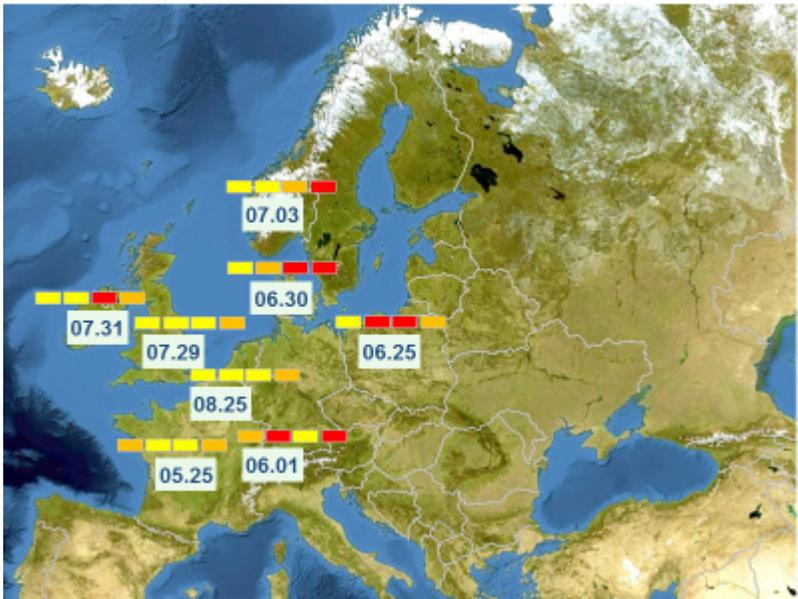
Figure 2. *Blight weather in May, June, July and August 2015. Low (yellow), medium (orange), high (red) risk*

Figure 3. *Date of first observation of late blight in more than 5 conventional, normally planted potato fields, 2016*
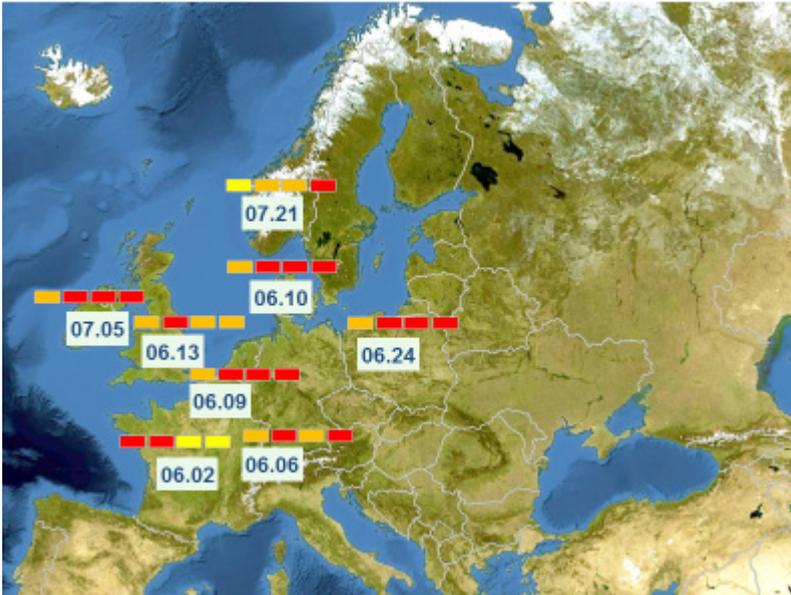
Figure 4. *Blight weather in May, June, July and August 2016. Low (yellow), medium (orange), high (red) risk*
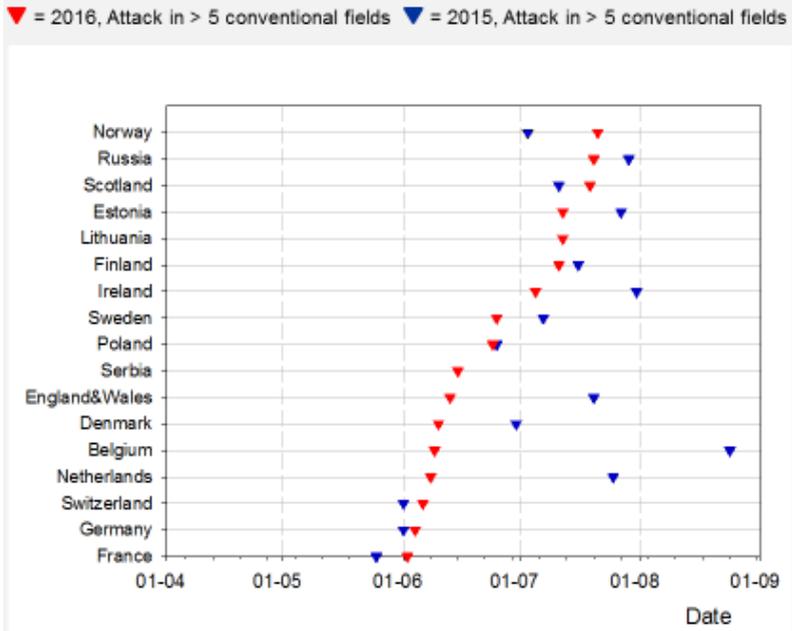
Figure 5. *Date when attacks were recorded in 5 or more conventional fields in 2015 (blue triangles) and in 2016 (red triangles).*

TUBER BLIGHT IN 2015 AND 2016

The level of tuber blight in 2015 was reported as low in all countries in Europe, except for some regions and some type of potatoes in Finland, Russia, Latvia and Germany where it was reported as medium. In 2016 the situation was similar in most countries but in some countries the problems were high (Finland, Latvia, Switzerland) (Figure 6).

Figure 6. *The level of tuber blight attacks (low, medium or high) in 2016 compared to normal*

INDICATIONS OF OOSPORES

In both 2015 and 2016, infections caused by oospores were reported in Sweden, Finland, Denmark and Lithuania (Figure 7).
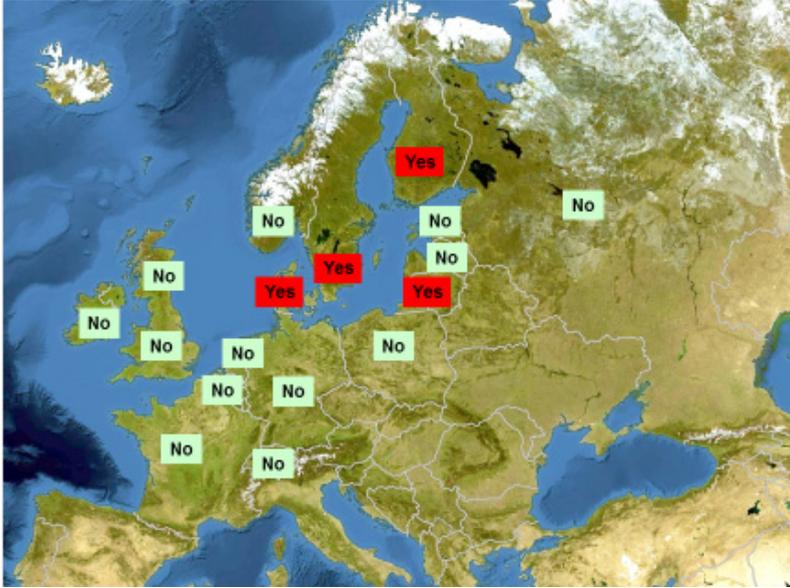
Figure 7. *Indications of oospores in Europe in 2015 and 2016.*

FUNGICIDES AND CONTROL STRATEGIES

In **Estonia** the first sprays were conducted in the middle of July 2015, in 2016 at the end of June. Fungicides were applied up to five times per season. Most commonly used active ingredients were propamocarb, mandipropamid and mancozeb. Most frequently used fungicides were Glory, Infinito, Revus and Dithane NT. In **Latvia** the number of fungicide applications in ware potatoes ranged between 3 and 5 (2015), 4 and 8 (2016). The conventional farmers do not wait until first symptoms appear. Always used systemic + contact fungicide. The main fungicides applied in ware and seed potatoes are: mancozeb, mefenoxam, propamocarb, dimethomorph, fluazinam, fluopicolid, cyazofamid and mandipropamid. In **Lithuania** on average four to six fungicide application is a common practice. First and sometimes second applications are done by contact fungicides, followed by two applications with systemic fungicides. The last one or two applications of the season are with contact or translaminar fungicides. In **Russia** the total number of fungicide treatments in 2015-2016 varied from 2-10. Farms producing potatoes for chips use fungicide applications more frequently than other potato-growing farms. The owners of allotment gardens use no fungicides. In **Poland**, the number of treatments for late blight in the plantations of general purpose was 1-5. On French fries dedicated plantations: 8-12, depending on the severity of the disease. The most common model of controlling late blight was a chemical protection of plants until the height of 15-20 centimeters with further continuation. This allowed performing 1-2 preventive treatments. A higher number was applied in the plantations dedicated to chips and French fries (2-

3). The most commonly applied active ingredients were: propamocarb-hydrochloride in combination with fenamidone or fluopicolide, metalaxyl + mancozeb, metalaxyl-M + mancozeb, cymoxanil + mancozeb. In **Serbia** in 2016, the most frequently used active ingredients for late blight control were: propineb, propamocarb-hydrochloride + fenamidone, cymoxanil, famoxadone + cymoxanil, fluazinam, metalaxyl + mancozeb, cyazofamid, mandipropamid + difenoconazol, metiram. The number of spraying were six to ten applications. In **Switzerland**, farmers control late blight by fungicide applications. At the beginning of the season, systemic fungicides are often used, afterwards they use protective or translaminar (or both combined) fungicides depending on the weather conditions and the late blight epidemic pressure. Farmers obtain such recommendations by their plant protection officer, the DSS PhytoPRE or the newspaper. In organic potato production, copper products are often used to control late blight (max. 4 kg/ha/year). There is also a PhytoPRE version for organic 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**, fungicide applications are started usually during the first week of July and continued to the beginning of September. Normally 5 to 8 applications are needed to keep late blight in control. Each season there are great regional differences in blight risk and therefore the number of applications is very variable between farms and geographical regions. In **Norway**, 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 based on warnings. 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. The number of sprays can be estimated to be about normal in 2015 and 2016. In **Denmark**, the main fungicides are Ranman Top (0.5 l/ha) and Revus (0.6 l/ha) in the standard spray programs starting last part of June. To a lesser extent Banjo Forte is also used. In situations where there is a need for curative action Proxanil (2.0 l/ha – 2.5 l/ha); propamocarb + cymoxanil) is used in combination with a protectant fungicide (Ranman Top or Revus). In **France**, in 2015, due to a very low late blight pressure, growers achieved a fair control right after emergence with contact fungicides. Later on, because the disease pressure was staying very low, growers were able to continue with longer delay between fungicide applications of simple protectant products. Very few translaminar and curative products have been used. The 2016 season was very different since late blight pressure was high starting at emergence. Short intervals of 4 to 5 days, with protectant and curative fungicides and products with efficient rainfastness were needed. Later in the season translaminar and curative activities of the fungicide applications were looked for in order to protect the crop. Important rainfalls and stormy rains in June conduced to difficulties for entering with tractors in some fields and some delayed treatments. In **Belgium** in 2015, the average number of fungicide applications (susceptible variety, mainly Bintje) was 13, which corresponds with an average interval of 8,8 days for the growing season (from min. 6 tot max. 14 days interval). In 2016, the average number of fungicide applications (susceptible varieties, mainly Bintje and Fontane) was 16, which corresponds with an average interval of 6,9 days for the growing season (from min. 4 tot max. 9 days interval). It has to be mentioned, however, that for the season 2016 (a) some applications could not be carried out when necessary, due to inaccessible fields in June and (b) as a result, more tank mixes (i.e. different commercial products) than usual were applied when conditions allowed again for spraying. In **the Netherlands**, most growers are using three or four different fungicides during the season. Staring with Acrobat, Curzate, Valbon or Revus followed by Infinito, Banjo Forte, Canvas and Ranman Top. On an average use of about 14 sprays over the years, in 2016 many growers spayed two times more. Farmers are growing more potatoes in

acreage over the years. Most of them use a DSS to support them in decision-making when to spray. But especially the bigger farmers don't differ much from a standard schedule. In **Germany** in 2015, the average number of fungicide applications was between 6 in the South and 12 in the North. In 2016, 7 to 8 spays in the South and 12-15 sprays in the North were necessary to control late blight. For the first application a systemic or local systemic fungicide was used. Then local systemic products (e.g. Revus, Revus Top, Infinito, Acrobat Plus, Valbon) were used. After flowering Ranman Top, Shirlan and fungicides containing mancozeb were commonly used. The spraying interval was according to DSS systems. In **England & Wales**, according to the most recent report available (UK pesticide usage survey report 263 using 2014 figures), 98.4% of ware crops were treated with fungicides with an average of 12 applications per crop. The most frequently applied active ingredients to ware crops were mancozeb/cymoxanil, fluazinam, cyazofamid, mandipropamid and cymoxanil. For seed crops, all those surveyed were treated with fungicide and received an average of 9 fungicide applications. The most frequently applied active ingredients were cymoxanil, cyazofamid, fluazinam. Cymoxanil/mancozeb and mandipropamid. Most fungicides are applied at a maximum of 7 day intervals. In **Scotland**, fungicide use and control strategies were similar to previous years. In **Ireland**, all commercial crops are subject to intensive fungicide control strategies, with fungicides applied to most crops from early rosette through to desiccation at seven day intervals. Only in prolonged periods of dry weather will the intervals be stretched to 10 days. Growers utilise all available chemistries. The selection of a fungicide is based on the development of the crop and the characteristics of the fungicide. Agronomists utilise the EuroBlight fungicide table to inform them of the different characteristics of a fungicide. The addition of cymoxanil to routine applications is becoming increasingly frequent especially under high pressure conditions.

POPULATION CHARACTERISTICS

In **Estonia**, in 2015 no large-scale monitoring was conducted. The small number of samples that were collected were tested all as mating type A1. In 2016 the monitoring showed the results of A1 being the dominant mating type with almost 75% of the population. In **Lithuania**, the last research about pathogen characteristics was done in 2012 by Runno-Paurson et al. (2015). Since that time further activities were not performed. In **Russia**, the majority of the studied *P. infestans* isolates, collected from potato fields, were of the A1 type (70%); the A2 type was reported only within 30% of the total number of isolates. All isolates were identified as of complex races (5-11 virulence genes). The majority of regions were characterized by phenylamide-sensitive isolates, except the Sverdlovsk region (>40% of phenylamide-resistant isolates). The Russian population is very diverse, most of the genotypes are unique and were not recognized by the SSR genotyping with common primers. In **Poland** in 2015, the symptoms of late blight on stems were registered for the 19% of investigated plantations. In one case, infection developed in the bottom of the plant 65 days after planting, and four days prior to symptoms on the leaves. The most common site of infection on the stems was the middle part of the plant and its apex. In 2016, symptoms of late blight on the stems was noticed on 36% of the observed plantations. The most common place of infection was the middle part of the plant and its apex. Recent data indicate that most of the *P. infestans* populations in **Germany** have a latent period between 48 to 72 hours. In **Switzerland** in 2015, a survey concerning *P. infestans* mating types and fungicide resistance was started. As the late blight pressure was only weak in 2015, only 20 isolates could be examined. 15 isolates belonged to mating type A2, three were mating type A1, one was infertile and one could not be clearly assigned. In general, a shift in mating type A2 was registered from 4% in 1997 (Knapova & Gisi, 2002) to 65% in 2007 (Gisi et al., 2011). In the small data set from 2015 the ratio of A2

raised to 75%. Isolates from the eastern part were more aggressive than those from the western part of Switzerland. 60% of the isolates were allocated to the genotype Blue 13 (EU_13_A2). Based on a MSN analysis the Blue 13 isolates were grouped close together which refers to a clonal reproduction. All isolates were sensitive to mandipropamid, almost all Blue 13 isolates were resistant or intermediate to mefenoxam. 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 2016 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 **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 are very important as an inoculum source. The population in **Denmark** is very diverse and proportion of the population is sexual recombining and forming oospores that give rise to early epidemics. In 2013 16 MLG out of 16 samples. In 2014 32 MLGs out of 32. In 2015 44 out of 60. Interestingly, a new clone appeared in Denmark in 2014 and 2015 – that was not yet recognised by EuroBlight – and given a name. The Mlg 54 group consists of 19 identical MLGs. Similar holds true for the other Nordic and Baltic countries – divers populations and no shared MLGs between countries. In **Norway**, the population of *P. infestans* is very variable with few dominant clones. In **England & Wales**, *P. infestans* genotypes 6_A1 and 13_A2 continue to be the dominant genotypes identified on samples taken from affected field sites which have been collected as part of the AHDB Potatoes late blight monitoring project across England and Wales. In 2015, the proportion of these genotypes was approximately 45% 6_A1 and 20% 13_A2. In 2016, the proportion was nearly 60% 6_A1 and 20% 13_A2. In **Ireland**, the population continues to be dominated by three clonal linages. The frequency of each of these changes between seasons. All three linages can be found in any given field.

USE OF DSSs
Several decision support systems for late blight forecasting and control are used in Europe (see Table below).

Country	DSS
Belgium	Improved Guntz-Divoux
Denmark	Blight Management
England, Wales, Scotland	Blight-Watch (Hutton criteria), Plant Plus & BlightCAST
Estonia	Estonian Crop Research Institute
Latvia	Plant Plus on some commercial farms
Finland	National Resource Institute: general LB warnings
France	Mileos®
Germany	PhytophthoraModel Weihenstephan, ISIP
Netherlands	Prophy, Plant Plus, Akkerweb (WUR model)
Ireland	Met. Service based on Irish rules (Bourke)
Norway	VIPS (Nærstad model)
Russia	Plant Plus, VNIIFBlight, Agrodozor
Sweden	Plant Plus, Blight Management (DK) & VIPS (NO)
Switzerland	Bio-PhytoPRE, PhytoPRE

ALTERNARIA 2015 & 2016

For long time *Alternaria* spp. was a minor problem in North and Western Europe. Since some years, more and more countries report an increasing occurrence of early blight in the fields. In the years 2015 and 2016 several European Countries observed severe infections with *Alternaria* spp.

EB disease observation and EB disease progress

The date of first observation of early blight symptoms in field trials 2015 is shown in Figure 8. The first symptoms occurred mid of June in Poland and Germany to mid of August in Belgium. In most regions one or two weeks later the disease epidemic started (Figure 9).

In 2016 the EB situation in Sweden and Belgium was similar to 2015. In Germany and Poland, the early blight infection occurred 2016 earlier than the year before (Germany: beginning of June, Poland end of May), but interestingly in most of the countries the epidemic started later than 2015. (Figure 10)

In Table 1 the EB specific disease development from May to September in different countries is shown. Till end of July in most European countries 2015 and 2016 the disease severity in the fields was lower than 20%. Only in Poland and Germany the disease severity was between 20 and 50% at this time. In several countries the EB disease progressed in August and reached more than 50% in Denmark, Germany and Poland.

EB: Identified Alternaria species

In most countries the *Alternaria* subspecies *Alternaria solani* and *Alternaria alternata* were identified on infected potato leaves (Tab. 2). In Denmark and Serbia only *Alternaria solani* could be detected. Overall the dominating species during the disease epidemic in 2015 and 2016 was *Alternaria solani* in most European countries.

Fungicide usage and fungicide resistance

The following active ingredients were used in different countries to control EB: mancozeb, azoxystrobin (QoI), chlorothalonil, boscalid (SDHI), pyraclostrobin (QoI) and difenconazole. According to the regional registration also mixtures of these active ingredients are registered. QoI´s and SDHI´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 QoI's has been reported for *A. solani* in potato (Pasche et al., 2004) and for *A. alternata.* The monitoring data from 2015 confirm the data from the previous year that in Germany, Belgium, Netherlands, Poland and Sweden the F129L mutation in *Alternaria solani* is very dominant. Additionally, in Austria, Denmark and Serbia F129L mutants were found in 2015 and 2016. The G143A mutation in *Alternaria alternata* was identified in isolates from Germany, Netherlands and Sweden.

SDHI mutants were found in Belgium (Landschoot et al., 2017) and Germany.

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

Figure 8. *First observation of early blight in 2015 in Europe*

Figure 9. *Start of the early blight epidemic in 2015 in Europe*

Figure 10. *Start of the early blight epidemic in 2016 in Europe*

Table 1. *EB specific disease severity 2016 in different European countries*

	disease severity				
	May	June	July	August	September
Finland	Low	Low	Low	Low	Low
Norway					
Sweden	Low	Low	Low	Low	Medium
Denmark			Low	Medium	High
Estonia					
Lithuania					
Scotland					
Northern Ireland					
England and Wales					
Belgium	Low	Low	Low	Medium	Medium
Netherlands	Low	Low	Low	Medium	Medium
Germany	Low	Low	Medium	High	High
Poland	Low	Low	Medium	High	High
Switzerland					
Czech republic					
France	Low	Low	Low	Low	Low
Serbia	Low	Low	Low	Medium	Medium
Russia		Low	Low	Medium	Medium

Legend:
- Low < 20%
- Medium 20 - 50%
- High > 50%
- no data, no eb

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

	A. solani	Jun	Jul	Aug	Sep		A. alternata	Jun	Jul	Aug	Sep
Finland	√			√	√						
Norway	√			√	√						
Sweden	√			√	√		√		√	√	√
Denmark	√		√	√	√						
Estonia											
Lithuania											
Scotland											
Northern Ireland											
Ireland											
England & Wales											
Belgium	√		√	√	√		√		√	√	√
Netherlands	√		√	√	√		√		√	√	√
Germany	√		√	√	√		√	√	√	√	√
Poland	√		√	√	√		√	√	√	√	√
Switzerland											
Czech republic											
France	√			√	√		√		√		
Serbia	√	√	√	√	√						
Russia	√			√	√		√		√	√	√

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IPMBlight2.0 :
using pathogen population information to improve late blight control

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INTRODUCTION

Controlling Potato Late Blight (PLB), caused by the pathogenic Oomycete *Phytophthora infestans,* remains a major challenge in Europe and worldwide. Recent estimates indeed rate the total cost of late blight at some 900 M€ a year in Europe alone, including the cost of losses to the disease and that of control measures enforced by growers and the whole potato industry (Haverkort et al., 2008). Several features in the biology and epidemiology of *P. infestans* qualify it as a re-emerging pathogen in many important potato growing regions of the world (Fry, 2015) and make late blight a definite threat to food security in many developing countries and to farmer income in developed regions.

Currently, late blight control is primarily based on numerous fungicide applications (Schepers et al., this volume). However, this strategy faces increasing concerns. The legislative pressure is strong to limit or ban access to some popular active ingredients, and pesticide use in general (Directive 2009/128/EC on the sustainable use of pesticides). Furthermore, reports of reduced efficacy and/or resistance to some active ingredients, including fluazinam, have been made during the past few years (Nielsen, 2014).

Some of these changes are correlative to large scale, and sometimes rapid genetic changes within European *P. infestans* populations, which prompted the set-up of Europe wide population surveys using FTA cards for sampling and SSR typing to analyse the genetic composition of local samples (Meier-Runge et al., 2014). Although this survey allows to gather rapidly detailed information on the structure of *P. infestans* populations, this information is not currently used to

estimate late blight risk, or exploited in late blight control DSSs. This is due in part to a major shortcoming, namely that the link between SSR genotypes and the phenotypes of the corresponding isolates/populations for biologically important traits, such as virulence to major R genes in potato cultivars, aggressiveness on susceptible hosts, or fungicide sensitivity, remains elusive. This is why the EuroBlight statement from the workshop in Brasov, 2015, recommended i) to continue and expand the monitoring of *P. infestans*, ii) to intensify the phenotyping of important genotypes and iii) that EuroBlight offer to participate in the development of new DSSs, and in the work for adaptation of existing DSSs to IPM2.0 (EuroBlight statement 2015, Brasov).

These recommendations lead to the IPMBlight2.0 project, funded by the C-IPM ERANET funded over the period 2016-2019. This paper will briefly present the objectives, structure and first achievements of this project. Other papers in these proceedings (e.g Hansen et al., this volume) will detail specific aspects of the activities.

IPMBLIGHT 2.0 - OBJECTIVES
The primary goal of IPMBlight 2.0 is to validate the IPM 2.0 concept, with potato late blight as a case study. IPM2.0 is a concept introduced and defined by Kessel et al. (2012) and tested in the DuRPh project (Haverkort et al., 2016). It relies on the fact that the use of resistant varieties are one of the most effective IPM measure (http://euroblight.net/control-strategies/best-practice/), and on the observation that it is as yet underexploited, in part because host resistance is often not stable across many years (Naerstad et al., 2007; Cooke et al., 2011). Although and increasing number of resistant cultivars are now released and made available in all market segments of the potato industry, and despite the existence of modern DSS operated from web platforms or mobile apps, late blight management is still often restricted to a repeated fungicide applications (up to 25 per season in some regions). Developing truly integrated strategies, that will take full advantage of all available options while providing better fungicide, but also host resistance stewardship, are thus needed more than ever. However, simply designing such strategies is not enough: to be sustainable and adopted, they must be tailored to the variability of *P. infestans* populations and their rapid evolution – which is the core of the IPM 2.0 concept. This in turn supposes that pathogen populations be monitored for both genotypes and phenotypes, including virulence, aggressiveness and fungicide sensitivity.

IPMBLIGHT2.0 – PROJECT ORGANISATION
IPMBlight 2.0 was designed around four WP, each dedicated to a specific goal but tightly interconnected (Figure 1).
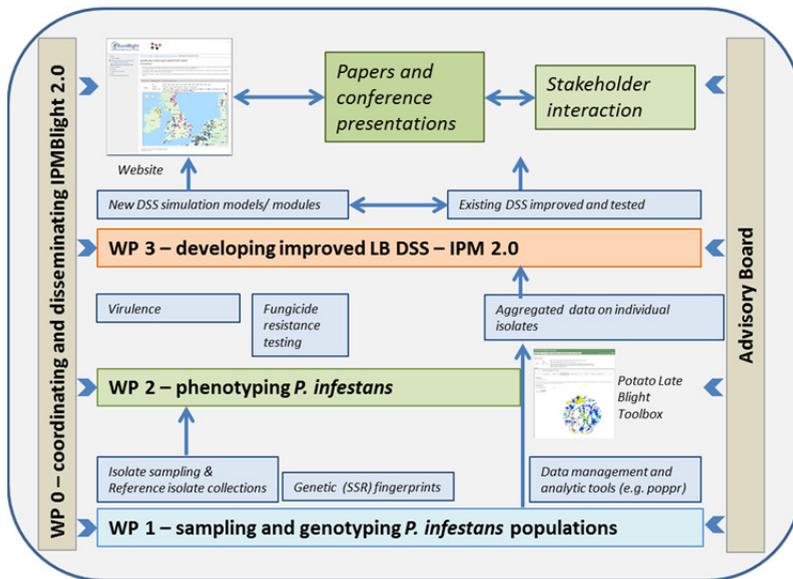
Figure 1. *IPMBlight 2.0 project structure.*

The project sets to analyze genotypic (WP1) and phenotypic (WP2) variation in reference collections of the pathogen sampled from sexual and clonal populations collected in partner countries, and to develop new DSS models while adjusting existing ones to offer risk assessment based on both epidemiological, weather-driven infection likelihood and pathogen phenotypes (WP3). The new DSS modules will therefore be able to best inform tactical choices ('should I spray now?') and strategic decisions ('can I trust this resistant cultivar? how can I adjust my spraying schedule accordingly?) for improved late blight control. WP 0 is dedicated to the administrative tasks, as well as the logistics of the project (meetings, etc.) and dissemination activities.

Confronting data from WP1 and 2 will allow in particular to answer the key question 'are genotypes reliable predictors of phenotypes?'. This is extremely important, since genotyping can now be done quickly and at a rather cheap price, whereas phenotyping biotests are much longer to perform, require the isolations of strains, and are quite costly. Finding a strong connection between genotypes and phenotypes would therefore allow to make rapid assumptions on population composition and characteristics, and hence to adjust in real time the DSS modules or parameters. The fact that many *P. infestans* populations, in particular from Western and Southern Europe, are structured as clones makes this hope plausible. However, IPMBlight 2.0 will also work with presumably sexual populations from Northern and northeastern Europe.

All protocols, data and information will be implemented in the EuroBlight information system, consisting of a Website (euroblight.net) databases and document repositories (for instance for collecting and storing reference protocols). The project therefore complements the annual

Euroblight survey, and provides extra reference material (live isolate collections, phenotypic data, new open-source DSS modules). It will also constitute a platform of reference European laboratories for *P. infestans* epidemiosurveillance and population analysis.

EARLY ACHIEVEMENTS...

Since the project started in the spring of 2016, only data from the first year are available, and not all of these have been processed entirely at the time of writing. Despite this, early achievements include:

- The collection of samples from all five partner countries (France, Denmark, Norway, Estonia, and the UK) confirm that western European populations of *P. infestans* (FR and UK) retain a clonal genetic structure, whereas Nordic populations (DK, EE and NO) appear sexually reproducing, with almost exclusively unique SSR genotypes.
- They also pointed out the presence within clonal populations of a new, apparently emerging clone, designated as 37_A2. The compilation of IPMBlight 2.0 and Euroblight survey data allowed to recognized that this clone, first spotted as an isolated outbreak in the Netherlands in 2013, has now spread to the whole Benelux, Northern France, UK, and some isolated locations in Germany, Switzerland and Adriatic countries.
- The comparison of aggressiveness between major clonal lineages collected within the IPMBlight survey suggest that there is extensive variability within lineages, but that 37_A2 tend to be among the most aggressive isolates present within the sampled populations (Fig 2)
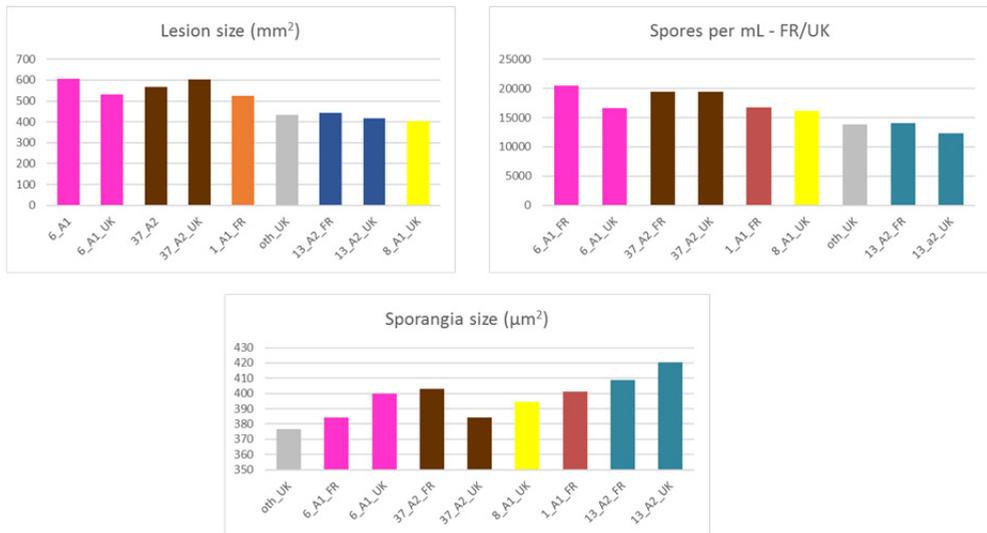
Figure 2. *Comparison of aggressiveness traits in current EU clonal lineages. The emerging 37_A2 clone appears to be highly aggressive.*

- The fungicide sensitivity assays carried out on IPMBlight 2.0 samples provide evidence that fluazinam insensitivity is developing within European populations of *P. infestans*. The data now need to be fully consolidated and matched with genotypic analyses.

- Trap nurseries were established in all partner countries in 2017, and in some of them in 2016, with a new differential host set including both 'historical' R genes from *Solanum demissum* and new resistance sources more recently introduced into breeding lines and cultivars. Again, the data are not fully analysed yet, but they reveal marked variation between hosts and locations.
- Finally, a re-coding of late blight DSSs in MatLab and in depth comparison of their performance with standard weather datasets has been made. A detailed account of this part of the work is given in another paper (Hansen et al., this volume).

… AND PRELIMINARY CONCLUSIONS

One year into the project is of course too early to decide to which extent it has been successful in reaching its objectives. However, the activities and results obtained this far are quite encouraging for the IPM 2.0 approach, on two main grounds:

- They demonstrate the value of an epidemiosurveillance scheme coupling fast genotyping and targeted phenotyping. This was evident in the ability of the network of labs involved in the project to i) spot the emergence of 37_A2 in its first year of real expansion outside its initial cradle, and ii) to generate the first comparative phenotypic data on this emerging lineage, relative to other major European clones. The fact that 37_A2 was identified as a highly aggressive lineage is worrisome. Recent evidence (Schepers et al., 2017) show that this genotype is most likely quite insensitive to fluazinam, which remains one of the staple pesticide used against late blight in Europe and elsewhere in the world. We now have to check whether our own data on the 37_A2 isolates in our collection confirm this insensitivity, and also what is the virulence profile of this lineage. The fast reaction to the discovery of this emerging threat is proof positive that a network of coordinated labs is essential for timely monitoring, but also controlling *P. infestans*.
- They also are instrumental in the development of improved management tools for better risk assessment. The comparison of existing DSS modules serves as the first step to design new software exploiting both the meteorological, but also the population composition data. This will make use of the EuroBlight IT platform, which plays a dual role: data storing and processing on one hand, result dissemination to scientists and end-users on the other hand.

This makes us confident that by the end of the project, this IT platform with enriched functionalities will further contribute to a better, more sustainable control of the late blight disease.

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Recent developments: late blight in Asia - AsiaBlight

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SUMMARY

The formation of a late blight network for Asia, AsiaBlight, was first proposed in 2014. In 2015, it was agreed that the initial activity should be generation of a coarse-scale map of the *Phytophthora infestans* population in Asia. This has been progressing in 2016 and 2017 with the assistance of the Inner Mongolia Potato E & T Center, Hohhot. FTA cards (funded by Bayer) have been distributed from Hohhot to contacts in ten Asian countries who have collected late blight samples and returned them to Hohhot for genotyping. In addition to FTA cards sent out under the auspices of AsiaBlight, late blight samples have also been collected on FTA cards elsewhere in Asia by other researchers. Although AsiaBlight is a project with minimal resources, it has achieved growing recognition, a degree of regional collaboration and limited but successful private-public partnership. The challenges in co-ordinating a late blight network without a dedicated budget across a large geographic region with limited co-operative links and many different potato and tomato regions are discussed and possible future activities considered.

KEYWORDS

Phytophthora infestans, China, SSR, population structure

INTRODUCTION

The success of EuroBlight has inspired the creation of other international late blight networks, notably USABlight and Tizón Latino. Although in recent years there have been a number of publications on the late blight population in Asian countries, relatively few of these reported nationwide studies and very few have used markers allowing comparison with populations in other parts of the world (Forbes, 2015). The aggressive genotype 13_A2 (Blue 13) has been detected in a number of Asian countries including China (Li et al., 2013b) and India (Chowdappa et al., 2015), but the extent of its spread is unknown and the implications of its presence are often not taken into account in late blight management. At a meeting in Nepal in late 2014, organised by the International Potato Center (CIP) with the National Potato Program of Nepal,

25 researchers representing 12 Asian countries agreed that there was a need for coordination of late blight research in Asia. A roadmap to create a proposal for a region-wide network of collaboration on potato late blight was developed and named AsiaBlight. This was further considered at a meeting in China in July 2015 where participants were strongly in favour of such an approach and the generation of a coarse-scale map of the *P. infestans* population in Asia was proposed as an initial activity. This would serve as a baseline for pathogen studies and underpin future endeavours of AsiaBlight to improve on-farm disease management. In September 2015, Indian scientists attending the 3rd International *Phytophthora* Symposium also agreed that co-operation on pathogen population change within AsiaBlight was needed. To progress this, in the absence of any specific funding or staff, in late 2015 Louise Cooke (recently retired from the Agri-Food & Biosciences Institute, Belfast) was approached to act as a voluntary coordinator for the initial AsiaBlight mapping project and started this role in January 2016. This paper reports progress since then.

PROGRESS TO DATE

The initial primary objective of AsiaBlight was to generate a coarse-scale map of the *P. infestans* population in Asia, but additional associated objectives were to demonstrate the potential of Public-Private Partnerships (initially between public sector research institutes and agrochemical companies) and to develop a team spirit among Asian partners in order to promote collaboration for future activities.

ORGANISATION OF SAMPLE COLLECTION

It was agreed that the collection of late blight samples should follow the EuroBlight model, with contacts in Asian countries being asked to collect *P. infestans* DNA from late blight lesions onto FTA cards (Whatman Classic FTA cards, with 4 sampling areas per card, 10-100 cards per country depending on potato production area). A time-line was proposed (Figure 1) which has served as a yardstick for measuring progress and demonstrates that the project is progressing more or less on as projected, albeit with a number of challenges.
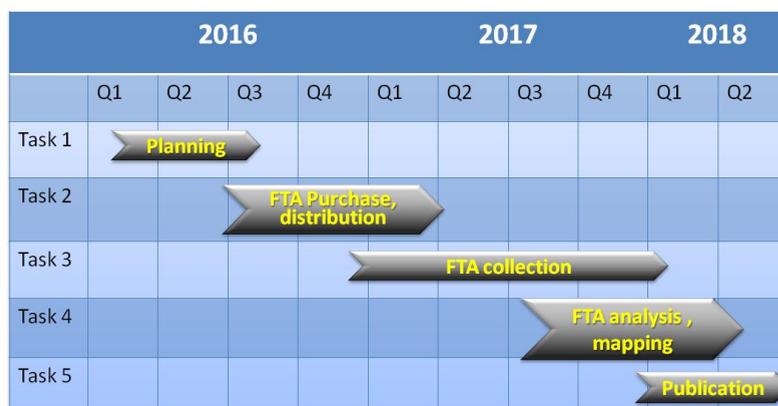
Figure 1. *Time-line for AsiaBlight initial project (a coarse-scale map of Phytophthora infestans in Asia) as proposed in January 2016*

The first challenge was to fund and source FTA cards and determine how they should be distributed. This was organised with the assistance of the CIP Office, Beijing. Bayer (Regions APAC 1 and APAC 2) agreed to fund the purchase of 500 FTA cards (obtained from a supplier in China) and Ruofang Zhang volunteered the assistance of her laboratory and staff in the Inner Mongolia Potato E & T Research Center to distribute the cards and to genotype the resultant *P. infestans* DNA samples.

Instructions for sample collection (based on the protocol developed by EuroBlight) and standard forms for detailing sample information (including site location, host, cultivar and disease level at sampling) were prepared. Contacts were requested to use one FTA card per site and to sample four separate actively sporulating lesions from each site where possible.

COUNTRY CONTACTS AND SAMPLING

Contacts were identified with the assistance of CIP scientists and other researchers. The late blight populations in China and the Republic of Korea were already being investigated in ongoing projects, so these countries were excluded from those to which FTA cards would be sent.

In July 2016, FTA cards, sampling instructions and sample forms were sent from Hohhot to contacts identified and willing to participate in Bangladesh, Georgia, India, Indonesia, Japan, Nepal, Taiwan, Tajikistan, Uzbekistan and Vietnam. Armenia had planned to take part, but the contact there had to withdraw because of changed responsibilities. After the cards had been sent out, it was found that samples could not be collected from Tajikistan and Uzbekistan owing to re-organisations and from India because of biosecurity legislation prohibiting pathogen DNA from being sent out of the country; this resulted in the loss of these cards. During May 2017, additional cards were sent from Hohhot to contacts in Georgia, Indonesia, Pakistan and the Philippines and in July 2017 to Tajikistan (contacts in Indonesia, Pakistan and the Philippines were Bayer personnel).

Initial attempts failed to find contacts able to participate in a number of countries including Armenia, Kazakhstan, Kyrgyzstan, Malaysia and Thailand.

In addition to the FTA cards sent out under the auspices of AsiaBlight, late blight samples were also being collected on FTA cards elsewhere in Asia by other researchers, who submitted them to the James Hutton Institute (JHI) for genotyping and mapping. These included Geert Kessel, Huub Schepers & colleagues from Wageningen University & Research (samples from Bangladesh, India, Indonesia, Myanmar, South Korea, Vietnam), Chris Ursell (samples from Java), Catherine Chatot (samples from Sri Lanka) and David Cooke (samples from Vietnam).

SAMPLE SUBMISSION

FTA cards with sampled *P. infestans* DNA were returned by the sample collectors to Hohhot along with completed sampling forms. Details of sampling and FTA cards returned to Hohhot date (October 2017) are shown in Table 1. Figure 2 shows the locations from which these samples and those collected by other researchers were obtained.

Table 1. *AsiaBlight FTA card sampling of Phytophthora infestans to October 2017*

Country	No. of FTA cards sent and when	Sampling status	Return of FTA cards to Hohhot
Bangladesh	25 (July 2016)	Samples collected from 25 potato crops, January 2017	Cards returned February 2017
Georgia	10 (July 2016) 10 (May 2017)	Samples collected from 10 potato crops, August 2016. Further sampling planned, summer 2017.	2016 cards returned September 2016. 2017 cards not yet returned.
Indonesia	10 (July 2016) 20 (May 2017)	Samples collected from 10 potato crops, October 2016. Further sampling planned summer 2017.	2016 cards returned December 2016. 2017 cards not yet returned.
Japan	10 (July 2016)	Isolates obtained from 10 potato crops (May and August 2016), isolates sampled onto FTA cards	Cards returned December 2016.
Nepal	25 (July 2016), 9 additional cards located locally.	Samples collected from 24 potato crops (November-December 2016, May-June 2017) and 10 tomato crops (February, June 2017).	Cards (34) returned July 2017.
Taiwan	10 (July 2016)	Isolates obtained from tomato crops (December 2014 to August 2016), sampled onto FTA cards.	Cards returned February 2017.
Vietnam	10 (July 2016)	Samples collected from 4 potato crops, February 2017, more samples to be collected later.	Cards (4) returned February 2017.
Pakistan	20 (May 2017)	Cards received.	Not yet returned.
The Philippines	10 (May 2017)	Cards received.	Not yet returned.
Tajikistan	10 (July 2017)	Cards received.	Not yet returned.

Figure 2. *Late blight samples collected in Asia from 2015-2017.*

DNA EXTRACTION AND GENOTYPING

DNA has been extracted from the FTA card samples from Bangladesh, Georgia, Indonesia, Japan, Taiwan and Vietnam and analysed using 12-plex SSR (Li et al., 2013a) with DNA from standard genotypes (provided by D.E.L. Cooke, JHI) included for reference. Some data are missing because of poor quality DNA where the late blight lesions were rather old with insufficient active sporulation. Standardisation of the allele sizing to allow identification of genotypes is in progress, but has proved challenging. A need for support in sizing and naming alleles has been identified and will be the subject of an online workshop by D.E.L. Cooke and possibly also a laboratory visit. For this reason it has not yet been possible to inform country contacts of the *P. infestans* genotypes identified in their samples.

CHALLENGES AND SUCCESSES

A number of challenges have been associated with the development of AsiaBlight:

- Asia is a large, very geographically dispersed and politically disparate region, with many different potato and tomato seasons.
- Co-operative links between Asian countries are limited.
- Countries differ greatly in their organisational structures, political attitudes to regional co-operation, plant health legislation and thus in their ability to participate.
- It has not been possible to identify contacts and get samples from all Asian countries that grow potatoes (or tomatoes) so a partial map will be developed.
- AsiaBlight relies on staff in Hohhot to send out and receive back FTA cards and on the goodwill and cooperation of local researchers to collect samples and return cards at their own expense; transferring cards within the region has often proved difficult and slow.
- In a number of cases cards have not yielded good quality *P. infestans* DNA because the need to sample active lesions has not been fully understood.
- The 12-plex SSR is a complex technique to implement particularly when following published protocols rather than learning hands-on. The two postgraduates who have worked on this in Hohhot have done an excellent job in getting the technique running there, but have moved on. There is a need for continuity and for support in genotype identification to generate publishable results by 2018 (as originally planned).
- AsiaBlight does not have its own funding and is benefitting from voluntary assistance, but providing continuity of funding and personnel is going to be important for the future.

Despite these challenges, there has been substantial progress over the past two years. AsiaBlight is becoming known as the late blight network for Asia and has demonstrated the value of Public-Private Partnerships. Other researchers working on late blight projects in Asia have proved keen to participate and have submitted data for inclusion in the mapping hosted by EuroBlight. Examination of preliminary SSR results from FTA cards sent out in 2016 (by D.E.L. Cooke) has indicated that the *P. infestans* genotype 13_A2 (Blue 13) is widespread in Asia. AsiaBlight has also acted as a stimulus for proceeding with an online virtual Workshop on allele-sizing to be given by David Cooke, which will include not only participants in Europe and China, but also Africa and South America.

NEXT STEPS

The 2017 sample collection is to be completed and all samples collected to date need to be genotyped (requiring some samples to be repeated because of missing data and the need for

standardization of allele sizing and genotype assignment). Only after the genotype identifications have been confirmed in the presence of standard genotype samples will it be appropriate to inform all those who submitted samples of results. It is planned to give AsiaBlight a web presence within the EuroBlight website, however, the co-operators agreement will be sought before genotype data are uploaded to the EuroBlight map. Cooperators will also be involved when results are written up for publication (it is proposed that all who submitted samples should be included as authors on any publication, subject to their agreement), which it was originally hoped would be achieved in 2018.

Several activities have been discussed to enhance AsiaBlight in the future, including i) finding a way to include researchers from India and other important potato-producing countries not sampled, involving more private sector partners, developing a second map with a focus on tomato and holding an AsiaBlight Workshop, the latter most likely to be hosted by China. Should additional significant funding be secured, other research areas are envisaged, such as the development of 'Blight Learning & Innovation Centers' (with field trials of fungicide efficacy, host resistance, pathogen studies and DSS) or the study of pathogen mutations or genotypes associated with reduced fungicide sensitivity.

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Late Blight in the USA – 2015 and 2016

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Late blight of potato and tomato has been sporadically important in the USA. It is not uncommon to have locally important but widely separated epidemics. Regional or national pandemics have been rare, but in the last several decades there have been two widespread pandemics. The first pandemic was caused by the introduction of the US-8 clonal lineage. US-8 is an especially aggressive lineage that is also largely unaffected by mefenoxam. The pandemic was limited to the eastern half of the USA in 1994, but in 1995 this lineage was found throughout all potato growing regions in the USA and also caused major damage in the western potato growing regions of the USA (Fry and Goodwin 1997; Johnson et al., 1997). The second pandemic was in 2009 when the US-22 clonal lineage was widely transported on infected tomato transplants to many locations in northeastern USA in June of 2009. This pandemic 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; Figure 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; Table 1). For example, the most common lineages differ in terms of their response to mefenoxam, a very effective oomycete fungicide against sensitive strains (Fry et al., 1979). Mefenoxam is ineffective against resistant strains (Goodwin et al., 1996; Matson et al., 2015). From the mid-1990s to 2009, most clonal lineages in the USA were largely resistant to mefenoxam (Fry et al., 2015) so growers in the USA 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). Additionally, lineages in the USA differ in terms of their pathogenicity to tomatoes. US-8 and US-24 are not good pathogens of tomatoes, whereas US-11 and US-23 are very good pathogens of tomatoes as well as potatoes. Thus knowledge of the lineage in a particular area provides crucially important information necessary to select the most effective management strategy.

While immigration of isolates into the USA has been the dominant evolutionary force influencing the *P. infestans* population in the USA there is evidence for the ephemeral existence of at least two sexual recombinant populations there. The first was detected in the 1990s in the Pacific Northwest and this recombinant population contained the US-11 clonal lineage (Gavino et al.,

2000). The second recombinant population was detected in New York State in 2010 and 2011 (Danies et al., 2014), but there has been no evidence that any of the individuals in this population has persisted (Figure 1).
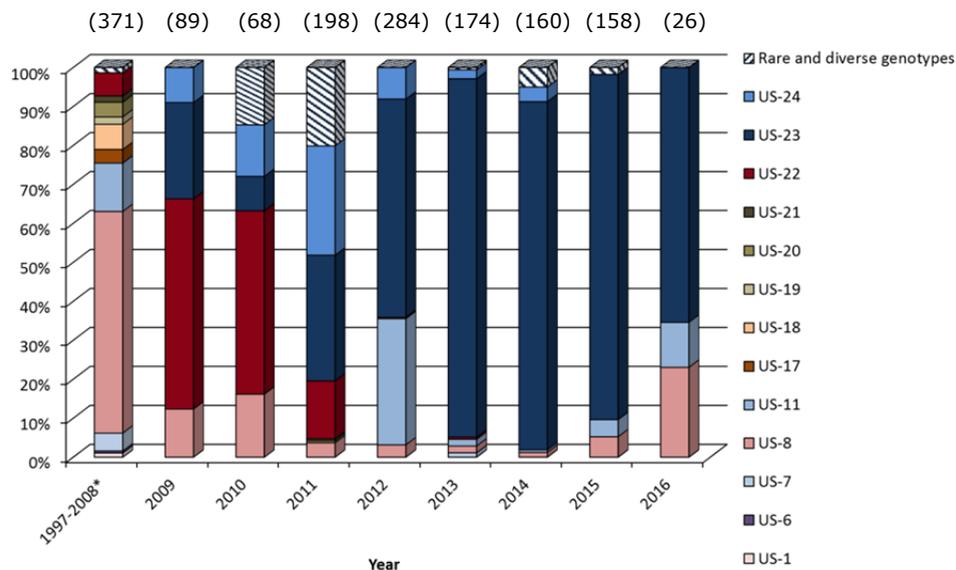
Figure 1. *Clonal lineages detected in the USA from 1997 – 2016. The data for 1997-2008 are from the Fry lab; Hu et al., 2012, and (Wangsomboondee et al., 2002); the data for 2009-2016 are from the Fry Lab, the Ristaino lab and the USAblight consortium. The sample size for each year is indicated in parentheses at the top of each column. This figure was prepared by Giovanna Danies.*

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

Because the populations in the USA has been dominated by so few clonal lineages, and because these lineages have known important different phenotypic characteristics, it is possible and useful to determine the lineage in near real-time. A group of plant pathologists from Florida, North Carolina, Maine, Maryland, New York, Pennsylvania, Washington and Wisconsin have agreed to facilitate the rapid identification of strains causing late blight in the USA. When an outbreak of late blight is discovered, the grower/extension agent, or other personnel send the

sample via overnight courier to Cornell for genotypic (microsatellite) analysis using the system developed by Li et al. (Li Y et al., 2013). The sample is received at Cornell in the morning, processed, sequenced overnight and interpreted the next day. The results can then be sent to the sender the day after receipt of the sample. Thus, the identification of the strain can be learned within 48 hours. Strain identification then informs the management tactics. The reports are also uploaded to the USABlight website (USAblight.org).

The US-23 lineage has dominated the *P. infestans* population in the USA since 2012, including 2015 and 2016 (Figure 1). The weather was quite favorable to late blight in many regions of the USA in 2015, and we received 158 samples from 19 different states, representing all regions of the USA. The vast majority of samples contained only the US-23 clonal lineage. However, US-8 was detected in four states in the Midwest and West. US-11 was detected in the West and US-24 was detected in the East. In 2016, the weather was generally drier over much of the USA, and late blight was much less prevalent, and we received only 26 samples. Again, US-23 was the most prevalent lineage (17 samples from four states). US-8 was again detected in the Midwest and West, and US-11 was detected in the West. (31 samples from the prairies of Canada were all US-23.)

The reasons for the dominance of US-23 over other strains have not been vigorously evaluated. However, two of its phenotypic characteristics could be very important. It is very aggressive on foliage and tubers (Danies et al., 2013), and it is pathogenic on both potatoes and tomatoes (Danies et al., 2013). Chance may also play a role, because this isolate has not been dominant in the Midwest of the USA (but has been prevalent in the prairie provinces of Canada), and it has not been dominant in western USA.

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Recent developments: late blight in Latin America

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Late blight is the most important disease in Latin America causing big productive losses and affecting food security. Therefore a group of researchers have decide to constitute the Tizonlatino network (https://tizonlatino.wordpress.com/), with the objective to share knowledge and protocols about the pathogen, the disease and its management, with the goal to advance in its sustainable control. This group is already performing studies to monitor and characterize the causal agent of late blight in different countries, disease management and control strategies using predictive systems.

Phytophthora infestans was originated in America and co-evolved with its host potato. Today, also new species of Phytophthora have been described in association with Solanaceae, this is the case of *P. andina* (Oliva, et al, 2010, Adler et al, 2004). *P. andina* and *P. infestans* attack potato, tomato and *S. muricatum*, but *S. betaceum* has only been associated with *P. andina* in Ecuador and Perú, while in Colombia, there is a mix of *P. andina, P. infestans* and probably intermediate genotypes (Forbes et al, 2013). On the other hand*, P. infestans* population shows a clonal population in some countries, such us in Colombia, Ecuador, Costa Rica, Peru and Chile, A1 mating type is described associated to potato but in Bolivia and Uruguay, mating type A2 is named. In Argentina, early in the 90's A2 was described in 89% of the population, and later in this decade A2 was the only one detected, but today, A1 is again predominant. Additionally, EC1 are dominant clonal lineage in Colombia, Ecuador and Perú, but in the last one, new lineages have been discovered in very high frequency, while, Chile used to be US1 the main population, but today the predominant is 2-A1, the same genotype described in Argentina. On the other hand, in México many studies has been performed about *P. infestans* characterization, host resistance and control, due to the high variability of pathogen genotypes and the presence of A1 and A2 mating type (Acuña et al, 2016).

Today, much work has been done combining different cultivar resistance and fungicide strategies, suggesting that the use of cultivars with reduced susceptibility to late blight can be managed with reduced fungicide rates and longer application intervals, thus offering more economical control of this disease, saving between 30 to 60% of the fungicide input. Most major genes known until now mainly come from *S. demissum*, however a number of new genes have recently been detected in other *Solanum* spp. such as *S. bulbocastanum*, *S. verucosum, S. stoloniferum, S. papita, S. venturi, S. microdontum, Solanum berthaultii and Solanum*

mochiquense. Much work has been performed introgressing R genes from the Mexican hexaploid species *S. demissum*, however, it is expected that there are more unidentified genes or genes interaction enhancing the resistance, therefore new source of resistance are being studied (Solano, et al., 2016, Solano et al., 2014, Diaz et al., 2003, Gabriel et al., 2007). Moreover, different studies have been developed by CIP about functionality of R genes and effector allele composition in Peruvian populations of *P. infestans* (Lindquist-Kreuzer et al., 2014) and the potato breeding program carried out by INIA Chile, has started a systematic crossing program to develop potato varieties with increased and more durable resistance, using molecular techniques to select segregants carrying multiple genes from crosses using R gene donor genotypes (Muñoz et al., 2015).

In addition, cultural practices are an important part of an integrated management program because it reduces the incidence and severity of the disease epidemic, thereby reducing yield losses and also, sometimes lowering the requirement of fungicides, especially in developing countries, because in these countries both the small scale farming (low yields) and the large scale farming (high yields) coexist (Mizubuti and Forbes, 2002).

Forecasting allows a better control of a disease and a more efficient use of fungicides. Today there is a great amount of weather information available and easy communication systems that makes the forecast an excellent decision support system (DSS) to develop an integrated pest management. These systems use different information and grade of complexity, according to the decisions makers (Acuña, 2007; Perez et al., 2016; Lucca and Rodriguez, 2015, Schepers, 2002, Fry et al., 1983). Some of them do not require technology like Hand-held DSS (HH-DSS) develop by the International Potato Center (CIP) to be used by small farmers in the Andes, which using only observation of the weather by the farmer and management, demonstrated similar performance than Simcast (Perez et al., 2016). Others are slightly sophisticated but easy to use by farmers, such is the case of the ones implemented by Argentina and Chile (Schepers, 2002; Acuña et al, 2007; Lucca and Rodriguez, 2015). In Chile, Late blight DSS is available since 2007, this system, today with 5000 users, utilize only weather information to do the warning, which is delivery to the farmers through a web page, SMS and e-mail (http://tizon.inia.cl). In a survey done about using the system, it shows that 42% of the farmers applied fungicide based on DSS information with 50% less spray compared to a schedule application (Bravo et al, 2016). Similarly, in Argentina an impact study about use DSS demonstrated 33% of monetary saving and spray reduction of 26% (Lucca and Rodriguez, 2015).

Thereby, warning systems are useful tools to develop integrated pest management, but the most important and fundamental is to consider what and how information is delivered to final users, It needs to be simple and easy to understand. Today, Tizon Latino network will start new studies focus in Late blight DSS as a tool to climate change adaptation in Latinoamerica.

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The Hutton Criteria: a classification tool for identifying high risk periods for potato late blight disease development in Great Britain

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SUMMARY

The Smith Period has been the national warning system for potato late blight in Great Britain (GB) for approximately 60 years, and it has not been assessed or revised since its inception. We assessed the performance of the Smith Period as a forecasting tool using Receiver Operator Characteristic (ROC) analysis on historical late blight outbreak- and weather-data from 2003-2014. The Smith Period was found to be a 'fair' diagnostic tool, with significant variation in performance across the different climatic regions of Great Britain. Based on these analyses and a series of controlled environment experiments, we developed a new forecasting system for late blight in GB – the Hutton Criteria. ROC analyses revealed the Hutton Criteria to be an 'excellent' diagnostic tool with a much improved uniformity in performance across GB. The Hutton Criteria have now replaced the Smith Period as the new national warning system for late blight in GB.

KEY WORDS

Phytophthora infestans, Smith Period, Hutton Criteria, Risk Criteria, Decision Support System

INTRODUCTION

Potato late blight remains a significant challenge to potato growers, but decision support systems can be used to inform growers of the risk of disease in order to optimise the timing of fungicide treatments. Each country generally has their own tailored DSSs for potato late blight, developed to suit the needs of their growers and varying in complexity, affordability, required inputs and outputs. The Smith Period is a set of temperature and relative humidity criteria developed in Great Britain (GB) to indicate high risk periods for potato late blight development. It is defined as two consecutive days where the minimum temperature is not below 10°C and there are at least 11 hours each day with a relative humidity ≥90% (Smith 1956-1). The aim of this study was to (1) evaluate how well the Smith Period has performed on recorded outbreaks from 2003 – 2014 across GB and (2) to test other models, guided by historical and experimental research, to determine whether alternative criteria offer significant improvements on the Smith Period.

MATERIALS & METHODS

The AHDB Potatoes funded Fight Against Blight (FAB) campaign has used a network of crop scouts across GB to sample and record potato late blight occurrences since 2003. The samples have been processed and recorded at The James Hutton Institute creating a FAB database of more than 2000 outbreaks recorded from 2003 -2014. Outbreak locations are reported by their post code district to provide anonymity to the grower. The Met Office provided the corresponding daily weather data, for April to September for each year, from a network of synoptic weather stations across GB, interpolated to 652 data points. The data comprised the daily minimum temperature and number of hours of relative humidity ≥90%, and was the data used by the AHDB Potatoes funded 'Blightwatch' system in that period to send risk alerts to growers based on the occurrence of Smith Periods in their post code district.

The potato late blight outbreak data was further subdivided based on nine defined climatic regions of GB (Figure 1).
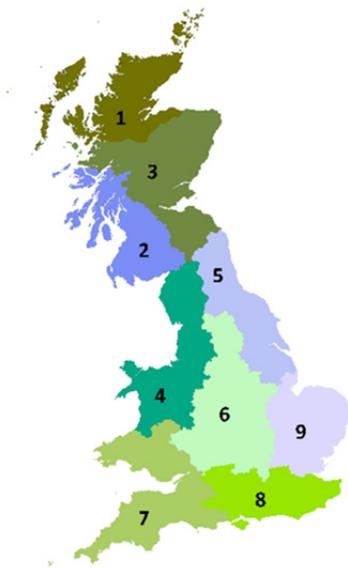
Figure 1.   *Climatic regions of Great Britain used in this study; (1) Scotland North, (2) Scotland West, (3) Scotland East, (4) England North west & Wales North, (5) England North East, (6) Midlands, (7) England South West & South Wales, (8) South East England (9) East Anglia*

Five alternatives to the Smith Period (trial models) were defined based on conclusions from previous controlled environment experiments, historic literature and feedback from industry. Each consisted of two consecutive days of the temperature and relative humidity criteria being met: (1) Min temp 8°C & 11 hours RH ≥90%, (2) Min temp 10°C & 6 hours RH ≥90%, (3) Min Temp 6°C & 11 hours RH ≥90%, (4) Min Temp 8°C & 6 hours RH ≥90%, (5) Min Temp 6°C & 6 hours RH ≥90% (Smith 1956-2, Crosier 1934).
Receiver operator characteristic (ROC) curves are a means of assessing the success of binary classifiers, often used to evaluate medical diagnostic tests (Forman 2002, Fawcett 2004, Heagerty

2000). In our data sets we want to investigate the presence or absence of an 'alert' prior to an outbreak. The data is assembled to provide for each day (day = 1-28) prior to outbreaks the proportion of outbreaks (x) which have received an alert (1) and which have not received an alert (0). Using the series of unique x values for each data set as threshold values we determine a series of false positive and true positive rate pairs to plot from which the ROC curve is constructed. The further to the top left hand corner a point lands on an ROC chart the better as it has a high true positive rate and a low false positive rate, and thus the more and ROC curve pulls to the top left hand corner, the better it's performance. Points and curves falling along the diagonal line of an ROC plot are the equivalent of a diagnostic tool guessing, as the false positive rate is equal to the true positive rate. We calculated the area under the ROC curve (AUROC) and used this to quantify the performance of the alert systems (Fawcett 2004, Forman 2002). As the same outbreak data set is used for the historic analysis and the trial models it allows a comparison of the resultant AUROCs when the only factors to change are the alert criteria. The AUROC is an accepted measure for comparing ROC curves and testing for significant changes by using their values in ANOVA's (Hanley McNeil 1982, Bradley 1997). The curves were calculated in XLStat, MatLab and the resultant data was further visualized and spatially analysed in ArcGIS.

RESULTS

The Smith Period and models 1-5 have general patterns of occurrence across Great Britain which should be considered when interpreting results. The contrast between the Smith Period and model 2 is of specific note (Figure 2). Lowering the relative humidity criteria of the Smith Period produces an increase in the frequency of alerts across the central areas of GB.
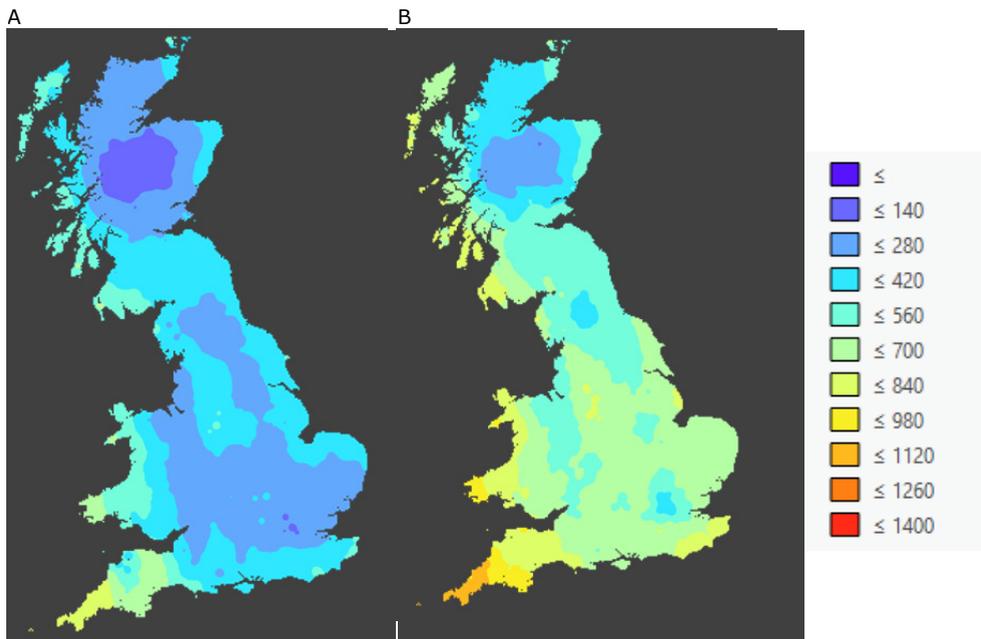
Figure 2. *Inverse distance weighted maps of the occurrence of (A) Smith Periods and (B) Model 2 across Great Britain from 2003 – 2014 from the 1st of April to the 30th of September*

ROC analysis ranks the Smith Period as a 'fair' classification tool with an AUROC of 0.686, and model 2 as an 'excellent' classification tool with an AUROC of 0.973 (Figure 3). AUROC results for the other trial models were 0.823, 0.849, 0.994, and 0.999 for models 1, 3, 4, and 5, respectively. It should be noted that the slight improvement in predictive accuracy for models 4 and 5 came at the expense of a much higher frequency of risk alerts: 7, 10, 16, 12, 24, and 30% of days prior to the reported outbreaks were classified as risk periods for the Smith Period and models 1 to 5, respectively. A comparison of the performance of the Smith Period and model 2 within the nine defined climatic regions showed a much greater spread in AUROC values for the Smith Period than for model 2 (Table 1). An ANOVA revealed highly significant differences in AUROC between regions [$F_{(8, 75)} = 3.62$, $p = 0.001$] and between years for the Smith Period. The results for model 2 did not show highly significant differences for regions.
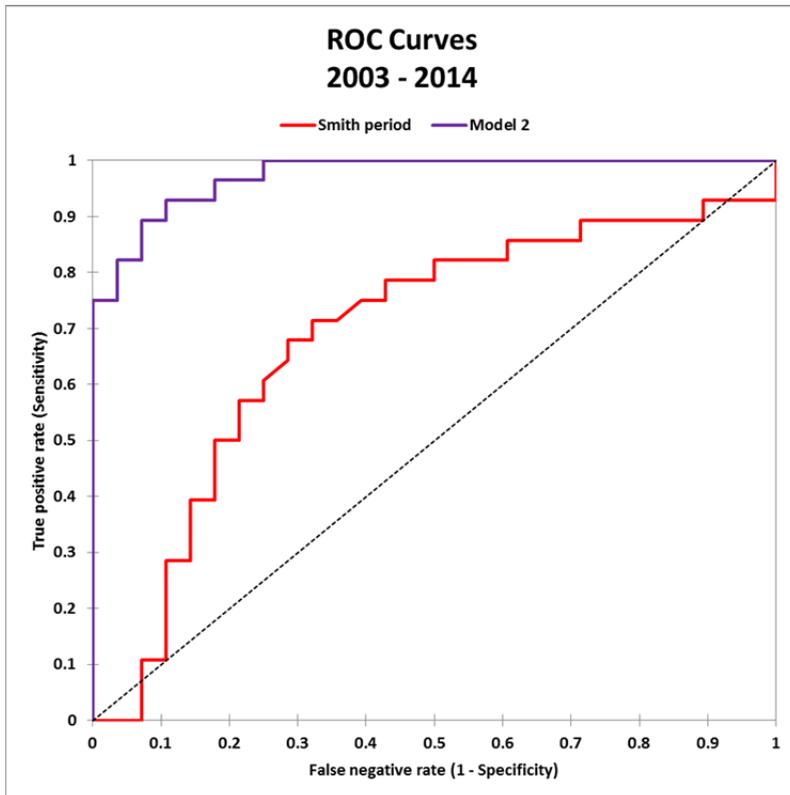
Figure 3. *Receiver operator characteristic curves for Smith Periods (red) and Model 2 (purple). These curves encompass the data for 28 days prior to >2000 reported potato late blight outbreaks from 2003 – 2014 across all of Great Britain. The Smith Period AUROC = 0.686 and Model 2 AUROC = 0.973*

Table 1. *Comparison of Area Under the ROC Curves for the Smith Period and Model 2 from 2003 – 2014 for Each Climatic Region*

	AUROC	
	Smith Period	**Model 2**
All of Great Britain	0.686	0.973
Climatic Region:		
Scotland West	0.225	0.915
Scotland East	0.740	0.946
England North East	0.684	0.976
England North West & Northern Wales	0.443	0.975
Midlands	0.628	0.987
England South West & Southern Wales	0.943	0.996
South East England	0.766	0.977
East Anglia	0.520	0.955

DISCUSSION

Our analyses revealed that the Smith Period has performed well across GB as a whole, but with a large degree of variation between climatic regions. A more uniform performance across GB would be desirable for a national warning system for late blight. It had been suspected previously from growers and evidenced in previous research (Chapman 2012), that the minimum temperature threshold was too high. Results from the trial models show an improvement of the low temperature models over the Smith Period, but not as great an improvement as was originally expected. Indeed there was no significant improvement in predictive performance between a minimum threshold of 8 or 6°C. Our experimental work provided evidence that lowering the relative humidity duration from 11 to 6 hours of ≥90% and maintaining the 10°C temperature threshold would lead to a marked improvement in predictive accuracy, and this was confirmed by the ROC analyses. Of the five trial replacement models, model 2 resulted in a significant improvement in overall predictive accuracy and uniformity in performance across the country, without a large increase in the frequency of risk alerts issued. These conclusions led us to select model 2 (the 'Hutton Criteria') as the replacement for the Smith Period. This growing season (2017) saw the Hutton Criteria implemented as the new national warning system for late blight on the AHDB Potatoes 'Blightwatch' website, and we will be assessing its performance using the above analyses together with the Fight Against Blight outbreak data for the 2017 season.

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Geodata to control potato late blight in Bangladesh

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Late blight (caused by *Phytophthora infestans*) is the most common and highly destructive, fungal disease in potato, tomato and other *Solanaceous* crops in Bangladesh. Annual potato yield losses due to late blight have been estimated at 25-57%. Late blight can be controlled but only by frequent and costly applications of fungicides. Nevertheless, control failures are common due to the challenging local fog periods.

The degree of control primarily depends on the composition of the local *P. infestans* population, the timing of the fungicide applications, crop development and disease pressure. The efficiency of late blight control can therefore significantly improve by informing farmers, in time, on predicted future infection events. In addition, the results from pathogen population monitoring may help farmers to choose the most efficient fungicide.

The GEOPOTATO project is developing and implementing a decision support service (DSS) in Bangladesh for an optimal control strategy of late blight in potato. The DSS will provide farmers with preventive spray advice when a late blight infection event is predicted to occur in the near future. Capacity building on integrated control of potato late blight helps farmers and advisors better understand disease development and management. Pathogen population monitoring has revealed wide spread occurrence of the metalaxyl resistant *P. infestans* clonal line EU_13_A2.

Satellite data are used to downscale weather forecasts and measure potato crop biomass, two important factors for late blight control. Various models combine and analyse the available information resulting in a timely spray advice provided to the farmers through SMS or voicemail messages. GEOPOTATO aims to become the preferred agricultural advice service for over

750,000 small Bengal farmers that collectively grow over 450,000 ha of potato during the "dry" winter season. The late blight alert service will be provided on a subscription basis during the potato growing season.

Forecasting the risk of late blight spread

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SUMMARY

Exposure experiments were conducted to assess the effect of solar radiation on the viability of detached *Phytophthora infestans* sporangia, and the probability of spore survival was analysed as a binary response using a binomial Generalized Linear Mixed Model (GLMM). Receiver operating characteristic curve (ROC) analysis and cross-validation were used to evaluate the global performance of the model for discriminating between viable and non-viable sporangia in the data. The model yielded an area under the ROC curve of 0.92 (95% CI = 0.90–0.93), signifying an excellent classification algorithm. The model was then tested as a forecasting system for potato late blight outbreaks using multiple years of outbreak data from across Great Britain, and achieved a prediction accuracy of 89% with an alert frequency of 1 in 7 days.

KEYWORDS

Inoculum, spore survival, *Phytophthora infestans*, disease risk, decision support system

INTRODUCTION

Solar radiation can have a major impact on the viability of detached (i.e., dispersing) fungal and oomycete sporangia, and thus the risk of between-field spread of disease (Bashi & Aylor, 1983, Belmar-Diaz et al., 2005, Kanetis et al., 2010, Mizubuti et al., 2005, Mizubuti et al., 2000, Olanya et al., 2011, Rotem et al., 1985, Sunseri et al., 2002, Wallin, 1953, Wu et al., 2000). The goals of this study were to: (1) quantify the effects of solar radiation dose on the viability of detached *P. infestans* sporangia belonging to the predominant genotypes in Great Britain (GB), (2) derive a simple binary classification model for predicting viable versus non-viable inoculum, and (3) validate the model as a tool for forecasting late blight outbreaks using 10 years of national-scale outbreak data from across GB.

MATERIALS AND METHODS

P. infestans sporangia of four different isolates belonging to the two predominant genotypes in GB (2 × 13_A2, and 2 × 6_A1) were exposed to ambient conditions at the James Hutton Institute, Dundee, Scotland (56°27'24.6"N and 3°04'10.2"W) between July and September 2016. The experiment was conducted on seven different days with exposures lasting 0, 1, 2, and 3 hours, at different times of the day. Sporangia were harvested by gently pressing lesions onto

round, 47mm diameter, 0.45µm pore, mixed cellulose esters membrane filters. The filter papers were attached to a screen comprised of muslin cloth connected to a wooden frame. The cumulative dose of solar (direct and diffuse) radiation received during exposure was determined using a Kipp & Zonen CMP3 Pyranometer, sourced from Campbell Scientific. Following exposure, the filter papers were removed from the screen and placed into a moist chamber to allow slow rehydration. Sporangia on filters were then transferred to water agar (1.5%) in petri-dishes and incubated at 18°C in the dark. Germination assessments were made after 24 h of incubation by counting at least 300 sporangia per replicate under a microscope (100x) and recording the number that had germinated or not. No distinction was made between direct or indirect sporangial germination.

A Generalized Linear Mixed Effects Model (GLMM) with a binomial distribution and a logit link function was used to estimate the probability that sporangia would be viable (germinate) based on the cumulative solar radiation dose during exposure. Our primary interest, however, was in developing a binary classification model in which the outcomes are labelled as positive or negative, or in this study, as viable or non-viable sporangia. In order to do so, the quantitative predictions (estimated probabilities of spore survival) of the model had to be converted to qualitative 'viable' and 'non-viable' labels. This required setting a 'decision threshold' or a 'cut-off value,' i.e., an estimated probability of survival above which sporangia were classified as viable, and below which sporangia were classified as non-viable. Receiver operating characteristic (ROC) analysis was used to evaluate the performance of the model as a binary classification system; an ROC curve was created by plotting the proportion of sporangia that were correctly classified as viable against the proportion that were misclassified as viable, for every possible cut-off value. To test the robustness of model predictions, the model was trained and tested using 10 repeats of 10-fold cross-validation. The accuracy of the model as a binary classification system was evaluated based on the area under the ROC curve (AUC), and a variety of statistical techniques were used to determine the optimal cut-off point for correctly classifying viable and non-viable sporangia.

The ability of the classification model to forecast the risk of between-field spread of disease was tested using historical late blight outbreak data from the AHDB Potatoes 'Fight Against Blight' campaign; these data comprised almost 2000 outbreaks from across GB, spanning 2005-2014. The model was driven using 1 km gridded solar radiation data (W m^{-2}) from the Climate hydrology and ecology research support system meteorology dataset for Great Britain (1961-2015) [CHESS-met]. A 28 day period prior to the date that each outbreak was reported was considered to be sufficient for relating weather conditions for survival of dispersing inoculum to the dates at which disease was first observed in the crop. On each day in that 28 day period, the classification model was used to 'forecast' if inoculum would be viable or non-viable. A prediction of viable inoculum on any day in that 28 day period was considered a successful forecast of that outbreak in this analysis. The number of forecasts in each 28 day period was calculated to determine the overall frequency of alerts.

RESULTS

We considered pathogen isolate as a grouping variable, but it did not have a significant effect on the probability of germination, nor on the relationship with radiation dose. The fixed effects portion of the final GLMM can be used to compute the model-based probability, P (on a continuous scale ranging from 0 to 1), of spore viability for a given cumulative solar radiation

dose, x: $P = 1 / (1 + \exp(-(2.37 - 0.45x)))$. The mean AUC (area under the empirical ROC curve) value of the model under the 10 × 10-fold cross-validation technique was 0.92 (95% CI = 0.90–0.93), signifying an excellent overall performance in discriminating between viable and non-viable sporangia in the experimental data (Figure 1). The best-performing cut-off (estimated probability) value for correctly classifying viable and non-viable sporangia was the point that minimised the straight line distance between the ROC curve and the upper left corner of the unit square, which is the point of perfect prediction: $P = 0.60$ (Figure 1). This resulted in an accuracy of 0.83 (95% CI = 0.82–0.85) in classifying sporangia in the experimental data.

The number and geographic distribution of potato late blight outbreaks varied greatly between years, ranging from 66 to 288 outbreaks, with a mean value of 182 outbreaks per year. The 'blight season' typically started in May, peaked in July, and ended in September. The binary classifier was able to correctly forecast 88.9% of outbreaks (mean value across all years) with a corresponding frequency of alerts of approximately 1 in 7 days classified as risk periods for between-field spread of disease (Figure 2). The mean length of time between the first alert in each 28 day window and the date at which disease was first observed and reported was 13.7 (SD±4.5) days.
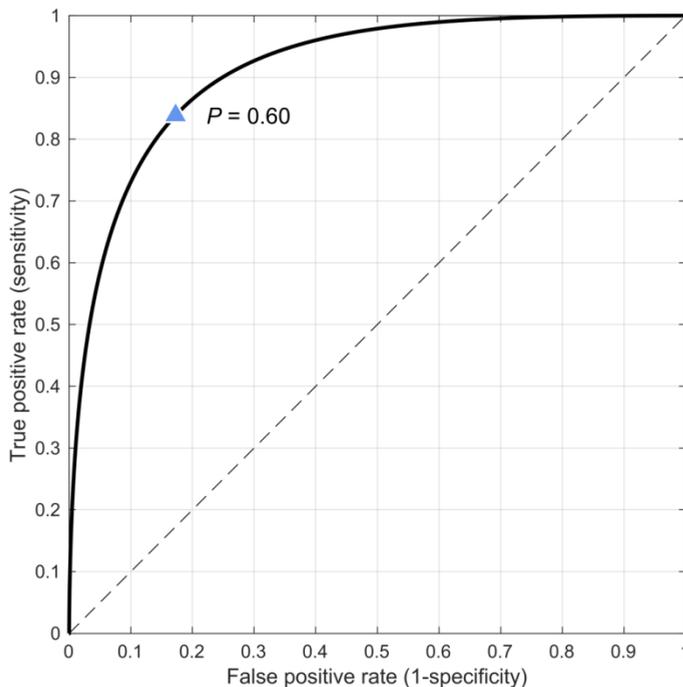
Figure 1. *Receiver operating characteristic (ROC) curve of the model for classifying spore survival outcome. The data marker shows the optimum cut-off probability value (P = 0.60) for classifying inoculum as viable or non-viable in the model. The model yielded an AUC of 0.92 (95% CI = 0.90–0.93)*

DISCUSSION

In this study we derived a simple logistic model for predicting the probability that detached (dispersing) *P. infestans* inoculum will remain viable after exposure to solar radiation. An important aspect of this model is the subsequent ease of computation; it requires only the cumulative dose of solar radiation as input, and it can be used with the aid of a basic calculator to predict the probability, P, of spore survival on a continuous scale ranging from 0 to 1. We also identified $P = 0.60$ as the optimum cut-off value for classifying sporangia as viable or non-viable in the experimental data. In this study we further extended our analyses beyond the norm for dose-response studies in aerobiology, which tend to focus solely on derivation of a dose-response curve or classification algorithm, and validated the binary classifier as a tool for forecasting outbreaks of disease using 10 years of national late blight outbreak data from across GB. The model achieved a predictive accuracy of 88.9% in forecasting late blight outbreaks, with a frequency of one risk alert per week. Although other meteorological risk factors could have been considered to improve the model, e.g., temperature and humidity, our goal was not to derive a comprehensive model of the environmental conditions underlying the survival of detached sporangia, but to produce a simple model that is both useful to and useable by growers and other practitioners in the agricultural sector. The resultant classifier could be easily integrated into existing decision support systems (DSS) for potato late blight that are currently implemented across Europe. To our knowledge, none of the widely adopted DSSs in Europe include any estimation of the risk of between-field spread of disease, and instead operate under the assumption of ubiquitous, viable inoculum throughout the season, and typically utilise a set of temperature and humidity rules to forecast the risk of infection (Cooke et al., 2011, Hansen, 2014). As our classifier for spore survival was derived using data on the predominant *P. infestans* genotypes in GB and Europe (13_A2 and 6_A1), it would serve as a useful complement to these systems, providing a binary (yes or no) forecast of the risk of between-field spread of disease that could be used to modify spray recommendations based on infection conditions only.
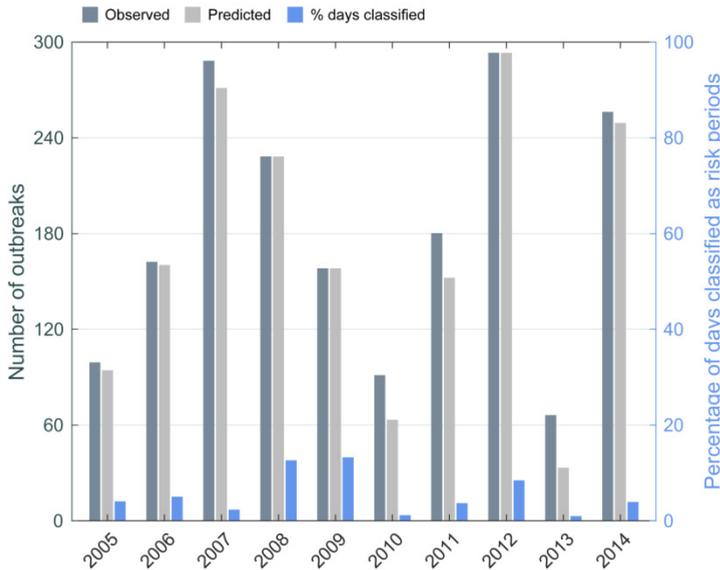
Figure 2. *Accuracy and frequency of forecasts of potato late blight risk using the binary classification model for spore survival. Dark grey bars show the number of observed late blight outbreaks each year, light grey bars show the number of outbreaks that were correctly forecast by the model, and blue bars show the percentage of days that received a risk warning.*

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66

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A new approach to the design of the VNIIFBlight decision support system used in the potato late and early blight management practice

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SUMMARY

A new version of the VNIIFBlight DSS has been designed to control both late and early blights of potato. This DSS uses combined information about the local climatic conditions, weather forecast, plant growth stage, disease resistance of potato cultivars, and fungicide characteristics. Depending on the combination of all these factors, one of three possible recommendations is proposed: 1) "Fungicide spraying is not required now"; 2) "The further fungicide spraying will be inefficient"; 3) "Fungicide spraying should be done" (with the indication of the list of fungicides which are the most suitable at this moment).

KEYWORDS

potatoes, *Phytophthora infestans*, Alternaria sp., decision support system, VNIIFBlight DSS, fungicide use, weather forecast, warning service

INTRODUCTION

Potato is one of the most important food products in Russia. Causal agents of various potato diseases represent a significant factor reducing the productivity and quality of potato. The most important and devastating diseases of the potato foliage and tubers are the late and early blights caused by the oomycete *Phytophthora infestans* and the fungi *Alternaria solani* and *A. alternata,* respectively.

In Russia both diseases are controlled mainly by fungicide applications. To date, the total number of registered fungicides intended to control late and early blights of potato reaches 45 (16 active ingredients). The most advanced Russian potato growers use routine spraying programs, in which fungicide treatments are applied with a regular interval; the choice of fungicides and the frequency of treatments are determined only by the cost of fungicides.

The known negative influence of fungicides on the human health and environment and their high cost resulted in a need to develop new technologies, which would provide a significant

optimization of the number of fungicide applications while maintaining the acceptable levels of potato production and quality. Commercial potato farms, which follow good agricultural practice, usually apply no more than 10 fungicides treatments.

The most important questions, usually asked by potato producers, are: "What should I do? When should I do it? Which conditions are the most suitable for a fungicide treatment?" It is known that fungicides required to control the late blight are effective only if they are applied shortly before the start of infection (Bødker and Nielsen, 2001). Many studies performed in advanced potato-producing countries were intended to optimize the number of protective treatments using various decision support systems (DSS), in which every treatment is determined by weather data and disease development simulators (Schepers et al., 2009; Kessel et al., 2010; Nielsen et al., 2010; Hansen et al., 2015). To date, the total number of DSS used in different countries is about 20. Among them, the most known systems are SimCast, NegFry, SimPhyt, Plant-Plus, ProPhy, Guntz-Divoux, PhytoPre, and China-blight (Fry et al., 1983; Schepers, 2004; Wander et al., 2006; Small et al., 2013; Hu et al., 2014). In some European countries, the use of DSS reduced the number of fungicide applications by 8-62% as compared with a routine scheme of treatments; these results were conferred in 26 of 29 tests (Schepers, 2004).

The first version of the VNIIFBlight DSS was intended to determine weather conditions favorable or unfavorable for the late blight outbreaks on potato (Filippov et al., 2015). The performed field trials showed that both routine scheme of fungicide application and the VNIIFBlight DSS provided an acceptable level of the late blight control under the conditions of a severe disease development. At the same time, the use of VNIIFBlight DSS resulted in a decrease of a fungicide input by 17-62% as compared to the routine program of treatment.

This paper includes a description and discussion of the approach used for the implementation of the VNIIFBlight DSS as the online decision support service (www.agropatrol.com) to control both late and early blights of potato.

THEORETICAL PROMISES AND METHODIC APPROACHES

A new version of the VNIIFBlight DSS was designed to control both late and early blights of potato. This DSS provides a solution of two tasks:
1. Determination of the most optimal dates for a fungicide application;
2. Provision of the choice of the most suitable fungicides.

Task 1. The calculation of the optimal dates for a fungicide application is based on several factors (Figure 1). To provide the possibility to determine "risky" days and recommended dates of fungicide applications against the late blight, we followed a standard five-day weather forecast.

The forecasted weather conditions are evaluated using the following two equations:

$$y_1 = -32.47 + 0.75x_1 + 0.41x_2 + 0.41x_3 + 0.27x_4 + 0.74x_5 + 0.30x_6 - 0.07x_7 - 0.16x_8 + 0.06x_9 + 0.01x_{10} + 2.88x_{11} + 1.98x_{12} + 1.98x_{13} + 1.79x_{14} + 0.53x_{15} \qquad (1)$$

$$y_2 = -31.34 + 0.63x_1 + 0.37x_2 + 0.42x_3 + 0.22x_4 + 0.65x_5 + 0.24x_6 - 0.06x_7 - 0.15x_8 - 0.13x_9 + 0.15x_{10} + 4.88x_{11} + 3.55x_{12} + 3.34x_{13} + 2.50x_{14} + 2.29x_{15} \qquad (2)$$

where $x_{1,2,3,4,5}$ and $x_{6,7,8,9,10}$ are the daily and night temperatures (C°), respectively, while $x_{11,12,13,14,15}$ describe precipitations occurred in the 1st, 2nd, 3rd, 4th and 5th days, respectively (yes/no).

If $y_1 < y_2$, then weather conditions are favorable for the late blight development. This model identifies two "images" of the weather, favorable or unfavorable for the re-infection and discrete outbreaks in the late blight development.

We also used some additional indices, which corrected the forecast depending on the climatic probability of late blight epidemics in different regions of Russia (<50%, 50-75%, and >75% of the seasons). The calculations were performed separately for two types of potato-growing technologies: no irrigation or sprinkling irrigation. The days of sprinkling are considered as rainy days (x_{11-15}).

The forecast for the first fungicide application against the late blight starts since the crop emergence. Every next fungicide treatment is applied according to the weather forecast, but with allowance for the level of disease resistance of a protected potato cultivar. In the case of resistant cultivars, it is possible either to reduce the dosage of active ingredients applied at "standard" intervals, or to increase the interval between applications keeping a standard concentration of active ingredients. According to the Russian legislation, one can increase intervals between sprayings, but cannot change the recommended dosage of a fungicide regardless of the cultivar resistance/susceptibility level. This DSS uses three levels of a cultivar resistance to the late blight (<5, 5-7 and 8-9 scores) corresponding to the 9-score scale, where 9 means the maximum resistance level). If a cultivar resistance does not exceed 5 scores, it is recommended to repeat the treatment within 7 days after the previous one. For potato cultivars which resistance level is 5-7 scores, the next treatment should be carried out within 10 days after the previous one. Finally, if the late blight resistance level reaches 8-9 scores (for example, cvs. Sarpo Mira or New York 121), the first and next applications are carried out according to the weather forecast, but only in the case, when one or more late blight nodes are observed on a field.

The treatment of potato against the early blight is recommended if the manifestation of this disease covers >50% of potato plants within the period between the end of rapid haulm growth and beginning of senescence.
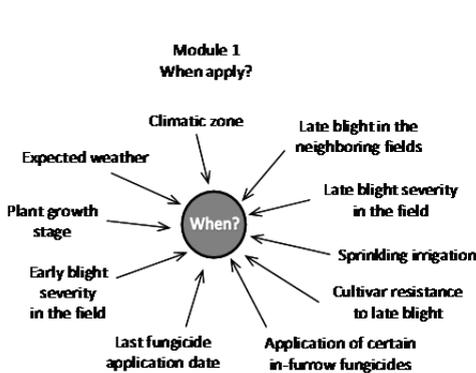
Figure 1. *Factors influencing on the choice of a time for a fungicide treatment against the late and early blights of potato.*
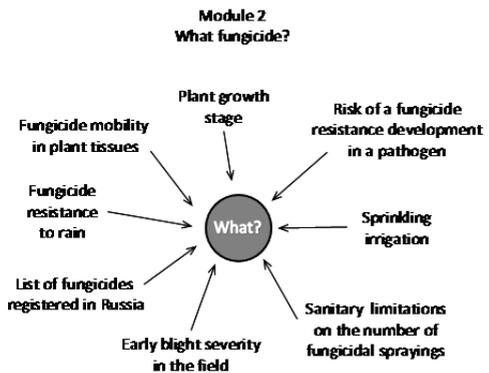
Figure 2. *Factors influencing the choice of fungicides to control late and early blights.*

Task 2. If a potato field is not protected with fungicides and the weather is favorable for the late blight, or the number of plants infected by the early blight exceeds 50%, then a fungicide application is recommended. The next question usually asked by potato growers is "What fungicides are the most suitable in this situation?" The proposed DSS provides an answer in the online mode using an interactive dialogue with a user.

The choice of fungicides provided by the program depends on a number of factors (Figure 2) including the risk of disease development, plant growth stage, fungicide's mode of action, anti-resistance strategies used to maintain a pathogen sensitivity to fungicides, and sanitary limitations concerning the number of fungicide applications per a season (according to the Russian Pesticide Regulations).

The VNIIFBlight DSS takes into account a different nature of the late blight and early blight development as well as their different sensitivity to the active ingredients of various fungicides. Under conditions favorable for either late blight or early blight, it recommends to apply fungicides efficient against each or both diseases. If the conditions are favorable for both late and early blights, it recommends to apply only fungicides effective against both diseases.

The VNIIFBlight program takes into account four potato growth periods:

1. Shoot emergence – beginning of a rapid haulm growth
2. Rapid haulm growth
3. End of a rapid haulm growth – start of senescence
4. Start of senescence – complete haulm destruction

Different stages require the use of different fungicides. In this DSS we used the Euroblight rating of fungicides. For example, VNIIFBlight chooses systemic fungicides to be used during a rapid haulm growth, but does not recommend their use at the later stages, since they are able to contaminate daughter tubers.

To prolong the active life of single-site fungicides, their use is restricted because of a cross-resistance occurring within FRAC fungicide groups. This fact is taken into consideration when a decision about the repeated fungicide applications is made (Filippov et al., 2016).

The VNIIFBlight system is based on the analysis of factors shown in Figs. 1-2 and proposes a user one of three possible recommendations: 1) "No fungicide spraying is required now"; 2) "Fungicide spraying should be done" (with the indication of the list of fungicides which are the most suitable at this moment); and 3) "The further fungicide spraying will be inefficient" (Figure 3).
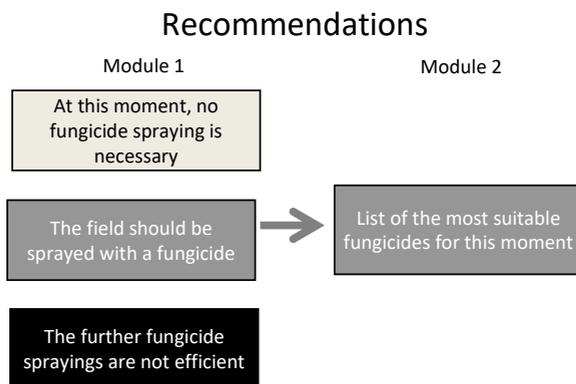
Figure 3. *Recommendations on the use of fungicides against the late blight/early blight.*

RESULTS

A new version of the VNIIFBlight DSS has been jointly developed and marketed by the All-Russian Research Institute of Phytopathology and the Agrodozor Ltd. The DSS is offered online for farmers and consultants (www.agropatrol.com). After authorization and a dialogue with the DSS, a user receives the following information:

1. The map of the region with the monitored potato fields (Figure 4). Fields are identified by spots of different colors. The red color informs about the necessity of a fungicide application.
2. Map of the region with the meteorological forecast for the late blight development in the monitored areas (Figure 5; red color means favorable conditions, green color means unfavorable conditions).
3. "Input/edit data" inset (Figure 6).

A user may input data concerning the situation at the monitored field and then receive the corresponding recommendations about the necessity to apply fungicide treatment, as well as the list of fungicides, which are the most appropriate at this moment.

CONCLUSION

A new version of the VNIIFBlight DSS has been developed to control both late and early blights of potato by a fungicide application. In the season of 2017, the field trials of a commercial online service to control the late and early blight of potato using this DSS version started at six potato farms located in the Moscow, Bryansk, Lipetsk, Belgorod, and Samara regions of Russia; the results of these trials will be available to the end of 2017. Next season we plan to expand the number of farms and regions involved.

Figure 4. Map of the region with the monitored potato fields. The color of fields (red or green) informs about the necessity of a fungicide application. Fields 1 and 3 are red, field 2 is green.
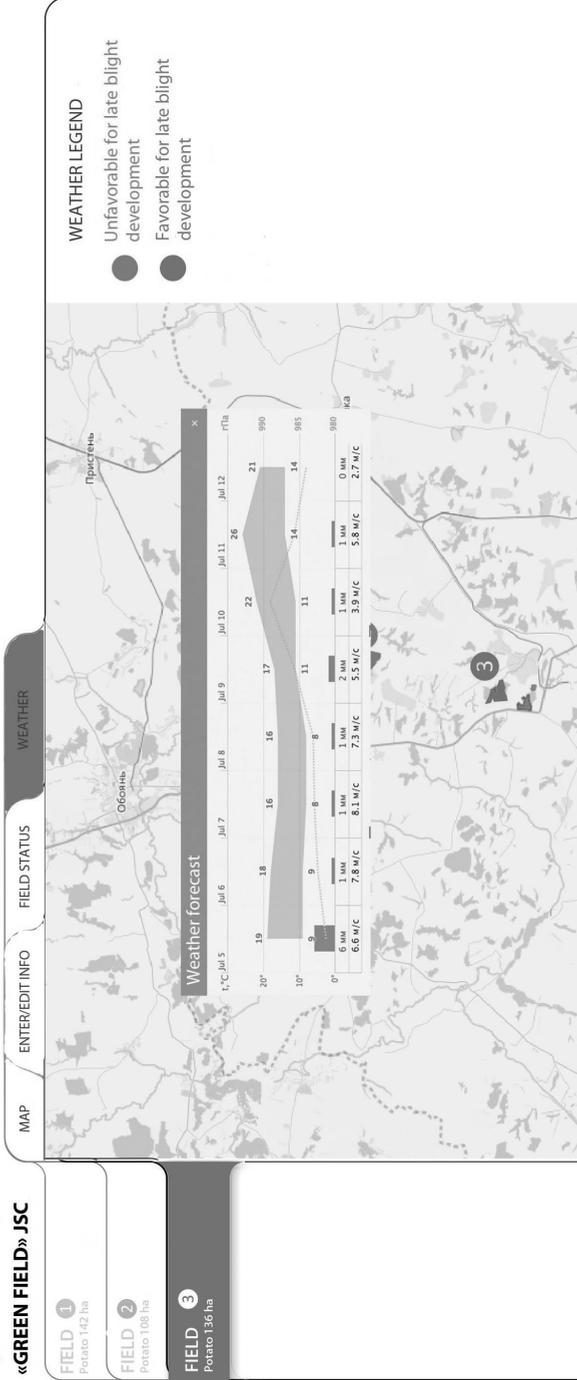
Figure 5. *Map of the region with the inset showing weather forecast.*

Figure 6. *"Input/edit data" inset providing recommended actions to control the late and early blights.*

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New Genomic approaches to study Phytophthora populations

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The center of origin of potato and *P. infestans* is Latin America, where the pathogen co-evolved with a large diversity of Solanaceous species. The first historical tragedy associated with *P. infestans*, the Irish potato famine, occurred 170 years ago. Even today, problems associated with *P. infestans* remain the main threat that affects potato and tomato crops around the world. In the decade of the 90's, late blight returns to be a global problem, causing epidemics in the potato production systems, due to migration and changes in the characteristics of the populations of the pathogen, which presented greater aggressiveness and resistance to fungicides causing loss of strength in commercial varieties. In Europe during severe blight years, up to 25 sprays were used per season in some countries (Hansen et al., 2009), and fungicide insensitivity was evolving in parts of Europe (Nielsen, 2014). Haverkort et al. (2009) estimated globally annual costs of € 5.2 billon including the costs of control measures. Late blight is considered a re-emerging disease encouraged by the increasing globalization of trade and climatic change. The disease has reached epidemic proportions in North and South America, and Europe due to the development of resistance to the fungicide metalaxyl in populations of the pathogen and the widespread occurrence of new more aggressive genotypes that are difficult to control (Kadish et al., 1990; Fry, 2015).

Nowadays, newly available capacity enables much greater precision in late blight management.

Potato is an important staple crop in Argentina. The most important losses of the crop are due to late blight control measures and losses of yield and quality associated with it. The South East of the Buenos Aires Province (SEBA), where the highest yields are obtained, the agro-ecological conditions are very conducive to late blight development. Currently, it is primarily controlled through frequent fungicide applications. Spraying programs are based on more or less fixed intervals, starting as early as 30 days after planting. Spraying frequencies may range from 7 - 10 days, depending on possible cultivar resistance, weather conditions, growth stage and active ingredient (Mantecón, 1998, 2000). As in many other countries, food companies and consumers are looking for more sustainable management and production technologies.

At National Agricultural Technology Institute (INTA), our research is focused on studies of the pathogen, its epidemiology and ecology, genetic improvement of the host and integrated pest

management. At the Mycology and Bacteriology Lab of Balcarce Research Station we developed, assessed and analyzed the impact of PhytoAlert, a decision support system (DSS) to control LB regarding disease control effectiveness, production costs and environmental impact during four consecutive growing seasons in SEBA.

PhytoAlert DSS was used to predict the critical moments for the development of late Blight since 2010 and the implementation of a preventive control strategy based on prevailing weather conditions (measured and forecasted), host resistance, and the degradation of fungicide.

PhytoAlert DSS improved the control of Late Blight by reducing fungicide use up to 50% and economic losses up to 47% and achieving a lower environmental impact (up to 48%) compared to a Calendar-based control system applied in the area (Lucca & Rodriguez, 2015).

We were also interested in epidemiological studies of *P. infestans* populations in Argentina and the region.

Genotypic diversity in *P. infestans* was historically assayed with several genotyping tools, from allozymes (Tooley & Fry, 1983), restriction fragment length polymorphisms (RFLP) (Goodwin et al., 1992), mitochondrial haplotypes (Danies et al., 2014, Carter et al., 1990), and microsatellites or simple sequence repeats (SSRs) (Li et al., 2013).

Recently GBS (genotyping by sequencing) (Elshire et al., 2011; Hansen et al., 2016) or gene sequencing (Dong et al., 2014, Goss et al., 2014) approaches have been used.

The development of Next-Generation Sequencing technologies (NGS) at reduced cost, allowed the use of these high throughput tools to answer important biological questions. Technological advances allow the generation and/or interrogation of massive amounts of genotype data and also to sequence genome of entire collections of microorganism.

In this sense, the Mycology and Bacteriology Lab (EEA INTA Balcarce) is working collaboratively with the Genomic Unit of Biotechnology Institute of INTA to carry out SSR genotyping and new available genomic strategies that can be applied to the study Phytophthora populations.

Genomic Unit (UGB.nCATG) of INTA is node of Argentine Consortium of Genomic Technology (CATG), a core laboratory oriented to the analysis of molecular markers and DNA sequencing that responds to internal and external demands of the public and private sectors, including the agricultural and livestock sectors, forestry, health, energy, environment, fishery, forensic, anthropological and judicial, in the areas of diagnosis, molecular epidemiology, prospection / discovery, breeding, regulation and control.
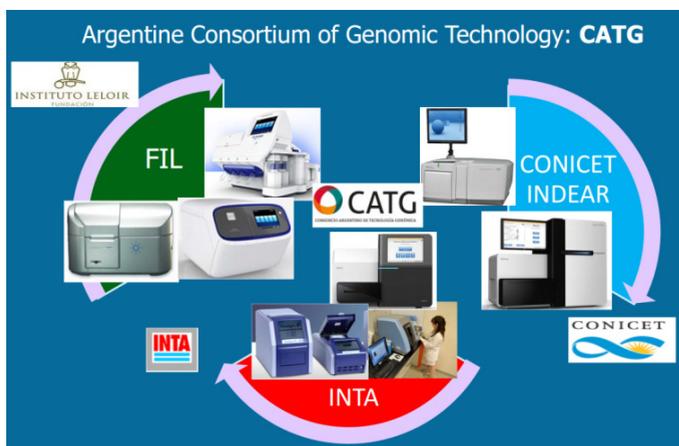
Installed capacities at INTA and accumulated experiences allow us to develop a wide spectrum of applications in the area of genomics using new technologies. UGB has specialized in genotyping techniques mainly targeting the breeding and molecular epidemiology sectors, focusing on target gene sequencing, complete sequencing of microbial, mitochondrial and plastid genomes, transcript sequencing and genotyping by sequencing.

The laboratory has ISO 17025 accreditation since 2012, being the only one in the country that has adopted quality standards for services in the area of genomics.
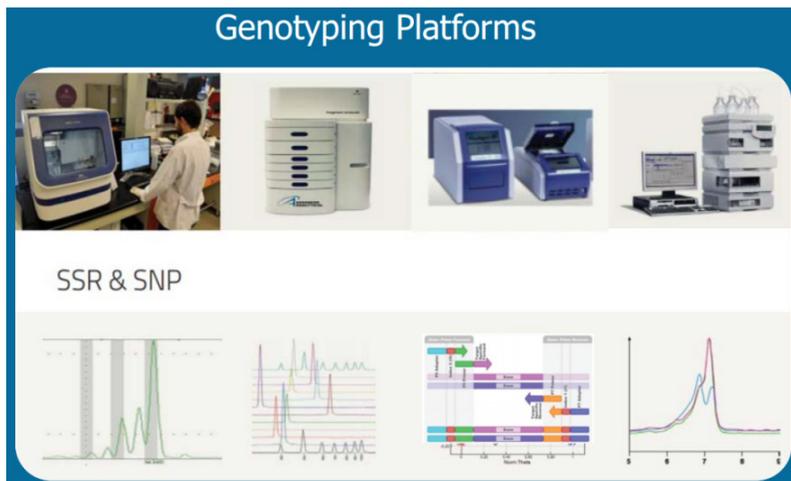
Training and outreach activities between partner countries, in particular for Late blight studies, with Tizón Latino Network are carrying out to share expertise and technical capacity in specific areas. Trainees carry out activities as learning about technologies, assay design, library construction and results analysis. Researchers specializing in the topics to be addressed advise them in the different stages of assay.

The Institute of Biotechnology also has a Bioinformatics Unit that provides the necessary infrastructure for data storage and for population dynamic studies.

The Bioinformatics Unit and its associated nodes are not only generating but also have experience in the processing and administration of previous data. The Bioinformatic Unit offers courses and internships and develops pipelines and free access bioinformatics tools for the analysis of NGS data. The close interaction between both Units allows improvements in protocols and adjustment of data analysis to obtain high quality results.

Our first epidemiological study of *P. infestans* in Argentina was carried out in SEBA by sampling, extraction of ADNg in FTA cards and genotyping of populations with an internationally agreed panel of 12 microsatellite markers (Li et al., 2013). Genetic studies showed that all isolates collected since 2007 to present belong to genotype 2_A1, although allelic variants were observed in the material evaluated.

A Latin American late blight network called Red Tizón Latino was launched in Bogotá, Colombia, in October 2014. In order to capture the genotypic variation of Phytophthora populations in Latin America, we developed a genotyping service to researches and companies of partner countries

of the network. We received samples from Chile, Brazil, Panama and Colombia from different Solanaceae hosts and analyzed them using a standardized 12 plex SSR genotyping (Li et al., 2013). The preliminary results had shown diversity of genotypes in Latin American Phytophthora populations. Each research group is analyzing in depth those preliminary results obtained to discuss them in the Third Workshop Tizon Latino 2018 to be held in Cusco, Peru, in the framework of World Potato Congress 2018 / ALAP 2018.

Based on our experience to adapt and develop new protocols, we suggest new genomic approaches to study Phytophthora populations.

TARGET SEQUENCING

Numerous papers report studies on specific regions, performed with Sanger sequencing. Currently, this robust technique is laborious and expensive to undertake epidemiological genomic studies that include numerous regions and isolates. Lange et al. (2014) proposed a protocol that allows the study of lots of regions in parallel on whole collections, using microfluidic units of Access Array (Fluidigm) technology that performs amplifications in sets of 48 samples per 48 regions amplified (2048) on a single chip, at a low cost per data point.

Amplicon sequencing is asayed in Illumina MiSeq system, obtaining read length to 2 × 250 base pairs.
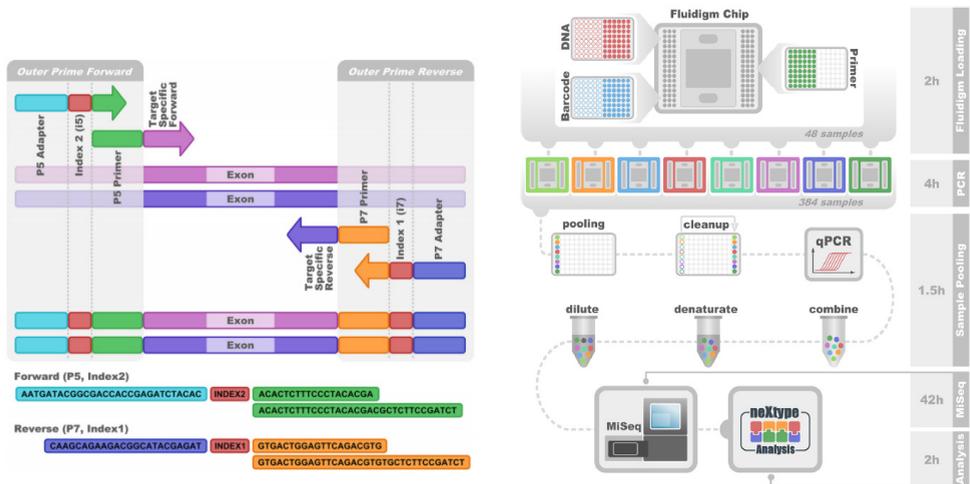
© Lange et al., 2014

DDRAD PROTOCOL – GBS

Different types of molecular markers are reported to date, and SNPs are currently the most widely used. Restriction enzyme genome-reduction methods, combined with Next Generation Sequencing (NGS), enable in a single assay to discover and genotype a large number of particular SNPs (in the order of thousands) of the study population. These methods are usually

called GBS, and allow different population studies to be carried out at a lower cost than using microarrays and without the need for previous molecular information on the species (Davey et al., 2011). The double digest Restriction Associated DNA sequencing strategy (ddRADseq, Peterson et al., 2012), based on genome digestion with a double enzyme, combined with efficient prediction of the enzyme pair and size selection (selection of DNA fragments by size) optimal for the species of interest, is one of the most promising methodologies of GBS.

The development of the methodology of ddRADseq for *P. infestans* was performed in the Genomic Unit based on Aguirre et al protocol.
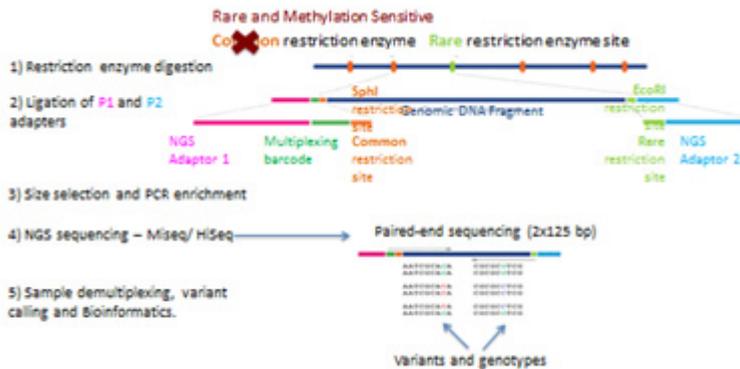
Aguirre et al., 2016

WHOLE GENOME SEQUENCING

P. infestans genome, was described by Haas et al. in 2009, this large and complex genome of 240 megabases (Mb) size results from a proliferation of repetitive DNA accounting for approximately 74% of the genome. It is a challenge for researchers to achieve protocols that allow obtaining of sequences at low costs and with results that allow the easy assembly of them.

First Draft Genome Sequence of the Pathogenic Fungus *Lomentospora prolificans* (formerly *Scedosporium prolificans*) was described recently. Sequencing and assembly of the fungus was performed using a combination of short, highly accurate Illumina reads and additional coverage in very long Oxford Nanopore reads (Luo et al., 2017).

These techniques are available in the genomic Unit and protocols can be assayed to obtained sequences of important isolates of *P. infestans* in Latin America.

The genomic approaches described in this work will be evaluated in the framework of different research projects of partner countries of Tizón Latino Network in order to improve understanding *P. infestans* populations in Latin America and to enable better late blight management. The newly genomic capacities of INTA are available for other research networks worldwide.

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Biologicals for the control of *Alternaria solani* under greenhouse and field conditions

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SUMMARY

In this study, the potential of some *Trichoderma* spp. and *Bacillus subtilis* as biological plant protection products has been observed in a first step. Therefor greenhouse trials and an annual field trial were performed. For the greenhouse trial, the potato plants were treated with different biologicals and one day later the plants were inoculated with *Alternaria solani*. In detail, the spore solutions of *T. asperellum*, *T. atroviride* and *T. harzianum*, but also commercial products like TrichoStar®, TrichoMix® and Serenade® were used for the biological control. TrichoStar® and TrichoMix® are mixtures of different *Trichoderma* spp. and Serenade® includes *Bacillus subtilis*. For the field trial with the variety Lady Amarilla, the same treatments as in the greenhouse and additionally *T. hamatum*, were applied in a 7 to 10-day interval. A reduction of early blight could have been observed for most of the treatments in the greenhouse and field trial.

KEYWORDS

Alternaria solani, early blight, early blight control, kernel infection, biological control, *Trichoderma* spp., *Bacillus subtilis*

INTRODUCTION

In recent years the increasing relevance of *Alternaria solani*, the pathogens which causes early blight on potato, leads to several discussions about the best way to control this disease. The most effective way of controlling this pathogen is the use of fungicides. Unfortunately, there is an increasing resistance development, regarding the two main fungicide groups for controlling early blight, the QoIs and the SDHIs due to their frequent use (Pasche et al., 2004; Leiminger et al., 2013; Gudmestad et al., 2013; Bauske et al., 2017). The upcoming question is now, if there are other possibilities to keep the plants healthy and in a second step to stop or at least slow down this resistance development. Referring to this, the biological control as an ecofriendly alternative method has been mentioned several times (Siameto et al., 2010; Soria et al., 2012). Already in 1932, the potential of *Trichoderma* spp. as biocontrol agent was reported by Weindling. There are already studies, which evaluated the antagonistic potential of some *Trichoderma* spp. e.g. against *A. porri* on onions or *A. solani* on tomatoes (Kamal et al., 2014;

Sobia et al., 2015). In this study the potential of different *Trichoderma* strains and some commercial biological products as biocontrol agents against *A. solani* on potatoes has been investigated. Therefor *in vivo* and field trials were performed.

MATERIALS AND METHODS

In vivo trial

Cultivation of Trichoderma strains

The strains, which were used in this trial were provided by the University of Szeged, Hungary. To cultivate the strains, they were put on ¼ PD-agar and incubated under daylight and 25°C for about two weeks.

Execution of the greenhouse trial

For the greenhouse trial the cultivar Kuras was used due to its high susceptibility to *A. solani*. The plants were cut to a three-leaf stadium and then treated with three different spore solutions of *Trichoderma* strains and the commercial products one day before inoculation with *A. solani*. The spore density for the *Trichoderma* spp. was 10^7 spores per ml. For the infection with *A. solani* one day after the treatments, a spore density of 10^3 spores per ml was used. In this trial three plants per treatment were used. Three neither treated nor inoculated control plants were also integrated. After the inoculation with the spore solutions *A. solani*, the plants had to incubate in the mist chamber for 48 h at 100% relative humidity and 20°C. After these 48 h a relative humidity of 70% was pursued until the end of the experiment. The infection with *A. solani* was assessed after two and seven days after inoculation (dai). For the visual assessment the rating schedule from Granovsky and Peterson (1954) was used. This trial was repeated twice.

Field trial

General information

For the first field trial in Freising - Weihenstephan, Germany, the cultivar Lady Amarilla from the early maturity group, known to be very susceptible against early blight was used. The plot size was 4m x 4,5m. The plots were treated with spore solutions of the single *Trichoderma* strains or the commercial products. Altogether the trial consisted of 9 biological treatments, one chemical reference (multisite fungicide) and an untreated control. The trial included four repetitions per treatment. Furthermore, a kernel infection was done to ensure an infection with early blight in the field.

Kernel infection

The used isolates were cultivated on SN-agar for two weeks under near UV-light (12h/12h). To generate the kernel inoculum, 150g of barley kernels were put in an autoclavable bag with 60 ml distilled water and closed with a buckler and rubber band. The bags were autoclaved twice. Half of an overgrown, about 2 weeks old SNA-plate was used to knead the kernels with the grown fungus, to ensure adherence of the conidia on the grains in the autoclaved bag. The infected kernels need to be incubated for four weeks under near UV-light (12h/12h), to support fungal growth. 5 g of the finished kernels were spread equally per m² between the potato rows.

Disease assessment

The observation of the disease progress started with emergence until death of the potato plants. In each of the four replications, 10 plants per plot were rated for disease progress of early blight. To exclude the influence of the surrounding plots, only the two rows in the middle of each plot were used for observation. For the visual assessment the potato plant was divided into three leaf levels (lower, middle, upper leaf level) in order to follow disease development. One leaflet per leaf level was examined to determine the percentage of necrotic leaf area. This rating was done by using a scheme for evaluation of the leaf necrosis in percentage from Granovsky and Peterson (1954).

RESULTS AND DISCUSSION

The results for the greenhouse trial showed a clear advantage for the chemical fungicide with 100% efficacy, whereas the biologicals reached efficacies from about 20% in average (Figure 1). Since it wasn´t the goal to get the same effects with the biologicals compared to the chemical fungicide, the potential of these tested substances is visible and need to be analyzed in more detail.
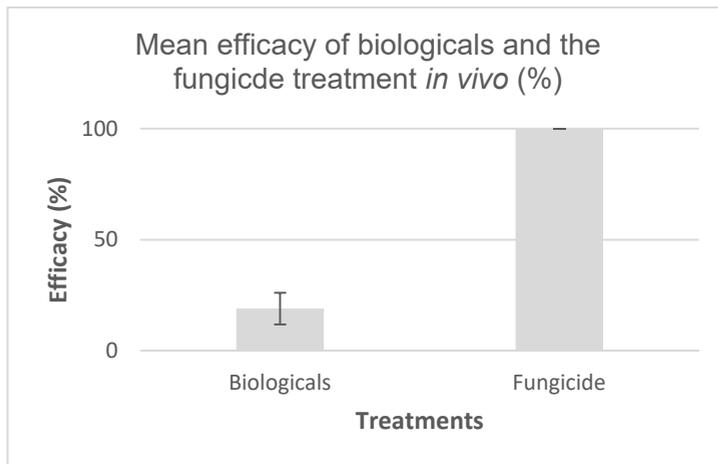
Figure 1. *Mean efficacy of biologicals and the fungicide treatment in vivo (%). For the biologicals, the average of all efficacies was calculated (n=6).*

Regarding the annual field trial, the results from the greenhouse were confirmed. The mean efficacy of the biological treatments at the beginning of the disease progression (11.08.2016) was nearly the same as for the fungicide treatment (44% and 52% respectively). One week later, a clear advantage for the fungicide treatment was observed (78%), but still the average of the efficacies from the biologicals was about 23% (Figure 2).
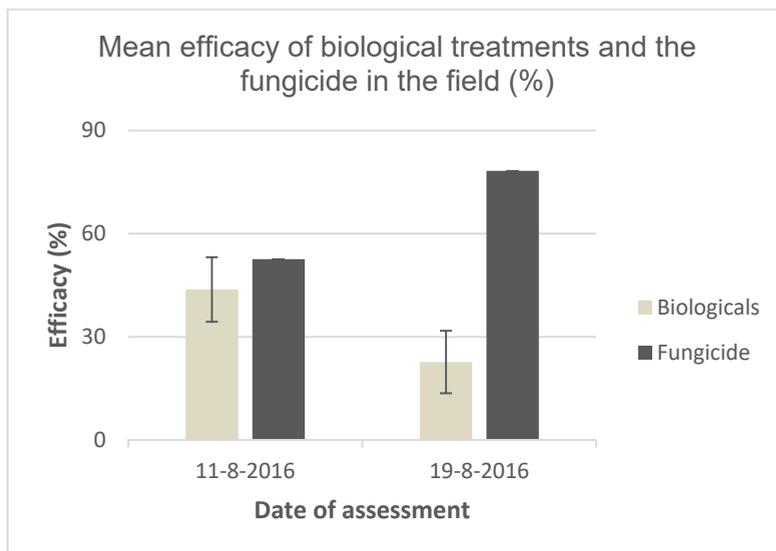
Figure 2. *Mean efficacy of biological treatments and the fungicide in the field (%). For the biologicals, the averages of all efficacies for the two dates were calculated (n=9).*

CONCLUSION

All in all, the potential for *Trichoderma* spp. and some other biological products to play a role in the control of early blight is visible. These biologicals cannot replace the chemical fungicides, but they could be a possibility to reduce the chemical treatments, by alternating use of biologicals and fungicides. Therefore, further research is necessary to end up with an optimized application strategy of these ecofriendly alternatives for conventional and biological farmers.

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Prevalence of QoI and SDHI fungicide resistance in *Alternaria solani* – the situation in Germany

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SUMMARY

Early blight (EB) caused by *Alternaria solani* is a highly destructive disease of potatoes. It is controlled by multiple preventive fungicide treatments during the growing season. QoI and SDHI fungicides are currently most effective but have a single site mode of action. The sensitivity of *A. solani* can be reduced by mutations in the genes of the target site of both fungicide groups. The F129L mutation in the Cyt b gene and mutations in the B, C and D subunits of the SDH are known to cause loss in sensitivity to QoIs respectively SDHIs (Pasche et al., 2005, Mallik et al., 2014).

1151 *A. solani* single spore isolates from the years 2005 to 2016 were screened for the presence of the F129L mutation and mutations in the SDH subunits B, C and D. F129L mutant isolates were first found 2009 in Southern Germany but until 2013 they could be detected in all potato growing areas in Germany. 2015 the percentage of mutant isolates within the population rose to almost 90% and they occurred in every location samples were taken from. *A. solani* SDHI mutants were first detected in 2013. 2014 they occurred in all potato growing areas in Germany but their number was low. This changed 2015 when approximately two-thirds of the surveyed isolates were SDH mutants and they were found in almost 90% of the locations.

In vitro and in vivo sensitivity tests showed a significant loss in sensitivity towards Azoxystrobin (QoI) and Boscalid (SDHI) in F129L respectively SDHI mutant isolates.

Considering the spreading and increase in the number of F129L and SDHI mutant *A. solani* isolates it is obvious that fungicide treatment strategies have to be adapted to maintain the effectiveness of QoI and SDHI fungicides against EB.

KEYWORDS

Alternaria solani, fungicide resistance, cytochrome b, F129L, QoI, SDHI

INTRODUCTION

Alternaria solani is the causative agent of early blight of potato (*Solanum tuberosum*). This very common disease, which can be found in most potato growing countries, can cause considerable

defoliation (Woudenberg et al., 2014). It typically reduces yields by ~20%, but yield reductions of up to 80% have been reported (Horsfield et al., 2010).

In Germany early blight occurs in all potato growing areas. It is controlled by multiple applications of protective fungicides during the growing season.

One of the most effective and therefore frequently applied protective fungicide agents is Azoxystrobin. It belongs to the class of the quinone outside inhibitors (QoIs), which bind to the quinone outside pocket of the cytochrome bc1 complex in the mitochondria and inhibits thus the electron transport in mitochondrial respiration. This single site mode of action implies a high risk of resistance development (FRAC Code List ©*2017) due to point mutations in the cytochrome b gene. In the USA only two years after the registration of Azoxystrobin in potato, Pasche et al. (2004) were the first to report of reduced sensitivity in *A. solani* isolates and demonstrated that this reduction is caused by the F129L mutation in the cyt b gene (Pasche et al., 2005). Leiminger et al. (2014) detected within the *A. solani* populations in Europe two types of cyt b genes which differ in their intro-exon structure.

Another effective protective fungicide is boscalid. It belongs to the class of succinate dehydrogenase inhibitors (SDHIs) which interfere with the mitochondrial respiration, too. They inhibit electron transport at complex II (succinate-dehydrogenase) by binding to the ubiquinone binding site formed by SDH subunits B, C and D. This single site mode of action also has a high risk of resistance development (FRAC Code List ©*2017). Point mutations leading to amino acid changes and reduced sensitivity of *A. solani* isolates can be detected in the gene sequences of all three subunits. Currently described mutations in *A. solani* are in subunit B: H278Y and H278R, C: H134R and in D: D123E, H133R (Mallik et al., 2014). In the USA boscalid was registered for the use in potato in 2005 (Mallik et al., 2014) and 2009 the first insensitive isolates occurred (Wharton et al., 2012).

In Germany Azoxystrobin and Boscalid were registered for the use in potato 2006 and 2008 respectively. The aim of this work was to survey the presence of the F129L mutation concerning the sensitivity towards QoIs and the presence of SDH subunit B, C and D mutants concerning the sensitivity towards SDHIs in German *A. solani* populations. Furthermore, the impact of the different mutations on the sensitivity of *A. solani* isolates towards Azoxystrobin respectively Boscalid was tested both in vitro (data not shown) and in vivo.

MATERIAL AND METHODS

Isolates
1151 *A. solani* single spore isolates from the years 2005 to 2016 were screened for the presence of the F129L mutation and mutations in the SDH subunits B, C and D. They were obtained from leaf samples from 214 locations from all potato growing areas in Germany. The samples were naturally infected and derived mostly from commercial potato crops but also from field trials. Up to ten isolates per sample were taken according to the method published by Leiminger et al. (2014).

DNA extraction
Genomic DNA of the *A. solani* isolates was extracted according to Leiminger et al. (2014).

Detection of the F129L mutation
Within *A. solani* populations in Europe two types of cyt b genes exist which differ in their intro-exon structure (Leiminger et al., 2014).

Therefore a standard PCR with two primer sets was used for the detection of the F129L mutation. Primer pairs As-Gf/r (Leiminger et al., 2014) and As-5f/r (Pasche et al., 2005) were used to amplify DNA segments containing the possible mutation site for genotype I respectively genotype II cyt b genes. The 207/214 bp products were separated by gel electrophoresis, excised, purified and sequenced (Leiminger et al., 2014). PCR for genotype I (As-Gf/r) was carried out according to Leiminger et al., 2014. PCR for genotype II (As-5f/r) was performed in a total volume of 20 µL containing 10x PCR buffer, 3,5 mM $MgCl_2$, 200 µM each dNTP (Fermentas), 0,5 µM As-5f primer, 1 µM As-5r primer, 1 U Taq DNA polymerase (SupraTherm, 5 U µL^{-1}, GeneCraft) and 50 ng genomic DNA as template. Cycling conditions were: Initial denaturation step at 95°C for 10 min, followed by 36 reaction cycles consisting of denaturation at 95°C for 1 min, primer annealing at 58°C for 30 s and DNA extension at 72°C for 30 s. After a final extension step of 72°C for 3 min, samples were cooled at 4°C.

Detection of SDH subunit B, C and D mutations
To detect possible mutations in the B, C and D subunits of the SDH complex, a standard PCR with three different primer pairs (SDHB-F/R, SDHC-F1/R2 and SDHD-F1/R2 (Mallik et al., 2014)) was used to amplify the almost complete respective genes. Each PCR was performed in a total volume of 25 µl containing 10x PCR buffer, 1,5 mM $MgCl_2$, 200 µM each dNTP (Fermentas), 0,6 µM forward/reverse primer, 1 U Taq DNA polymerase (SupraTherm, 5 U µL^{-1}, GeneCraft) and 50 ng genomic DNA as template. Cycling conditions for the SDHB gene were: initial step of 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min. After a final extension step at 72°C for 7 min, samples were cooled at 4°C. For the SDHC and D gene the duration of the final extension step was reduced to 45 s. The 1.082, 570 and 607 bp products were separated by gel electrophoresis, excised, purified and sequenced according to Leiminger et al. (2014).

In vivo fungicide sensitivity assay
The effect of the presence of the F129L and SDHI mutations in *A. solani* isolates on the fungicide efficacy of Azoxystrobin and Boscalid was determined in greenhouse trials with inoculated potato plants as described by Leiminger et al. (2014). The sensitivity tests for Azoxystrobin included a subset of six wild-type and five F129L isolates, the tests for Boscalid three wild-type, one B-H278R, two new, not yet described C and one C-H134R SDH mutant isolates.
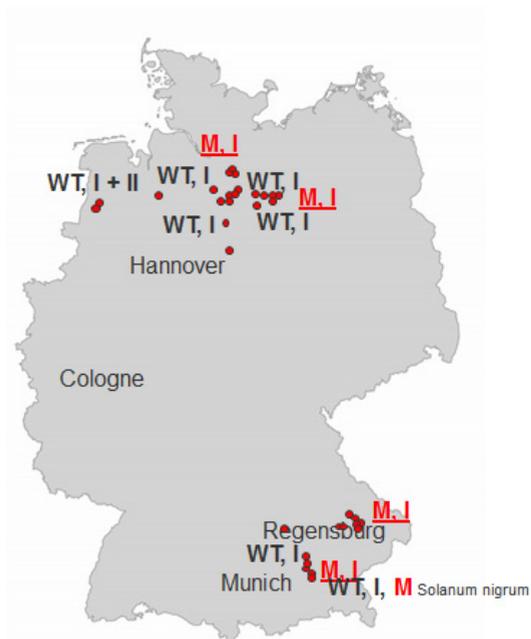
Figure 1. *Occurrence of A. solani F129L mutant isolates in Germany, 2015. Map of locations screened for F129L mutants. Unlabeled dots indicate the presence of genotype II mutant isolates in a location, circles the absence. M, I = genotype I mutants detected. WT, I/II = wild-type genotype I/II detected.*

RESULTS

Presence of F129L mutants in the Alternaria population in Germany

All fifty-five isolates screened for the F129L mutation from 2005 to 2008 were wild-type and with one exception genotype I. They originated from forty locations, most of them in Southern Germany but to a smaller part in Northern and Western Germany, too. In 2009 39 isolates from 21 plots in Southern and Northern Germany were screened and the first two F129L mutant isolates found in one location in Southern Germany, both of them genotype II. In the following year the F129L mutation could be detected in two more locations in Southern Germany, 2011 in eight. 2012 F129L mutant isolates occurred additionally in three locations in Northern Germany and for the second time since 2006 genotype II wild-types in one location each in Southern and Northern Germany. 2013 the first mutant isolates were found in Eastern and Western Germany, so the F129L mutation was present in all potato growing areas in Germany now. Until 2012 F129L mutant isolates were always genotype II but 2013 the first genotype I mutant isolates could be detected. They derived from three different plots in Northern Germany. In total 266 isolates from 24 locations were screened and 72 mutants found in 20.

Of the 67 *A. solani* isolates surveyed 2014 20 had the F129L mutation and 18 out of 30 plots situated in all potato growing areas in Germany were affected. In 2015 a strong increase in the occurrence of the F129L mutation could be observed (Figure 1). 170 isolates out of 196 carried the mutation. They were detected in all 34 plots located in Northern and Southern Germany.

Genotype I mutants occurred in two places each in Northern Germany and Southern Germany. Wild-types could only be found in 7 places at all, one of them for the third time in 10 years a genotype II wild-type isolate. It was also possible to isolate F129L mutants from leaves of *Solanum nigrum* plants which grew nearby a potato crop. The situation in 2016 was similar to 2015: F129L mutant isolates were detected in all 26 plots surveyed and they predominated. Genotype I mutants occurred in three different locations in Northern Germany.

Sensitivity of A. solani isolates towards Azoxystrobin
In vitro plate tests with technical grade Azoxystrobin showed significantly reduced conidia germination rates in F129L mutant isolates (Leiminger et al., 2014). Therefore the fungicide efficacy was tested in vivo in greenhouse trials with inoculated potato plants, too (Figure 2). F129L mutant isolates showed a significantly reduced sensitivity compared to wild-types. At a concentration of 100 µg technical grade Azoxystrobin/ml fungicide spray solution (= field concentration) the efficacy was reduced to 74 to 43% in mutants, whereas wild-type isolates had efficacies around 95%.
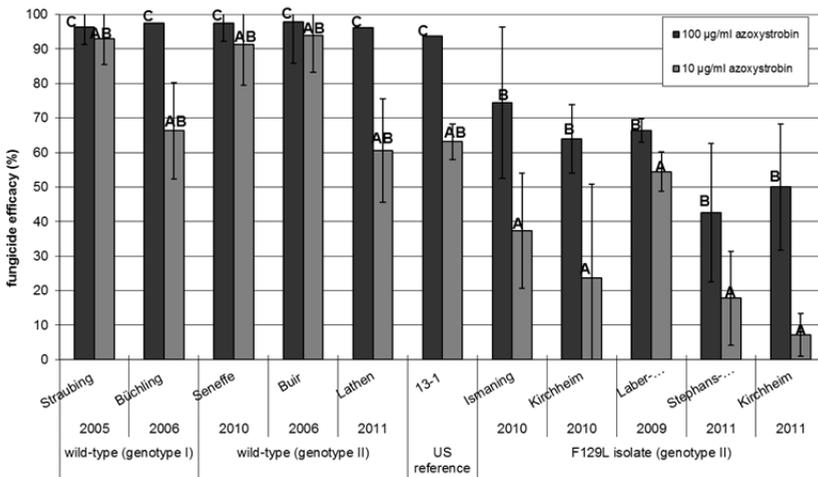
Figure 2. *Fungicide efficacy of Azoxystrobin against German wild-type (n = 6) and F129L (n = 5) Alternaria solani isolates. In vivo greenhouse tests (mean of two replications) with concentrations of 10/100µg technical grade Azoxystrobin/ml fungicide spray solution. Disease severity was rated 1 week after artificial inoculation. Columns with the same letter are not significantly different (Tukey's b test, P = 0_05). Vertical bars indicate standard deviation.*

Presence of SDH mutants in the Alternaria population in Germany
The isolates screened for the F129L mutation were used to survey the presence of SDH mutants in the *Alternaria* population in Germany, too. Within the 297 isolates of the years 2005 to 2012 no SDH mutants could be detected. The first mutants occurred 2013 (Fig.: 3A) in two locations in Western Germany (subunit B: H278Y and subunit C: H134R)) and one location in Northern Germany (subunit C: H134R). 2014 the C-H134R mutation was detected in two locations in Eastern Germany and a new mutation in subunit C, not described in the literature yet, in one plot in Southern Germany (Fig.: 3B).

The situation in 2015 (Fig.: 3C) showed similar to the development concerning the presence of the F129L mutation a dramatic increase in the occurrence of SDH mutant *A. solani* isolates. 134 of the 196 screened isolates showed a mutation in one of the three SDH subunits. 30 out of 34 locations were affected. The predominant mutation was C-H134R but B-H278Y and B-H278R could also be detected as well as the new C and D-D123E mutants. All isolates with a SDH mutation possessed the F129L mutation additionally.

In all of the 26 locations the samples derived from in 2016, SDH mutants were detected. Wild-type isolates only in 4. C-H134R was the predominant mutation but B-H278Y occurred, to a lesser part, too. B-H278R, the new C and D-D123E mutants could not be found in 2016.

A.   **B.**   **C.**
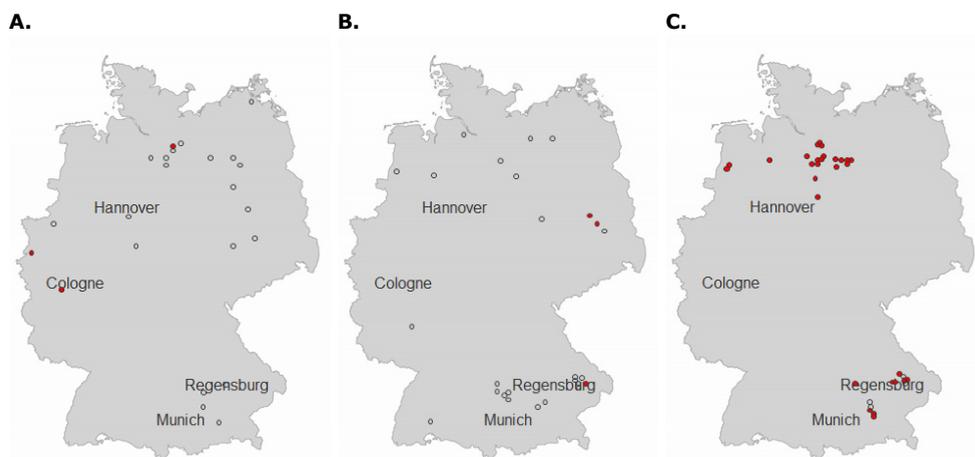
Figure 3. *Occurrence of A. solani SDH mutant isolates in Germany, 2013 (A), 2014 (B) and 2015 (C). Map of locations screened for SDH mutants. Dots indicate the presence of mutant isolates in a location, circles the absence.*

In vivo sensitivity of A. solani isolates towards Boscalid

The three wild-type, one B-H278R, two new C and one C-H134R SDH mutant isolates used for the in vivo sensitivity tests derived from the years 2014 and 2015. At a concentration of 100µg technical grade Boscalid/ml fungicide solution, which matches the field application dose, the SDH wild-type isolates showed a fungicide efficacy of nearly 100%. The mutants were within a range of 98 to 66%. Only the C-H134R isolate differed significantly (Tukey-b, α=5%) from the wild-types.

CONCLUSION

QoI and SDHI fungicides are important tools for the control of early blight caused by *A. solani* in potato. Their single site mode of action holds a high risk of resistance development (FRAC Code List ©*2017) caused by point mutations in the genes encoding the binding sites of the fungicides/ubiquinone. To prevent a fast development, selection and spreading of less sensitive mutants it is important to adapt the fungicide treatment strategy. Therefore it is necessary to get information about the current situation concerning the occurrence of *A. solani* F129L and SDH mutant isolates.

In Germany the first F129L mutant isolates were detected in 2009, three years after the registration of Azoxystrobin for the use in potato, in Southern Germany. Until 2014 the F129L mutation could be found in all potato growing in Germany. Almost all mutant isolates were genotype II, whereas most wild-type isolates were genotype I. 2013 the first genotype I mutants occurred and their number increased until 2016. In 2015 the percentage of wild-type isolates decreased dramatically, almost 90% of the screened isolates were F129L mutants and they were detected in all locations surveyed. Only in 17,6% of the locations wild-type isolates were found. The situation in 2016 was similar.

Sensitivity tests with German *A. solani* F129L mutant isolates in vitro (Leiminger et al., 2014) as well as in vivo showed a significantly reduced sensitivity towards Azoxystrobin compared to wild-types with fungicide efficacies between 74 and 42%.

The first *A. solani* isolates with mutations in the SDH complex were detected in 2013, five years after registration of Boscalid for the use in potato, in North and West Germany. In the following year SDH mutants turned up in East and South Germany, the South German isolates being a new not yet described C mutant. In both years the percentage of mutants within the total amount of screened isolates and the number of affected locations was low. 2015 the situation changed completely: Approximately two-thirds of the surveyed isolates were SDH mutants and they were found in almost 90% of the locations. C-H134R was the predominant mutation but the new C was also detected as well as both known subunit B mutations and D-D123E. 2016 mutants were detected in all surveyed locations, the main mutation being C-H134R and to a lesser part B-H278Y.

SDH C-H134R, C new and B-H278Y mutants tested in vivo for their sensitivity to Boscalid had a reduced sensitivity within a range of 66,5 to 98,3% fungicide efficacy.

A. solani isolates with reduced sensitivity to Azoxystrobin and Boscalid are therefore present in all potato growing areas in Germany and their part in the *A. solani* population is high. Spraying strategies have to be adapted to this situation.

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Control of early blight by the use of SDHI fungicides

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SUMMARY

Regarding the increasing amount of SDH-mutated *Alternaria solani* isolates, it´s necessary to get a deeper knowledge about the influence of these mutations on fungicide efficacy of different SDHIs (Succinate dehydrogenase inhibitors). Therefor all three stages of sensitivity-testing were performed: *in vitro* trials (calculation of the EC50 values), *in vivo* trials (greenhouse trials with boscalid) and last but not least field trials (fungicide efficacy of different SDHIs under field conditions with two potato varieties). For the field trials it was also essential to get a targeted infection with mutated *A. solani* isolates to observe the real interaction between the fungicide and the mutated field isolate on the field. Therefor the kernel infection with two mutated isolates (both H134R) and one SDH-wildtype isolate was used. All in all, a decreasing fungicide efficacy has been observed in all three stages of the testing. Interestingly different fungicide performances were observed regarding the kernel infections with the two mutated isolates (both H134R). In the field trial it was also reassured that the variety still plays an important role in integrated pest management.

KEYWORDS

Alternaria solani, early blight, SDHI fungicides, control of early blight, SDH-mutation, fungicide resistance, kernel infection, fungicide sensitivity testing

INTRODUCTION

In recent years the increasing relevance of *Alternaria solani*, the pathogens which causes early blight on potato, leads to several discussions about the best way to control this disease. The most effective way of controlling this pathogen is the use of fungicides. There are two main fungicide groups, the QoIs and the SDHIs. Beside the shift in fungicide sensitivity of *A. solani* against the QoIs (Leiminger et al., 2013; Pasche et al., 2004), there is also an increasing resistance development against the SDHI Boscalid reported in the last view years (Gudmestad et al., 2013, Bauske et al., 2017). In case of the SDHIs, the decreasing fungicide efficacy can be traced back to some mutations in the subunits of the succinate dehydrogenase of *A. solani*, named B-H278R/Y, C-H134R, D-D123E and D-H133R. The upcoming question is now, if and in which dimension these mutations have an influence on the efficacy of SDHI-fungicide treatments. To answer this question *in vivo* and field trials were performed with different SDH-mutated and wildtype isolates. *In vitro* trials were already performed for these isolates.

MATERIALS AND METHODS

In vivo trial

For the greenhouse trial the cultivar Kuras was used due to its high susceptibility against *A. solani*. The plants were cut to a three-leaf stadium and then treated with different amounts of boscalid (0; 0,1; 1; 10; 100µg boscalid/ml) one day before inoculation with the fungus. In this trial three plants per treatment were used. Three neither treated nor inoculated control plants were also integrated. After the inoculation with the spore solutions of the different *A. solani* isolates, the plants had to incubate in the mist chamber for 48 h at 100% relative humidity and 20°C. After these 48 h a relative humidity of 70% was pursued until the end of the experiment. The experiment was performed with three SDH-wildtype isolates, two B-H278R mutants, two C-H134R mutants and two isolates with a not yet described mutation in the subunit C. The affection with *A. solani* was assessed after two and seven days after inoculation (dai). For the visual assessment the rating schedule from Granovsky and Peterson (1954) was used. The trial was repeated three times for most of the isolates. Two isolates were only repeated twice, due to growing problems.

Field trial

General information

For the field trial in Freising - Weihenstephan, Germany, the cultivars Maxilla from the late maturity group, known to be susceptible, and Lady Amarilla from the early maturity group, known to be very susceptible against early blight were used. The plot size was 4m x 4,5m. The trial consists of four different treatments with commercial products, which differ in their active ingredient and an untreated control. The trial included four repetitions per treatment. Furthermore, a kernel infection with three different *A. solani* isolates were done on the one hand to ensure an infection with early blight and on the other hand to have separated SDH- wildtype and –mutant *A. solani* populations in the field. So, for the kernel infection one SDH-wildtype isolate and two SDH-mutants were used.

Kernel infection

The used isolates were cultivated on SN-agar for two weeks under near UV-light (12h/12h). To generate the kernel inoculum, 150g of barley kernels were put in an autoclavable bag with 60 ml distilled water and closed with a buckler and rubber band. The bags were autoclaved twice. Half of an overgrown, about 2 weeks old SNA-plate was used to knead the kernels with the grown fungus, to ensure adherence of the conidia on the grains in the autoclaved bag. The infected kernels need to be incubated for four weeks under near UV-light (12h/12h), to support fungal growth. 5 g of the finished kernels were spread equally per m² between the potato rows. The trial was split into three parts, one for the wildtype inoculation, one for the mutant 1 and another one for mutant 2, to minimize an intermixture of the different isolates by wind or rain splash.

Disease assessment

The observation of the disease progress started with emergence until death of the potato plants. In each of the four replications, 10 plants per plot were rated for disease progress of early blight. To exclude the influence of the surrounding plots, only the two rows in the middle of each plot were used for observation. For the visual assessment the potato plant was divided into three leaf levels (lower, middle, upper leaf level) in order to follow disease development. One leaflet per

leaf level was examined to determine the percentage of necrotic leaf area. This rating was done by using a scheme for evaluation of the leaf necrosis in percentage from Granovsky and Peterson (1954).

RESULTS AND DISCUSSION

In all three stages of sensitivity testing (*in vitro*, *in vivo* and in the field) there was a clear tendency that SDH mutations in *A. solani* have a negative effect on the sensitivity to SDHI-fungicides (Tab.1).

Table 1. *Overview of different sensitivity factors regarding SDH- wildtype and –mutant isolates (means). n=number of isolates. *rAUDPC of untreated control in the wildtype inoculation: 0,49; in the mutant-inoculation: 0,57. In this table, only the results for the variety Lady Amarilla are described.*

	SDH-wildtype isolates	SDH-mutated isolates
In vitro (EC50 values with Cantus)	0,1µg/ml (n=9)	154 µg/ml (n=20)
In vivo (Fungicide-efficacy with boscalid)	99% (n=3)	69% (n=6)
In the field (rAUDPC)* (with Cantus)	0,37 (n=1)	0,49 (n=2)

Furthermore, the detected fungicide efficacy in the field differed between the two mutant inoculations, although they have the same mutation C-H134R.

In addition, in this first field trial, the four different SDHI-fungicides, which were used for the experiment, also showed a variability in the fungicide-efficacy both for the wildtype and the mutant inoculation.

CONCLUSION

In vitro, *in vivo* and in the field, a reduced SDHI-fungicide efficacy was observed. To confirm these results, especially from the annual field trial, a second (and third) field experiment needs to be done.

It was also observed in the annual field trial that even if there´s a decreasing SDHI-fungicide efficacy, there is a high dependency on the active ingredient of each fungicide.

All in all, the three stages of sensitivity testing give a deeper knowledge about the raising loss of fungicide-efficacy and the epidemiology of the pathogen.

Due to the fact, that mutations in the Succinate dehydrogenase of *A. solani* lead to a decreasing fungicide-efficacy even in the field and it is important to get a deeper knowledge about the efficacy of different SDHIs regarding the increasing and changing SDHI mutant population in European potato growing areas.

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Timing the application of fungicides to control potato early blight (*Alternaria solani*) in multi-location field trials in Denmark

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SUMMARY

Field experiments were carried-out at three locations in Denmark (i.e. Flakkebjerg, Sunds and Billund) in order to evaluate different spray strategies to control potato early blight. The treatments evaluated in the experiments were: (1) untreated, (2) a standard application in which fungicide application started at row closure, (3) starting fungicide application at the onset of first symptoms (First Symptoms), (4) starting fungicide application at 14 days after onset of first symptoms (Late Application), (5) fungicide application according to the maturity of the potato (Maturity-Based Model), (6) Modified TOMCAST, in which first spray was done at 330 physiologic days (Pdays) and subsequent spray was done according TOMCAST Disease Severity Value (DSV) threshold, and (7) TOMCAST + Maturity-based model, in which we combined the TOMCAST DSV and Maturity-Based Model. In general, our results showed that starting spraying from onset of symptoms could provide sufficient early blight control comparable to standard practice without any yield penalties. Starting application of fungicide later in the season resulted in lower disease control. The Modified-TOMCAST, Maturity-Based Model and TOMCAST + Maturity-based models controlled early blight effectively in all the experimental locations. In general, our results showed that low area under disease progress curve (AUDPC) values were associated with the fungicide treatments compared to the untreated. However, fungicide applications which started at 14 days after first symptoms resulted in lower disease control compared to the standard and other fungicide treatments. Starting application at the onset of symptoms resulted in low AUDPC blight control comparable to the standard treatment.
The decision support models showed a potential for optimizing the control of early blight by better targeting fungicide applications.

KEYWORDS

Early blight, Decision Support System, Control strategies, Maturity-based models, TOMCAST model, Physiologic age.

INTRODUCTION

Early blight, caused by *Alternaria solani*, has become an important disease in recent years in Denmark. The disease is mainly seen in the starch potatoes in August to September and can cause substantial yield losses of 7-20% annually.

Currently, none of the potato varieties being grown in Denmark is completely resistant to early blight (Abuley et al., 2017); thus, fungicide application is widely used to control early blight in Denmark (Abuley and Nielsen, 2017).

There has been a lot of discussion in Denmark about how to control the disease. One of the common control practices is to spray at a 14-day interval three to four times with difenoconazole or pyraclostrobin + boscalid. Because of the high prevalence of fungicide resistant F129L mutants, azoxystrobin is only used in maximum one application. However, there is no agreement on when to start the first spraying. Thus, our first objective was to determine the best time to start fungicide application to control early blight.

Recently, we published some Decision Support System models (DSS) (i.e. TOMCAST and Maturity-based models) to control early blight (Abuley and Nielsen, 2017). However, these trials were conducted at one location (Flakkebjerg). The second objective of this study was, therefore, to evaluate these models at different locations. Should the models perform well in multi-location field trials in Denmark, it would be made available to the farmers for the control of early blight.

MATERIALS & METHODS

General aspects of the experiment

In co-operation with the Danish Advisory Service (SEGES), we conducted field experiments at Flakkebjerg Research Centre (AU), Sunds and Billund (SEGES) in the 2016 growing season. The experiment at Flakkebjerg Research Centre (Western Zealand) was artificially inoculated on 29th June with barley kernels infested with *A. solani* as described previously (Abuley et al., 2017). The experiments at Sunds and Billund were situated in farmers' fields in Western Jutland, which is the main potato-growing region in Denmark. The experiments in Sunds and Billund were not artificially inoculated with *A. solani.*

The experiment was laid out as a randomized complete block design (RCBD) with four replications at all experimental locations. The plot size was 7 m x 3.75 m in all experimental locations. The potato variety Kuras was used at all experimental plots. Kuras is a late maturing variety that covers more than 50% of the area with starch potatoes in Denmark. The variety, even though is susceptible to early blight, is known to develop early blight slowly (i.e. slow blighting type resistance) (Abuley et al., 2017).

The treatments investigated were as follows:

1. Untreated
2. Standard (4x Signum WG). Here full dose (i.e. 0.25 kg/ha) of the fungicide Signum WG (pyraclostrobin+boscalid) starting from row closure at 14-day intervals.
3. First Symptoms (4x Signum WG). In this treatment, full dose of Signum WG was applied four times in the season starting from the onset of early blight lesions at 14 days intervals.
4. Late Application (4x Signum WG). Fungicide application (full dose) was started 14 days after first symptoms were observed.

5. Maturity-Based Model. This treatment recommended fungicide application according to the age-dependent susceptibility of the potato plant (Abuley and Nielsen, 2017).
6. Modified-TOMCAST model. In this treatment, first fungicide treatment occurred after 330 physiologic age/days (P-days), and subsequent sprays followed the accumulation of 20 TOMCAST DSV. Full dose of Signum WG was applied whenever fungicide application is required like described in Abuley and Nielsen (2017).
7. TOMCAST + Maturity-Based model. In this treatment, we combined the TOMCAST and the Maturity-based models as described in Abuley and Nielsen (2017).

Weather data and model implementation
Hourly readings of temperature, relative humidity and leaf wetness were taken from the nearest weather station. The physiologic age of the potatoes was determined from 50% emergence using the physiologic age equation described by Sands et al. (1979). The leaf wetness data and temperature during the hours of leaf wetness were used to run the TOMCAST model as described in Abuley and Nielsen (2017).

Disease assessment and statistical analyses
At each experimental location, we assessed the percentage covered by early blight on each plot on a weekly basis. The assessment data were used to calculate the area under the disease progress curve (AUDPC) according to Shaner and Finney (1977) to compare the treatments. The starch yield was assessed for each treatment as described in Abuley and Nielsen (2017).
The disease (AUDPC) and yield (hkg starch/hectare) were statistically analyzed by using ANOVA in R statistical software (R Core Team, 2016).

RESULTS & DISCUSSION
The AUDPC values were significantly different between the treatments ($p < 0.001$). In general, the fungicide treatments had low levels of early blight compared to the untreated (Figure 1), which suggests that fungicide application is important to control early blight.

It is apparent from Figure 1 that fungicide application according to the Modified-TOMCAST model resulted in the lowest AUDPC values at all three locations. There was a clear indication that starting fungicide application 14 days after first symptoms resulted in lower disease control and significantly higher AUDPC compared to starting at row closure or from the onset of the disease (Figure 1). The conclusion from this observation that for effective early blight control fungicide application should start at the onset of first symptoms. Previous reports (e.g. Campo-Arana, 2007; Abuley and Nielsen 2017) have also found that effective control comparable to a standard application can be achieved when fungicide application starts from first symptoms.

The use of physiologic age to time the application of fungicide instead of first symptoms was also effective in our models. Previous reports have shown that the time of first symptoms is dependent on the physiologic age of the potato plant (Pschiedt and Stevenson, 1988). The physiologic age model is a simple model driven only by temperature; thus, it can be a good alternative to scouting for first symptoms. In these field trials, all the weather-based models (i.e. Modified-TOMCAST, Maturity-Based Model and TOMCAST + Maturity-Based Model) were based on the physiologic age of the potato as determined by the temperature-based physiologic model.

Spraying fungicides according to the Maturity-based model reduced early blight attack significantly at all locations. We observed sufficient early blight suppression when we combined TOMCAST and Maturity-Based model (Figure 1). In general, the models provided appreciable levels of fungicide use than the standard application.

Even though we saw significant differences in the AUDPC values of the treatments, the starch yields were not statistically different at Sunds and Billund (Figure 2). In Flakkebjerg, however, the Modified-TOMCAST, Maturity-based model and TOMCAST+Maturity-Based models resulted in the highest starch yields, which were statistically different from the starch yield in the untreated plots (Figure 2).
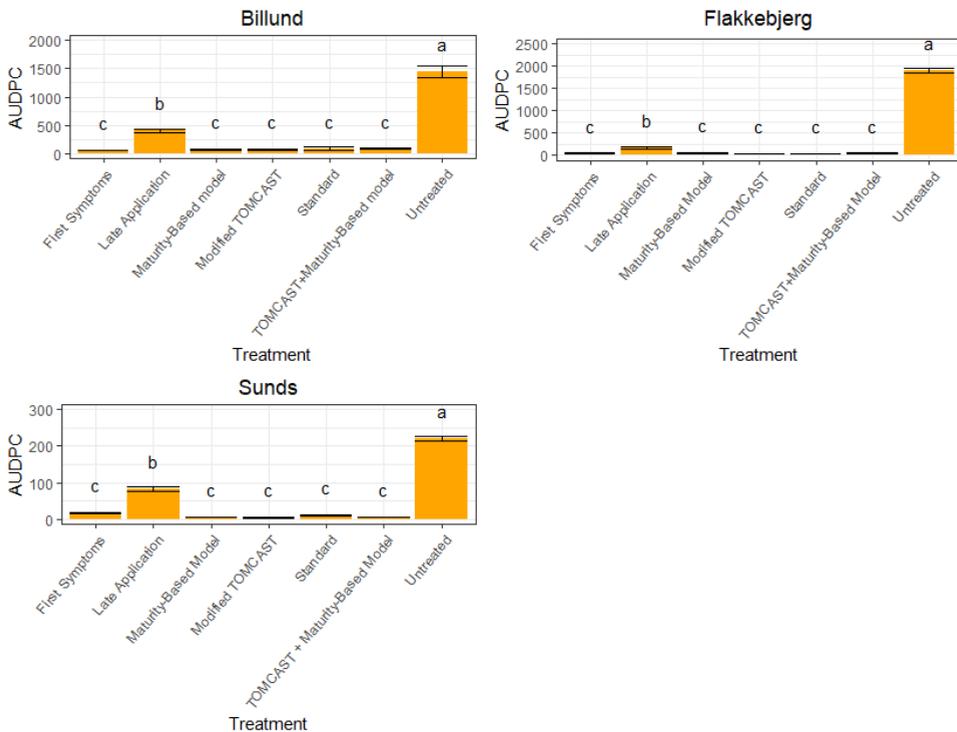
Figure 1. *The area under disease progress curve (AUDPC) as function of fungicide application at the three experimental location (Flakkebjerg, Sunds and Billund). The bars represent the mean values of four replicates. Bars followed by the same letters are not significantly different and vice versa (α=0.05, Tukey honest significance difference). See text for explanation of treatments.*
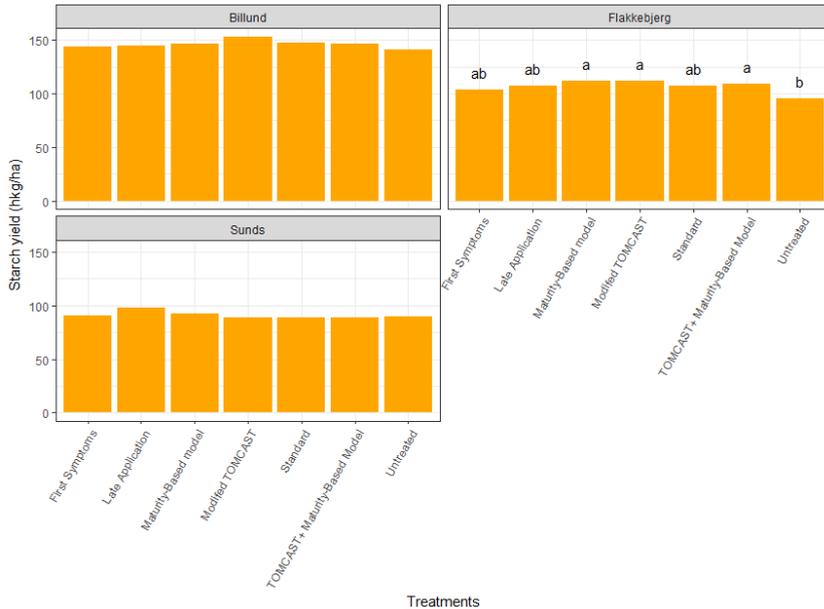
Figure 2. *Starch yield (hkg/ha) for the trials at the three experimental locations (Flakkebjerg, Sunds and Billund). Means followed by the same letter within a column are not significantly different (α=0.05, Tukey's Honest significant differences). See text for explanation of treatments.*

CONCLUSION

These experiments showed that the initial spray in the control of early blight can start at the onset of symptoms and still provide sufficient early blight control compared to the non-treated control plots without any yield reduction. Delaying the application of fungicide to i.e. 14 days after onset of symptoms resulted in weaker disease control. The Modified-TOMCAST, Maturity-based and TOMCAST + Maturity-based models controlled early blight effectively at all the experimental locations. The models show a potential for optimizing control of early blight by better targeting fungicide applications. The trials will be continued in 2017.

ACKNOWLEDGEMENTS

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Efficacy of fungicides to control early blight: results of EuroBlight experiments to calculate decimal ratings

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SUMMARY

Early blight is becoming an increasing problem in potato crops. Early blight is caused by *Alternaria* species, predominantly *A. solani*. In agricultural practice, early blight is controlled by applying fungicides in the second half of the season. Up until July 2017 the ratings of the fungicides to control early blight were based upon expert judgement from independent researchers. At the EuroBlight workshop in Brasov it was proposed that decimal ratings for fungicides for the efficacy table should be calculated after field experiments have been carried out over two years in three European countries, in accordance with the agreed experimental protocol. Ratings for fungicides with specific activity against *Alternaria* were tested using a 14-day spray interval whereas late blight fungicides with such activity were sprayed at 7-day intervals. Furthermore, some control strategies that used both groups of fungicide were also included. In July 2017 the first decimal ratings were published on the EuroBlight web site. This paper describes the set-up of the trials and the procedure for awarding a decimal rating.

KEYWORDS

Alternaria solani, early blight, potato, *Solanum tuberosum*

INTRODUCTION

Early blight caused by *Alternaria solani* is the second most important foliar disease of potatoes after potato late blight. The potato crop needs to be protected from *A. solani* by spraying fungicides regularly during the second half of the growing season. It is important to use fungicides that effectively protect leaves against this disease.

Some fungicides are approved specifically to control early blight. Some fungicides used to control potato late blight also contribute to the control of early blight. Until July 2017 the ratings of the fungicides to control early blight listed on the EuroBlight website were based upon expert judgement from independent researchers. At first the ratings scale was 0 to +++, in which 0 was no efficacy and +++ was excellent efficacy. In 2013 at the EuroBlight workshop in Cyprus it

was decided to adapt the scale to 0 to ++++. Nevertheless, the need was felt to base the ratings on experiments rather than expert judgement.

To evaluate the effectiveness of fungicides a harmonised protocol was developed at the Brasov workshop. It was proposed that the ratings of fungicides for the EuroBlight table should be calculated using the results from a minimum of six field experiments, carried out over two years in three European countries, in a system similar to that used to generate the fungicide ratings for potato late blight. In 2015 the first three experiments were carried out in Germany, Denmark and the Netherlands. In 2016 another three experiments were made in the same countries. The effectiveness of fungicides against early blight was compared by measuring the protection of leaves using standard 14- or 7-day spray schedules (these spray schedules do not necessarily comply with the product label recommendations). Protection against early blight derives from the protectant and/or curative properties of the active ingredients. The dose rates tested were the highest preventative doses registered in Europe. The results of the trials were used to re-evaluate the effectiveness of fungicides to control early blight. This report describes the protocols used and the analysis of the efficacy of fungicides to control early blight during the second half of the season.

MATERIAL AND METHODS

Experiments
In both 2015 and 2016 three experiments were carried out in Denmark, Germany and The Netherlands. The fungicides were sprayed at 7- or 14-day intervals or as a combined spray strategy. Combined spray strategy treatments comprised two or more fungicides, at least one of which was sprayed with a 7-day interval and one with a 14-day interval. The spray applications coincided with those of the other treatments. Spray strategy treatments starting earlier or ending later are allowed in the experiments but a rating is not permitted. Table 1 gives the number of treatments in each experiment carried out in 2015 and 2016.

Table 1. *The number of treatments included in the EuroBlight early blight fungicide rating experiments according to spray strategy applied.*

Fungicide	2015			2016		
	D	DK	NL	D	DK	NL
14-day interval	5	4	3	6	5	5
7-day interval	7	7	7	8	8	8
Combined strategy	2	2	2	2	2	2
Reference[1]	3	3	3	3	3	3

[1]: the reference included an untreated control and mancozeb (1500 g active ingredient/ha) in a 7- or 14-day spray interval.

Spraying to control early blight started approximately at the end of flowering. In Denmark and Germany the last spray application date was the same regardless of the 7- or 14-day spray interval, whereas in the Netherlands the last spray application for the 14-day interval was 1 week before the last spray application for the 7-day interval.

During the growing season the percentage foliar blight severity caused by *Alternaria* spp. was assessed at weekly intervals. To evaluate the epidemic, the Area under the Disease Progress Curve (AUDPC) was determined. The number of days from the first to the last disease observation varied for each experiment therefore StAUDPC values were calculated by dividing the AUDPC value by the number of days between the first and last disease observation. For each fungicide within any one experiment the same number of days was used. The StAUDPC is an indicator of the efficacy of the fungicides during the whole growing season and is the basis for calculating a decimal rating.

Statistics

Each experiment was laid out as a randomised complete block design with one treatment factor, the fungicides being tested, and four replicates. A mixed model analysis (REML) was performed on StAUDPC values calculated per experimental plot. REML analysis was used because not every fungicide was present in all six experiments. A mixed model consists of fixed treatment terms (here fungicide) and random block terms (here experiment, block and plot; formula 1).

$$stAUDPC_{ijkp} = \mu + E_i + B_{ij} + \beta_k + P_{ijp},$$

(1)

where

μ	=	overall mean
E_i	=	effect of experiment $i \sim N(0, \sigma_E^2)$
B_{ij}	=	effect of block j within experiment $i \sim N(0, \sigma_B^2)$
P_{ijp}	=	effect of plot p within block $B_{ij} \sim N(0, \sigma_P^2)$
β_k	=	effect of fungicide k

Based on the mean StAUDPC (mstAUDPC), ratings for the effectiveness of the fungicides to control early blight were calculated, according to formula 2.

$$ER_k = 5 \frac{MAX(y) - y_k}{MAX(y)},$$

(2)

ER_k	=	efficacy rating of the fungicide k to control potato early blight during the whole growing season.
y	=	mstAUDPC
MAX (y)	=	mstAUDPC of the fungicide with the highest mstAUDPC determined in the series of experiments, i.e. the untreated control.

RESULTS

At the 2017 EuroBlight workshop in Aarhus some deviations from the original protocol (that had been produced at the Brasov workshop) were discussed and accepted:

- The untreated control will be used as the reference, instead of mancozeb
- Combined spray strategies can be rated if they fit within a 7- and 14-day spray schedule

The adapted protocol is described in the appendix to this paper. The results of the dedicated trials in 2015 and 2016 led to the first EuroBlight table in which fungicides were assigned decimal ratings. The table was published on 31 July 2017 on the EuroBlight website http://euroblight.net/alternaria/early-blight-fungicide-table/early-blight-fungicide-table-new/

DISCUSSION

In the past a decimal rating system was set up to assess the efficacy of fungicides to control potato late blight, later the system was extended to tuber blight as well. New decimal ratings are published regularly on the EuroBlight website. In the mean time early blight caused by *Alternaria* became a more important disease of potatoes. The need to expand the decimal rating system also to include early blight was recognised.

In the *Alternaria* subgroup meeting at Munich in 2014 a more dynamic ratings system for fungicide efficacy in controlling early blight was discussed. A draft protocol for a trial was prepared. The protocol was presented at the EuroBlight meeting in Brasov in 2015 and accepted. In 2015 three experiments were carried out, followed by another three in 2016. In Aarhus in 2017 the first results were presented, but the fungicides were not identified, only codes were supplied. A number of fungicides met the criteria to allow a rating to be awarded. The ratings are based on non-transformed StAUDPC values. The main advantage is that ratings are determined using a system that is more objective than that used to produce table ratings up until the Brasov meeting in 2015. Another advantage is that there is scope for future, more effective fungicides to be rated higher than 4, the maximum until 2016. Furthermore, ratings once given are not fixed, thus relative changes in the effectiveness of fungicides can be made apparent. It was agreed at the Aarhus meeting that as soon as new ratings are calculated from trials and are approved the fungicide table on the EuroBlight website will be updated. In fact the new ratings were published on 31 July 2017.

The ratings proposed are exclusively based on the results of the trials carried out according to the EuroBlight protocol. The ratings are calculated for the highest fungicide doses registered in Europe. In agricultural practice lower dose rates are and will be used. The ratings do not reflect the fungicide efficacies when lower dose rates are used. Initially the reference treatments were expected to be mancozeb sprayed at a 7-day or 14-day interval. However, in Aarhus in 2017 it was decided that the untreated control should be the reference treatment for the early blight experiments. The advantage of the untreated control being the reference is that the rating for the untreated is by default 0, i.e. no efficacy to control early blight. In contrast, if mancozeb had been chosen as the reference a rating would have to have been awarded by expert subjective opinion, not based on experiments. For potato late blight a fungicide reference was inevitable because the disease progress curve for the untreated control is normally very different from those of the fungicide treatments. For early blight the epidemic usually starts later in the season and the gap in disease severity between fungicide treatments and the untreated is considerably smaller.

Details of the experiments can be found on the EuroBlight website.

CONCLUSIONS

The experiments were set up to calculate decimal ratings for fungicides controlling early blight.
- The untreated control was introduced as the reference and rated 0 by default.
- The decimal rating is scaled from 0 to a maximum of 5.
- A rating is based on a minimum of six experiments and is calculated using the StAUDPC values.
- A rating can be awarded only when the fungicide is registered somewhere in Europe.

- The first early blight fungicide table was published on 31 July 2017 and comprised one fungicide sprayed with a 14-day spray interval, five fungicides sprayed using a 7-day interval and two combined strategies. In these two cases one of the fungicides was sprayed at a 7-day and the other fungicide at a 14-day interval. The time frame for test fungicide application should be the same for all treatments.

ACKNOWLEDGEMENT

ADAPTED PROTOCOL FOR THE EVALUATION OF THE EFFICACY OF FUNGICIDES TO CONTROL EARLY BLIGHT

The protocol for testing effectiveness to control early blight was prepared by Huub Schepers, Jürgen Leiminger, Bent Nielsen, Hans Hausladen, Jan Spoelder, Jozefa Kapsa, Pieter Vanhaverbeke, Dani Shtienberg and Bert Evenhuis and is published below.

Purpose/aim of trials

To compare the "Effectiveness against early blight" by measuring the protection of leaves against infection by early blight resulting from the application of a fungicide according to this protocol. This spray schedule is not necessarily related to the label recommendations. This protection originates from the protectant and/or curative properties of the active ingredients. EPPO guideline PP 1/2 (3) (revised in 1996) describes the standard requirements of the field trial.

Specific additional requirements:

- A susceptible local ware or starch potato variety. The growth habit of the cultivar should be recorded, i.e. determinate or indeterminate growth.
- Potato late blight is controlled in a weekly scheme using fungicides with no efficacy to control early blight. For instance start with mandipropamid and end the spray schedule using cyazofamid.
- Preferably the experiment is carried out with natural infection. However if conditions are less suitable inoculation may be carried out with *A. solani*-infested grain kernels on the soil within the plot. The artificial inoculation is carried out 3 days before the first spray until 7 days after the first spray. When the inoculation is not successful it will be repeated.
- Misting is permissible, when conditions are exceptionally dry.
- Each treatment consists of applications of the fungicide to be tested regardless of the limited application numbers on the label.
- First spray depends on local conditions, but needs to be applied before the start of the epidemic and should be timed approximately at 7-8 weeks after crop emergence.
- Crop growth stage should be recorded on the days that the trial is sprayed. The BBCH key should be used.
- Untreated plots are part of the field experiment. In 2017 the untreated control was accepted as a reference for future trials but also retrospectively for earlier experiments.
- A reference treatment (two variants) is part of the field experiment i.e. 1500 g a.i. mancozeb. Sprayed in a 14-day interval and in a 7-day interval. From 2017 onwards the

mancozeb references are not necessarily part of the experiments, an untreated control is mandatory.

- Spray frequency is every 7-days (+/- 1 day) or every 14-days (+/- 1 day), to be chosen by the participant sponsors. A spray strategy with more than one fungicide is allowed, even if this means that one fungicide is sprayed with a 7-day interval and the other using a 14-day interval. The time frame of the spray applications should be the same for all treatments.
- The efficacy of the early blight fungicide(s) was to be compared to one of the two reference treatments accordingly. However, from 2017 onwards the efficacy is compared to the untreated control, allowing also spray strategies to be included in the trials.
- The number of sprays depends on the early blight epidemic and the spray interval chosen.
- Dose rate is the highest dose registered in Europe
- Assessment: every week (or more frequently when necessary) of plots by rating the % leaf area with symptoms. To assess early blight we recommend using the assessment key in the EPPO-guideline PP 1/263 (1).
- Desiccation: timing and method according to GAP.
- It is not strictly necessary to harvest the trial.
- A method for determining the rating for the "EuroBlight Fungicide Table" will be proposed when six successful trials (two seasons x three trials) have been carried out by independent research institutes in at least three different growing regions/countries in Europe. The proposed methodology will be agreed by independent researchers and the agrochemical manufacturers and where possible will be used to analyse data from registration trials, in which the relevant standard products are included. In this way a robust dataset will form the basis of the rating given for the "Effectiveness against early blight".

N.B. A successful trial is one that is carried out strictly according to this protocol and sufficient early blight is observed in the plots (>10% foliar severity in the worst treatment). The rating is set by determination and comparison of the AUDPCs of the six successful trials. A validation of this method will have to be carried out with existing trial data to find out whether a linear, a logarithmic or another transformation has to be carried out on the data.

Mancozeb: essential tool for sustainable protection of potato against early blight (*Alternaria* spp.*)*

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SUMMARY

In recent years Applied Plant Research International, part of Wageningen University Research (Wageningen U.R.) and UPL Europe Ltd (UPL) have demonstrated that mancozeb is effective on the 13_A2, 6_A1 and 33_A2 genotypes of late blight (*Phytophthora infestans*). In 2015 research was extended to study the efficacy of mancozeb on early blight (*Alternaria* spp.) and in particular *Alternaria solani* with the F 129 mutation associated with strobilurin resistance.

Initial laboratory research was conducted by Wageningen U.R. to study the efficacy of the fungicides, azoxystrobin, boscalid +pyraclostrobin and mancozeb on the spore germination of 15 *A. solani* isolates collected between 2006 and 2014. The results indicated that there was no shift in sensitivity to mancozeb within the *A. solani* populations tested but it was shown that some of the *A. solani* isolates had reduced sensitivity to azoxystrobin and to a lesser extent to boscalid+pyraclostrobin.

The objective of the 2016 field research by Wageningen U.R. was to verify the efficacy of difenoconazole, boscalid+ pyraclostrobin and mancozeb in controlling different genotypes of *A. solani*. The trial area was broadcast with *Alternaria* infected wheat kernels, a mixture of 95% wildtype and 5% F129L type and disease assessments were carried out regularly. All treatments were effective until 9th September (15 days after the last spray). In order to assess the *Alternaria* genotype and the presence of the F129L mutation, leaves with *Alternaria* spp. lesions were collected on September 17th and genotyped. No significant shift of the *A. solani* genotype was found where mancozeb was used compared to the untreated control. Where boscalid+pyraclostrobin or the same followed by difenoconazole was sprayed, significantly more F129L types were found compared to the untreated control.

A field trial was conducted by UPL in potatoes in the Netherlands in 2015. The objective was to determine the efficacy of selected fungicides in controlling *Alternaria* spp. The fungicides tested were mancozeb, azoxystrobin, boscalid+pyraclostrobin and difenoconazole, Results demonstrated that mancozeb provided significantly better control than the reference boscalid+pyraclostrobin programme. No significant differences were observed between other treatments.

Mancozeb has been registered for more than 60 years and due to its multi-site mode of action, it has consistently maintained its efficacy against both *A. solani* and *P. infestans* and remains an important component of fungicide resistance management programmes.

KEYWORDS
A. solani, fungicide, resistance, mancozeb, genotype, sensitivity

INTRODUCTION
Late blight, caused by *P. infestans*, is the most important disease in potato production but early blight, *A. solani* is regularly found in potato fields in the second half of the season. Fungicide products commonly used for *P. infestans* control programmes are based on products that contain actives such as boscalid, pyraclostrobin, azoxystrobin, difenaconazole and mancozeb. It is recognised that some fungicide products used to control *P. infestans* also exhibit some control of *A. solani*, especially those that contain mancozeb.

Resistance of *A. solani* to azoxystrobin has been reported in the United States (Pasche and Gudmested, 2008). This resistance is associated with the F129L mutation. This mutation has also been reported in Germany (Leiminger et al., 2014), and one isolate with the F129L mutation has been found in the Netherlands (Evenhuis et al., 2013).

A. solani isolates were collected from field experiments in the Netherlands during the period 2006 to 2014 and stored in liquid nitrogen at Wageningen U.R. In 2015 at the request of UPL a laboratory experiment tested the efficacy of boscalid+pyraclostrobin, azoxystrobin and mancozeb to control 15 isolates of *A.solani*.

Following on from the 2015 laboratory study a field trial was carried out by Wageningen U.R. in 2016 to test the efficacy of selected fungicide programmes to control a mixture of *A. solani* isolates (wild genotype and F129 genotype) at the request of UPL.

A field experiment with different fungicide programmes was conducted in 2015 by UPL investigating the control of *Alternaria spp.*

LABORATORY RESEARCH (2015)
Testing the efficacy of fungicides on the spore germination of *A. solani,* research conducted by Wageningen UR.

MATERIALS AND METHODS
To establish EC_{50} values using selected fungicides a dose rate series was determined. The efficacy of boscalid 26.7% + pyraclostrobin 6.7% w/w and azoxystrobin 250 g/l was tested at 0.01, 0.1, 1, 10 and 100 ppm. Mancozeb (75% w/w) was tested at 0.1, 1, 10, 100 and 1000 ppm. The ppm values were adjusted to the dose rate of the active ingredients. In the case of boscalid+pyraclostrobin the dose rate was added up. The fungicides at the appropriate dose rate were added to cooling Water Agar and poured into Petri dishes. To boscalid+ pyraclostrobin and azoxystrobin 100 mg / l SHAM dissolved in methanol was added, regardless of the dose rate tested. SHAM is a known inhibitor of the alternative oxidase (AOX) pathway that has been

suggested as a possible mode of QoI resistance in vitro in other fungi, therefore it was added to the medium.

A selection of 15 *A. solani* isolates was taken from the isolates that had been collected between 2006 and 2014 by Wageningen UR.

The inoculum density was set at approximately 10,000 sporangia per ml and the spore suspension was sprayed on to the agar plates containing the fungicides. The plates were incubated for 6 hours at room temperature (20°C) under day light conditions. After warm incubation, the plates were transferred to a dark chamber held at 4°C until germination assessments were carried out.

The germination rate of *A. solani* was established by counting the number of germinated and non-germinated spores under a light microscope. The spores were considered germinated when the length of the spore tube was at least the same as the diameter of the spore. The percentage germination was calculated by division of the number of germinated spores with the total number of spores counted multiplied by 100.

The experiments were replicated two times and each replication consisted of one Petri dish containing spores of a known *A. solani* isolate. Fungicide sensitivity was measured as the concentration at which spore germination was inhibited by 50% relative to the untreated control (EC_{50} value) and was determined for each isolate. Analysis of variance on $Log10$ (EC_{50}) was made using GENSTAT 17th Edition.

RESULTS

This experiment was designed to establish the EC_{50} values of fungicides to control different *A. solani* isolates. The results are presented in Figures 1-7.
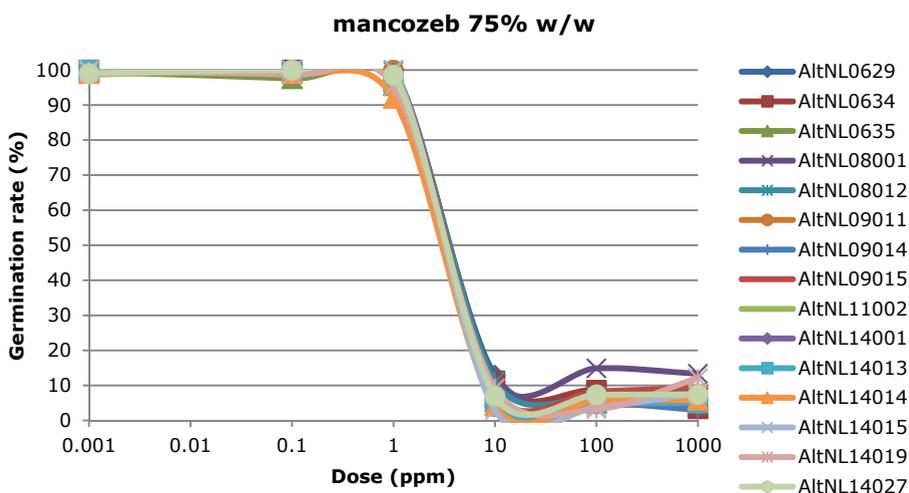
Figure 1. *Dose response effect of mancozeb to control 15 isolates of A. solani. The untreated control was placed at 0.001 ppm.*

boscalid 26.7% + pyraclostrobin 6.7% w/w
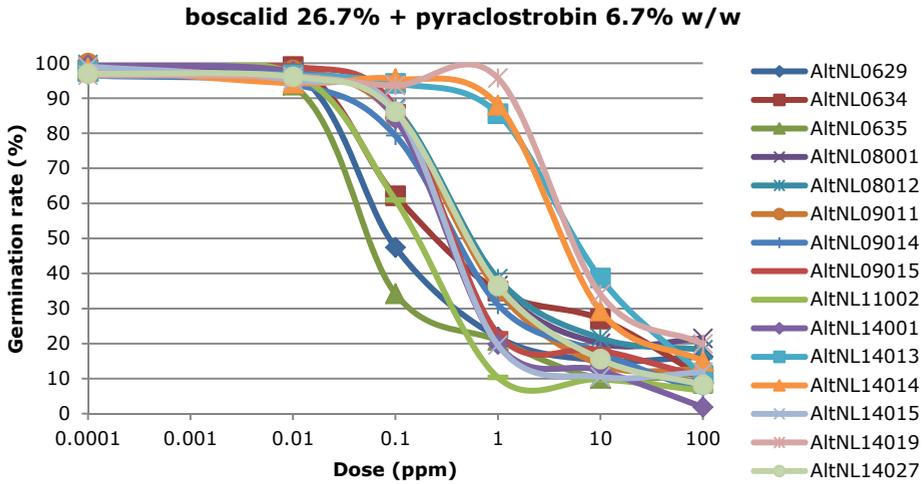
Figure 2. *Dose response effect of boscalid+pyraclostrobin to control 15 isolates of A. solani. The untreated control was placed at 0.0001 ppm.*

azoxystrobin 250 g/l
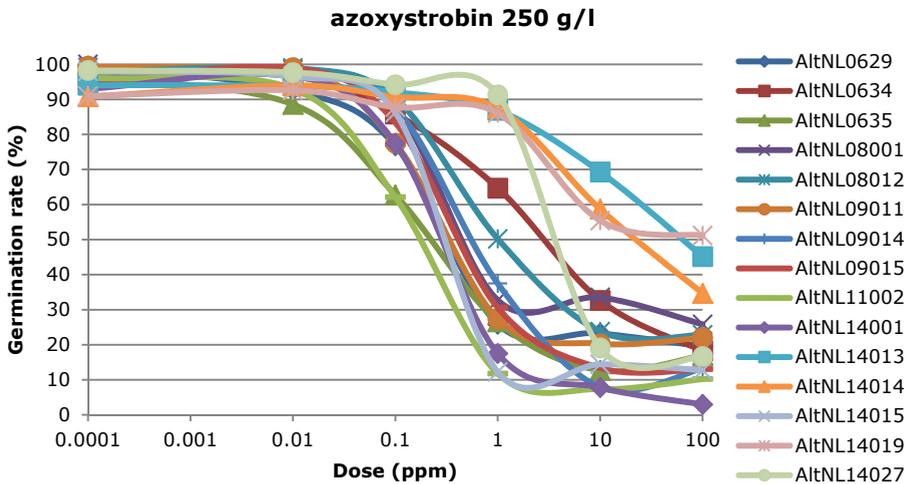
Figure 3. *Dose response effect of azoxystrobin to control 15 isolates of A. solani. The untreated control was placed at 0.0001 ppm.*
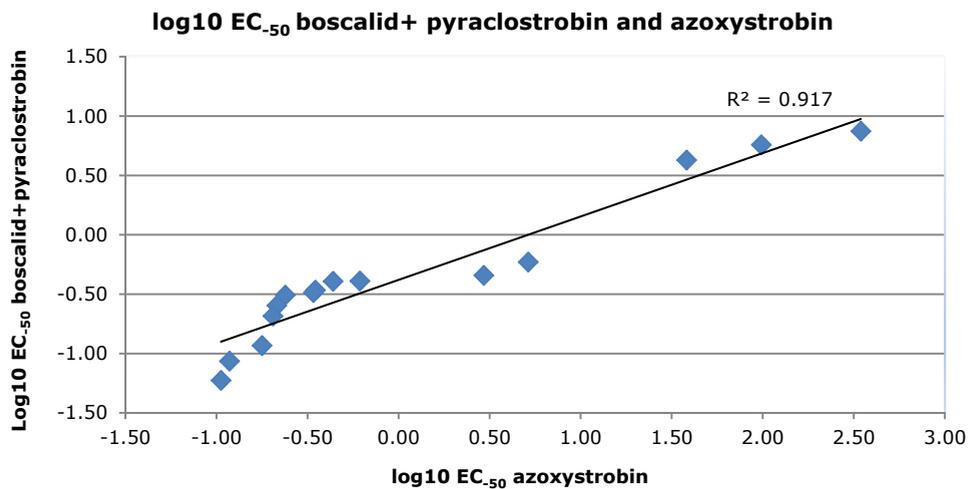
Figure 4. *Correlation between EC$_{-50}$ values for spore germination of boscalid+pyraclostrobin and azoxystrobin.*
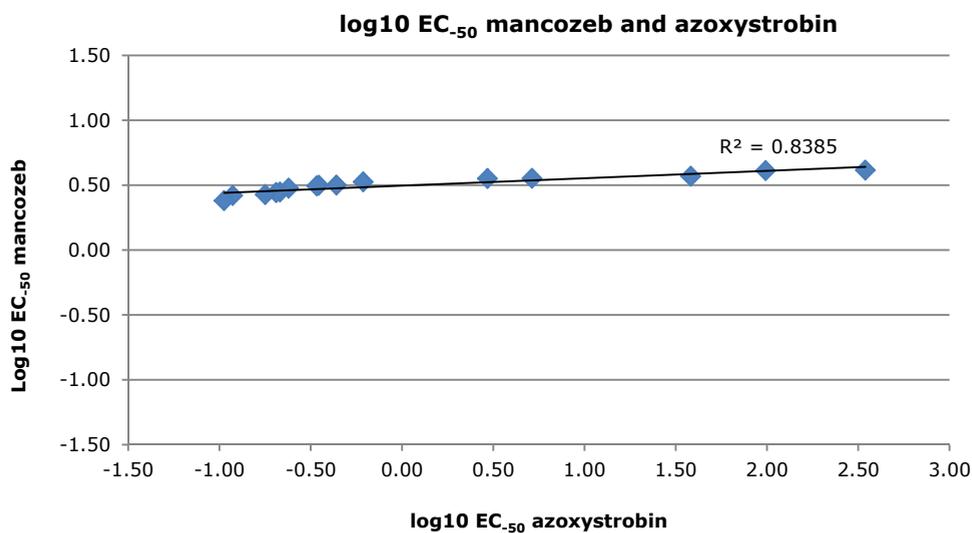
Figure 5. *Correlation between EC$_{-50}$ values for spore germination of mancozeb and azoxystrobin.*
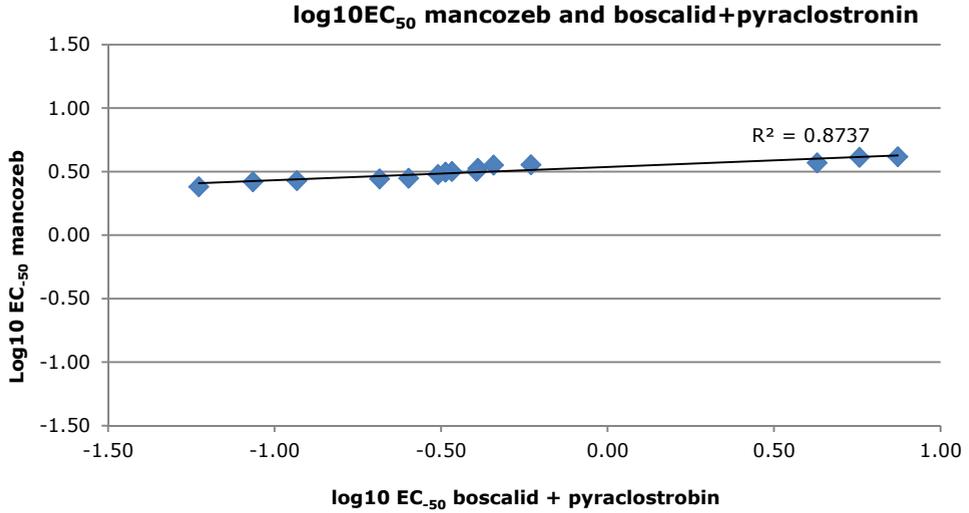
Figure 6. *Correlation between EC_{-50} values for spore germination of mancozeb and boscalid+pyraclostrobin*
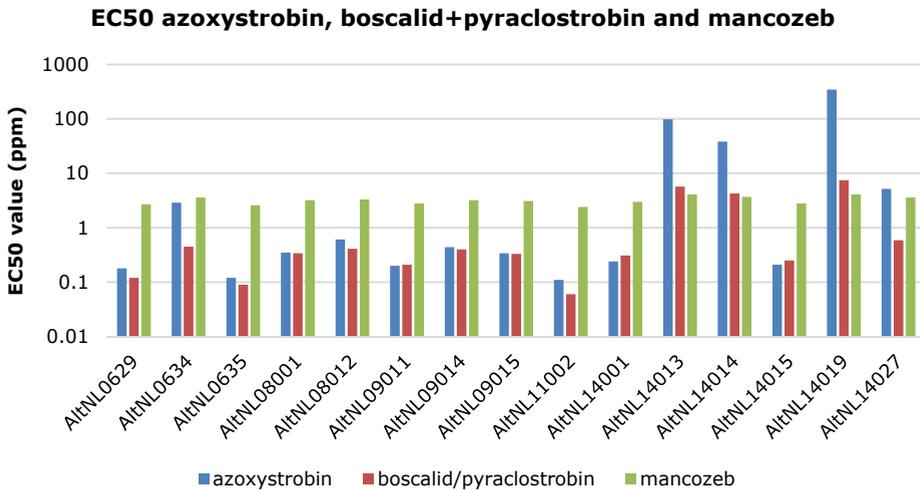
Figure 7. *EC_{50} Values (ppm) of the fungicides used, showing the 15 Isolates of A. solani, in order of sampling year*

DISCUSSION AND CONCLUSION

Within the *A. solani* isolates tested the sensitivity to mancozeb did not significantly differ, although the EC_{50} values for mancozeb in most cases were higher than for azoxystrobin and boscalid+pyraclostrobin. The EC_{50} values for mancozeb were very consistent this indicates that there was no shift in sensitivity to mancozeb within the *A. solani* population tested.

The results suggest that some of the *A. solani* isolates became less sensitive to azoxystrobin and to a lesser extent to boscalid+ pyraclostrobin particularly for the 2014 samples.

From the data there appears to be some correlation in the sensitivity of *A. solani* isolates to the different fungicides tested. This does not necessarily mean that this is caused by cross resistance, it may be caused by fitness of the *A. solani* isolate. You would expect an isolate that more readily germinates to do so under any circumstances compared to a less fit isolate. The slope of the line is much steeper when boscalid + pyraclostrobin and azoxystrobin are compared (Figure 4) than when the comparison is made with mancozeb (Figures 5 and 6). No significant difference in mancozeb sensitivity was found between the *A. solani* isolates thus cross resistance, between mancozeb on one hand and azoxystrobin or boscalid+pyraclostrobin on the other is not likely. For both boscalid+pyraclostrobin and azoxystrobin the EC_{50}-value differs significantly between isolates tested. In this case isolates less sensitive to boscalid+pyraclostrobin seemed to be also less sensitive to azoxystrobin, suggesting the possibility of cross resistance or sensitivity.

FIELD STUDY (2016)

Efficacy of fungicides to control different early blight genotypes, research conducted by Wageningen U.R.

MATERIALS AND METHODS

Fungicide applications were carried out using a trial site sprayer with Airmix 110.04 nozzles. Nozzles were hanging approximately 50 cm above the foliage. Sprayings were carried out based on 300 l/ha. Potato plants were sprayed for the first time when they reached a height of 20-30 cm when rapid growth started. Specific sprays to control *Alternaria spp.* commenced mid-July 2016. Haulm killing was carried out on 30 September 2016, despite natural senescence of the crop. In Table 1 the fungicides used and dose rates are presented. Treatment A is the untreated control and H the reference treatment chosen. Spray strategies are given in Table 2.

Table 1.   *The fungicides used and the applied dose rates.*

Code	Active ingredient	Dose rate (L or kg/ha)
N	difenoconazole 250 g/l	0.5
S	boscalid 26.7% + pyraclostrobin 6.7% w/w	0.2
P	mancozeb 80% w/w	2.0

Table 2.   *Spray strategies and date of application.*

Treatment	Product	12-7	19-7	26-7	2-8	9-8	16-8	23-8
A	UTC	-	-	-	-	-	-	-
B	S	S	-	S	-	S	-	S
C	P	P	P	P	P	P	P	P
D	P + S	P+S	P	P+S	P	P+S	P	P+S
E	N	N	-	N	-	N	-	N
H	S or N	S	-	S	-	N	-	N

For inoculation a selection of 2 *A. solani* isolates was made. AltNL03003 was isolated in 2003 and belongs to genotype 1 and is a wild type. AltNL15002 belongs to genotype 2 and possesses the F129L mutation. Both isolates were grown on wheat kernels separately. A mixture of 95% wildtype and 5% F129L type Infested kernels were broadcasted in the field on 15 July 2016.

To assess *Alternaria spp.* genotype and the presence of the F129L mutation 8 leaflets with *Alternaria* spp. lesions were sampled per plot. Samples were taken four times during the season, in the last week of August and in the first three weeks of September. Only the last sample was genotyped. The samples were air dried and stored in Petri dishes until further processing. At the laboratory monospore cultures were made on agar. Mycelium was scraped from the agar plate after incubation. DNA was extracted from the mycelium. The genotype involved was assessed by carrying out two PCR described by Pasche et al., 2004 and Lieminger et al., 2015. The PCR product was extracted from the gel and sequenced. The nucleotide order was assessed and the presence of the F129L mutation was established according to the publication of Lieminger et al., 2015.

Statistics: analysis of variance on the parameters was made using GENSTAT 18th Edition. The experiment was carried out with four replications in a randomised block design. Each replication consisted of a plot. Transformation of data was carried out when necessary.

RESULTS

Field assessments
On 22 July 2016 no *Alternaria* was found. A week later on 29 July *Alternaria* was present in all plots at a low disease severity rate of 0.001% (data not shown). From 5 August onwards the *Alternaria* epidemic started (Figure 8). After 17 September it was not possible to assess *Alternaria* because it was too much entangled with natural senescence to distinguish.
Disease severity of all spray strategies was significantly lower than the untreated control. Based on AUDPC, the efficacy of treatments D, E and H were significantly better than treatments B and C.
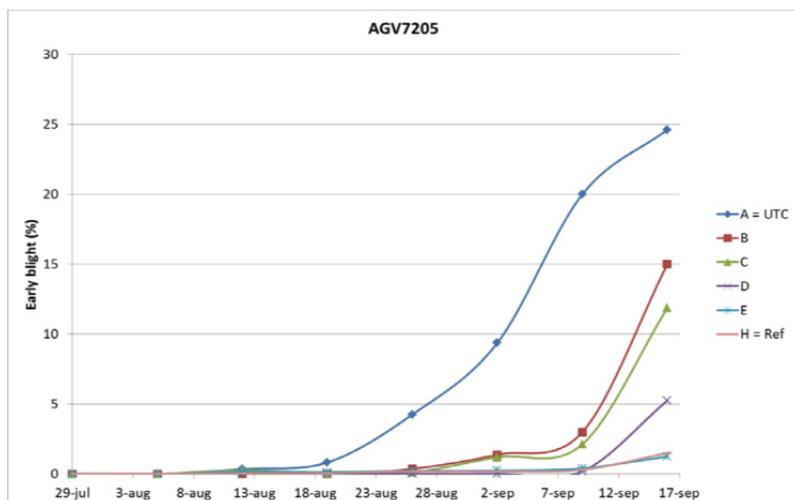
Figure 8. *Alternaria disease severity (%)*
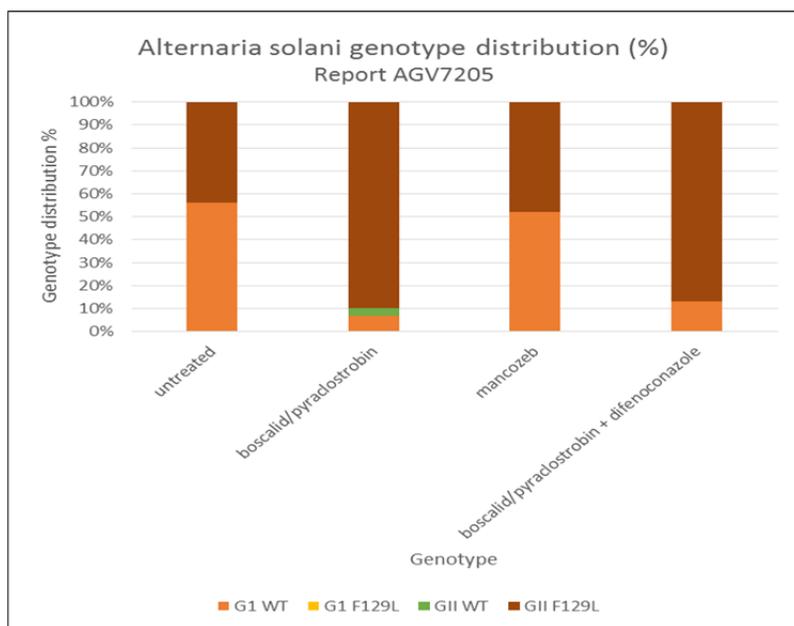
Figure 9. *A. solani genotype distribution (%)*

Genotyping

The results are presented in Figure 9 no significant shift of the *A. solani* genotype was found when mancoze*b* was used compared to the untreated control. When boscalid+pyraclostrobin (B) or boscalid+pyraclostrobin followed by difenoconazole (H) was sprayed significantly more F129L types were found compared to the untreated control.

DISCUSSION AND CONCLUSION

A field experiment was carried out in potato cultivar Agria. The *Alternaria* epidemic developed late despite artificial inoculation. The weather in June was conducive for *P. infestans* and not so much for *Alternaria*. *P. infestans* was controlled with cover sprays using fungicides which have no known efficacy to control *Alternaria*.

Alternaria severity in the untreated control was significantly higher than all other treatments indicating that the inoculation was successful. Furthermore all spray strategies effectively controlled *Alternaria*, although the efficacy varied between strategies.

Disease severity of treatment B and C increased more towards the end of the season compared to treatments D, E and H. The last spray application was 23 August and the increase was observed from 9 September onwards. Interestingly when mancozeb was added to boscaclid+pyraclostrobin (or v.v.) the efficacy to control Early Blight increased compared to the products used solo. However the efficacy to control *Alternaria* was still less than using difenoconazole or the combination boscaclid+pyraclostrobin followed by difenoconazole.

The field was inoculated with wheat kernels with 2 *Alternaria* genotypes. Genotype I (GI)) is wild type and Genotype II (GII) possesses the F129L mutation. Predominantly these two genotypes were found in the field (Figure 9). Only 1 isolate found was Genotype II, wild type. No Genotype I isolates were found possessing the F129L mutation. In the untreated control 57% of the isolates were Genotype I and wild type. The experiment was inoculated with 90% Genotype I wildtype. This could suggest that the F129L mutation was already present in the natural *A. solani* population at the location of the field experiment. Alternatively GII might be more aggressive than GI and therefore was found back more frequently.

Leaves were picked at four times during the season. *A. solani* was isolated only from the leaves picked at the last sampling date. Leaves of the other sampling dates were stored under dry conditions at room temperature and could be used for additional assessments.

When boscaclid+pyraclostrobin or boscaclid+pyraclostrobin followed by difenoconazole was sprayed, significantly more GII F129L *A. solani* types were found than in the untreated control and when mancozeb was sprayed. This suggests that selection towards GII F129L occurred under influence of spray strategies B and H. It is known that the F129L mutation causes a reduced sensitivity to QoI fungicides. One of the active ingredients, pyraclostrobin is a strobilurin, QoI fungicide.

FIELD STUDY (2015)

Control of *Alternaria spp*. in potatoes in the Netherlands, research conducted by UPL

MATERIALS AND METHODS

Fungicide applications were carried out using a AZO sprayer with Teejet 110.02 nozzles. Nozzles were hanging approximately 50 cm above the foliage. Sprayings were carried out based on 300 l/ha. Potato plants were sprayed for the first time at BBCH stage 67 against *Alternaria* at that point no *Alternaria* infestation was visible. In total 4 applications were made: 24 July, 31 July, 07 August and 19 August 2015. In Table 3 the fungicides used and dose rates are presented. Treatment 13 is the untreated control (untreated strips) were regularly situated within the trial.

Table 3.   *Fungicide actives used and the applied dose rates.*

Code	Active ingredient	Dose (L or kg/ha)
1	mancozeb 75% WG	1.3
2	mancozeb 75% WG	1.6
3	mancozeb 75% WG	2.0
7	boscalid 26.7% + pyraclostrobin 6.7% WG	0.2
8	difenoconazole 250 g/l EC	0.5
9	azoxystrobin 250 g/l SC	0.25
13	Untreated	

Table 4.   *Alternaria severity*

Trt No.	Treatment Name	Rate	Rate Unit	7-Aug		19-Aug		28-Aug	
1	mancozeb 75% w/w	1.3	kg/ha	0.001	a	0.02	a	2.35	bc
2	mancozeb 75% w/w	1.6	kg/ha	0.001	a	0.04	a	1.06	bc
3	mancozeb 75% w/w	2.0	kg/ha	0.000	a	0.02	a	0.28	c
7	boscalid + pyraclostrobin	0.2	kg/ha	0.033	a	0.35	a	3.95	b
8	difenoconazole	0.5	l/ha	0.000	a	0.22	a	1.30	bc
9	azoxystrobin	0.25	l/ha	0.000	a	0.20	a	2.00	bc
13	untreated strips			0.002	a	0.45	a	6.63	a

The infestation is this trial was natural. No laboratory determination of *Alternaria* was made, but the infestation symptoms appeared to be *A. solani*. After August 28th no further assessments were possible, due to natural senescence of the crop.

Results were evaluated using Agricultural Research Manager (ARM), version 2016. The experiment was carried out with four replications in a randomised block design. Each replication consisted of a block. No transformation of data was carried out.

DISCUSSION AND CONCLUSION

A field experiment was carried out in the potato cultivar Innovator. The *Alternaria* epidemic developed late and the severity in the untreated control stayed low. Potato late blight (*P. infestans*) was controlled with cover sprays using fungicides which have no known efficacy to control *Alternaria*.

All treatments showed a significantly lower infestation of *A. solani* compared to the untreated control. A dosage effect of mancozeb was clearly visible although this was not significant, the highest dosage of 2.0 kg/ha showing the lowest infestation of *A. solani*. The control with mancozeb 2.0 kg/ha was significantly better than the reference product boscalid+pyraclostrobin 0.2 kg/ha. No significant difference was obtained between other treatments.

CONCLUSION

Mancozeb has been found to be effective against all genotypes of *A. solani*. When mancozeb was used no shift in the *A. solani* genotypes was found compared to the untreated control as opposed to boscalid+pyraclostrobin and azoxytrobin where a difference was observed. It can be

concluded that mancozeb is an essential tool in managing fungicide resistance of populations of *A. solani* that have been identified in the past and present.

Studies in recent years have also demonstrated that mancozeb is efficient on all genotypes of *P. infestans*. As with *A. solani* mancozeb does not cause any change in the composition of the populations of the two pathogens thus maintaining the natural equilibrium between the two main potato diseases.

Due to its "multi-site" mode of action on foliar diseases, mancozeb remains a key active for sustainable protection of the potato crop and is essential in helping to prolong the efficacy of fungicides with single site mode of action.

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Optimizing the Use of Curative Late Blight Fungicides

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SUMMARY

Fungicides that can act curatively (within the incubation period of pathogen development) are an increasingly important component of late blight (*Phytophthora infestans*) control strategies. This study aims to produce a simple decision aid for the use of products with curative activity by growers and agronomists. Interim results, which form the basis of the decision aid, are presented here. Data from glasshouse bioassays with a representative curative fungicide (fluopicolide + propamocarb), a susceptible variety (King Edward), and an isolate belonging to an aggressive genotype suggest that curative control declines 24 hours after infection, with little benefit gained from curative treatments applied 40 hours or more post infection. However, results from field trials suggest that varietal resistance is a significant modifying factor: curative treatments sprayed 43 hours after infection significantly reduced lesion number for the varieties Cara and Sarpo Mira but not the more susceptible King Edward.

KEYWORDS

Phytophthora infestans, curative fungicides, decision support, varietal resistance, control strategies

INTRODUCTION

In northern Europe late blight of potato is controlled by routine applications of fungicides, usually at no greater than 7-day intervals (Hansen et al., 2016). All fungicide applications are intended as prophylactics, i.e. to prevent the establishment of infections within the crop. However, several active ingredients (a.i.s) of commonly used fungicide formulations have some mobility *in planta*, and can act curatively. Curative activity is defined as pathogen control that occurs post infection, but before the development of visible symptoms (Ivic 2010). Whilst it is inadvisable to use late blight fungicides solely as curatives, curative activity is an important component of many late blight spray programs – particularly when fungicide treatments are scheduled immediately following periods when the risk of infection is very high.

P. infestans has a rapid life cycle and the efficacy of curative fungicides declines as the pathogen develops, meaning there is a short 'curative window' in which they offer good control (Pirondi et al., 2017). Timing is therefore of great importance, with mistimed applications unlikely to contribute to effective disease control. However, growers and agronomists currently have somewhat limited information to guide them when considering their use of curatives. Published ratings for curative activity are provided in the EuroBlight table (Bain. 2016), and whilst these are of great utility they are nevertheless qualitative, and are derived from subjective opinion. Additionally, there is some evidence that the curative window can be modified by factors that alter the rate at which leaf tissue is colonized by *P. infestans* (Genet et al., 2001) but which factors are of greatest importance and the extent to which they attenuate or enhance curativity is not well understood.

The purpose of this project is to produce a simple decision aid that can be used to support the use of curatives in integrated control of late blight. The final decision aid will incorporate some of the major modifying factors, such as temperature, pathogen lineage (Cooke et al., 2014) and the varietal resistance of the individual crop. Of key importance is that the aid is (i) based on empirical data, and (ii) applicable to the field situation. This paper briefly describes some of the methods used to gather this information.

MATERIALS AND METHODS

The curative fungicide Infinito (Bayer CropScience; 62.5 g fluopicolide + 625 g propamocarb l^{-1}) was applied at the recommended field dose of 1.6 l ha^{-1} in 200 l water in all bioassays and field trials described below. This product was selected as a representative 'good' (++ rated) curative fungicide using the information in the EuroBlight fungicide table. Artificial inoculations used spore suspensions of *P. infestans*, adjusted to 10^5 sporangia ml^{-1}. These suspensions were prepared from 7 day-old infected leaflets. Each inoculation site received a 20 µl droplet, placed on the adaxial leaf surface, avoiding large veins.

Curative threshold bioassays

Foliage was collected from 7 week-old, glasshouse-grown King Edward (foliage resistance rating 3) potato plants. Leaf discs (12 mm diameter) were cut from this material using a cork borer. The discs were then placed within a 170 mm x 170 mm Perspex frame, into which holes had been drilled, each frame accommodating 64 discs. Cut edges were covered by Parafilm strips leaving a 1 cm^2 area of tissue exposed. Discs were then individually inoculated with 20 µl droplets of *P. infestans* (isolate 2012_9922C, isolated from Great Britain) sporangial suspension. Inoculated discs were sealed within transparent plastic boxes lined with damp tissue paper. Boxes were in turn placed within a controlled climate chamber (16h / 8h day-night cycle, 18^oC). At timings corresponding to 4-hour intervals between 8 and 72 hours post inoculation, selected frames were removed from the climate chamber and treated with Infinito using an AZO compressed air precision sprayer. Frames were returned to incubation conditions immediately following treatment. Seven days from the initial inoculation frames were assessed for disease development on discs. A disc that was completely necrotic or showed signs of sporulation was classified as a successful infection, whilst one that showed no symptoms or small arrested lesions was classified as effective control.

Varietal resistance field trials

Potato plants of varieties King Edward, Cara (foliar resistance rating of 5) and Sarpo Mira (7) were grown in small propagation pots within a poly-tunnel for approximately 7 weeks. When high risk weather was forecast (Smith criteria met) plants were transported to a trial field where a late blight epidemic was in progress. Plants were placed within open trays on ridges and were left exposed for 2 hours. The plants were then sealed within plastic sheeting and placed within a climate chamber (16h / 8h day-night cycle, 18°C). After two days incubation, 12 plants per cultivar were treated curatively and returned to the climate chamber. Seven days after exposure to inoculum, the number of late blight lesions per plant was counted. The trial was repeated at a later date with the following modifications: cultivars King Edward and Cara were used, and three separate Infinito treatment times were included: 1, 2 and 3 days post exposure.

RESULTS

Curative threshold bioassays

Figure 1 shows data for three runs of the leaf disc bioassay with the same isolate (9922c). At early time points (8 – 24 hours) curative sprays generally offered good control on the leaf discs, protecting between 60 – 100% of discs. This control is then rapidly lost from 24 – 40 hours, and at time points greater than 40 hours curative sprays rarely prevented more than 30% of infection sites from developing into lesions. The data are best described by a sigmoid curve ($R^2 = 0.67$) with the formula $y = 0.89 / (1 + e^{[-0.1 * \{x - 30.9\}]})$.
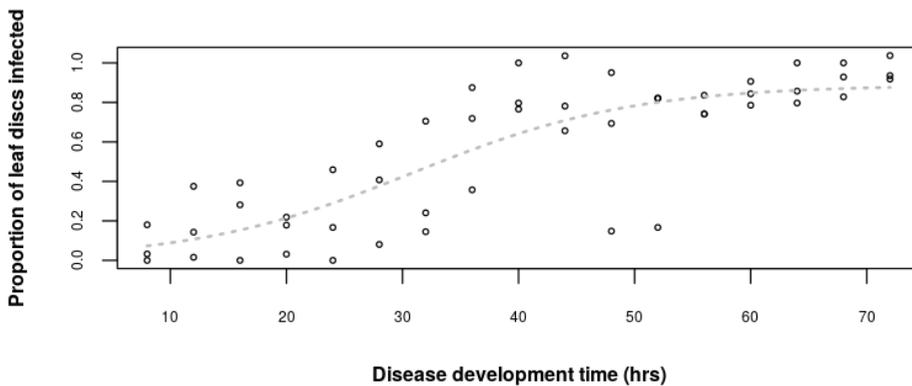
Figure 1. *Proportion of leaf disc infection (n=64) in relation to curative fungicide timing (hours at 18 °C from inoculation to treatment with Infinito). Data from three runs of the bioassay are shown, all using isolate 2012_9922C (genotype 13_A2).*

Varietal resistance field trials

In the first experiment using small plants grown in a polytunnel that were later exposed to field inoculum at 43-hour post-infection, curative fungicide treatment was effective on the two more resistant cultivars (Cara and Sarpo Mira) but not the most susceptible cultivar (King Edward) (Figure 2A). In the repeat experiment treating the susceptible King Edward (3) curatively after more than 1 day gave a lack of control similar to no fungicide, however there was a significant benefit from Infinito applied to Cara both 2 and 3 days after infection (Figure 2B).
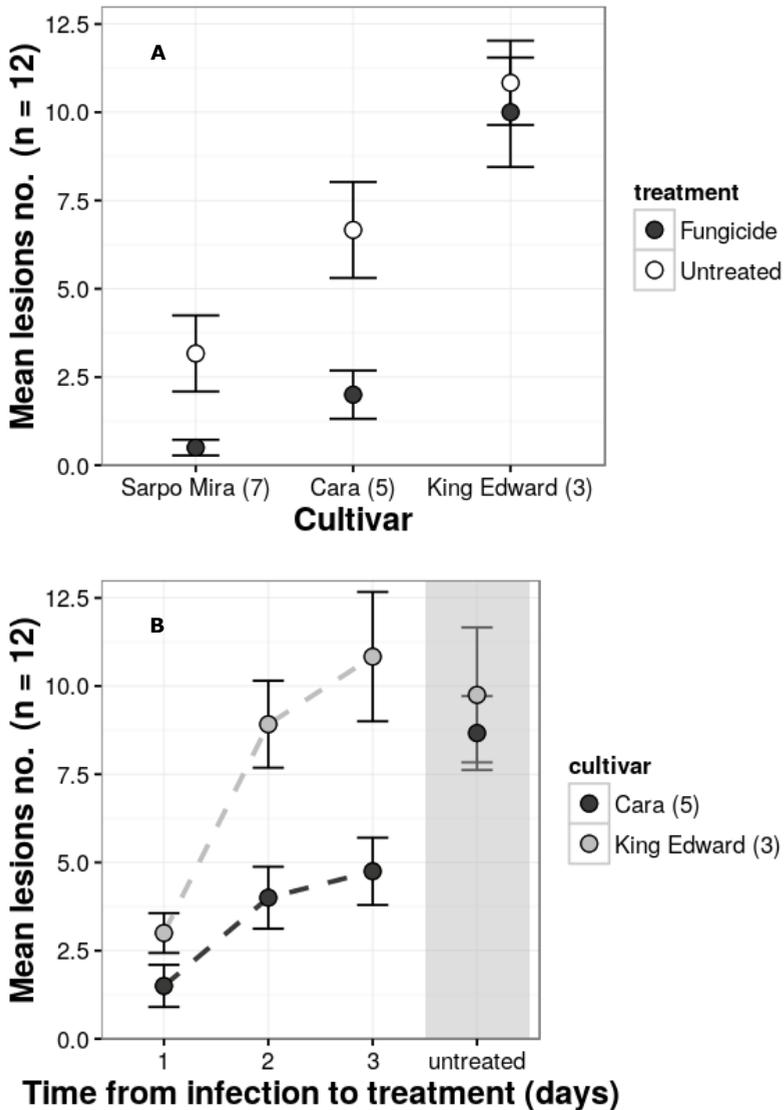
Figure 2. *Mean lesion count ± SE on plants exposed to natural inoculum for 2 hours and subsequently sprayed with curative fungicide after 2 days (43 hours) incubation at 18 °C (A) or after 1, 2 or 3 days incubation at 18 °C (B).*

DISCUSSION

An integrated management program is most likely to be effectively implemented if practitioners have a range of flexible tools which in combination with personal experience and knowledge of local conditions can inform decision making (Barzman et al., 2015). Data presented here will help form the basis of the decision aid and it is envisaged that the final aid will be used in the UK in conjunction with existing support systems such as the Hutton Criteria.

Results from this investigation confirm that curative activity is time limited, particularly on cultivars that are very susceptible to infection by *P. infestans*. The results of the small plant varietal resistance field trials support the inclusion of crop variety within the decision aid, as varieties with higher foliar resistance ratings appeared to have an extended time window for curative control. However, it is not clear if this is generally applicable or is specific only to the tested cultivars. This is being investigated further. Quantitative resistance to *P. infestans* is probably based on a range of difference mechanisms (Poland et al., 2009), which may vary between cultivars. It is conceivable that some of these may not impact on the rate at which the pathogen colonizes tissue, and so would be not act as a modifying factor on the curative effect.

It has been demonstrated previously that air temperature acts as a major modifying factor (Genet et al., 2001) with temperatures that are sub-optimal for pathogen development extending the time period from infection to treatment over which a curative treatment gave good control. Temperature data from the experiments carried out will allow the final decision aid to operate in thermal time, which should greatly enhance its infield utility. Pathogen lineage is another potential modifying factor, with some more aggressive lineages displaying a more rapid life cycle (Cooke et al., 2012; 2014); the described bioassay has been repeated with a less aggressive genotype (data not shown) and aggressiveness differences will be taken account of in the final decision aid.

ACKNOWLEDGEMENTS

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DuPont™ Zorvec® disease control: A novel tool for the control of late blight in potatoes

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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 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*. Because of the unique new mode of action a new Frac classification has been issued (Frac classification 49).

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) 20 minute rainfast.
This combination of attributes allows oxathiapiprolin to provide consistent and reliable disease control, even under the most severe conditions. The combination of attributes gives also a 3 till 4 day longer protection when compared to the current product used to control *Phytophthora infestans.*

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. With launches of the product in all parts of the globe and a registration expected in Europe soon* broad practical experience is a fact. DuPont Zorvec® is recommended for preventative use in mixture with another fungicide belonging to another group. For this reason the product will be markted in twinpacks and followed by ready to use mixtures.

*At the time of writing the abstract, a registration for Zorvec Enicade® has been obtained in Ireland. In July 2017 Zorvec Enicade® was added to the EuroBlight table.

Can potassium phosphite be integrated in late blight control strategies in starch potato?

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INTRODUCTION

Potato late blight is a serious disease that requires intensive and repeated fungicide treatments to avoid yield loss. We have earlier showed that potassium phosphite combined with reduced doses of fungicides provide good protection against both late blight and tuber blight. In more resistant starch potato cultivars potassium phosphite alone may provide sufficient protection (Liljeroth et al., 2016). Despite a very low toxicity of phosphite there are concerns about residue levels in harvested tubers. Currently the MRL for phosphite in ware potato is 30 mg/kg (EU commission regulation). In this study we report continued field results from starch potato treated with phosphite and analysis of residue levels in the products, i.e. starch, fiber and protein, after processing the potato tubers.

MATERIALS AND METHODS

Field trials with traditional fungicide programs were compared with treatments with potassium phosphite alone or in combination with fungicides in a randomized block design with four blocks during two years. The first treatment was applied during the third week of June. In the standard fungicide program the treatments were applied 12 times (T1-T12) with 7-day interval at recommended dose. The fungicides Revus (T1, T3), Infinito (T4, T5, T6) and Ranman Top (T2, T10, T11, T12) were alternated. Phosphite (Proalexin) was applied at all treatment occasions and the full dose rate was 5 L/ha Proalexin. In the combination treatment with 7-day interval reduced doses of the fungicides (50 or 25% of full dose) was used in combination with 50% dose of phosphite. In the combination treatment at 14-day interval full or half doses of the fungicides were combined with full dose phosphite so that in total the same amounts were applied as in the 7-day interval treatment. The most commonly grown starch potato cultivar Kuras was used in the experiments. Each plot was five rows of 10 m length, from which the middle three rows were harvested. Late blight was visually scored weekly and early blight was scored at the end of the season. At harvest samples were taken for tuber blight assessment. Tubers were processed and the resulting starch, fiber and protein fractions were analyzed for presence of phosphite and phosphate with ion chromatography.

RESULTS AND DISCUSSION

We found that applying potassium phosphite provided almost as good protection against late blight as the conventional fungicide program. However, we found a tendency to lower starch content in tubers from plots treated with phosphite alone that needs to be investigated further. Applying combinations between fungicides and phosphite at half recommended dose or at full dose but applied at longer interval, 14 days instead of normally 7 days, gave as good or better protection than conventional fungicides at recommended dose with 7-day interval (Figure 1).

These treatments also gave the highest average tuber yield and starch yield although not significantly different from the conventional treatment. The amount of tuber blight was very low and no differences between treatments could be detected. The infection rate of early blight was more than 25% lower in treatments involving phosphite compared to treatments with late blight fungicides only (p=0.004). In harvested tubers we found residues of phosphite ranging from 25-100 mg kg^{-1} tuber depending on the rate of application. However, in the processed starch product the level of phosphite residues was below the detection limit of about 1 mg kg^{-1} starch. In the protein and fiber fractions low amounts of phosphite were found. Preliminary calculations indicate that about 80% of the phosphite found in tubers at harvest will end up in the process water during processing.
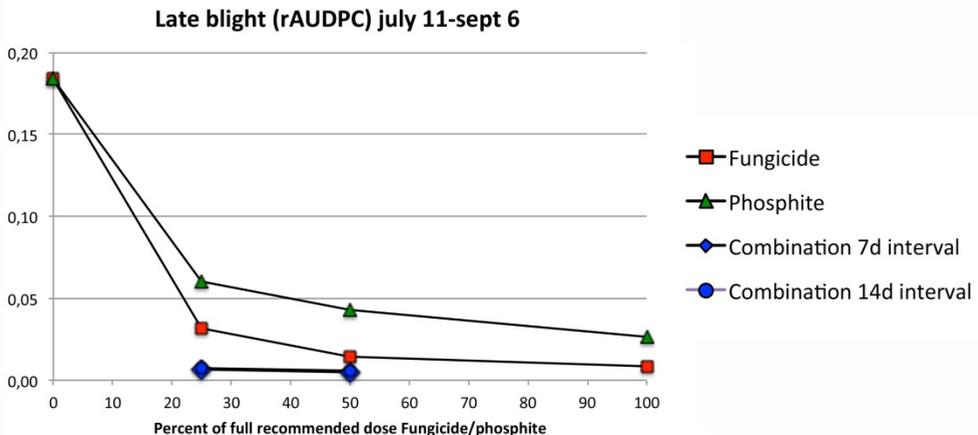
Figure 1. *Late blight severity expressed as relative area under the disease progress curve (rAUDPC) in a field trial 2016 with the starch potato cultivar Kuras. Dose-response curves of phosphite applied alone at 7-day interval, a fungicide strategy where Revus, RanmanTop and Infinito was alternated at 7-day interval and two treatments where reduced doses of the fungicides were used in combination with potassium phosphite at 7 or 14-day interval.*

In conclusion, potassium phosphite provided good control of late blight in starch potato in Sweden. Potassium phosphite used in combination with reduced doses of fungicides provided as good control as full dose fungicides with maintained yield. Also the combination applied at 14-day interval gave similar level of late blight control and yield compared to a traditional fungicide program with 7-day interval. While residues of phosphite were found in harvested tubers no residues were found in the starch fraction, and only low levels were found in fiber or

protein fractions after processing. Potassium phosphite may, provided that it can get approved, be considered as a low-toxicity alternative treatment against late blight in starch potato and it may also reduce the infection rate of early blight.

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Predicting the combined efficacy of host resistance and fungicides

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SUMMARY

Integrating strategies for the control of late blight caused by *Phytophthora infestans* on potato, such as epidemic rate limiting host resistance combined with reduced fungicide doses, has been demonstrated to successfully decrease foliar disease severity. A simple multiplicative survival model (MSM) was devised to predict disease severity on cultivars with differing disease resistance treated with different fungicide doses. AUDPC data were obtained from field experiments testing cultivar by fungicide treatment combinations. The effectiveness of the host resistance was expressed as the proportion of disease remaining on each of the more resistant cultivars compared with the most susceptible cultivar. The effectiveness of fungicide treatment was calculated using data from the most susceptible cultivar to parameterise a dose response curve function. This allowed calculation of the proportion of disease remaining at any given dose. The MSM model was then used to predict the AUDPC of other variety by fungicide dose combinations. A regression line fitted to the observed and predicted data explained 68% of the variation in AUDPC. Aspects of experimental design which could be addressed to improve predictive value are described.

KEYWORDS

Late blight, *Phytophthora infestans*, foliar blight, host resistance, fungicides, integrated control, prediction, multiplicative survival model, MSM

INTRODUCTION

Cultivar resistance, in combination with reduced fungicide input, has been shown to successfully reduce foliar late blight severity (Fry, 1978, Kirk et al., 2001, Kirk et al., 2005, Nærstad et al., 2007, Bain et al., 2011). A shift in the late blight population in GB towards more aggressive and virulent *P. infestans* genotypes, including 13_A2 and 6_A1, resulted in the foliar resistance ratings of several cultivars being downgraded from resistant (e.g. Cara with a rating of 7 in 2010) to moderately resistant (Cara with rating of 5 in 2012) (Lees et al., 2012). Sufficiently large differences in foliar resistance between cultivars are a key part of integrated control;

however, 99% of the potato area in GB consists of cultivars with a resistance rating of 5 or below.

Two broad types of simple models have been widely reported in the literature to determine the efficacy expected from the joint action of pesticide mixtures: the additive dose model (ADM) and the multiplicative survival model (MSM) (Morse, 1978). The MSM has been used to describe the joint efficacy of two active ingredients applied simultaneously and the joint action of host genes (Bliss, 1939, Gisi et al., 1985, Grimmer et al., 2015). It has also been used to determine whether particular mixtures are synergistic or antagonistic (Gisi, 1996). Synergy or antagonism may exist between varietal resistance and fungicide dose for control of late blight where disease control provided by this "mixture" is higher, or lower, than would be predicted from the disease control achieved from the cultivars and fungicide tested individually. The aim of this paper was to determine whether a simple model, based on multiplicative survival principles, could predict the joint action of fungicide dose and host resistance combinations.

MATERIALS AND METHODS

Field experiments
In 2010 and 2011, four experiments were conducted to determine the effectiveness of integrated control treatments incorporating reduced fungicide inputs and cultivar resistance to control foliar late blight during rapid canopy growth: two were conducted in Ayrshire, Scotland and two in Ceredigion, Wales. Experiments were laid out in a randomised split plot design with four replicates. Each sub-plot consisted of either King Edward (foliar late blight resistance rating 3), Cara (5) or Sarpo Mira (7) and was four rows wide by c. 3m long, with seed spacing determined by tuber size. All foliar assessments were done on the centre two rows of each sub-plot. Treatment fungicide applications were started as soon as plants started to meet within the rows or earlier if late blight risk was high. One fungicide (Revus; 250g/L mandipropamid: full label rate 0.6 L/ha) was applied at 0, 25, 50, 75 and 100% of the recommended label dose at 7-day intervals. A maximum of five test fungicide applications were applied across sites. Dithane NT (mancozeb 75% w/w) at 2.0 Kg/ha was applied once treatment applications were completed.

Table 2. *Cultivars, fungicide treatments and GB foliar blight resistance ratings for all cultivars included in rapid canopy experiments in 2010 and 2011.*

Year	Doses applied (proportion of the full recommended label rate)	Spray interval	Cultivar (foliar blight resistance rating)		
2010 and 2011	0.00, 0.25, 0.50, 0.75 and 1.00	7 days	King Edward (3)	Cara (5)	Sarpo Mira (7)

Experimental sites were inoculated on 12 July 2010 and 3 July 2011 (Cilcennin) and 12 July 2010 and 8 July 2011 (Auchincruive) with one or several *P. infestans* isolates (genotype 13_A2). At Cilcennin, fungicides were applied in 250 litres of water per hectare using a hand held Oxford Precision Sprayer operating at 2.0 bars (200 kPa) through F02-110 flat fan nozzles. At Auchincruive, fungicides were applied in 200 litres of water per ha using a tractor-mounted, modified AZO compressed air sprayer, operating at 3.5 bars (350 kPa) to give a medium/fine

spray quality using Lurmark F03-110 nozzles. The percentage leaf area destroyed by foliar blight was assessed at regular intervals during the epidemic using a modified version of the keys of Large (1952) and Anon (1976). Data were collected as the percentage of leaf area affected by foliar late blight and used to calculate the Area Under the Disease Progress Curve (AUDPC).

Predicting the effectiveness of host resistance and fungicide combinations
To determine whether the observed levels of late blight (as the AUDPC) could be used to predict the effectiveness of host resistance and fungicide combinations, a function was developed incorporating a simple exponential function describing the fungicide dose response curve (Paveley et al., 2000). Data derived from the dose response curve of the most susceptible cultivar, King Edward, were used to calculate the parameters *b* and *k* using FITNONLINEAR in Genstat. The parameters were then used in function (1), which was derived to predict the effect of host resistance and fungicide dose combinations from trial data based on the principles of the multiplicative survival model (MSM):

$$(1) \qquad D = D_o \left| \left(\frac{D_r}{D_s} \right) (1 - b(1 - e^{-kP})) \right|$$

D is the predicted level of disease for the appropriate test cultivar and fungicide dose, D_o is the untreated AUDPC of the standard cultivar (in this case the most susceptible cultivar King Edward). For the first analysis, D_s is the untreated AUDPC for the standard cultivar, D_r is the untreated AUDPC for the partially resistant test cultivar and *P* is the proportion of the fungicide dose (e.g. 0.25 for ¼ of the recommended label dose, 1 for the full recommended label dose). The constants *b* and *k* were calculated using the dose response curve for the standard cultivar (King Edward) in each experiment individually as described previously. Observed disease severity was linearly regressed against the predicted severity as advocated in Piñeiro et al. (2008). All analysis was done in Genstat 16th Edition (VSN International Ltd, UK).

RESULTS AND DISCUSSION

Foliar late blight progress in the experiments in 2010 and 2011
There were four contrasting foliar late blight epidemics in 2010 and 2011 (Figure 1). At the SRUC site in Scotland in 2010, there were 14 Smith periods during the season; 5 in July, 4 in August and 5 in September. Differences between cultivars, however, were less pronounced with the largest contrast between untreated cultivars and only small differences between fungicide-treated cultivars regardless of dose applied. At the ADAS site in Wales there were fewer Smith periods in 2010, with 4 in July, 4 in August and 3 in September giving a total of 11 for the growing season. The epidemic was more severe and there was greater separation of cultivars, particularly where fungicides had been applied. In 2011, the epidemic was more severe at the Scottish site. There were 11 Smith periods during the season; 3 in July, 6 in August, 1 in September and 1 in October. Differences between the untreated cultivars were clear, however, fungicide applications to Cara substantially decreased foliar blight to levels achieved on Sarpo Mira treated with fungicides. At the Welsh site, 7 Smith periods were reported during the growing season: 2 in July, 4 in August and 1 in September. Again, fungicides substantially decreased the epidemic on all cultivars at all doses tested and this has been demonstrated under GB conditions previously (Bradshaw and Bain, 2007, Bain et al., 2011, Bain et al., 2013).
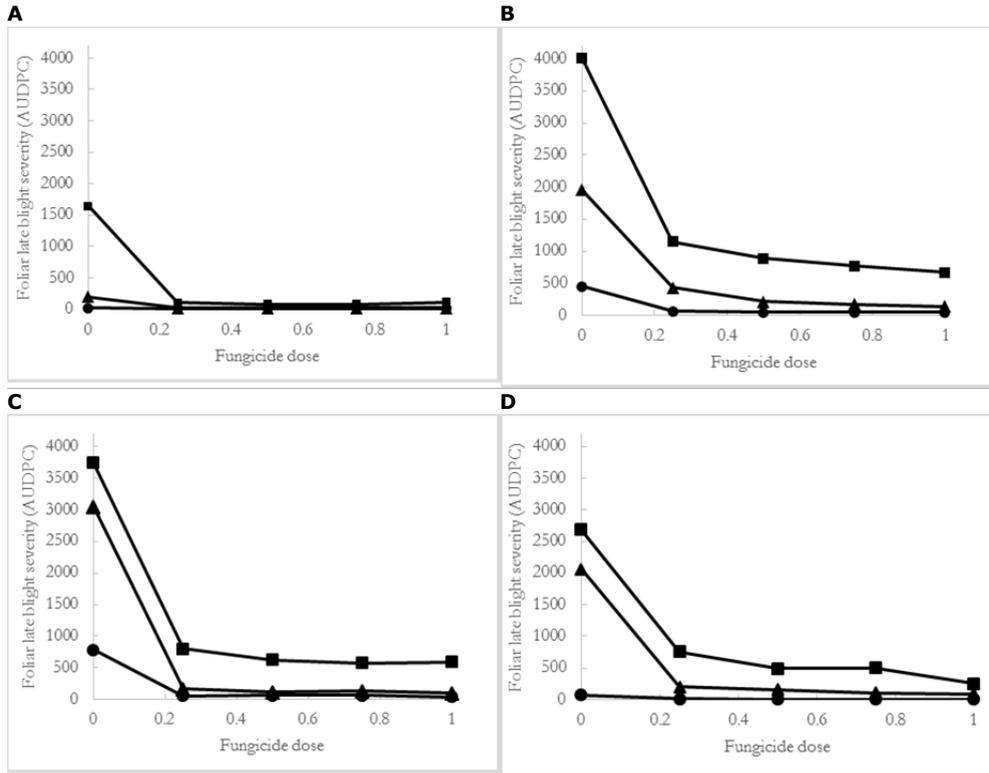
Figure 1. *Progress of foliar late blight (as the AUDPC for each fungicide dose and cultivar combination) in experiments conducted in 2010 and 2011. A = SRUC in 2010, B = SRUC in 2011, C = ADAS in 2010 and D = ADAS in 2011. To distinguish between cultivars, the following applies: King Edward (■), Cara (▲) and Sarpo Mira (●). Fungicide dose is the proportion of the full recommended rate (e.g. 1 = the full recommended label rate for a single application) applied.*

Determining predictive value using MSM principles

Generating up to date information on the likely contribution of host resistance and fungicide dose combinations to disease control is necessary, particularly following the dominance of more aggressive *P. infestans* genotypes. In Great Britain, the dominance of 13_A2, one of the newer aggressive genotypes, resulted in the re-rating of cultivars, including Cara, from highly to moderately resistant (Lees et al., 2012).

The analysis suggests that there is a good relationship between the observed and predicted values, with 68% of the variation accounted for (Figure 2). However, most of the predicted values were overestimates of actual disease observed. Mancozeb was applied to all treatments including untreated plots once treatment fungicide applications were completed, to allow differences to develop between treatments prior to desiccation. Mancozeb application has been shown to require 8 to 10 days before it slows established epidemics (Fry et al., 1979). Disease in untreated plots ranged from 3% to 78% on King Edward, 0.9 to 49% on Cara and 0.1% to 3% on Sarpo Mira immediately prior to mancozeb application, therefore the timing of this

application, relative to the epidemic, would be different for each treatment. The effect of mancozeb on the epidemic growth rate on King Edward will be reduced compared to other treatments where disease was less established, leading to bias in predictions. Such bias could not be excluded from this analysis, but can be avoided for future experiments.

It has been demonstrated previously that the rank order of cultivars exposed to *P. infestans* remains similar with or without fungicide treatment, however, the contribution of host resistance to disease control is lower where plants remain unprotected by fungicide in the presence of these more aggressive strains (Bain et al., 2009). Therefore understanding the effectiveness of fungicide dose and cultivar combinations is necessary if accurate information is to guide agronomists or be incorporated into decision support systems.
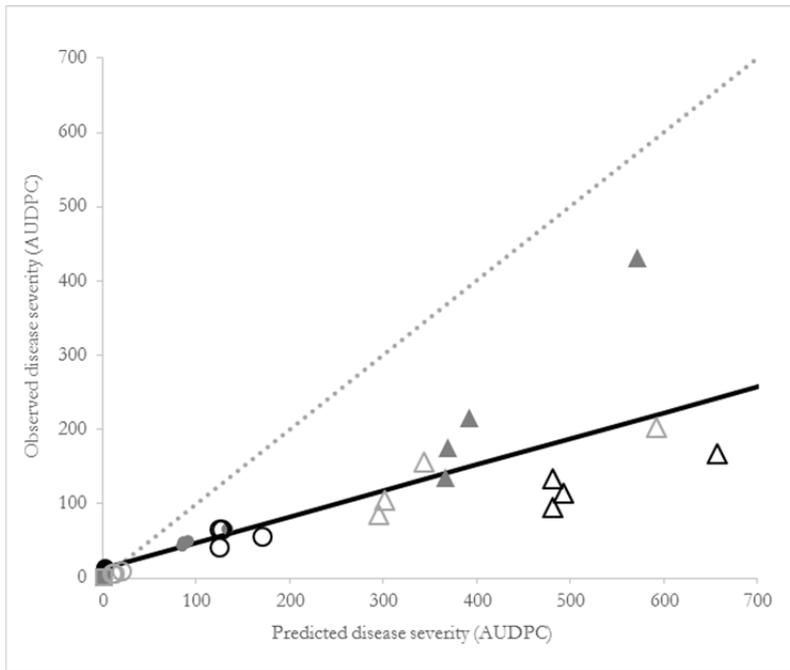
Figure 2. *Observed AUDPC plotted against predicted AUDPC for each dose and cultivar combination from 2 years of field experiments (R^2 = 0.68). Data from individual years are shown as black (2010) and grey (2011). Sites are separated by solid markers (SRUC) and open markers (ADAS). Cultivars can be distinguished as Cara (▲) and Sarpo Mira (●). Dotted line shown has a slope of one. The solid line is the regression of predicted vs observed AUDPC values. Observed and predicted untreated AUDPCs are not included, as well as observed and predicted data derived from the standard cultivar King Edward.*

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Integration of pathogen and host resistance information in existing DSSs – introducing the IPMBlight2.0 approach

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INTRODUCTION

Potato Late Blight (PLB) caused by *Phytophthora infestans* is still a major problem for potato production in Europe (Schepers et al., 2018, this proceeding). Milder winters in Northern Europe are resulting in volunteer plants that may act as inoculum sources the forthcoming season. Sexual recombination is causing early infections from oospores in some regions. Intensive and widespread use of popular cultivars is causing pathogen adaptation to host resistance that might lead to higher fungicide use (Cooke et al., 2011). A stringent execution of the EU Legislation 1107/2009, reduces the access to a wide array of active ingredients with different mode of actions. This increases the risk of fungicide resistance as stated in the EuroBlight statement from the EuroBlight workshop in Aarhus 2017. These trends are causing a real threat to the EU goal of a sustainable control of potato late blight and reductions in the pesticide use in general (Directive 2009/128/EC on the sustainable use of pesticides). EuroBlight recommends best management practices in potato and it is clear that the use of resistant varieties is one of the most effective IPM measures (http://euroblight.net/control-strategies/best-practice/). Host resistance is often not stable across many years as pathogens may adapt and overcome resistances in specific varieties (Hansen et al., 2005; Cooke et al., 2011). The EuroBlight monitoring of the *P. infestans* population in Europe since 2013, mainly using SSR genotyping, highlighted how dynamic, regional and host specific these populations evolves and spreads in Europe. With the introduction of the IPMBlight2.0 project (Andrivon et al., 2018, this proceeding) more isolates will be phenotyped for fungicide resistance, virulence and aggressiveness enabling linkages between genotype and phenotype. EuroBlight also seeks collaboration with breeding companies and the official variety-testing network (VCU) to evaluate and document the type and level of resistance in commercial cultivars across Europe. This is the prerequisite for IPM2.0 in potato as defined and introduced by Kessel et al. (2011) and tested in the DuRPh project (Haverkort et al., 2016) and in the AMIGA project (Kesssel et al., 2017, in

press). There is a need to introduce more complete PLB IPM strategies which use host plant resistance as the backbone for PLB control, aims to deliver total PLB control and to prevent *P. infestans* from breaking the host resistance whilst at the same time using as few chemical inputs as possible. This is the goal of the next level of IPM - IPM2.0 for potato late blight control allowing for a much more durable exploitation of host plant resistance, cheaper PLB control and a strongly reduced burden on the environment (Kessel et al., 2011).

In a statement from the EuroBlight workshop in Brasov, 2015, EuroBlight recommended i) to continue and expand the monitoring of *P. infestans*, ii) to intensify the phenotyping of important genotypes and iii) that EuroBlight offers to participate in the development of new and the adaptation of existing PLB Decision Support Systems (DSSs) to IPM2.0 as defined above (EuroBlight statement 2015, Brasov). These recommendations led to the C-IPM ERANET funded IPMBlight2.0 project 2016-2019 (Andrivon et al., this proceeding) covering all aspects mentioned in the statement. This paper will present results from the first step towards the implementation of the IPMBlight2.0 approach into a DSS modelling framework.

METHODS
Weather based blight risk sub-models from six existing European DSSs were programmed in the MATLAB modelling framework, and the blight risk was calculated using weather data from across Europe. In this paper we show results from simulations with data from a Danish weather station for two years, 2015 and 2016. Observations from the Danish late blight disease surveillance network were used to evaluate the model outputs, and results from one trap nursery were included in a simulation to demonstrate the potential of including the IPM2.0 approach on one of the DSSs calculation of a control strategy.

Sub-models
The weather based sub-models selected for this exercise were: Infection pressure (Blight Management, DK), Effective Blight Hours (EBH (Irish rules, IR)), Infection risk (Naerstad model, NO), Critical day (WUR Blight, NL), Produced spores (Mileos, FR) Risk 1-4 (Hutton Criteria, UK). The blight risk sub-models from each DSS were implemented using the MATLAB programming environment, enabling run of all sub-models with weather data from selected weather stations and years. All DSSs are described on the EuroBlight website (http://euroblight.net/control-strategies/dss-overview/). A reference humidity model was introduced as:
- Humid hours were calculated as occurring when Rh≥88% or leaf wetness ≥30 min/hh or precipitation ≥0,2 mm/hh.
- The cumulative total of humid hours was then calculated across three days (72 hours).

Weather data
Historical, hourly weather data were collated from DK, NO, UK, FR, IR and NL for the years 2014-2016. Weather data included were: Wind speed [m/s], Temp [°C], Rh [%], Leaf wetness [Min/hh], Precipitation [mm] and Solar radiation [MJ/m²].

Climate Data Interface - CDI
All the selected sub-models use Rh as an estimate of humid conditions for sporulation or as an estimator of leaf wetness for infection except the Naerstad model, which needs data on leaf wetness. Rh sensors are very sensitive for erosion of calibration. To quality control the weather data for the IPMBlight2.0 modelling exercises, we developed a "Climate Data Interface" (CDI)

using the MATLAB programming environment. This component quality controls (QC) all data and writes the QC summary and elementary statistic in dedicated Excel files for further analysis. If consecutive missing values were fewer or equal to five hours values were estimated using a linear interpolation procedure.

Climate Data Interface operations and calculations:
- Number of missing values for each variable separately (when) (by station/ year / month)
- Interpolation of missing data if subsequent missing data <6 hours
- Min and max of weather variables (by station / year / month)
- Median, mean, min, max and std of the top 200 Rh hourly measurements. (by station /year / month)
- Mean Rh during hours with precipitation >0,5 mm, global radiation<0,5 MJ/m² and wind speed <5 m/s (by station / year / month)
- Estimated leaf wetness based on standard variables (several methods)

Calculation of blight risk for six weather based blight risk sub-models
The blight risk was calculated for the six sub-models using controlled weather data. This exercise enabled comparison of model outputs and analysis of blight risk in Europe across regions and years.

IPMBlight2.0 approach – assumptions and simulation with the Danish DSS, Blight Management
To demonstrate the implications of introducing the IPMBlight2.0 approach in one of the DSS we did a simulation exercise introducing an abstract "ghost" variety, but running the model with real weather data and results from the local trap nursery:
- Introduce a "ghost" variety carrying R8 and one effective but unknown R-gene in a simulation with actual 2017 weather data and real data from a trap nursery in the North Jutland region of Denmark.
- Monitor the regional *P. infestans* population for the presence or emergence of virulent strains against R-gene differentials and varieties in the trap nursery present at AKV Langholt, North Jutland region, 2017.
- Use the Blight Management DSS (DK) to simulate a control strategy according to the IPM2.0 approach for the "ghost"variety and compare results with a conventional control strategy.
- Calculate the fungicide use as the Treatment Frequency Index (TFI) as the number of normal dosages used in the simulated control strategy e.g. use of two time half dosage counts as one (1.0).
- Calculations were done for a susceptible starch cultivar and a resistant cultivar with original settings (Table 1) and, a resistant cultivar with use of the IPMBlight approach (Figure 6)

Trap Nursery
In 2017, ten trap nurseries were established in five EU countries in the frame of the IPMBlight2.0 project. The nurseries included all the Black's differential set and some cultivars with known resistances: R1, R2, R3, R4, R5, R7, R8, R9, R10, R11, Bintje, Alouette, Carolus, Robijn, Sarpo Mira, Toluca, Coquine Irna, Kelly Irna, Makhaï Irna. Additional varieties were included at AKV Langholt, Dronninglund, Denmark e.g. Anouk (ware), Kuras (starch), Nofy (starch) and PL11-0111 (Starch). For this paper, results from this nursery were used for the IPMBlight2.0 modelling exercise. Disease severity [%] was scored ten times from 4 July to 13 September. A trap nursery data management system was developed to store and analyse the data. The goal for this system is to i) evaluate the level and type of host resistance in the differentials and

additional cultivars tested using the EucaBlight approach (Hansen et al., 2007) and ii) use the data as input to the modelling work in the project.

RESULTS

Quality control of weather data
Several data sets tested with the CDI failed the quality control e.g. missing data for several days no leaf wetness or global radiation, only precipitation on daily basis or significant change in the offset of Rh measurements. In all the six sub-models analysed, Rh is a key variable for calculating the risk of sporulation and/or other parts of the life cycle. The Rh threshold used in the models varied from 85 to 90%. From one station, all Rh measurements were below 90%. Running the models with these data resulted in zero, or very low weather based blight risk. From the analysis of data from another weather station it was obvious that the sensor calibration was corrupted, (approximately 7% too low), but was then changed with a new sensor in July 2015 (Figure 1). For data from a third station the Rh measurements dropped suddenly and dramatically approximately 4% in July 2015, and this (too low) calibration stayed for the subsequent 2 years (Figure 2). Looking at data from many stations in North Europe across a number of years and several sensor types the level of Max Rh values should be in the range of 97-99%.
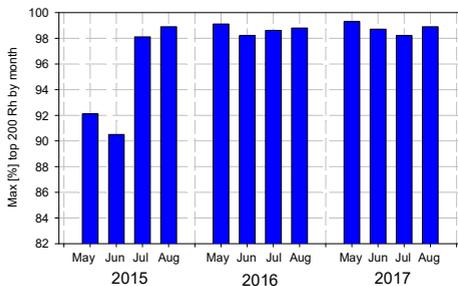
Figure 1. *Max Rh by month for hourly weather data at EU station A*
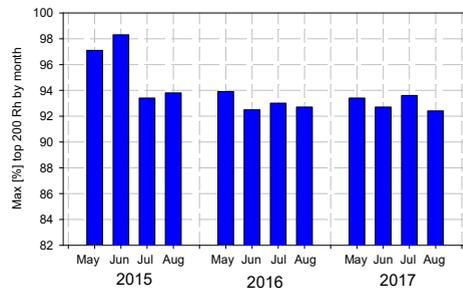
Figure 2. *Max Rh by month for hourly weather data at EU station B*

Comparison of blight risk sub-models and late blight development
Output from the six blight risk sub-models is visualised in the same graph together with the reference humidity model, including the date of first observed PLB attack in Denmark (red letter A) and date when PLB was recorded in the region for the first time (red letter B) (Figure 3 & Figure 4). The blight risk estimations are supplemented with biological data from Denmark, 2014-2017:

- Date when late blight was recorded in 5 or more conventional fields (marked A)
- Date when late blight was recorded for the first time in the region, less than 50 km distance from Dronninglund (marked B)
- Dates of first spray recommended in a susceptible and a resistant variety calculated with the Danish DSS.
- The number of sprays calculated for a susceptible and a resistant variety for the standard period 1 June to 30 September, using real data on late blight appearance from DK and from

the region as given in Table 1. Resistance level and the regional observation of late blight influences the calculations of the dose rates in the model.

Table 1. *Potato late blight appearance and DSS calculations for Denmark and Region Dronninglund, 2014-2017. See text for detailed explanations.*

Year	Denmark	Dronninglund, Region North Denmark			No of sprays, 1 June – 30 Sept.	
	Date of PLB in the Country (A)	Date of PLB in the region (B)	Recommended date for first spray, susc. variety	Recommended date for first spray, resis. variety	Susceptible variety	Resistant variety
2017	16 June*	16 June*	8 June	22 June	12,75	9,50
2016	10 June*	27 June	15 June	15 June	13,50	9,00
2015	22 June	6 July	22 June	29 June	12.00	8.00
2014	1 June*	17 June	6 June	6 June	14.00	9,75

* indications of oospores

Under Danish conditions crop emergence is on average from 20 May to 1 June in the south and approximately one week later in the North of Denmark. Early infection from oospores is assumed to occur during crop emergence. Attacks originating from infected tubers normally take place around 15-30 June under normal conditions.

In 2015, blight was found in Denmark in five or more conventional fields on 22 June and in the Dronninglund region on the 6 July. All sub-models indicated a weather based blight risk in early May – before crop emergence and therefore not relevant for any early control actions. The month of May was relatively humid and mean temperatures were often below 10°C. Most models use a lower temperature threshold of 10°C and despite humid conditions the blight risk was therefore calculated as low until early to mid June – after a rise in temperature (Figure 3). There is a good correspondence between the blight risk sub-models for the indication of high blight risk e.g. the high infection pressure from the DK model is corresponding very well with several consecutive critical days as indicated by the WUR blight sub-model. Max risk is calculated during the same periods for more or less all sub-models (Figure 3). For Mileos, the risk is indicated as a build-up of produced spores, but again, the number of consecutive days with high spore loads corresponds well with high risk indicated by the other sub-models.

In 2016, temperatures in May were higher than the previous year and crop emergence was about a week earlier. Blight was found in Denmark in five or more conventional fields on 10 June in the Mid-South of Jutland (some of these fields with indications of infections from oospores) – and in the Dronninglund region on the 27 June. All sub-models indicated a weather based blight risk in late May, and in the Mid-South of Denmark (same infection peak) this coincided with more than 40 mm of rain during crop emergence. Early infections from oospores might be the reason for early observations of blight on young plants in this region. Due to later crop emergence on the North of Denmark this region most probably experienced an "escape" situation of the combination of crop emergence, high infection pressure and rain. All the sub-models predicted blight risk 5-10 days before blight was actually found in this region.
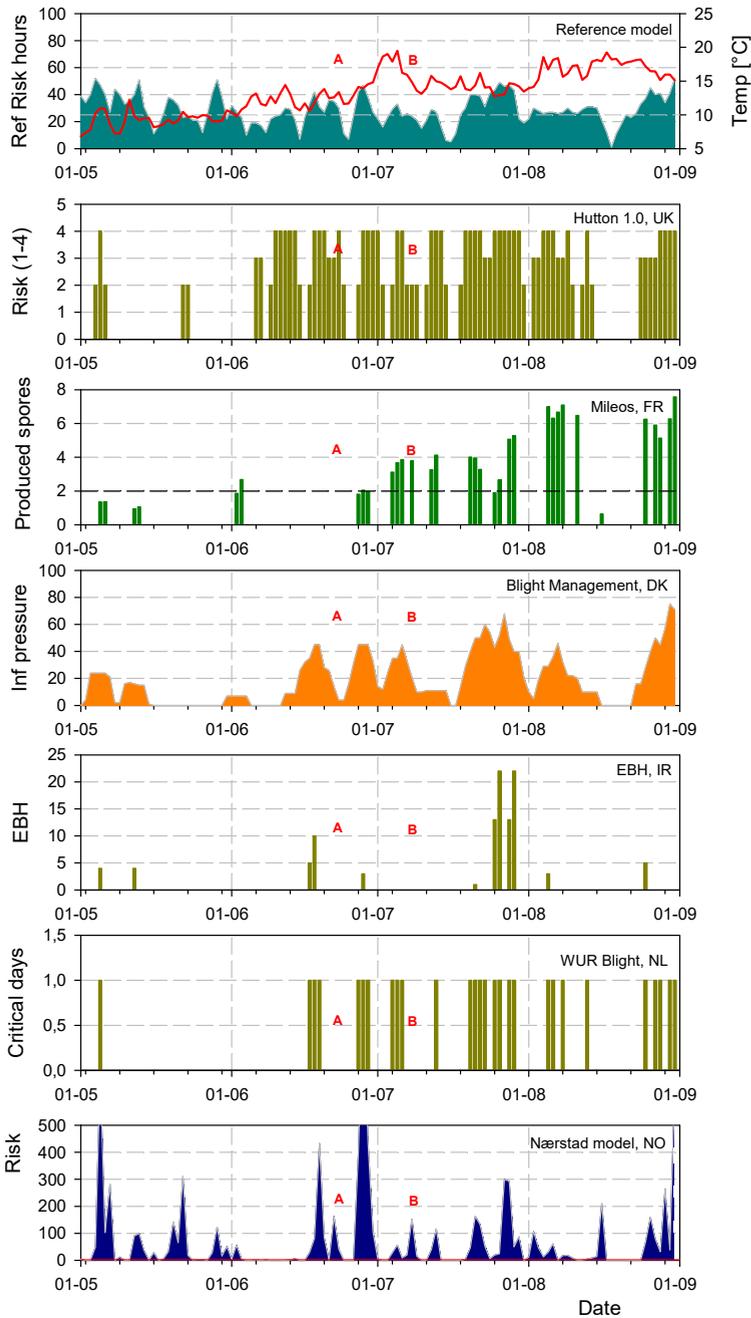
Figure 3. *Outputs of six blight risk sub-models and a reference model using weather data from Dronninglund, DK, 2015. The Letter A indicates the date when late blight was first recorded in Denmark. The letter B indicates when late blight was found in the Dronninglund region. For the Reference model the daily mean temperature is indicated on the right Y-axis.*
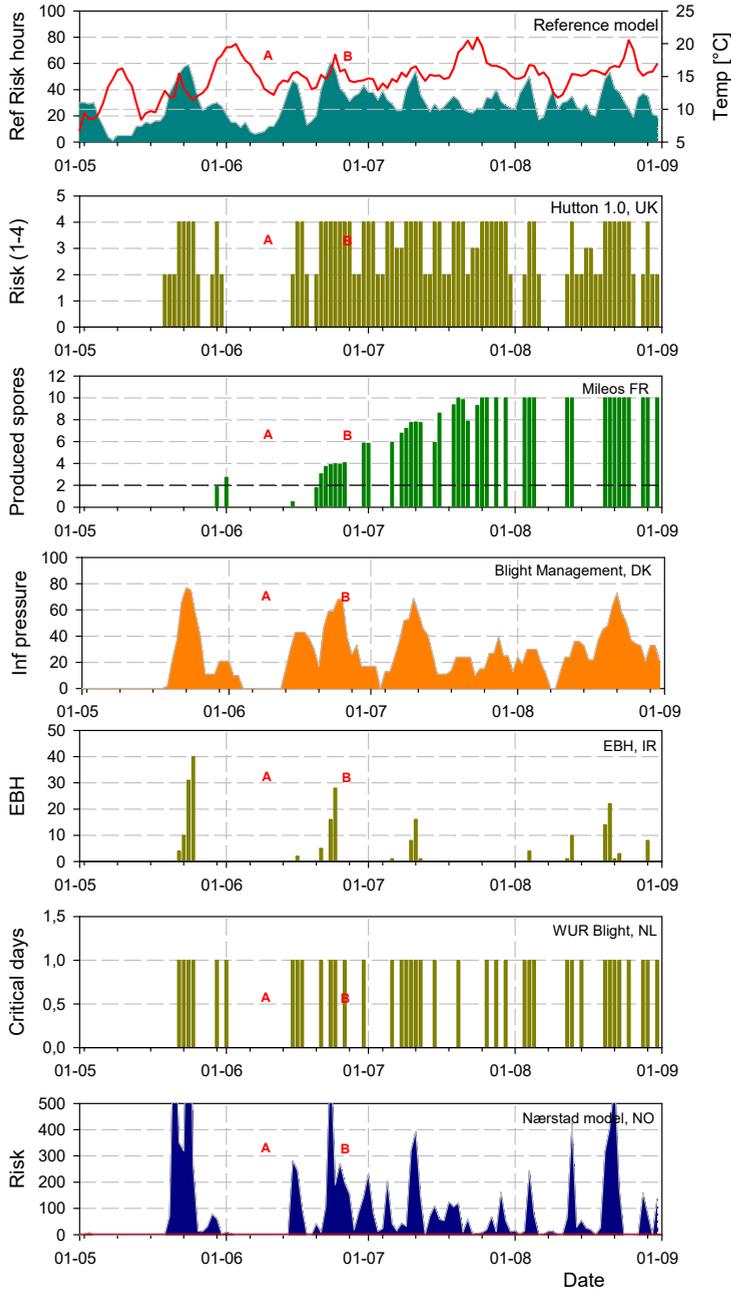
Figure 4. *Outputs of six blight risk sub-models and a reference model using weather data from Dronninglund, DK, 2016. The Letter A indicates the date when late blight was found in Denmark. The letter B indicates when late blight was found in the Dronninglund region. For the Reference model the daily mean temperature is indicated on the right Y-axis.*

Trap Nursery data

In the trap nursery at Dronninglund, PLB was recorded for the first time on 4 July (Figure 5). First symptoms of late blight in Sarpo Mira was recorded on 21 August. The genetic basis of late blight resistance in 'Sarpo Mira' is highly complex, consisting of at least five different R genes that confer qualitative and quantitative resistance to late blight (Rietman et al., 2012). The rapid disease development in untreated Sarpo Mira in this trial is considered to be due to a general high stress load from not only blight but also a mix with *Alternaria* spp. and general senescence that was difficult to separate from each other. For all differentials and varieties tested, the rate of disease development (slope of the curves) was high indicating a less effective horizontal resistance. Kuras is the most popular starch potato variety in Denmark covering approximately 60% of 25-30.000 ha of starch potatoes. Fifteen years ago, late blight was often found in Kuras in August. In recent years, attacks in Kuras were recorded early in the surveillance network – indicating that the *P. infestans* population in Denmark has changed and now overcome resistances in Kuras. The variety Nofy is a new variety expected to replace Kuras and PLB was observed a month later than in Kuras. In a new clone, PL11-0111, late blight was not observed at all during the season, indicating the potential of exploiting effective resistance in late blight control.
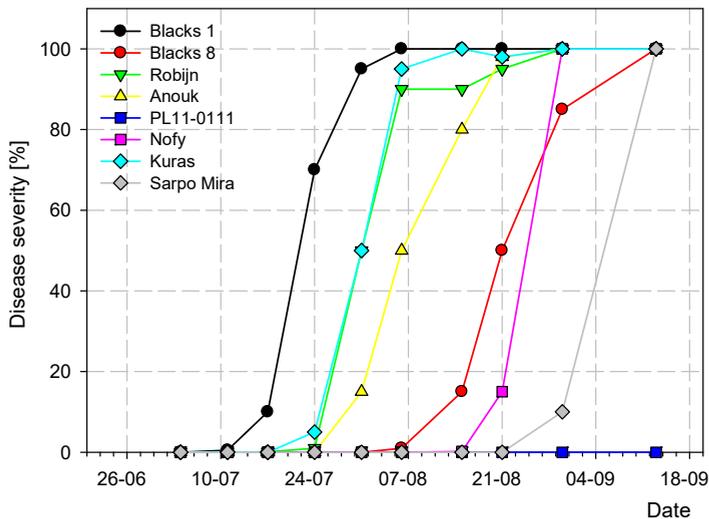
Figure 5. *Disease progress in selected differential clones and varieties tested at Dronninglund, Denmark, 2017. See text for detailed information.*

Simulation with a DSS based on the IPMBlight2.0 approach

Calculations with the Danish DSS were used to indicate how the implementation of the IPMBlight2.0 approach would influence the control in a "ghost variety" carrying R8 and an unknown but effective R gene (Fig 6). Infection pressure is given in yellow-orange gradient area, left y-axis (0-20 representing low, 20-40 medium and >40 units as high risk). Markers indicate

the calculated recommended dosage of preventive fungicide i.e. Revus/Ranman. The red markers indicate time and dosage recommended for a strategy with weekly sprays. The light grey areas indicate when late blight appeared in the region and dark grey when late blight appeared in the field / trial. The red arrows indicate when a first spray is recommended in a resistant cultivar according to a current standard control strategy (top graph). This strategy results in a TFI of 9,5 (see also Table 1). Using the IPMBlight2.0 approach the preventive spray should be applied when R8 is eroded in the trap nursery (6 August). The assumption is that the R8 and the unknown R gene will protect the crop. When one of the R genes are broken, fungicides must be applied to protect durability of the remaining R gene. In the given example the resistant variety was treated from 10 August to 29 September in weekly intervals: 4 times 0,5 normal dosage, 2 times 0,75 dosage and one time 0,25 dosage totaling 3,75 normal dosage (TFI=3,75).
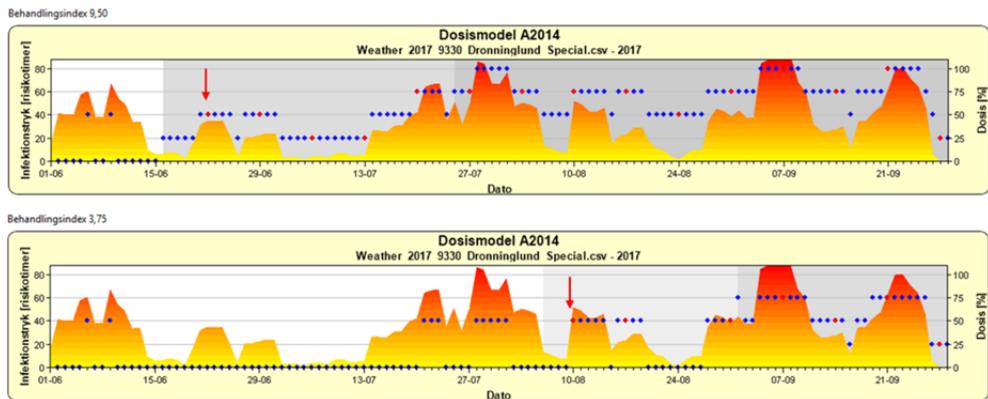
Figure 6. *Calculation of a control strategy using weather data from Dronninglund 2017. Top graph indicates a current control strategy in a resistant cultivar resulting in fungicide consumption of 9,5 normal dosages of a contact fungicide. Bottom graph indicates a control strategy in a resistant cultivar based on the IPMBlight2.0 approach resulting in fungicide consumption of 3,75 normal dosages of a contact fungicide. See text for further explanation.*

DISCUSSION

During the EU.NET.ICP concerted action (1996-2002) six different decision support systems for the control of late blight were tested in European validation trials in 2000 and 2001, Simphyt, Plant-Plus, NegFry, ProPhy, Guntz-Divoux/Milsol and PhytoPre+2000 (Hansen et al., 2002). It was concluded that all DSSs effectively controlled PLB at the same level as routine treatment, but with less fungicide input. It was also concluded that it was difficult to compare whole systems and it was recommended to analyse and compare systems on sub-model level. This was taken up by the ENDURE project (Hansen et al., 2010) and now again in this exercise. The goal in the IPMBlight2.0 project is to analyse, not only the six blight risk sub-models, but also how the different DSSs calculates a control strategy. Special focus will be on the DSS inclusion of host resistance and pathogen information. Innovative ideas on this were developed by Wageningen University and published in several publications based on the DuRPH, the AMIGA

project and recently the GEOPOTATO project (Kessel et al., 2011; Haverkort et al., 2016; Kessel et al., 2017; Kesssel et al., this proceeding).

The first step in the analysis of the weather based blight risk sub-models was to check the quality of collated weather data. We noticed that the (lack of) quality of weather data used for running PLB sub-models is a problem. Input of low quality weather data might lead to wrong advice. If DSSs fail the growers may be reluctant to use the DSS again, or any DSS – even if bad weather data subsequently were identified as the cause of the wrong advice. When we then ran the models with quality controlled weather data, results indicate that more or less all DSSs indicate blight weather conditions accurately. The differences in the full DSSs include different steps and inclusion of other sub-models to go from weather based blight risk to a recommendation for control. Issues in this set of sub-models and/or "decision rules" can be: when to start chemical control, which fungicides to use (type and dosages), whether to use weekly intervals and variable dosages or variable intervals and full dosage, how to take resistance into account and how to present and visualise results.

The IPMBlight2.0 project will try to broaden out these very promising results on IPM2.0 to several existing DSSs in Europe as well as giving open access to test and evaluation of existing DSS sub-models in countries or regions where DSSs are not used today. The next step in the IPMBlight2.0 project will be to implement the six blight risk sub-models as well as supplementary sub-models as a series of interoperable web applications on the EuroBlight IT platform. On this platform, interfaces will be built to the trap nursery data management system, the EuroBlight pathogen monitoring data and the fungicide information that feeds into the EuroBlight Fungicide Table. In total, this will enable simulations of control strategies that analyse different innovative approaches and utilize excessive amounts of data on European level. Based on experience and results from this collaborative modelling framework, existing DSSs in partner countries will be adjusted. The improved DSSs will be tested in field experimental trials in the forthcoming years. The EU.NET.ICP project concluded that it was probably not possible to build one DSS for whole Europe. IPMBlight2.0 recognises this conclusion to be valid, but at the same time suggest to adapt its work to EU calls to the scientific community (and stakeholders) on sharing of data and software, adopt the open science ideas, implement and optimize e-infrastructures to support science and link up with the European Open Science Cloud Agenda (EOSC Declaration).

In October 2017, the Commission adopted a report addressed to the European Parliament and to the Council on the sustainable use of pesticides Directive (2009/128/EC) which takes stock of progress made by Member States on a range of topics. One key finding in the report is that Integrated Pest Management (IPM) remains underused by Member States. This is despite the fact that the number of EU-approved low risk/non-chemical pesticide substances has doubled since 2009. Compliance at individual grower level is not being systematically checked by Member States. When revising their National Action plans, Member States need to improve their quality, primarily by establishing specific and measurable targets and indicators for a long-term strategy for the reduction of risks and impacts from pesticide use (Report From The Commission To The European Parliament And The Council). In the recent EuroBlight statement from the Aarhus Meeting, EuroBlight offered to test innovative ideas and strategies through participatory actions. EuroBlight is also expressed its willingness to play an active role in the assessment of more complex and integrated blight control systems. All IPMBlight2.0 partners are also a part of EuroBlight and the EuroBlight network recognises that the value and visibility of its databases

and web tools can be further enhanced to the benefit of a range of stakeholders. The integration of the IPMBlight2.0 activities and data with the EuroBlight existing data and tools is a step in this direction.

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Stability of late blight resistance of potato hybrids with diverse genetic background

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SUMMARY

Nine clones of interspecies potato hybrids and cv. Nayada recommended for cultivating on the territory of the northwestern Russia were tested for nine years under different infection loads in the field and following twice repeated artificial infection with a highly aggressive isolate of *Phytophthora infestans*. Stable resistance to late blight (LB) in the field through the entire period of observations was registered only in clone 99-4-1, meanwhile the responses of other genotypes varied depending on the year of the test. The results of the tests under natural and artificial infection when compared by the average degree of consistency (Kendall's W = 0.43) did not concur perfectly indicating that a comprehensive assessment is necessary. In the best characterized clones 171-3 and 99-4-1 comprising the genetic material of *S. anigenum*, *S. demissum* and *S. stoloniferum*, only two race-specific resistance genes were found: *R1* and *R3b* presuming that further study of the genetic nature of LB resistance in the interspecific hybrids is on the agenda.

KEYWORDS

Phytophthora infestans, Solanum section *Petota*, interspecific hybrids, durable resistance.

INTRODUCTION

The potato collection maintained in the N.I. Vavilov Institute of Plant Genetic Resources (VIR) comprises more than 120 potato hybrid clones developed and recommended to breeders as valuable sources of agronomic traits. Most of these hybrids were bred in the 1990s and have been propagated clonally till nowadays. These hybrids are maintained in the Pushkin experimental plots (VIR) located in the North West of Russia, where potato late blight (LB) outbreaks occur nearly every year. Complexity and high diversity of the races in the Pushkin population of *Phytophthora infestans* has been established both by phenotypic and molecular analysis of its structure (Kuznetsova et al., 2016; Sokolova et al., 2017; Zoteyeva, Patrikeeva, 2010). We selected nine hybrid clones with diverse genetic background and compared their

phenotype and genetic profiles in order to examine the efficacy of LB resistance in the hybrid clones from the VIR collection and to pinpoint the combinations of the genes for race-specific resistance to *P. infestans* (*Rpi* genes) apparently providing for durable LB resistance.

MATERIALS AND METHODS

Potato genotypes tested for LB resistance
Nine clones of interspecific potato hybrids and cv. Nayada as a resistant standard were assessed for LB resistance in the field under conditions of natural infestation and in the laboratory assays. These clones originate from different parental lines and carry the genetic material from two to five tuber-bearing *Solanum* species, such as *S. alandiae, S. andigenum, S. bulbocastanum, S. demissum, S. polytrichon* (syn. *S. stoloniferum*)*, S. simplicifolium* (syn. *S. microdontum*) or *S. stoloniferum*, which were used as donors of LB resistance (Table 1). We initially selected the clones with haulm and tubers that resembled in the largest the cultivated potatoes and manifested high LB resistance scores (7-8). All tested genotypes belong to the middle maturity group.

LB resistance test
Field trials were carried out at the Pushkin Experimental Station of VIR (St. Petersburg) through cropping seasons 2008-2016. Ten plants of each clone per plot in two repetitions were planted in a randomized design into a common field of the potato collection. Observations started when first LB symptoms appeared on cv. Bintje used as a susceptible standard. The area of leaves affected by LB was scored 4-5 times during each cropping season and used for calculating the area under the disease progress curve (AUDPC). AUDPC values from each single experiment were transformed into the relative AUDPC (rAUDPC) score as described by Fry (1978). Maximum disease induced damage in potato foliage and tuber yields were assessed at the end of each cropping season.

The laboratory test with detached leaves was carried out in IP according to EuroBlight protocol Version 1.2 (euroblight.net). Three leaves per clone in two repetitions and cultivar Santé as a susceptible control have been infected with the complex race N161 (the IP collection) combining the virulence genes 1 to 11, A1 mating type and a high aggressiveness (Kuznetsova et al., 2016). The experimental data for LB resistance were transformed into 1-9-point scores.

Screening for of Rpi genes
Clones of hybrid clones and cv. Nayada were screened in ARRIAB with sequence characterized amplification region (SCAR) markers for seven genes: *R1*, *R2/Rpi-blb3*, *R3a*, *R3b*, *RB/Rpi-blb1=Rpi-sto1*, *Rpi-blb2* and *Rpi-vnt1.3* using the PCR protocols described elsewhere (Fadina et al., 2017).

RESULTS

Climatic conditions and LB emergency during nine growth seasons
Weather conditions for the growing seasons 2008-2016 were not equally favorable for potato plants and LB development. Through the whole period, the summer air temperatures exceeded the average long-term values, sometimes by 5-6.6°C (June 2013, July 2010, 2011, 2014, August 2015). The amount of precipitation in the summer months varied significantly. For

example, in June 2009 and 2010, July 2015 and 2016 and in August 2008, 2009, 2012 and 2016, precipitation increased by 1.5-2 times as compared to the average monthly rate. Nevertheless, there were periods of lack of moisture - June 2011 and 2015, July 2008 and 2014, August 2015. Obviously, such instability of weather conditions strongly affected the patterns of both plant growth and development and LB emergence and spread. Upon the combined results of evaluating the defeat by LB of the entire field collection of potato hybrids comprising 120 clones, we established that the epiphytotic LB development took place in 2008 and 2016. Less sweeping but nevertheless as serious plant damage was noted in 2013. In 2009, 2011, 2012 and 2015, LB developed more slowly, and many cultivars and potato hybrids were in time to form a high yield. In 2010 and 2014, LB was registered on susceptible cultivars and some hybrids only at the very end of the growth season.

LB resistance of interspecific potato hybrids in the field trials

In both years of epiphytotic LB development (2008 and 2016), the progress of disease commenced as soon as the first damage symptoms were observed on cv. Bintje: 78 and 67 days after planting, respectively. The weather conditions favorable for the rapid LB development contributed to disease spread, in 5-6 days (apparently corresponding to the first cycle of the pathogen development), lesions appeared on cv. Nayada and eight hybrid clones. Clone 40-2000 was vividly affected much later: in 18-20 days from the beginning of the observations. In both epiphytotic years, LB progressed at different rate on the leaves of hybrid clones: most rapidly in clones 117-2 and much slower in clones 171-3 and 40-2000. In the years of moderate disease development, the disease progress and the extent of damage of nine hybrid clones and cv. Nayada considerably differed from the indices registered during epiphytoty. The values of rAUDPC for three-year study ranged from 0.05 to 0.53 (Table 1). The Friedman's ANOVA rank test indicates significant year-by-year variation in rAUDPC values in field tests (χ^2Friedman = 6.95> χ^2 (o.o5) = 5.99). In the years of epiphytotic LB manifestation, the minimal rAUDPC values were noted in clones 40-2000 and 171-3. In the years of moderate development, six clones:

40-2000, 91-19-3, 117-2, 171-3, 99-4-1 and 160-17 - produced similar rAUDPC values (Table 1). Variations in the rAUDPC indices in different years of testing are confirmed by statistical analysis. Kendall's coefficient of concordance W of 0.24 indicated weak coherence in year-by-year potato response to LB infection.

Table 1. *LB resistance and average productivity of potatoes with diverse genetic background*

Clone	Background	*Solanum* spp. in hybrid pedigree	*Rpi* assessed with SCAR markers	r AUDPC			DL	Yield, g/plant
				2008	2015	2016		
117-2	F1	*tbr, dms, aln*	*R2/Rpi-blb3, R3b, Rpi-blb2, Rpi-vnt1.3*	0.47	0.13	0.53	5 MS	550-1160
25-1-2007	BC1	*tbr, dms, aln*	*R1, R3b, Rpi-blb2*	0.33	0.23	0.33	5 MS	480-1000
99-4-1	BC2	*tbr, dms, adg, sto*	*R1, R3b*	0.23	0.16	0.28	5.5M S	475-860
171-3	F2 BC1	*tbr, adg, ryb, dms, sto*	*R3b*	0.12	0.16	0.19	6 MR	400-800
160-17	F2 BC1	same	*Rpi-blb2*	0.23	0.16	0.43	nd	660-1150
194-4т	BC2 (Fn)	*tbr, adg, phu, dms, sto*	*R3b, RB/Rpi-blb1*	0.27	0.34	0.45	5 MS	550-1400
34-5-2003	Fn	*tbr, adg, ryb, phu, dms, sto*	*Rpi-blb2*	0.36	0.21	0.30	5 MS	400-930
91-19-3	Fn	*tbr, adg, ryb, acl, blb, sto*	nd	0.23	0.13	0.41	nd	370-1160
40-2000	BC2F2 × BC2F1	*tbr, adg, dms, sto, plt, sml*	*R1, Rpi-vnt1.3*	0.05	0.13	0.25	4 MS	520-1200
cv. Nayada		*tbr, adg, phu, dms, vrn, sto*	*R1, R2/Rpi-blb3, Rpi-blb2*	0.27	0.23	0.45	nd	620-1100

acl — S. acaule, adg — S. andigenum, aln — S. alandiae, dms — S. demissum, phu — S. phureja, plt — S. polytrichon, ryb — S. rybinii, sml- S. simplicifolium, sto — S. stoloniferum, tbr – S. tuberosum, vrn — S. vernei. DL – detached leaflet test, nd — no data.

The extent of maximum damage by LB in the tested set of potato hybrid clones was assessed at the end of the growth period using the 1-9 scale, where 9 is the absence of lesions. The value of this index varied from 1 to 9 in clones 117-2, 25-1-2007, 194-4t and cv. Nayada (Figure 1). By the time of harvesting, in other six hybrid clones under study 30% or more of the leaf surface stayed unaffected by the disease. As far as this trait was concerned, clone 99-4-1 was the most durable: through nine years of observations, over 50% of the leaf surface remained unaffected (Figure 1). In clone 171-3 for 8 consecutive years of observations no more than by 25% of leaf surface was affected; however, by the end of the 2016 growth period, it was affected much stronger. Clone 40-2000 manifested lesser LB lesions by the end of growth during epiphytotic periods in 2008 and 2015 than in 2012 and 2013 (Figure 1). ANOVA proved a significant effect of the "year of tests" factor on the LB damage severity in the tested clones (F = 15.3> 3.4).

The results of our long-term field experiments under the conditions of the North-West Russia concur with the data from the earlier tests of the same potato genotypes under other soil and climatic conditions. Thus, in the tests in 1990s run on the island of Sakhalin, which is very similar in its weather conditions to the valley of Toluca (Mexico), clones 91-19-3 was not affected by LB by the harvesting time, while the standard cultivars completely perished. Clone 99-4-1 was highly resistant to LB under high infection conditions in the tests performed in Belarus (Kozlov, Rogozina, 2014).
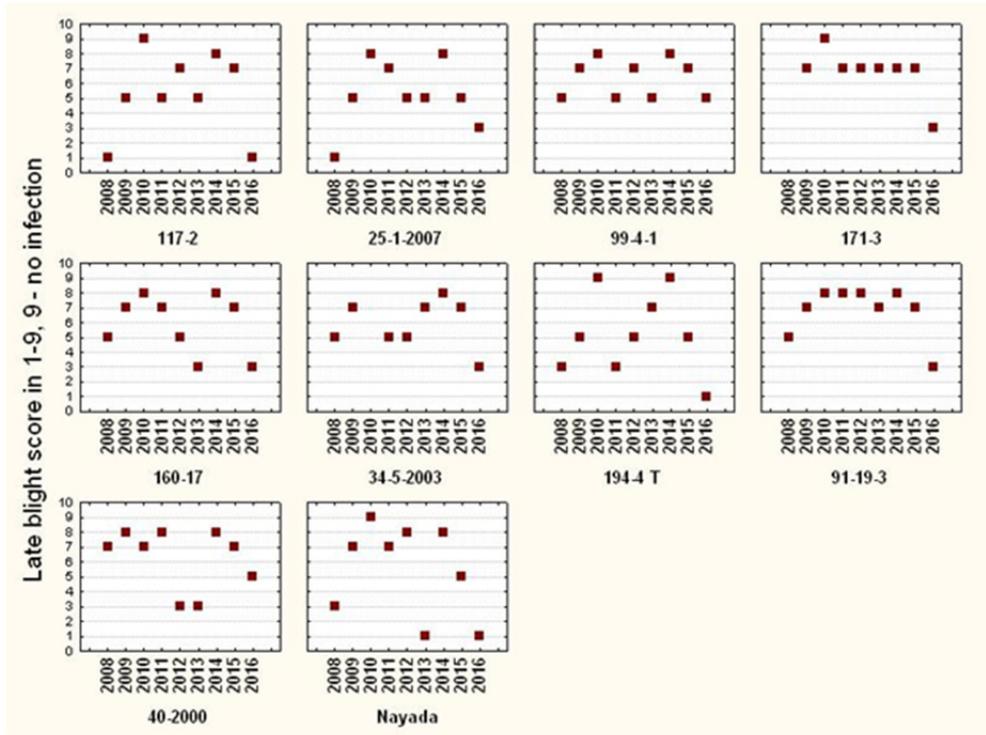
Figure 1. *Maximum damage caused by LB in nine hybrid clones and cv. Nayada in the 2008-2016 trials*

Laboratory tests for LB resistance

Based on the results of a two-year assessment of LB resistance of hybrid clones under the artificial infection, all investigated genotypes, except for clone 171-3, were classified as moderately susceptible. Clone 171-3 and cv. Sarpo Mira (currently a reference for high LB resistance) were found to be moderately resistant (7 MR). It is noteworthy that in contrast to Sarpo Mira, which is the late-season cultivar in the northwestern Russia, clone 171-3 belongs to the middle maturity group cultivars. These estimates of LB resistance under artificial infestation did not perfectly match the results of field trials: the Kendall's W value was equal to 0.43, probably due to a higher infection load in the laboratory tests and lack of competition between different *P. infestans* isolates colonizing potato plants in the field.

On the average, the consistency of LB resistance indices obtained in the field trials and laboratory tests with detached leaves was not high confirming the previous reports for wild species (Rogozina et al., 2010; Sharma et al., 2013). It follows that assessments for two to three years only in the field or only in the laboratory are not sufficient for evaluating the actual LB resistance.

Productivity

The average productivity of potato plants varied depending on the potato genotype and the year of testing. The most noticeable variation was observed in clones 194-4 and 91-19-3, wherein the weight of tubers collected from one plant varied by 2-3 times. Instability of productivity was also noted in cv. Nayada recommended for the northwestern Russia (Table 1). The link between LB damage and productivity was not strong: the final score of disease severity and tuber yield did not correlate (the Spearman's coefficient $r = 0.26$, $p < 0.001$). Plant productivity is a complex trait, and its manifestation depends on many factors. Two-way ANOVA indicated that the factor "year of trial" significantly affected the tuber yield. Obviously, in addition to LB, both other diseases and weather conditions significantly influenced the final tuber yield as confirmed by evaluating the productivity of clones 25-1-2007, 91-19-3 and 171-3: here the yield was higher in 2015, under heavy LB, than in the absence of disease in 2010.

Resistance genes

According to the SCAR marker analysis, nine potato clones and cv. Nayada comprised various patterns of one to four *Rpi* genes (Table 1). The relationship between LB resistance and *Rpi* gene profile of these potato clones was not evident and must to be studied further. Durable resistance of clone 99-4-1 to LB apparently involves the genes other than the *Rpi* genes registered in this study. This clone is one of the most probable sources of race non-specific LB resistance. Clone 171-3 is another promising target for gene mining: while this genotype considerably exceeds other hybrid clones by LB resistance, the marker analysis comes out with a single gene *R3b.*

DISCUSSION

Under the current changes in *P. infestans* populations, most clones of interspecies hybrids became more susceptible to the disease: of nine clones under study, durable LB resistance was found only in clone 99-4-1. Clones of different pedigrees probably differ in their defense mechanisms, such as resistance to infection penetration in clone 40-2000, slow pathogen development in the tissues of clone 99-4-1, or resistance to initial infection and its further spread in clone 171-3. Plant defense reaction in response to pathogen infection involves two defense systems (Jones, Dangl, 2006). The *Rpi* genes play a crucial role in defense as their products, e.g., receptor kinases, recognize pathogen effectors and respond to the changes in biochemical processes of plants affected by the invading pathogen. In the 20th century, the priorities of breeding potatoes for LB resistance changed dramatically: first, breeders employed the initial material comprising the *Rpi* genes of *S. demissum* and few other *Solanum* species, next, they turned to the *Rpi*-free genetic material providing for so-called horizontal LB resistance and finally, in the last decade, breeders focused again on the *Rpi* genes in a wider taxonomic context (Govers, Struik, 2009; Sliwka and Zimnoch-Guzowska, 2013). Discovery of *Rpi* genes with a broad resistance spectrum, such as *RB/Rpi-blb1, Rpi-blb2 and Rpi-vnt1* became a spectacular prerequisite for breeding potatoes resistant to numerous pathogen races due to pyramiding the effective *Rpi* genes. Using the methods of conventional breeding, several cultivars and advanced breeding lines have been developed, which carry four to seven *Rpi* genes (Khavkin et al., 2014; Kim et al., 2012; Rietman et al., 2012). The transcriptome analysis when employed for an in-depth study of the genetic nature of LB resistance of potato cultivars and non-tuber-forming *Solanum* species, revealed over 400 expressed *Rpi* genes in resistant forms of cultivated potatoes (Frades et al., 2015). In this regard, the detection of few *Rpi* genes in clones 171-3 and 99-4-1 presumes that LB resistance of these genotypes involves other genes than *R1* and *R3b* most probably transferred from *S. demissum*.

CONCLUSION

Clones of interspecific potato hybrids highly LB resistant in the 1990s tests became today more susceptible to the disease. Of nine clones under study, durable LB resistance was found only in clone 99-4-1. In clones 99-4-1 and 171-3 bred on the basis of potato cultivars and *S. andigenum*, *S. demissum* and *S. stoloniferum* accessions from the VIR collection, the already known genes *R1* and *R3b* are obviously complemented by other as yet unknown *Rpi* genes

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http://euroblight.net/fileadmin/euroblight/Protocols/Detached_Leaf_Test.pdf

New data on early blight of potato and tomato caused by a complex of large-spored *Alternaria* species in Algeria

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SUMMARY

Potato and tomato occupy an important place in the Algerian agriculture. These two crops are highly threatened by abiotic and biotic stresses. The surveys carried out in different areas of production since 2010 show that after the downy mildew, early blight was very damaging particularly in areas where the climatic conditions were favourable to the development of the pathogen. From 2012 to 2015, a total of 247 isolates having morphological and cultural characteristics of *Alternaria* were obtained from 12 major growing regions of potato and tomato located in the center, east, west and south of Algeria. On the basis of the morphological characteristics of the isolates (large conidia not catenulated with long beak), 156 isolates belonging to the *A. porri* group were selected for further identification and characterization. Molecular studies based on a PCR using specific primers detected two main species *A. solani* and *A. linariae* whose isolates were obtained from samples showing the symptoms of early blight. Sequential analysis of the Calmodulin gene confirmed the existence of *A. linariae* in the potato and tomato samples. In addition, these analyzes detected among the isolates of *A. solani*, isolates belonging rather to the species *A. grandis* whose samples are also from potato and tomato. Finally, the sequential analyses of the Rpb2 gene have revealed the existence of three isolates belonging to the species *A. protenta* among the isolates initially identified as *A. solani*. Pathogenicity tests carried out by artificial inoculation showed that all isolates belonging to the four species : *A. solani*, *A. linariae, A. grandis* and *A. protenta* isolated from potato and tomato fields cause the typical early blight symptômes on detached leaflets and whole seedlings of the two hosts with varying levels of attack. These results confirm the ability of *A. grandis* to also attack tomatoes while it is traditionally found on potato. We show especially for the first time the presence of *A. linariae* on potato culture while this species was always described on tomato. These new data oblige us to reconsider the parasitic specialization of *Alternaria* species with large conidia on solanaceae particularly on potato and tomato.

KEYWORDS

Early blight, potato, tomato, prevalence, large-spored *Alternaria* spp, parasitic specialization

INTRODUCTION

Potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) are the most important and useful member of the family solanaceae. They represent the first vegetable crop in terms of acreage and production in Algeria (Chehat, 2008). In 2013, Algeria occupied the second rank after Egypt in the production of potatoes in Africa. The national potato production in the last decade (2003-2013) has increased from 1 879 918 to 4 400 000 tonnes for an increase in the acreage under cultivation from 88 660 to 140 000 hectares. Similarly during the same periode the yield has increased significantly from 212 to 314,3 Qx/ha (FAOSTAT, 2015). These two crops are distributed differently in some areas, and are conducted according to different cultural practices. The potato which is considered as field crop occupies significant acreage in several regions of the country from north to south and from east to west. On the contrary, tomato is cultivated in the north part of Algeria on smaller surface areas and most often under plastic house as in the Biskra region. Both crops can exist side by side and can follow each other in many coastal regions of the country.

Among the fungal diseases infecting potato and tomato crops, early blight which is a widely distributed disease can cause significant economic yield losses (Pscheidt and Stevenson 1986; Rotem 1994). Early blight epidemics are particularly severe in tropical countries during warm and wet seasons (Batista et al., 2006; Mantecon, 2007). Nevertheless, the disease is becoming more severe in all regions partly due to warmer temperatures (Kapsa, 2008). The early blight symptoms of tomato and potato most frequently observed take two forms : more or less large spots often with yellowing around them and many more spots often quite small. These symptoms have been attributed to several *Alternaria* species : *A. alternata* when the conidia are small and catenulate, *A. solani* on potato and *A. linariae* (= *A. tomatophila*) on tomato when the conidia are large and solitary. With the molecular markers, several species morphologically identical with small conidia like *A. alternata, A. arborescens, A. infectoria* and *A. tenuis* have been reported on both host plants (Simmons, 2000). Likewise, several species morphologically similar with large conidia have been also reported on potato (*A. solani, A. grandis* and *A. protenta*) (Simmons, 2000 ; Orina et al., 2009 ; Gannibal et al., 2014 ; Landschoot et al., 2016) and on tomato (*A. linariae, A. solani* and *A. grandis*) (Simmons, 2000 ; Rodrigues et al., 2010). In Algeria, a complex of three species *A. solani, A. linariae* and *A. alternata* were reported to be the causal agents of the potato and tomato early blight (Ayad, 2014 ; Bessadat, 2014, 2016). In two recent publications, it was reported for the first time in Algeria the presence of *A. protenta* on potato (Ayad et al., 2016) and *A. grandis* on tomato (Bessadat, 2016). This indicates the complexity and the difficulty to assign a given species to a symptom and/or to the morphological characteristics of the conidia.

The objectives of the present study were to identify large-spored pathogenic *Alternaria* species on the basis of molecular, cultural and morphological characteristics and to clarify their pathogenicity on their respective potato and tomato hosts. The cultural, morphological and molecular characterization were based on 156 isolates collected during 4 years (2012-2015) on various potato and tomato fields in different bioclimatic zones of Algeria. To better highlight the different species present in Algeria, the sequences of different genomic regions such as ITS region, Calmodulin and RPB2 genes were analyzed. In addition, the parasitic specialization of *A. linariae* and *A. protenta* newly identified on potato were verified by artificial cross inoculations under conditions very similar to those in the field.

MATERIALS AND METHODS

Prospection, sampling and Isolation
Surveys were carried out in the period 2012 to 2015 and sampling was performed in 12 potato and tomato-growing regions from north (10, 16, 42) to south (03, 07, 39) and from east (21,24) to west (02, 27, 29, 44) of Algeria (Fig.1). More than 247 samples with typical early blight symptoms (dark, elongated or circular lesions with concentric rings surrounded by a yellow halo) were collected for isolation. Lesions with typical early blight symptoms were cut off from infected leaflets, desinfected and placed on potato dextrose agar medium at 22°C. The isolates obtained were purified by monospore culture on potato dextrose agar medium. The occurrence and the prevalence of the disease were evaluated by the percentage frequencies of the *Alternaria* species responsible of the early blight in these two potato and tomato crops and in the different growing regions.
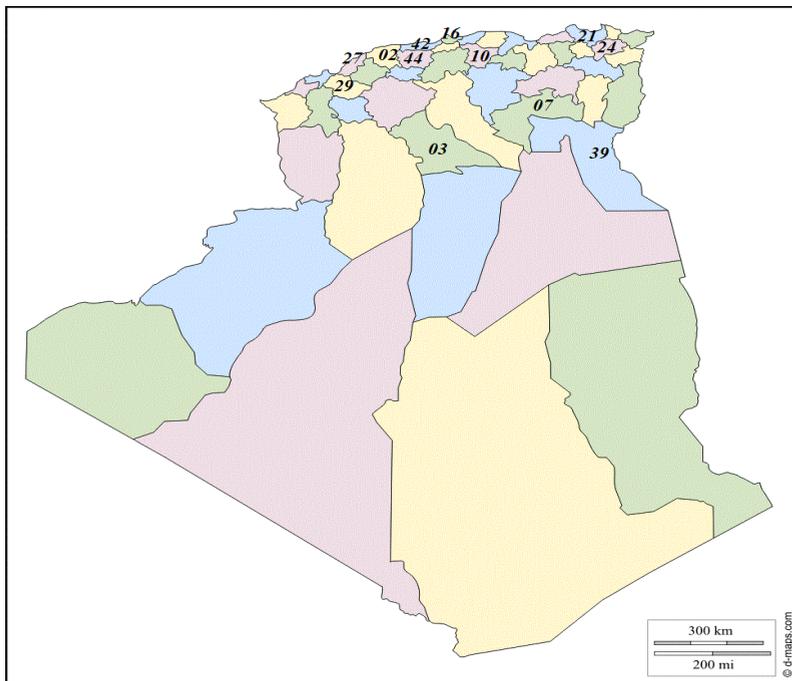
Figure 1. *Geographical localization of the potato and tomato- regions prospected*

Identification of large-spored Alternaria species using molecular markers
This study was carried out on the 156 isolates previously selected. Total genomic DNA was extracted according to the method described by Goodwin and Lee (1993). PCR using specific primers of *A. solani*/*A. grandis* group and *A. linariae* was performed. The primer pairs ITS1/ITS4 that gives good amplifications on the *Alternaria* species were used (White et al., 1990 ; Garde et al., 1991). All the DNA extracts of the 156 isolates were amplified. Two primer pairs OAsF7 and OAsR6 specific to both species *A. solani* and *A. grandis* for the amplification of the 164 bp of

the gene encoding Alt a1 and OatF4 and OatR2 which are specific to *A. linareae* for the amplification of the 438 pb of the gene encoding for the Calmodulin were used (Gannibal et al., 2013). These preliminary identifications were completed by PCR/RFLP using restriction enzymes *HaeII* and *RsaI* to differentiate between the two species *A. solani* and *A. grandis*. Of the 156 isolates identified by PCR and PCR/RFLP, 25 isolates were selected and their DNA was amplified at the Calmodulin loci. The PCR products were sent to GATC Biotech (Germany) for sequencing. To confirm the presence of *A. protenta* among the *A. solani* isolates, the locus Rpb2 of all *A. solani* isolates was amplified. All the sequences of the 25 isolates obtained after sequencing were analysed by the BLAST tool and compared with those in the NCBI databases. Sequence alignment was carried out with the software phylogeny.fr ("One Click").

Morphological characterization of large-spored Alternaria species
Of the 25 isolates previously identified by molecular tools, 15 isolates were morphologically characterized. The morphological characteristics used for the identification of the 15 isolates were based on the following criteria : hyphal width, body conidia length and width, beak length, number of horizontal and vertical septa. The measurements of the conidia were compared with those in the literature (Simmons 2007).

Pathogenicity of large-spored species on their respective potato and tomato hosts
To confirm the pathogenicity of the large spored *Alternaria* species, inoculations on detached leaflets and on whole plants were performed on susceptible varieties of tomato (Marmande and St Pierre) and potato (Spunta and SarpoMira). A collection of 7 *A. solani*, 3 *A. linariae*, 3 *A. grandis* and 2 *A. protenta* isolates was used. These isolates were selected for their ability to sporulate. Spore suspensions have been prepared from 15 days old cultures whose sporulation was checked with Malassez hemocytometer. The final concentrations of the suspensions were adjusted to 10^4 conidia/ml. The inoculations were made by depositing drops of 20 µl on detached leaflets and by spraying the whole plants. The disease severity was evaluated by using a visual rating scale from 1 to 9 expressing the extension of necrosis according to Duarte et al. (2013). Statistical analyzes of the results were made with Statistica 6.0 software which enable analysis of variance.

RESULTS AND DISCUSSION

Pré-identification of large-spored Alternaria species
The 247 isolates obtained were divided in two groups on the basis of the morphological characteristics : the first group (91 isolates) with abundant sporulation and small catenulate conidia typical of the section *Alternata*, the second group (156 isolates) with large, solitary, beaked conidia typical of the section *Porri*. These isolates were selected under a binocular stereomicroscope and microscope and compared with those in the literature (Simmons, 2007). The small-spored group have not been included in our study.

Molecular characterization and identification of large-spored Alternaria species
The preliminary tests carried out for the identification of the 156 isolates selected on the basis of morphological characters (large conidia with long beak) and analyzed by the universal primers specific for both groups of *Alternaria* species (*Alternaria solani A. grandis* OAsF7/R6 and *A. linariae* OATF4/R2) have confirmed the presence of these three species on potato crop in Algeria. These species were already reported in Algeria by Bessadat (2016) and around the

world by Gannibal et al. (2012), Rodriguez et al. (2009), Landschoot et al. (2017). Further species identification was conducted in order to differentiate betwen *A. solani* and *A. grandis* isolates on the one hand, and to confirm the identification of *A. linariae* on the other. PCR/RFLP by amplifying the locus Calmodulin followed by restriction enzyme digestion (*HaeII* and *RsaI*) of the PCR products has resulted in the identification of 43 *A. linariae* isolates, 92 *A. solani* isolates, 12 *A. grandis* isolates and 6 isolates which showed an anormal restriction enzyme digestion. The sequencing of 22 isolates representing each of the three species identified previously and isolated from the two host plants collected in the 12 surveyed regions gave the phylogenetic tree shown in Figure 2. The first cluster contains the *A. linariae* isolates that are distinct from the other two clusters regrouping isolates previously identified as *A. solani*, the second cluster includes *A. solani* isolates, and the third cluster regroups the *A. grandis* isolates. Finally, the sequencing of the Rpb2 locus carried out on the second cluster which include *A. solani* isolates has identified 3 isolates as *A. protenta* two of which were isolated from potato and one was isolated from tomato (Fig.3).

Morphological characterization of large-spored Alternaria species

Of the measurements of the hyphal width, the body conidia length and width, the beak length and the number of horizontal and vertical septa of the four species (*A. solani, A. grandis, A. linariae* and *A. protenta*) previously identified by molecular tools are indicated in Table 1.

Figure 2. *Cluster analysis of sequence at the Calmodulin loci*

Figure 3. Cluster analysis of sequence at the Rpb2 loci

Table 1. Morphological characteristics of 15 isolates identified as A. solani, A. grandis, A. protenta and A. linariae

Morphological characteristics	Species			
	A. solani	*A. grandis*	*A. linariae*	*A. protenta*
Hyphal width	4.23–7.57µm	4.40–8.74µm	3.39–8.37µm	3.40–6.67µm
Body conidia length	46.23–80.86µm	36.17–97.15µm	41.08–108.27µm	28.14–93.90µm
Body conidia width	13.47–25.54µm	10.66–21.70 µm	8.47–18.12µm	10.30–22.73µm
Beak length	30.86–114.40µm	61.28–186.57 µm	56.84–224.78µm	34.24–130.4µm
Number of horizontal septa	1–9	1–7	0–3	0–8
Number of vertical septa	1–12	2–11	1–19	1–10

The morphological characteristics of 15 isolates with large conidia showed high variability between them. In addition, there was an overlapping in the characters of the isolates in the shape and the size of the conidia. The morphological characteristics of the isolates did not clearly differentiate between the four large-spored *Alternaria* species currently reported on potato and tomato crops. Therefore, morphological characters were not sufficient for clear distinction between the four large spored *Alternaria* species. Even for some species like *A. solani*, *A. grandis* and *A. protenta*, the molecular characterization was not sufficient for their distinction; only nucleotide polymorphism at selected loci (Rpb2) allowed specific identification between the isolates of the two species *A. grandis* and *A. protenta*.

Occurrence and prevalence of the large-spored Alternaria species in Algeria

The survey carried out through the 12 growing regions of potato and tomato showed the presence of 4 large-spored *Alternaria* species in Algeria, i.e., *A. solani, A. grandis, A. linariae* and *A. protenta* at variable levels (Figure 4). *A. solani* is the most common species in Algeria with 63%. This species has a significant isolation frequency in the south (30.40%) and a relatively small frequency in the east (14.86%) and in the center and the west (8.78%) (Figure 5). The isolation frequencies of the four species identified in Algeria vary also with the crop. *A. solani* attacks both crops potato and tomato, but it is more prevalent on potato (50.60%) than on tomato (12.15%). These results are consistent with those obtained by Bessadat, (2014) which estimate the incidence of the early blight to 79.63%.
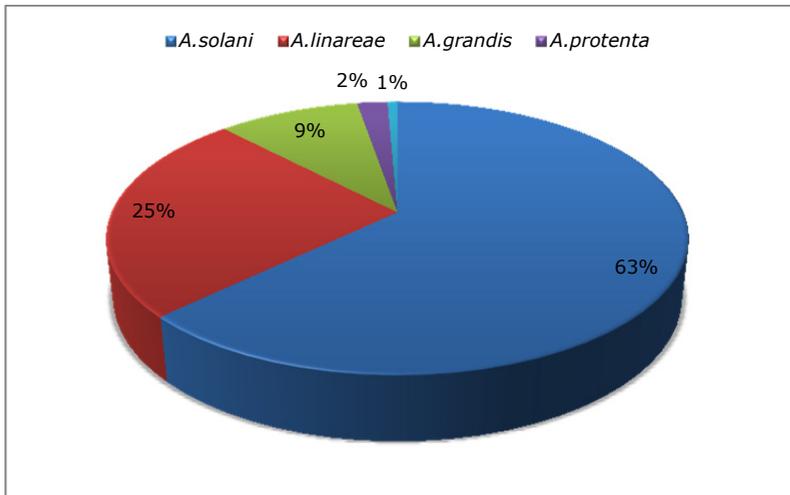
Figure 4. *Distribution of Alternaria spp. in Algeria on potato and tomato*

A. solani was considered for long time as the main species responsible for the potato and tomato early blight, however other species such as *A. grandis* et *A. linariae* have been recently reported respectively on potato and tomato (Simons, 2000 ; Rodrigues, 2009). *A. linariae* was isolated from potato for the first time in Algeria with an isolation frequency of 25% (Communication personal, 2016), whereas *A. grandis* was found on tomato (Bessadat et al., 2016). The isolation frequencies of *A. linariae* were 11.48% in the center, 8.10%, in the south, 4.72% in the west and 0.67% in the east, in addition this species is more prevalente on tomato (20.29%) than on potato (4.71%). *A. grandis* has a distribution percentage of 9% and an isolation frequency of 4.72%. in the center and the west of Algeria. This species was found on potato (6.08%) and on tomato (3.37%) (Figure 6). Another species *A. protenta* was characterized through sequencing the Rpb2 gene. Two isolates from potato and one isolate from tomato were identified as *A. protenta* (Ayad, 2016). These results were confirmed by artificial cross inoculations in order to check the pathogenicity of the isolates.
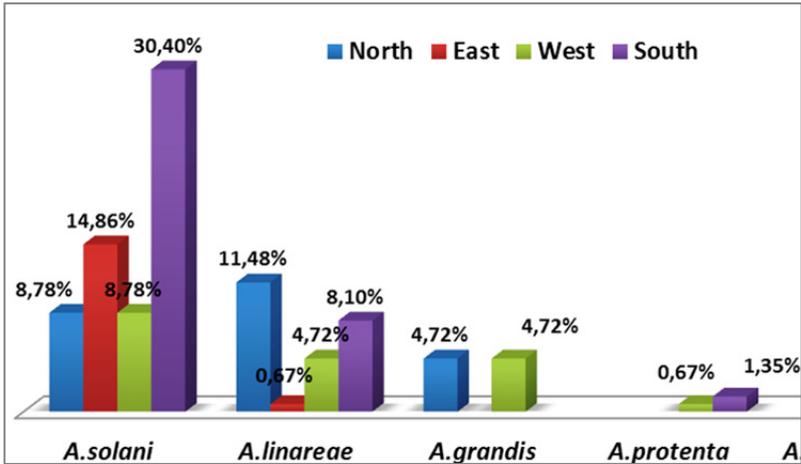
Figure 5. *Distribution of Alternaria spp. in Algeria according to the potato and tomato growing areas*
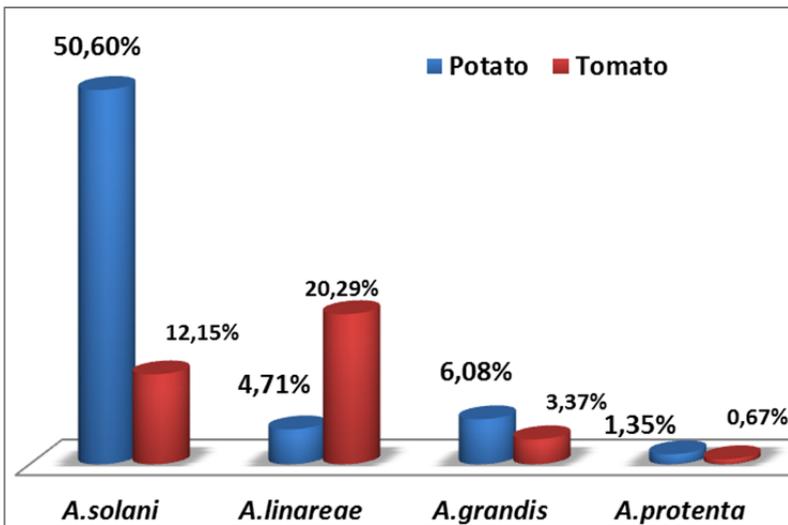
Figure 6. *Distribution of Alternaria spp. in Algeria according to the potato and tomato crops*

We therefore confirm the presence of other *Alternaria* species on potato other than *A. solani* particularly *A. grandis* and *A. protenta* which have been reported by other authors such as *A. grandis* in Europe (Landschoot et al., 2017), South America (Rodrigues et al., 2010), and Algeria (Bessadat et al., 2016). In contrast, we report for the first time in Algeria the presence in the field of *A. linariae* on potato and possibly in the world, and *A. protenta* on potato and tomato.

Aggressiveness and parasitic specialization of Alternaria species on their hosts

The high degree of aggressiveness obtained on whole seedling plants of potato and tomato, shows that the three *Alternaria* ssp. (*A. solani*, *A. grandis* and *A. linariae*) isolated from potato or tomato are aggressive on both host plants (Figs. 7 and 8). These behaviors are also in agreement with those realized *in vitro* conditions by Gilbert and Webbs (2007), and Rogrigues (2009) for *A. linariae* and *A. grandis* respectively on tomato and potato (Figure 9 and Figure 10). The same results were also reported by Rodrigues et al. (2010), Gannibal et al. (2014) and Woudenberg (2014) for *A. solani* on potato and tomato. We confirmed that *A. grandis* is formerly considered as responsable of early blight of potato and also tomato in Algeria as showed by Bessadat (2016). In this study we showed that *A. linariae* is pathogenic on potato in field conditions. These results allow us to confirm the absence of parasitic specialisation in the three pathogens with respect to their two hosts: potato and tomato.
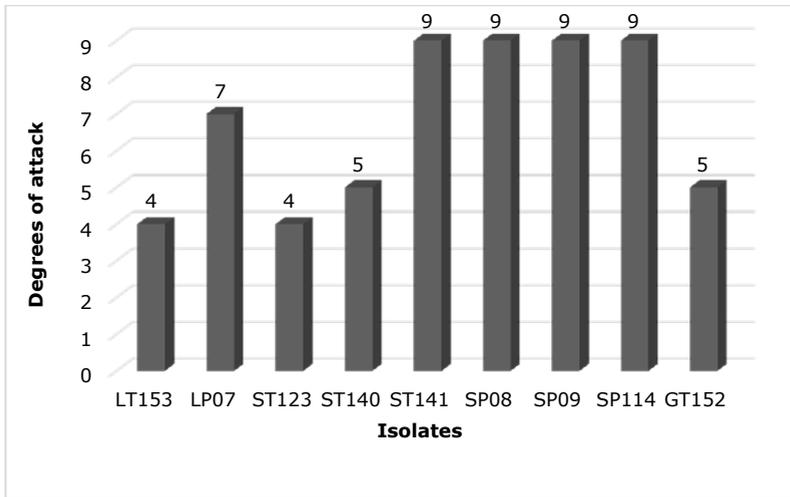
Figure 7. *Degrees of attack of Alternaria spp. isolates on potato (S: A. solani; L: A. linariae; G: A.grandis; P: potato; T: tomato)*
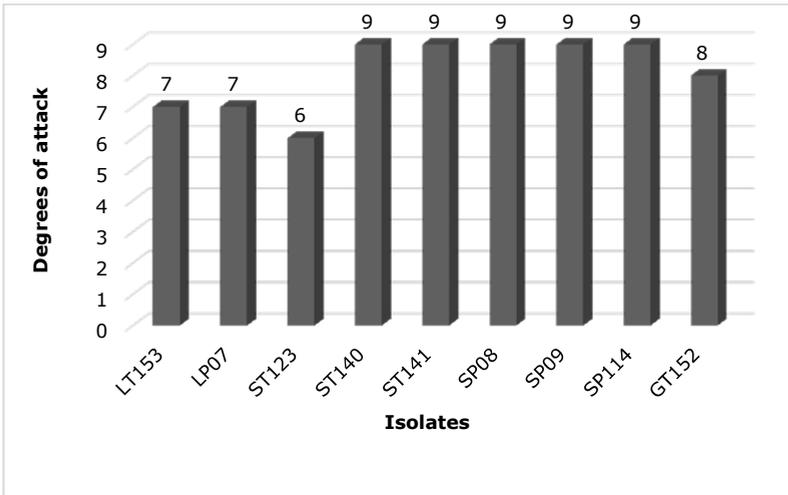
Figure 8. *Degrees of attack of Alternaria spp. isolates on tomato (S: A. solani; L: A. linariae; G: A.grandis; P: potato; T: tomato)*

Figure 9. *Symptoms on detached leaflets and whole plants inoculated by P02 isolate (=A. linariae isolated from potato)*

Figure 10. *Symptoms on detached leaflets and whole plants inoculated by P09 isolate (=A. grandis isolated from potato)*

CONCLUSION

The *Alternaria* large-spored species associated with potato and tomato early blight in Algeria were inventoried. *A. solani*, *A. linariae* and *A. grandis* are the common species found on potato and tomato. *A. protenta* was reported on potato and tomato for the first time in Algeria and possibly in the world. Morphological characterization remains insufficient for the distinction of *A. solani*, *A. linariae*, *A. grandis* and *A. protenta*. The identification of these four *Alternaria* species has to be confirmed by molecular markers. Beside *Alternaria alternata* species group, *A. solani* is the most common large spored species on potato; however others species with large conidia such as *A. linariae*, *A. grandis* and *A. protenta* can also infect potato in field conditions in Algeria. *A. solani* and *A. linariae* are the most common species on potato and tomato respectively in all potato and tomato growing regions of Algeria. *A. protenta* and *A. linariae* were found on potato for the first time in Algeria and have been pathogenic on this crop. Our results indicate that *A. linariae* is not specific to tomato as previously reported in the literature since it has been isolated under field conditions from potato for the first time in Algeria and possibly in the world. Also *A. grandis i*s not specific to potato.

ACKNOWLEDGEMENTS

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Alternaria spp. and *Colletotrichum coccodes* in potato leaves with early blight symptoms

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ABSTRACT

Colletotrichum coccodes and *Alternaria* species are plant pathogenic fungi affecting different organs of potato, tomato, and some other plants. Species-specific primers were applied for the survey of *C. coccodes* and *Alternaria* spp. on affected potato leaves collected in different regions of Russia. All studied *Alternaria* pathogens were present in blighted leaves alone or in the combinations, such as *A. alternata + A. solani, A. infectoria + A. alternata, A. solani + A. infectoria*. *A. alternata* (*sensu lato*) was the most frequent species revealed in 50% of tested potato leaves (alone or in combination with other species). *A. solani* and *A. infectoria* were revealed in 30 and 13% of samples, respectively. The presence of a DNA region specific for *C. coccodes* was detected in DNA samples isolated from potato leaves collected in the Northern Ossetia, Kostroma region, and Mariy El Republic.

KEYWORDS

Colletotrichum coccodes, early blight, *Alternaria* spp*.,* leaf blight, potato diseases, anthracnose, black dot

INTRODUCTION

Alternaria species cause diseases of numerous economically important host plants, including potato, tomato, cereals, etc. Common symptoms of *Alternaria* infection are necrotic lesions on leaves, which are primarily concentric and are often surrounded with yellow chlorotic tissue. In recent years characterized by warm and dry summer seasons, early blight became widespread in the central and southern parts of Russia and in Europe and became one of most important diseases of potato being inferior to the late blight. Three *Alternaria* species were found in infected potato leaf tissues in Russia: *A. alternata* (in this report we consider *A. tenuissima*, *A. arborescens* and *A. alternata* as the same group, designated *A. alternata* sensu lato), *A. solani,* and *A. infectoria* (Orina et al., 2010, Gannibal, 2007, Elansky et al., 2012).
Colletotrichum coccodes (Wallr.) S. Hughes is a plant pathogenic fungus affecting different organs of tomato, potato, and a wide range of other plant species. In the case of potato tuber

infection, the fungus causes so-called black dot disease. This disease results in a peel exfoliation, significantly worsens the appearance of tubers, and causes water losses during storage. The black dot disease is observed in the majority of world potato-producing regions; the corresponding yield losses for susceptible cultivars may reach 30% (Johnson and Miliczky, 1993; Johnson 1994; Tsror et al., 1999).

Black dot development on leaves causes formation of necrotic lesions similar to early blight or brown spot symptoms (Johnson and Miliczky, 1993). According to US researchers, who collected leaves with early blight or brown spot symptoms (caused by *Alternaria solani* and *A. alternata*), in some years *C. coccodes* represented up to 5-10% of strains isolated from leaves (Tymon et al., 2016). The development of the black dot disease on leaves and other above-ground parts of plants causes mass development of spores, which then infect other plant organs and other plants with drops of rain or irrigation water. The purpose of this study was the PCR-based investigation of *C. coccodes* and *Alternaria* sp. occurrence in potato leaves with early blight or brown spot symptoms collected in European Russia.

Another practically important issue is a differentiation between *A. alternata* s.l. and *A. solani* due to their different resistance to fungicides, virulence on cultivars, and optimal growth temperature (Pobedinskaya et al., 2012, Kapsa, 2008). In this study we applied PCR approach and species-specific primer sets for the survey of early blight agents (*A. solani*, *A. alternata* and *A. infectoria*) on affected potato leaves collected in different regions of European Russia.
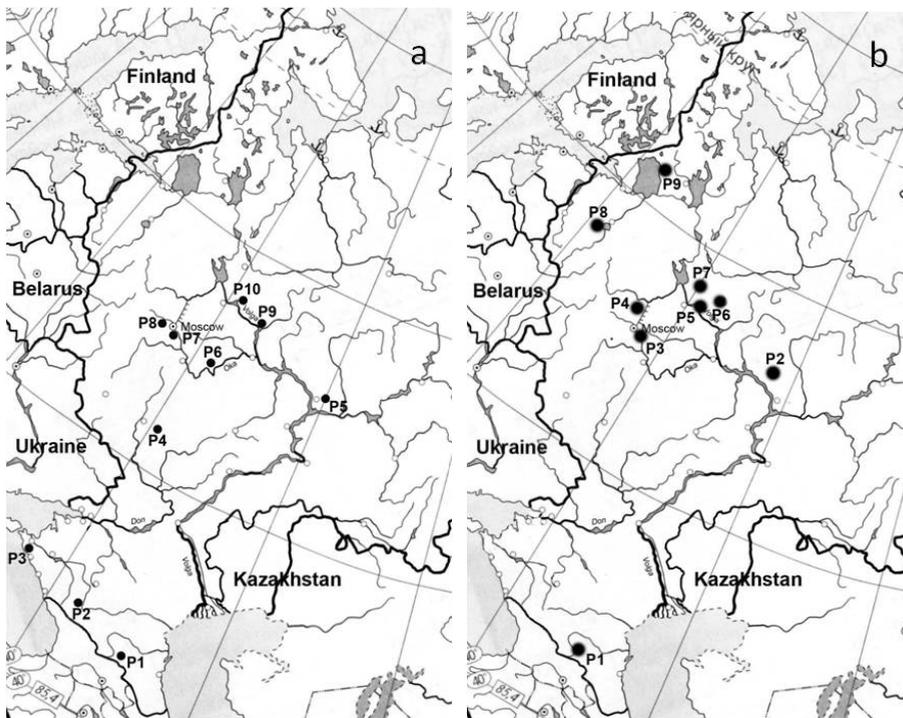
Figure 1. *Collection sites of infected potato leaves for: a) Alternaria tests (see Table 2), b) C. coccodes tests (see Table 3).*

MATERIALS AND METHODS

Sample collection. Potato leaves with clear manifestation of infection were collected from commercial fields and homestead plots in 17 sampling sites located in nine different regions of European Russia (Figure 1; Tables 2, 3). Samples were collected in August-September, when potato tubers reached a marketable size, but top parts of plants still remained green. During sampling, only one leaf per plant was collected, and the distance between the sampled plants was 5 m or more. For each sampling site, 20-25 green and non-wilted leaves with clear dark necroses, similar to early blight lesions, were collected. The leaves were immediately put into 70% ethanol to prevent the development of secondary mycobiota on dead tissues.

DNA extraction. DNA was extracted from the whole simple leaflets with one or multiple necroses by crushing samples in CTAB DNA extraction buffer (0.5 M NaCl, 10 mM Tris-HCl [pH 7.5], 10 mM EDTA, 2% [w/v] CTAB) using liquid nitrogen as described by Kutuzova et al., 2017. DNA concentration was determined spectrophotometrically at 260 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The final concentration of extracted DNA was adjusted to 50 ng/µl. All DNA samples were stored at −20°C.

PCR amplification. Isolated DNA was first amplified using ITS1F and ITS4 primers (Table 1), which selectively amplify DNA of the most of ascomycetes and basidiomycetes. Only samples with successful PCR reaction were used in the further work. A selective amplification at the next stage of the study was provided by the use of species-specific primers (Table 1, Figure 2).

The total PCR reaction volume was 25 µl. Reaction mixture contained 1 µl of DNA template, dNTP (200 µM), primers (0.2 µM of each, Table 1), 1.5 U of Taq polymerase and reaction buffer (Promega Corp., Madison, WI). In the case of negative control, 1 µl of milliQ water was used instead of fungal DNA. Amplification was performed using a Biometra T1 cycler (Biometra, Germany). Thermal cycling conditions included an initial denaturation step at 94°C for 3 min followed by 30 cycles of denaturation (94°C for 30 s), 30-s annealing at specific temperature (Table 1), elongation (72°C for 45 s), and a final elongation stage (72°C for 5 min). PCR products were electrophoretically separated on 1% agarose gel supplemented with ethidium bromide (0.5 µg/ml) in a 0.5× TBE buffer at 100 V for approximately 1 h, then visualized and recorded using an ImageStore 7500 UV transilluminator (UVP Inc., Upland, CA).

Table 1. *List of primers used in the study*

Primer	Nucleotide sequence	Annealing temperature, ºC	Specific for species	Reference
ITS4/ITS1F	5'-TCCTCCGCTTATTGATATGC 5'-CTTGGTCATTTAGAGGAAGTAA	54°C	Fungi	White et al. 1990
				Gardes and Bruns 1993
Cc1NF1/ Cc2NR1	5'-TGCCGCCTGCGGACCCCCCT 5'-GGCTCCGAGAGGGTCCGCCA	66°C	*C. coccodes*	Cullen et al. 2002
ITS5/MR	5'-GGAAGTAAAAGTCGTAACAAGG 5'-GACCTTTGCTGATAGAGAGTG	50°C	*A. alternata*	Kokaeva et al., 2017
ITS5/SR	5'-GGAAGTAAAAGTCGTAACAAGG 5'-CTTGGGGCTGGAAGAGAGCGC	56°C	*A. solani*	
Inf.pr/ Inf.obr	5'-GACACCCCCCGCTGGGGCACTGC 5'-GGTTGGTCCTGAGGGCGGGCGA	56°C	*A. infectoria*	

RESULTS

Our study showed the occurrence of *C. coccodes* and *Alternaria* species in green potato leaves with dry necrotic lesions. *A. alternata* appeared to be the most common species revealed in samples from 10 fields located in eight different regions of Russia. This species was found in 50% of samples and was present in all studied regions (Table 2). *A. solani* was identified in 30% of samples and was found in all studied regions except the Krasnodar region. *A. infectoria* was detected in 13% of samples and did not present in samples from the Stavropol and Ryazan regions.
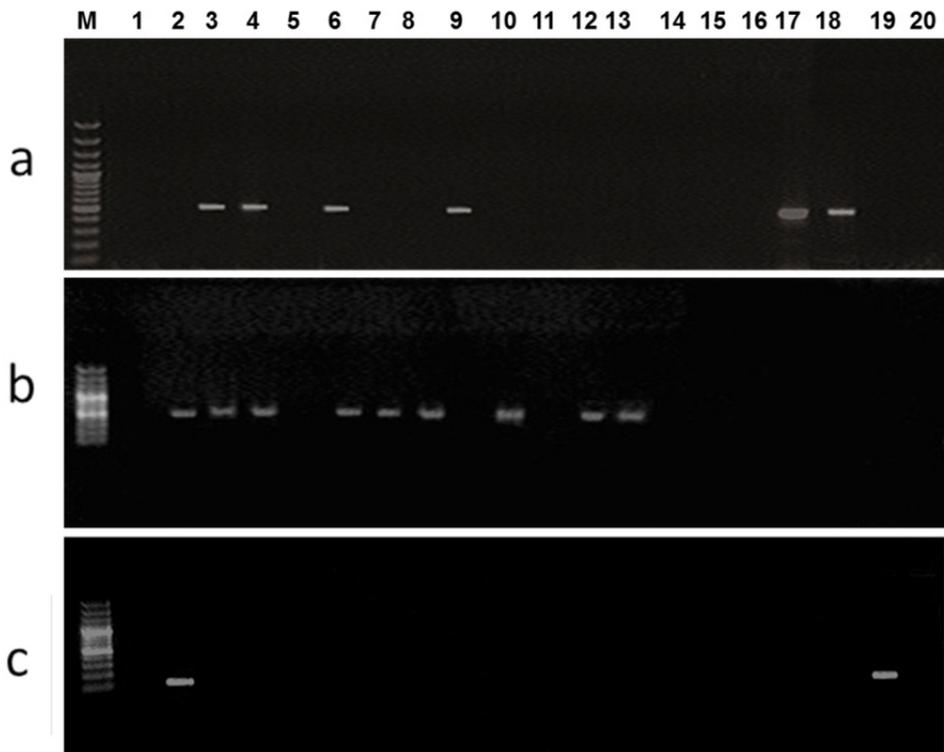
Figure 2. *Identification of Alternaria spp. using species-specific primers. a - amplification with ITS5-MR (A. alternata), b - amplification with ITS5-SR (A. solani), c - amplification with Inf.pr-Inf.obr (A. infectoria) M – DNA ladder 1kb., 1 – negative control, 2-20 - DNA samples of tested leaves.*

A simultaneous presence of two species in one host sample was considered separately (16% of samples). The co-occurrence of *A. solani* and *A. alternata* was revealed in 9% of samples from all regions except the Ryazan and Krasnodar regions. The combination of *A. alternata* and *A. infectoria* was revealed in 4% of samples. The co-occurrence of *A. solani* and *A. infectoria* was detected only in 3% of samples. Finally, no early blight agents were found in 23% of samples with blight and necrotic symptoms. None of the tested samples contained all 3 target *Alternaria* species.

C. coccodes was revealed in five samples from the North Ossetia, Kostroma region, and Mariy El Republic (Table 3). In the North Ossetia, plants of the sampled field were green and actively vegetated; only single leaves with dry necroses were observed. In the Mariy El Republic and Kostroma region (Strelnikovo), samples were collected at the end of the vegetation season; plants were strongly affected with early and late blights and the haulm started to wither.

DISCUSSION

The presence of *Alternaria* spp. was examined in 306 potato samples. According to the data of other authors, *A. solani* is indicated as the main causative agent of early blight of solanaceous crops (Dang et al., 2015). Nevertheless, in this study small-spored *A. alternata* (sensu lato) was found in a larger number of samples. Large-spored *A. solani* prevailed on potato leaves collected in the Moscow region, but was not detected on potato in the Anapa district of the Krasnodar region.

There is an ongoing discussion about the importance of *Alternaria* species for the early blight disease. Some researchers are convinced that only *A. solani* is pathogenic (Turkensteen et al., 2010). In this case, *A. alternata* may be a saprophyte, which colonizes leaf lesions caused by *A. solani,* and, therefore, represents a secondary infection. Nevertheless, some authors postulated the pathogenicity of *A. alternata* (Droby et al., 1984, Zheng et al., 2015). In our previous studies we showed the ability of *A. alternata* to infect potato and tomato leaves and the different virulence of *A. alternata* to various cultivars (Kokaeva et al., 2015, Kudryavtseva et al., 2017). This paper confirms that *A. alternata, A. solani,* or *A. infectoria* alone are capable to induce the disease. The possibility of *A. infectoria* to cause early blight on potato leaves was also shown in Iran (Ardestani et al., 2010). Some American researchers (Tymon et al., 2016) also isolated strains of the *A. infectoria* group from potato leaves.

Table 2. *The occurrence of pathogenic Alternaria species on potato leaves from different regions of Russia*

Region	Sampling site (Fig.1a)	Number of samples analyzed[a]	Number of samples containing DNA of:						
			A.s.[b] only	A.alt. only	A.inf only.	A.s.+ A.inf. together	A.s. + A.alt. together	A.alt. + A.inf. together	No Alternaria DNA
Republic of North Osetia-Alania, Vladikavkaz district	P1	8	0	1	0	0	5	2	0
Stavropol region, Kislovodsk city	P2	23	2	14	0	0	1	0	6
Krasnodar region, Anapa district	P3	8	0	4	2	0	0	2	0
Voronezh region, Panino district	P4	28	8	12	1	2	3	2	0
Ryazan region, Kasimov district	P5	12	3	6	0	0	0	0	3
Tatarstan republic, Kazan district	P6	78	10	25	2	0	3	0	38
Moscow region, Lyubertsy district	P7	25	9	8	2	1	3	0	2
Moscow region, Odintsovo district	P8	67	21	17	5	2	9	1	12
Kostroma region, Makarovo district	P9	15	0	11	1	1	1	1	0
Kostroma region, Minskoe village	P10	42	3	17	4	2	2	4	10
Total		306	56	115	17	8	27	12	71
Total %			18%	37%	6%	3%	9%	4%	23%

[a] *Number of samples with positive results for the ITS 1F and ITS4 primers.*
[b] *A.s. – A. solani, A.alt – A. alternata s.l., A.inf. – A. infectoria.*

A considerable part of samples demonstrated a simultaneous presence of the studied species with the prevalence of the complex of *A. solani* and *A. alternata*. Several studies showed that *A. solani* and *A. alternata* could be isolated simultaneously from the lesions with typical EB symptoms (Bäßler et al., 2004; Latorse et al., 2010). In some of these studies, a high pathogenicity of the *A. solani – A. alternata* complex is discussed (Leiminger and Hausladen 2012, 2013). *Alternaria* pathogens were not detected in 23% of tested potato samples with clear early blight symptoms; probably, these symptoms were caused by other fungal pathogens, such as *Cladosporium* sp., *Colletotrichum coccodes,* etc.

Table 3. *Occurrence of C. coccodes DNA in the collected samples potato leaves*

Sampling site (Figure 1b)	Regions	Number of samples analyzed[a]	Number of samples containing DNA of C. coccodes[b]
P1	North Ossetia, Mikhailovskoe village	10	1
P2	Mariy El Republic, Yoshkar-Ola city	12	3
P3	Moscow region, Lyubertsy district	24	0
P4	Moscow region, Dmitrov district, Rogachevo village	3	0
P5	Kostroma region, Kostroma district, Strel'nikovo village	12	1
P6	Kostroma region, Susanino district	3	0
P7	Vologda region, Gryazovets district, Rostilovo village	12	0
P8	Novgorod region, coast of Il'men lake	9	0
P9	Karelia, Lyaskela village	11	0
	Total (potato)	**96**	**5**

[a] *Number of samples with positive results for the ITS 1F and ITS4 primers.*
[b] *Number of samples with positive results for the Cc1NF1and Cc2NR1 primers.*

The performed study demonstrated the occurrence of *C. coccodes* on potato leaves collected in different regions of European Russia. The affection of leaves and above-ground stem parts of potato by *C. coccodes* was observed after the artificial inoculation (cuticle injury by a sand blaster with the subsequent incubation under high-moisture conditions), and was followed by high yield losses (Nitzan et al., 2006; Johnson, 1994, Mohan et al., 1992). Such injury of live green leaves caused formation of necrotic lesions similar to early blight symptoms, but without concentric rings. The edges of necroses were often surrounded by the yellowing, and the further disease development resulted in a leaf wilt (Johnson and Miliczky, 1993). Aggressive isolates were able to infect intact leaves (Andrivon et al., 1998). During harvesting, spores may migrate from leaves to damaged tubers and infect them. Therefore, potato tuber protection requires application of effective systemic or translaminar fungicides on senescent haulm. The chemical desiccation of top parts of potato plants before harvesting may be also helpful.

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188

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Needs oriented treatment against *Alternaria solani* in ware potato

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SUMMARY

Despite a very low incidence of early blight in the middle part of Sweden during seven years of monitoring (2010-2016) ware potato crops are often treated with fungicides on a regular basis. Strobilurins, often in combination with boscalid, are commonly used. In starch potato grown in the southern part of Sweden there are, however, often severe epidemics of early blight and fungicide treatments are necessary to avoid yield losses. The epidemic usually starts in end of August. The use of difenoconazol has increased during the past years in those areas. However, the haulm of ware potato is usually killed in the end of August/beginning of September, which is at the same time as when the epidemics normally start, while starch potato is grown until October in the southern part of Sweden. Therefore, we think there are reasons to dispute the need for fungicide treatments on a habitually basis in ware potato, especially in the middle part of Sweden. Two years of field trials showed that the yields were not higher in plots treated with either strobilurins alone or in combination with an SDHI fungicide (boscalid) compared to the untreated control plots. Fungicide treatments may as well be excluded in the southern Sweden in ware potato cultivars with partial host resistance, such as cvs Folva, Asterix and Ovatio, as indicated by results from three field trials in 2013 and 2015. In 2017, we performed further field trials both in the middle part and in the south of Sweden and preliminary analysis indicate similar results as earlier years, *i.e*. fungicide treatment does not increase yield in ware potato.

The substitution F129L (strobilurin resistance) is dominating in the southern population of *A. solani* and occurs in about half of the population in the middle part of Sweden. The efficacy of boscalid may have decreased in the southern part of Sweden since the efficacy of Signum was not as high in 2016 as previous years. Analyses are ongoing to unveil this issue. We suggest increased efforts to apply IPM including host resistance and plant strengtheners in combination with minimized use of fungicides.

KEYWORDS

Alternaria solani, early blight, fungicide sensitivity, plant strengthener

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 about the objective of the subgroup. The group approved that the objective is to **increase the knowledge** of early blight especially in

- Monitoring of fungicide sensitivity
- Phenotypic characterization of the pathogens
- Decision Support System
- Host resistance

These topics are the basics for the development of an IPM system to control EB and provide information to support national development.

2. ACTIVITIES

The EuroBlight Alternaria group updated the list of protocols. The new protocol concerning the "Characterization of SDHI mutation" was added.

At the moment 19 different protocols for isolation, spore production, artificial inoculation (greenhouse and field) and molecular detection of different *Alternaria* species are on the website 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*)
- + Characterization of SDHI mutation

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

Together with the fungicide rating group the protocol for EB fungicide trials to provide a decimal rating for *Alternaria* fungicides was discussed. In comparison to the previous protocol an untreated plot is the reference for the decimal rating. The control plots have to be included in the randomized field trial.

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

4. HOMEPAGE

All relevant publications (*Alternaria* on potato and tomato) will be uploaded and updated on the EUROBLIGHT homepage.

5. NEW INITIATIVES

In 2015 the members of the subgroup decided to initiate a cooperating project dealing with the spread of the QoI fungicide resistance of *A. solani* in European potato growing areas. The project "Monitoring of sensitivity to fungicides (QoI) of *A. solani* isolates in Europe" started in 2015 with a limited number of *Alternaria solani* isolates in different locations/countries and was expanded in 2016. The aim is to publish the data about the "Prevalence of QoI Resistance in European *Alternaria solani* population".

Further the group discussed following Integrated Pest Management tools to control early blight:

- Cultivar resistance (maturity group)
- Healthy seed tuber
- Crop rotation
- Controlling weeds and volunteer potatoes
- Nutrition deficiency (Nitrogen,..)
- Fertilization (Calcium cyanamide)
- Reduction of biotic and abiotic stress
  (E.g. Aphids, drought, ….)
- Diagnostic
- DSS
- Biologicals
- Chemical application

Also recommendations for the integrated and sustained fungicide strategies with the background of an increasing loss of fungicide sensitivity of *Alternaria* isolates were discussed. The discussion will continue at the next EB subgroup meeting.

Report of the Control Strategies Subgroup meeting on 17 May 2017

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1. *ALTERNARIA* BLIGHT

1.1 Changes to the early blight fungicide efficacy table
Three action points from the Brasov meeting were addressed. **1.** A new ratings table containing objective and trials-based efficacy ratings has replaced the previous table comprising subjective ratings. The positioning of the new table solves the issue of poor links in the EuroBlight website to the early blight fungicide table. **2.** The dose rates of the fungicides with ratings generated from the early blight efficacy ratings trials are included in the new table. **3.** Comments on insensitivity risk are now included in the footnotes to the table.

There is one outstanding action point from the Brasov meeting: the inclusion of a footnote explaining the transgression of product labels, in terms of number of fungicide applications, in the ratings trial protocol.

1.2 Alternaria subgroup
Proposal: The *Alternaria* subgroup was requested to take charge of any action points appropriate to early blight aspects of EuroBlight. The subgroup should address action points from the Brasov workshop onwards (Agreed).

2. *PHYTOPHTHORA* BLIGHT

2.1 Changes to the late blight fungicide efficacy table
Prior to the Aarhus workshop Syngenta requested that the EuroBlight fungicide experts give ratings (0 to +++) for the co-formulation of mandipropamid + cymoxanil (Carial Flex) based on the experts' experience with this product and also information provided by Syngenta. These scores were added to the table.

The provisional leaf blight efficacy rating of 4.6 was assigned to [zoxamide + dimethomorph (Presidium)] + fluazinam and included in the late blight table. The rating is provisional because it is based on five trials, not six.

At the Aarhus meeting it was stated that none of the fungicide registration dates listed in the table had been questioned and therefore no updates were required.

2.2 Ratings trials

Up to the time of the Aarhus meeting there had been no reports made to EuroBlight of reduced efficacy for specific fungicides in relation to their EuroBlight ratings. Any future reports should be addressed to Huub Schepers and include supporting evidence.

No host resistance elicitors were included in the 2015 or 2016 trials for efficacy ratings because none were submitted for inclusion by companies.

Proposal: Tank mixes of fungicide and adjuvant should be included in the EuroBlight efficacy table (Not agreed).

In 2017 the late blight leaf blight efficacy trials will be in DK, NL and UK.

2.3 New initiatives and developments

The Best Practice guides are to be revised, for Europe initially. Volunteers for this task were requested and Ruairidh Bain and Faye Ritchie agreed to assist Huub. The completed European-centred guides, containing more detail and better quality information, will be put on the EuroBlight website. Subsequently the guides will be adapted for other continents. Ivette Acuña and Jorge Andrade-Piedra offered to help with this.

3. GENERAL POINTS

All of the protocols previously available on the two older websites need to be transferred onto the new EuroBlight website and then updated if necessary. This is required not only for members of EuroBlight but to facilitate the sharing of protocols with researchers in other blight networks. At the Aarhus meeting there was a request specifically for the fungicide rainfastness protocol.

Issues remain over the links on the EuroBlight website to the websites for Africa Blight, Asia Blight, Tizon Latino and US Blight and also the amount of information about these four other networks on the EuroBlight website.

Detailed information for the eleven Best Practice items in the Control Strategies section of the website was absent.

4. RECORD OF FUNGICIDE TABLES

The most up to date versions of the late blight and *Alternaria* fungicide efficacy tables should be accessed via the EuroBlight website. The fungicides tables in this paper are a record of the tables as at September 2017.

GENERAL COMMENTS ABOUT THE RATINGS TABLE FOR LATE BLIGHT FUNGICIDES

Ratings are intended as a guide only and will be amended in future if new information becomes available. Ratings for leaf blight control 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 – 2015). Ratings for tuber blight control are also based on results from dedicated 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). Ratings for leaf blight and tuber blight control are each calculated from the results of a minimum of six EuroBlight field trials. There are few products with decimal ratings for tuber blight control compared with earlier subjective ratings but the 0 to +++ ratings can be obtained from the previous workshop proceedings.

All other ratings in the table are on a 0 to +++ scale. These ratings are derived from non-EuroBlight field experiments and experience of the performance of products when used in commercial conditions.

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 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.

GENERAL COMMENTS ABOUT THE RATINGS TABLE FOR ALTERNARIA FUNGICIDES
See the footnotes to the table. The scores for individual products are not additive for mixtures of active ingredients. The dose rates are those used in the six EuroBlight field trials necessary to determine the leaf blight ratings. The ratings given are for fungicides with an efficacy against early blight currently registered in several EU countries and are for commercially available products. The ratings are NOT for the active ingredients themselves.

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.

198

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.

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 2017)

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			
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]	+(+)	+++	contact/translaminar	2005
mandipropamid+difenoconazole (0.6)	4.0	++	+(+)		+++	+[6]	+(+)	+++	contact/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								contact/translaminar+contact	2013
(zoxamide+ dimethomorph) +fluazinam (1.0 + 0.4)	4.6[9]								contact/translaminar+contact	2015
mandipropamid+cymoxanil (0.6)	4.4	++	+(+)		+++	++	+(+)	+++	contact/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

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						
propamocarb+cymoxanil (2.0)					+(+)	++(+)[7]	++(+)					systemic+translaminar	2011
propamocarb-HCl+**fenamidone** (2.0)	2.5	+(+)	++		++(+)	++	++				+++	systemic+**translaminar**	1998
propamocarb-HCl+**fluopicolide** (1.6)	3.8	++	++	3.9	+++	++	++(+)				++(+)	systemic **+ translaminar**	2006
oxathiapiprolin (0.15)		++(+)	++(+)		+++	++	++(+)				+++	systemic	2017

Footnotes to Late Blight Fungicide Table

See caveats listed in the section entitled 'General comments about the ratings table for late blight fungicides'

[1] *Includes maneb, mancozeb, propineb and metiram.*

[2] *See text for comments on phenylamide resistance.*

[3] *Based on EuroBlight field test in 2006-2015.*

[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 ++*

[9] *A provisional rating based on five EuroBlight experiments.*

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 *: See section on phenylamide resistance. Isolates of P. infestans have been found in parts of Europe resulting in lower field efficacy of fluazinam. 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.*

Early Blight Fungicide Table Efficacy of fungicides for the control of early blight caused by Alternaria solani and Alternaria alternata (as at September 2017)

Product	Efficacy rating [1,2]		
	14-day interval	Strategy	7-day interval
Spray interval 14 days			
mancozeb 2.0	1.7	-	-
Spray strategy[3]			
(zoxamide + mancozeb)[a] 1.8 + azoxystrobin[b,5] 0.5[4]	-	3.7	-
(zoxamide + mancozeb)[a] 1.8 + difenoconazole[b] 0.5	-	3.9	-
Spray interval 7 days			
mancozeb 2.0	-	-	2.5
zoxamide + mancozeb 1.8	-	-	2.8
fenamidone[5] + propamocarb 2.0	-	-	2.2
fluazinam + azoxystrobin[5] 0.5	-	-	3.1
dimethomorph + mancozeb 2.0	-	-	2.9

[1] Ratings for Alternaria are based on results from EuroBlight field trials during 2015-2016, and only compounds included in these trials are rated for Alternaria. The scale for Alternaria is a 0-5 scale.

[2] The ratings are intended as a guide only and will be amended in future if new information becomes available.

[3] The active ingredients were sprayed in a spray strategy with a 7 day interval ([a]) or a 14 day interval ([b]).

[4] azoxystrobin was sprayed at label rate which is 0.5 for DK and DE, and 0.25 for NL.

[5] Alternaria solani isolates that are less sensitive to QoI-fungicides have been isolated from potato plants in Europe. Therefore resistance management strategies should be implemented (see FRAC web site for details). Ratings will be lower where fungicide insensitive strains are present.

Disclaimer: Whilst every effort has been made to ensure that the information is accurate, no liability can be accepted for any error or omission in the content of the table or for any loss, damage or other accident arising from the use of the fungicides listed herein. Omission of a fungicide does not necessarily mean that it is not approved for use within one or more EU countries. The ratings are based on the national dose rate label recommendation for a particular product. Where the disease pressure is low, intervals between spray applications may be extended and, in some countries, fungicide applications are made in response to nationally issued spray warnings and/or Decision Support Systems. It is essential therefore to follow the instructions given on the approved label of a particular early blight fungicide appropriate to the country of use before handling, storing or using any early blight fungicide or other crop protection product.

Posters

Characterization of different methods of mating type determination in *Phytophthora infestans*

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Characterization of different methods of mating type determination in *Phytophthora infestans*

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Introduction: *Phytophthora infestans* (Mont.) de Bary is one of the most devastating potato pathogens worldwide. This heterothallic organism can reproduce both sexually (producing oospores) and asexually (producing sporangia). Sexual propagation takes place between two mating types designated A1 and A2 and influences the population structure. When this kind of reproduction occurs, genetic variability in *P. infestans* populations increases having major implications for disease control. Over the years, different methods of the mating type determination were developed. We validated different available markers (W16, S1, PHYB) [1, 2, 3] for *P. infestans* mating type and compared of the obtained results with data from the classic pairing test.

Tab. 1. Details of *P. infestans* isolates from different locations used for mating type determination by pairing test and PCR reactions.

	Isolate name	Origin	Collection year	Genotype	Mating type assessment method				
					Another laboratory	Pairing test	W16	S1	PHYB
1	EC-1[1]	Ecuador	2007	EC-1	A1	A1	A1	A1	A1
2	3394[1, 3]	Ecuador	-	P. andina	A1	-	A2	A1	A1
3	3/1/02[4]	Hungary	2002	-	A1	A1	A1	A1	A1
4	TV580[1,3]	Mexico	-	-	A1	A1	A2	A1	A1
5	80029[1,3]	Netherlands	1980	-	A1	A1	A1	A1	A1
6	88069[1,3]	Netherlands	1988	-	A1	A1	A1	A1	A1
7	05194[3]	Netherlands	2005	-	A1	A1	A1	A1	A2
8	MP1444[3]	Poland	2011	-	-	A1	A1	A1	A1
9	08-P15-12[5]	France	2008	-	A2	A2	A2	A2	A2
10	08-P37-01[5]	France	2008	-	A2	A2	A2	A2	A2
11	08-N-91[5]	France	2008	-	A2	A2	A2	A2	A2
12	09-P15-07[5]	France	2009	-	A2	A2	A2	A2	A2
13	13/04/02[1, 3]	Hungary	2002	-	A2	A2	A2	A2	A2
14	90128[3]	Netherlands	1990	-	A2	A2	A2	A2	A1
15	88133[1,3]	Netherlands	1988	-	A2	A2	A2	A2	A1
16	MP821	Poland	2006	-	-	A2	A2	A2	A2
17	MP1358[3]	Poland	2011	-	-	A2	A1	A1	A1
18	MP1487[3]	Poland	2012	-	-	A2	A2	A2	A2
19	USD 618	UK	2007	-	A2	A2	A2	A2	A1
20	07/39[1, 6]	UK	2007	13_A2	A2	A2	A2	A2	A2
21	3928A[4]	UK	2006	13_A2	A2	A2	A2	A2	A2
22	74001[3]	Netherlands	1974	US-1	A1	A1	A2	A1	A1
23	IPO-0[1, 3]	UK	2007	US-1	A1	A1	A2	A1	A1
24	7818[1, 3]	UK	-	US-1	A1	A1	A2	A1	A1
25	87000[1, 3]	USA	-	US-1	A1	A1	A2	A1	A1
26	9440507	USA	1994	US-1	A1	A1	A2	A1	A1

[1] isolates for which PCR products of marker W16 were sequenced
[2] isolates were received from The Sainsbury Laboratory, UK
[3] isolates were received from Francine Govers and Rob Weide – Wageningen University, Netherlands
[4] isolates were received from József Bakonyi
[5] isolates were received from Roselyne Corbière, INRA, Fance
[6] isolates were received from David E. L. Cooke – James Hutton Institute, Scotland, UK

Material and Methods: Two groups of *P. infestans* isolates were studied: 146 Polish isolates collected in year 2011 [4] and 26 control diversified isolates from eight countries (Tab. 1). A pairing test (Fig. 1) was performed by crossing the isolates with the standard isolates of A1 (7818) and A2 (90128) mating types on the rye A agar media according to the method described by Zarzycka [5]. Markers W16, S1, PHYB were evaluated using a PCR method (Fig. 2). W16 fragments were digested with the restriction enzyme *Bsu*RI. PCR products of W16 marker were sequenced for 13 isolates by external company. W16 products for three isolates were sequenced after cloning.
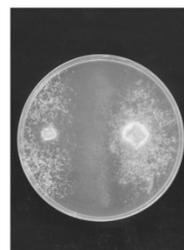
Fig.1. Pairing test of *P. infestans* on Petri dish on the rye A agar media.

Tab. 2. Results of the mating type determination in group of 146 Polish *P. infestans* isolates from year 2011.

Mating type assessment method				Number of isolates	(%)
Pairing test	W16	S1	PHYB		
A1	A2	A2	A1	1	0.7
A1	A1	A1	A2	6	4.1
A2	A1	A1	A1	5	3.4
A2	A2	A2	A1	8	5.5
A1	A1	A1	A1	87	86.3
A2	A2	A2	A2	39	
Total				146	100

Results:

• **Pairing test:** confirmed the results for control *P. infestans* isolates obtained from other laboratories (Tab. 1). Among 146 Polish isolates, the results for all methods were consistent for 126 isolates (86.3%) (Tab. 2)

• **Marker W16:** all isolates of genotype US-1, TV580 and isolate *P. andina* were misidentified as A2 (Tab. 1). In 146 Polish group six isolates were wrongly assigned (Tab. 2). W16 sequences from genotype US-1 and *P. andina* clustered together with isolates of A2 mating type (Fig. 3)

• **Marker S1:** a DNA fragment was observed for isolates of A1 (Fig. 2). Results for 26 control isolates were in agreement with the data from pairing test, except the isolate MP1358 (Tab. 1). For the Polish isolates from year 2011 data exactly corresponded with the data for marker W16 (Tab. 2)

• **Marker PHYB:** a DNA fragment was observed for isolate of A2 (Fig. 2). The discrepancies between pairing test reached 23 isolates among all tested isolates of *P. infestans* (Tab. 1, 2)
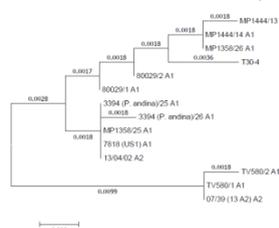

Fig. 3. The phylogenetic relationships among the *P. infestans* W16 sequences analysed by programme MEGA6 using the maximum likelihood (ML) method and bootstrap with 1000 replications.

Fig. 2. Agarose gels with the PCR products of markers: A – S1, B – W16 and C – PHYB. Lanes: 1 – 9440507, 2 – 74001, 3 – 87000, 4 – 7818, 5 – 90128, 6 – 09-P15-07, 7 – 13/04/02, 8 – USD 618, 9 – 80029, 10 – EC-1, 11 – TV580, 12 – *P. andino*, N– negative control.

Conclusion: To characterize the structure of *P. infestans* populations and to improve management of the late blight disease, it is important to determine mating type precisely. While the pairing test is time and labour-consuming, none of the PCR tests proved to be absolutely consistent with the results of pairing test. However, after validation and excluding of some populations, such as US-1, markers W16 and S1 can be applied with satisfying rate of correct scores (95.9% in Polish population from year 2011).

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Phenotypic and genotypic characteristics of Belgian isolates of *Phytophthora infestans* in 2013-2016

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Phenotypic and genotypic characteristics of Belgian isolates of *Phytophthora infestans* in 2013-2016

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Summary

A total of 385 isolates of *Phytophthora infestans* were collected in the southern part of Belgium (Wallonia) in several potato fields, volunteers and dumps during the years 2013-2016. Most of isolates were tested for several phenotypic characteristics, such as mating type, virulence and sensitivity to metalaxyl. The isolates were analyzed using standardized 12 plex Euroblight SSR genotyping (Euroblight monitoring). Genotypes were determined by comparing fragment sizes with isolates previously genotyped.
Clone 13_A2 (Blue-13) made up 50% of the samples and was resistant to Metalaxyl. The second most prevalent clone (1_A1) was found at a 22% frequency. The frequency of the clone 6_A1 increased from 3 to 10% of the population. The two clones were sensitive to metalaxyl. Two new clones were detected in Belgium in 2016 at low frequency: 37_A2 and 39_A1. Lastly, the genetically diverse 'Others' samples decreased from 31 to 4% of the population. Clones 13_A2 and 1_A1 had virulence profiles more complex than clones 6_A1 and Others.

Materials and Methods

- Single-lesion isolates were obtained by placing pieces of infected tissue on tuber slices of a susceptible potato cultivar (Bintje). Pure cultures were obtained by transferring small pieces of mycelium growing on the upper side of the potato slice on pea agar medium.
- The mating type was tested by growing isolates on rye agar with the known references strains of the A1 and A2 mating types. After 7-14 days incubation at 16°C, the presence or absence of oospores was recorded under a microscope.
- Virulence patterns were determined using Black's differential set of potato clones, each having one of the R1-R11 pathotype-specific resistance genes.
- The floating potato leafdisc method was used to determine metalaxyl sensitivity. Isolates were tested on 0.1, 1, 10 and 100 mg metalaxyl/ml. Isolates sporulating on water containing 10 and 100 mg/ml were rated as resistant, those on 1 mg/ml were rated as moderately resistant and those that sporulated only on water and 0.1 mg/ml were rated as sensitive.
- The isolates were analyzed using standardized 12 plex Euroblight SSR genotyping (Euroblight monitoring). Genotypes were determined by comparing fragment sizes with isolates previously genotyped (Cooke, James Hutton Institute).

Results

Among the 381 tested isolates, 55% were A2 mating type and 45% were A1 mating type. From 2013 to 2016, among the 351 genotyped isolates, 70 to 95% of the population comprised known clonal lineages. The remaining samples were genetically diverse genotypes (and named « Others »). The most prevalent clones were 13_A2 (60% in 2016), 6_A1 and 1_A1. Two new clones were observed in 2016 (37_A2 and 39_A1) but at very low frequency. Clone 33_A2 still observed in 2015 was not encountered in 2016. Two isolates of BE-clone were observed in Belgium only in 2016. (http://euroblight.net)

All clones are present in each walloon province but clone 1_A1 was most prevalent in Hainaut province (West) with 50% of the population. In the south east (Luxembourg province) more than a third of population was genotypes named Others. In total, 334 isolates were screened for resistance to metalaxyl. All 13_A2 clones were resistant whereas 1_A1 clones and 6_A1 clones were sensitive to Metalaxyl. 95% of Others clones were sensitive.
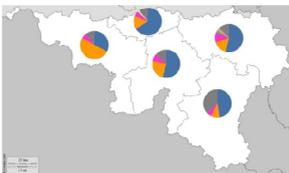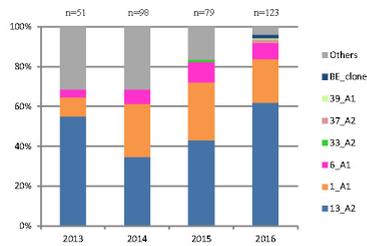
All known virulence genes were found in belgian isolates. During 4 years, 31 different races were found among 199 isolates but 4 races were the most prevalent : 1-2-3-4-5-6-7-8-9-10-11 (20%), 1-2-3-4-5-6-7-8-10-11 (15%), 1-2-3-4-5-6-7-10-11 (13%) and 1-3-4-7-10-11 (9%). Clones 13_A2 and 1_A1 had virulence profiles more complex than clones 6_A1 and Others.

	13_A2	1_A1	6_A1	Others
1-2-3-4-5-6-7-8-9-10-11	40	5	0	0
1-2-3-4-5-6-7-8-10-11	9	5	0	0
1-2-3-4-5-6-7-10-11	11	13	0	2
1-3-4-7-10-11	1	3	3	10
1-3-4-7-8-10-11	0	0	5	4

Monitoring French populations of *Phytophthora infestans* 2013-2016 on potato reveals local changes in genotypic structures

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Monitoring French populations of *Phytophthora infestans* 2013-2016 on potato reveals local changes in genotypic structures

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Introduction

The oomycete *Phytophthora infestans* is renowned for its fast and dramatic changes in the genotype occurrence and for its capacity to adapt with strong impact for durable management of potato late blight.

From 2013 to 2016, surveys were conducted in the French major production areas to investigate genotypic dynamics of *P. infestans* populations, in order to characterize the rapid adaptation and mechanisms driving their displacements.

P. infestans collection

Isolates were randomly collected in commercial fields and experimental trials from single lesions and squashed onto FTA cards.
- ➤ A total of 1162 isolates were sampled in **western** and **northern** France over four years; and in **eastern** France in 2016.
- ➤ Some isolations were also performed to obtain alive isolates for biological assays, from the same lesions.

Genotypic structure

Analysis was performed at 12 microsatellite loci, according to Cooke *et al.* (2012), Li *et al.* (2013) and euroblight.net. It revealed :
- ❖ Clonal lineage structure and low genotypic diversity.
- ❖ **EU_13_A2** : predominant lineage over the temporal and spatial scales. Moreover it showed many sub-clonal variants.
- ❖ Variations in the distribution of the major lineages according to years and regions
  - ➤ **EU_6_A1**: high frequency in the West, but declined each year (54% in 2013 to 28% in 2016). The major decline is notable in the North (2% in 2016).
  - ➤ **EU_37_A2** : emergence of this new lineage in 2016 in the North (33%) and East (10%).
  - ➤ **EU_1_A1** : an old lineage which persists across years and regions, with a higher frequency in the North than in the West.
  - ➤ Eastern region : different from the two others with greater diversity and **EU_36_A2** emergence.
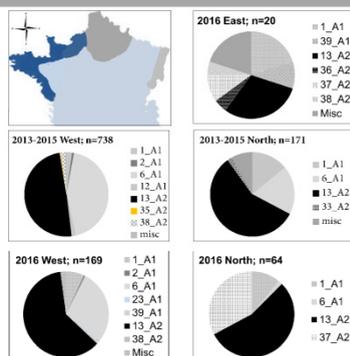
Phenotypic fitness

Aggressiveness traits of major clonal lineage isolates from 2016 were evaluated on cv. Bintje detached leaflets, incubated at 15°C dark / 18°C light (16h). Traits were compared at 5 days post-inoculation on 10 leaflets per isolate.
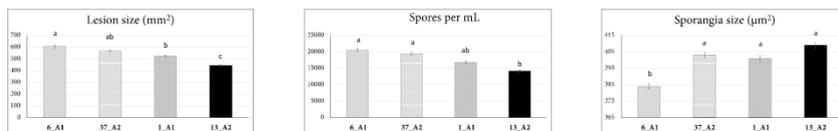
- ❖ **EU_6_A1 isolates** (n = 10) were the most aggressive one:
  - ➤ large lesions and abundant sporulation
  - ➤ with the smallest sporangia size.
- ❖ **EU_37_A2 isolates** (n = 8) were similar to 6_A1 for lesion size and sporulation, but with bigger sporangia.

- ❖ **EU_13_A2 isolates** (n = 14) were the least aggressive with
  - ➤ smallest lesions and fewer sporulation
  - ➤ with big sporangia size.
- ❖ **EU_1_A1 isolates** (n = 9) were intermediate.

Discussion

- ❖ The 4-year extensive sampling revealed that French *P. infestans* populations have undergone recent and strong changes over time and locations.
- ❖ Local adaptation to environmental factors: important impacts on dynamics of the pathogen.
  - ➤ In western France, as in the UK, climatic conditions (moderate T°C, high humidity) could favored the EU_6_A1 lineage.

- ➤ The widespread occurrence of the EU_13_A2 lineage and its persistence suggests its high potential to adapt to various agro-ecosystems. Moreover, its larger sporangial size could provide the lineage advantages such as better survival or infection efficiency.
- ❖ Future investigations are crucial to explain and possibly predict the invasive success of new lineages, as EU_36_A2, 37_A2, and their epidemic and fitness potential.

Variable temperature response in Algerian *Phytophthora infestans* EU_23_A1 isolates from tomato

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Variable temperature response in Algerian *Phytophthora infestans* EU_23_A1 isolates from tomato

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Introduction

In the north-center of Algeria, tomato is an important crop, grown during summer in fields and during winter under plastic tunnels. The tomato crops are often grown in close proximity to potato.
On these two plants, *Phytophthora infestans*, the causal agent of late blight, is a very serious threat, but little is known about the local *P. infestans* population on tomato.

➢ A survey was thus carried out in commercial tomato fields around Algiers, between 2010 and 2016 (predominantly in 2014 and 2015), to determine the genotypic structuration of *P. infestans* population on this crop.

➢ The response of this population to temperature, a major abiotic factor for disease development, was evaluated.

Sampling locations

77 tomato isolates of *P. infestans* were collected from the main production areas around Algiers and Tipaza (100 km West of Algiers) on November and December 2014 and 2015. Some were also sampled in experiment trials and plastic tunnels from 2010 to 2016.

SSR analysis

16 microsatellite markers (Cooke *et al.*, 2012; Li *et al.*, 2013) revealed that tomato population of *P. infestans* is composed of **2 clonal lineages** :

➢ **EU_23_A1** is the dominant lineage

➢ **EU_2_A1** : sampled in experimental trials from 2010 to 2014.

➢ Two "other" isolates (A1 mating type) were also collected in 2010 and 2011 .
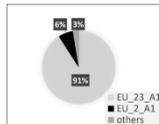
Frequency of *P. infestans* clonal lineages within the isolate collection from tomato in Algeria

➢ Structure analysis (K=5) showed that the isolate collection was split into 4 clusters, of which 2 corresponded to **EU_23_A1:**
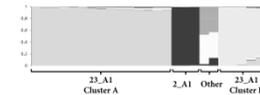
➢ **Cluster A** with isolates mainly sampled from 2014 to 2016;
➢ **Cluster B** with a majority of 2014 isolates.
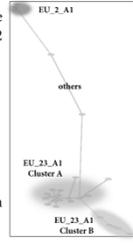
Minimum Spanning Tree

Temperature effect on mycelial growth on pea medium

Isolates were tested at five constant temperatures : **10°C, 15°C, 20°C, 25°C** and **30°C** (3 replicates per isolate and T°).

➢ 19 isolates of EU_23_A1: 10 from Tipaza and 9 sampled around Algiers;
➢ 3 isolates used for comparison :
   ➢ two EU_2_A1 isolates: 1 from tomato, 1 from potato
   ➢ one EU_13_A2 isolate from potato

Colony diameter was assessed after 10 incubation days.

❖ Temperature response profiles varied markedly between isolates, but did not sign EU_23_A1 clusters or locations.
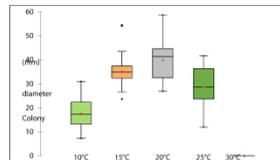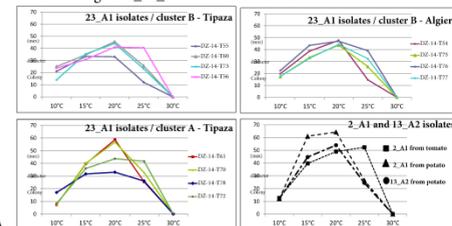
Colony diameter (mean, minimum, maximum values) of 19 isolates of *P. infestans* EU_23_A1 lineage on pea agar medium, at 10, 15, 20, 25 and 30°C.

❖ The optimal temperature for mycelial growth was :
   ➢ 20°C for 11 isolates, as for the 2_A1 and 13_A2 isolates from potato;
   ➢ 25°C for 3 isolates, and for the 2_A1 isolate from tomato;
   ➢ 15 °C for 5 isolates.

❖ The 23_A1 isolates grew generally faster at 15°C than 25°C.

❖ The minimum mycelial growth was at :
   ➢ 10°C for 15 isolates, as for the 2_A1 and 13_A2 isolates;
   ➢ 25°C for 4 isolates.

❖ At 10°C, colonies of 23_A1 isolates (especially from cluster B) were larger than those of 2_A1 and 13_A2 isolates.

❖ None of the isolates grew at 30°C.

Discussion and perspectives

❖ Our study revealed a clear host specialization of EU_23_A1 isolates on tomato in Algeria. EU_13_A2, the dominant clonal lineage on potato, was not detected on tomato.

❖ The north-center Algerian 23_A1 population on tomato is composed of two main clusters.

❖ Isolates showed variable thermal responses and some isolates developped well at 25°C. However, *in vitro* mycelial growth at five constant temperatures did not allow to differentiate the two clusters within EU_23_A1.

❖ These results, which need now to be confirmed *in vivo* on plants, may reflect an adaptation of some EU_23_A1 isolates to high temperatures which occur during summer.

❖ Tomato cultivated in summer represents a potential host for specific populations of *P. infestans* with a preference for high temperatures and grow well above 20°C.

❖ In Algeria, the warm period becomes longer than the cold period; these results have thus to be considered in the context of climate change.

Structure of *Phytophthora infestans* population in north-western Algeria from 2008-2014

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Structure of *Phytophthora infestans* population in north-western Algeria from 2008-2014

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INTRODUCTION

Late blight caused by *Phytophthora infestans* is the most serious disease of potato worldwide.

In Algeria the disease is very common on potato, but has also been reported on tomato in some areas of the country.

During 2008-2014, the late blight has reached epidemic proportions in many potato-growing areas of the north west of Algeria, an emerging potato production region. Consequently, heavy yield losses were recorded despite the excessive use of fungicide.

In order to understand the population of pathogen in north west Algeria, a total of 161 *P. infestans* isolates collected on potato and tomato from 2008-2014 were characterized for the mating type, the level of metalaxyl sensitivity (n=92) and their genotypic diversity with microsatellite markers (n=117).

MATERIALS AND METHODS

Samples were collected during 2008-2014 from potato crops grown in the field and tomatoes grown in the field and greenhouses, located in different sites in north west Algeria (Fig 1).

Figure 1: The Survey map indicating locations where tested isolates were taken during 2008 to 2014 in north-west Algeria

• The **mating type** of the isolates was determined by pairing them with reference isolates of A1 and A2 mating type on pea medium. After 11-15 days of incubation at 15°C, plates were examined microscopically for the presence of the oospores in the hyphal interaction area between the isolates paired.

• The **sensitivity to metalaxyl** was determined by the isolates' ability to grow and sporulate on potato leaf discs at different concentrations (0, 10, 100 mg/L). Isolates sporulating on the discs floating on water containing 100 mg/L metalaxyl were rated as resistant (R), those on 10 mg/L were rated as intermediate (I) and those that sporulated only on water were considered as sensitive (S).

• The **genotypic diversity** was analyzed using simple sequence repeats (SSR) markers: Pi02, Pi4B, G11, Pi04, Pi63, Pi70, D13, SSR2, SSR4, SSR6, SSR8 and SSR11.
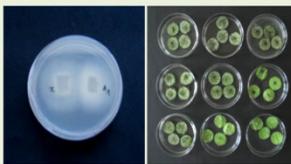
Figure 2: Mating type test   **Figure 3:** Metalaxyl sensitivity test

RESULTS

• A mating type assay showed that 70 % isolates were A2 mating type and 30 % were A1 mating type. Both mating types were sometimes found in the same field.

• A high percentage of resistance to metalaxyl (89%) among isolates was detected. Metalaxyl resistant phenotype was present in both mating types with a higher percentage among A2 mating type isolates.
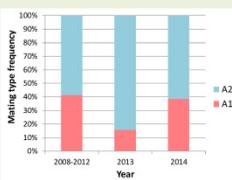
Figure 4: Frequency of mating types (A1, A2) among *Phytophthora infestans* isolates from north-western Algeria during 2008-2014
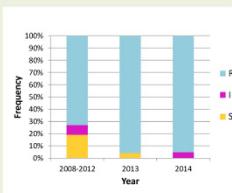
Figure 5: Metalaxyl resistance among *Phytophthora infestans* isolates from the northwestern Algeria during 2008-2014

• SSR analysis of *P. infestans* population showed a low genotypic diversity. Genotype 13_A2 was the predominant in the population with a frequency of 67% followed by 2_A1 (21%) and 23_A1 (5%). Genotype 23_A1 was detected only in tomato and potato isolates collected in 2013 and 2014.

• Several sub-clonal variants were observed in the 13_A2 population in Algeria.   The 13_A2_2 sub-type was most commonly recovered from 2008 to 2014.
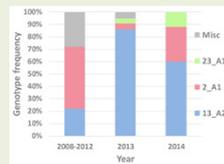
Figure 6: Genotype frequency of *Phytophthora infestans* isolates collected from north-western Algeria during 2008-2014.
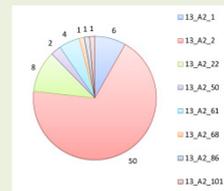
Figure 7: Sub-group frequency analysis of the genetic 13_A2 lineage of *Phytophthora infestans* isolates collected from north-western Algeria during the period of 2008 to 2014

CONCLUSIONS

• *Phtophthora infestans* population in north-western Algeria is mainly composed of the A2 mating type isolates associated with the clonal lineage 13_A2 and A1 isolates of 2_A1 and 23_A1.

• The coexistence of both mating types in most of the sampling sites means that sexual reproduction and the production of oospores may occur in this region.

• The high level of metalaxyl resistance in *P. infestans* population suggests that the use of metalaxyl formulations should be carefully planned in late blight management in Algeria.

This study is a preliminary contribution to the worldwide effort to characterize *P. infestans* and it provides some information on the pathogen population in strategic region of Algeria. Further investigations are required to establish a complete structure of the entire population of this pathogen, especially on tomato and thus complete the map of all the production areas.

Screening Irish *Phytophthora infestans* populations for mutations associated with CAA resistance

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Introduction

Late blight caused by *Phytophthora infestans* remains the most destructive disease of Irish potato crops (Fig 1). As weather conditions conducive to it's spread and development are often prevalent throughout the growing season intensive control measures are routinely applied. Due to limited host resistances in the most widely cultivated varieties, these control measures are heavily reliant on the routine applications of fungicides.

While a range of active ingredients belonging to different fungicide families are currently available for the control of *P. infestans*, ensuring their continued availability and effectiveness is essential. Key to achieving this includes monitoring *P. infestans* populations for the potential development of resistance. Here we describe the development of a high resolution melt curve assay for the detection of mutations in the *PiCes3A* gene associated with CAA resistance (Blum et al., 2010).

Fig 1. Late blight continues to cause significant damage to Irish potato crops

Materials & Methods

- Sensitivity of a selection of Irish *P. infestans* isolates to the CAA fungicide mandipropamid determined both *in vitro* and *in planta,* and partial sequence analysis of *PiCes3A*.

- Individual 147bp fragments of *PiCes3A* representing different mutations associated with CAA resistance in oomycetes identified by Blum (2012) and including wild-type synthesised as G-Blocks (Integrated DNA Technology) and cloned into pCR4-TOPO.

- Presence of different mutations confirmed using a high resolution melting (HRM) assay using an unlabelled probe and asymmetrical PCR. Ratio of different plasmids used to represent different allele frequencies.

Results & Discussion

- Irish contemporary populations, dominated by clonal linages EU8_A1, EU5_A1, EU6_A1 and EU13_A2 remain sensitive to the CAA fungicides.

- No mutations associated with CAA resistance identified in historical Irish *P. infestans* DNA collection (1981-2008).

- HRM assay using an unlabelled probe and asymmetrical PCR is able to detect potential changes in PiCes3A associated with CAA resistance (Fig 2).

- Differences in melt curves between 1:1, 2:1 and 2:1 ratio of wild-type:mutant detectable (Fig 2) allowing application to field strains.

- Application of HRM assay using *P. infestans* stored on FTA cards ongoing
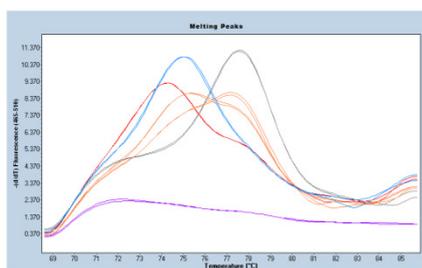
Fig 2. HRM analysis of *PiCes3A* fragments incorporating mutations associated with CAA resistance. Grey: G1105 (wildtype); Blue: A1105; Red: V1105; Orange: 1:1 and 2:1 G1105: A1105

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Analysis of fungal diversity on *Solanum dulcamara* and *S. tuberosum* leaves by sequencing of cloned PCR-amplified ITS rDNA

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Potato is attacked by various fungal pathogens. Mycobiota associated with diseased bittersweet nightshade (*Solanum dulcamara* L.) and potato (*Solanum tuberosum* L.) leaves has been investigated by rDNA cloning. In total, 33 fungal species belonging to 26 genera have been identified. Fungal species from leaves of *S. dulcamara* included *Alternaria alternata, Aureobasidium pullulans*, *Boeremia exigua*, *Botrytis cinerea*, *Cladosporium cladosporioides, C. herbarum*, *C. tenuissimum*, *Colletotrichum acutatum, C. gloeosporioides*, *Coniothyrium fuckelii*, *Fusarium oxysporum*, *Phoma herbarum*, *Phytophthora infestans, Thanatephorus cucumeris,* and *Thielavia basicola*. Fungal species detected on *S. tuberosum* leaves included *Alternaria alternata*, *A. solani*, *A. infectoria, Cladosporium cladosporioides, C. herbarum, C. tenuissimum, Colletotrichum coccodes, C. gloeosporioides, Epicoccum nigrum, Fusarium* sp., *Mycosphaerella* sp., *Phoma* sp., *Phytophthora infestans*, *Rhodotorula glutensis, Tolypocladium inflatum*, *Saccharomyces* sp., *Cryptococcus* sp., *C. tephrensis*, *Dioszegia crocea*, and *Hannaella oryzae.* Mycobiota of healthy *S. tuberosum* leaves was studied as the control and included *A. alternata*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *C. herbarum*, *Cryptococcus wieringae*, *Hymenoscyphus scutula*, *Mycocentrospora acerina*, *Phaeohelotium epiphyllum*, *Phaeosphaeria culmorum*, *Plectosphaerella cucumerina*, and *Zalerion arboricola*.

Plant-fungal interactions may occur in various ways. Plant leaves represent a home for a variety of organisms, such as fungi, which form the mycobiota on a leaf surface (phylloplane), non-pathogenic fungi inhabiting plant tissues (endophytes), phytopathogenic fungi, and saprotrophs utilizing weakened or dying tissues.

Identification of leaf-associated fungi is performed using several methods. Cytological microscoping or electron microscoping provide a direct detection of fungal structures on or within plant tissues. Isolation of axenic cultures of fungi on culture media makes it possible to identify the fungal species and their biological features. Finally, fungi may be also identified by some molecular-genetic methods. In this case, no culturing approaches are required, and the identification of non-cultivated species is possible (Chiang et al., 2001, O'Callaghan et al., 2006).

To date, phytopathogenic fungi infecting Solanaceae crops have been well-studied, but little attention has been paid to the mycobiota of plant leaves without any visible symptoms of infection. Wild Solanaceae plants that may play an important role as a source of infection, were also underexplored. Bittersweet nightshade (*Solanum dulcamara* L.) belongs to the most widespread wild Solanaceae species of Central Russia. This perennial liana may represent a natural late blight depositary (Cooke et al., 2002; Flier et al., 2003).

Hollomon (1967) characterized fungal species inhabiting the surface of potato leaflets by the flushing of leaflets followed by the isolation of pure cultures. He revealed the presence of *Alternaria tenuis* Nees, *Aspergillus* sp., *Aureobasidium pullulans* (de Bary) G. Arnaud*, Botrytis cinerea* Pers., *Cladosporium herbarum* (Pers.) Link*, Epicoccum* sp., *Fusarium* sp., *Gliocladium* sp., *Mucor* sp., *Penicillium* sp., *Phoma* sp., *Pythium* sp., *Stemphylium* sp., *Verticillium* sp.

In this study, fungi associated with potato and bittersweet nightshade leaves were characterized by molecular techniques without their isolation of pure cultures.

MATERIALS AND METHODS

Plant material. *Solanum tuberosum* leaves with blight spots were collected in July-August at commercial fields located in four different regions of Russia (Figure 1). *S. dulcamara* leaves with visible fungal affection symptoms were collected in August 2016 in the Botanical garden of the Lomonosov Moscow State University. Leaves were sampled and put individually into 50-mL tubes with 70% ethanol and stored at +4 ºC.

Pathogen-free seed tubers of 2 potato varieties (Siren and Zhukovskiy ranniy) were planted in 2016 in the Botanical garden of Lomonosov Moscow State University. This area represents an upland surrounded by multi-storey residential areas and located within 7 km from the center of the Moscow city. The nearest potato/tomato plantations and small garden plots are located about 17 and 10 km away from the sampling area, respectively. Leaves without visible symptoms of fungal affection were collected in July 2016. Sampled leaves were instantly frozen at −75°C prior DNA extraction.

Figure 1. *Location of sampling sites. P1, Republic of North Ossetia-Alania; P2, Kislovodsk (Stavropol region); P3, Voronezh region, Panino district; P4, Moscow region, Lyubertsy district.*

DNA extraction, amplification and cloning. Total DNA was extracted from each of affected leaves according to the standard CTAB protocol (Kutuzova et al., 2017). Amplified rDNA fragments included a part of the 18S gene region, internal transcribed spacer ITS1, 5.8S gene, internal transcribed spacer ITS2, and a part of the 28S gene region. Universal ITS1f и ITS4 primers and the standard PCR protocols were used (White et al., 1990; Gardes and Bruns, 1993). Amplicons of the required length were extracted from electrophoretic gels using a CleanUp kit (Evrogen Ltd, Russia), inserted into a pAL-TA vector (Evrogen Ltd., Russia), and used for the transformation of *E. coli* (Dh5α strain) cells according to Inoue et al. (1990). The resulting clone library was examined by the restriction analysis of the amplified DNA insertion using MspI restriction endonuclease (Figure 2). Based on the obtained results, different restriction profiles of the insertion were selected. Plasmid DNA was extracted from a sample selected according to the Lee and Rasheed protocol (1990), and nucleotide insertions were sequenced. In total, 113 clones from diseased potato leaves, 39 from *S. dulcamara*, and 26 from healthy potato leaves were sequenced.
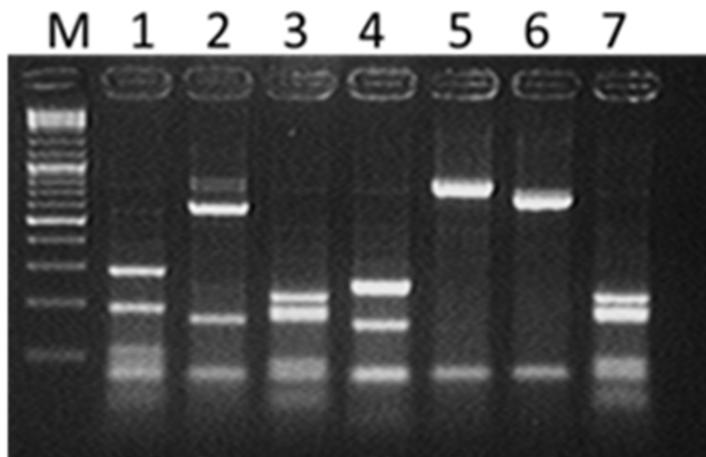
Figure 2. *Restriction digestion of different clones from DNA of a blighted potato leaf sample. 1, Cladosporium cladosporoides; 3, 7, Cladosporium tenuissimum; 2, Alternaria alternata; 4, Tolypocladium inflatum; 5, Rhodotorula glutensis; 6, Hanaella oryzae; M- 1 kb DNA ladder.*

RESULTS

The cloning and sequencing of total DNA isolated from blighted and spotted potato leaves collected in four regions of Russia resulted in the identification of 20 fungal taxa from 13 genera: *Alternaria alternata* (Fr.) Keissl, *Alternaria solani* Sorauer, *Alternaria infectoria* E.G. Simmons*, Cladosporium cladosporioides* (Fresen.) G.A. de Vries*, Cladosporium herbarum* (Pers.) Link*, Cladosporium tenuissimum* Cooke*, Colletotrichum coccodes* (Wallr.) S. Hughes*, Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.*, Epicoccum nigrum* Link*, Fusarium* sp., *Mycosphaerella* sp., *Phoma* sp., *Phytophthora infestans* (Mont.) de Bary, *Rhodotorula glutensis* (Fresenius) F.C. Harrison*, Tolypocladium inflatum* W. Gams, *Saccharomyces* sp., *Cryptococcus* sp., *Cryptococcus tephrensis* Vishniac, *Dioszegia crocea* (Buhagiar), and *Hannaella oryzae* (Nakase & M. Suzuki) F.Y. Bai & Q.M. Wang. Among 113 clones sequenced, 46 and 35 belonged to the *Cladosporium* and *Alternaria* genera, respectively. Only *Alternaria alternata* was revealed in samples from all sampling sites, while other species varied between regions.

The following 11 fungal taxa were revealed by the cloning and sequencing of DNA extracted from healthy potato leaves: *Alternaria alternata*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cryptococcus wieringae* Á. Fonseca, Scorzetti & Fell, *Hymenoscyphus scutula* (Pers.) W. Phillips, *Mycocentrospora acerina* (R. Hartig) Deighton, *Phaeohelotium epiphyllum* (Pers.) Hengstm., *Phaeosphaeria culmorum* (Auersw.) Leuchtm, *Plectosphaerella cucumerina* (Lindf.) W. Gams, and *Zalerion arboricola* Buczacki. Some species, such as *A. alternata, C. cladosporioides,* and *C. herbarum,* were revealed on both healthy and infected potato leaves.

In the case of bittersweet nightshade leaves, the following 14 fungal and oomycete taxa were revealed: *Alternaria alternata*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Cladosporium tenuissimum*, *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum gloeosporioides*, *Cryptococcus* sp., *Mycosphaerella* sp., *Phialophora* sp., *Phoma*

herbarum Westend., *Phoma* sp., *Phytophthora infestans*, and *Thielavia basicola* Zopf. Some fungal species (*Alternaria alternata*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Cladosporium herbarum* and species of the genus *Cryptococcus* sp.) presented on both healthy potato leaves and affected bittersweet nightshade leaves. The majority of isolated species were typical pathogens affecting potato and tomato plants. We also found species atypical for solanaceous plants or those, which have not previously been observed in Russia.

Thus, 21 species were revealed on the leaves of *S. dulcamara* and *S. tuberosum* grown in the botanical garden apart from agricultural fields, and some of these species were pathogens of Solanaceae plants. Some of the species were first revealed on Solanaceae plants in Russia, but were known as pathogenic in other countries. *Hymenoscyphus scutula*, *Phaeohelotium epiphyllum*, and *Zalerion arboricola* were first detected on potato leaves.

The air layer near the soil surface contain propagules of soil fungi, as well as fungal species from the water surface, plants, and various buildings. The flora of the Botanical garden, where the bittersweet nightshade and healthy potato were grown, includes a wide variety of plant species, which also influenced on the composition of the detected mycobiota. Airborne spores could also present on the surface of leaves and, therefore, were included in the analysis.

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EPIDEMIOLOGY AND RISK FACTORS OF POTATO LATE BLIGHT IN ALGERIA

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SUMMARY

Our study aims to determine the risk factors involved in the development of potato blight in Algeria. For this aim, we monitored the development of the disease during three years (2013, 2014 and 2016) in three regions (Ain Defla, Chlef and Mostaganem). Herein, the epidemiology of the disease was evaluated by validating two predictive models with analysis of the disease history. Rain appears to play an important role in the development of epidemics, it offers the moisture required for the pathogen propagules, and also provides a favorable temperature and ideal luminosity. Both predictive models have demonstrated an ability to predict the disease, and they should be adjusted according to the risk period defined by our work to be more accurate.

KEY WORDS

Late Blight, Potato, *Phytophthora infestans,* epidemiology, Weather conditions.

INTRODUCTION

The potato late blight caused by the oomycete; *Phytophthora infestans* (Mont.) de Bary, is the major problem of potato and tomato crops worldwide. Annual total losses resulting from this disease are estimated to be approximately 6 million $; including yield losses and fungicide treatment [1].

In Algeria, considerable losses of production are recorded following the first severe epidemic of the 2007 season. Losses continue after this epidemic, by increasing the treatment cost. A single fungicide treatment before this epidemic was sufficient to control the disease.

Any control strategy must take into account the climatic factors, the varietal level resistance and the characteristics of the pathogen to be successful. The interaction between these factors makes the disease control more complex leading to excessive and inadequate treatment [2].

This complexity has oriented the control strategy towards the development and the use of decision support system (DSS) [3].

The objective of our study is to define risk factors, which may lead to epidemiological situations. The result of this work will be exploited to develop a specific DSS for the Algerian crop condition.

MATERIALS AND METHODS

This work was carried out in three regions (Ain Defla, Chlef and Mostaganem) for a period of three years (2013,2014 and 2016), in the seasonal crop from mid-December to May. The variety studied is Spunta, which is susceptible and occupies about 60% of the cultivated area.

Each year, the date of first observation of late blight, the critical climatic periods that precede the outbreak of epidemics, the total number of treatment and date of planting were noted.

We evaluated the predictive capacity of two models (Smith period and NegFry) to generate warnings following critical periods determined by monitoring. Evaluation was done, by calculating the number of days which separate the date of the epidemic outbreak and the date of warning generated by the two models and then we compare the first warning of NegFy model with the first observed symptoms.

For the NEGFRY model we only considered the second model, which describes the effects of host resistance and climate on the development of *Phytophthora infestans* on potato. The decision on when to apply fungicides is based on cumulative of blight units (30 blight units for the susceptible variety Spunta).

RESULT

1. monitoring of late blight between 2013 and 2016 in Algeria
During the three years of study, two epidemics were recorded in 2013 and 2016 with a percentage of infection exceeding 90%, while in 2014 the disease severity was only 1% due to a drought season. All epidemics outbreaks were recorded following a rainy period. The first symptoms appeared in the mid-February and early March period, characterized by low and sometimes glacial temperatures (average daily temperature below than 15 °C), limiting the development of epidemiological situations. The epidemiological phase was observed generally during the month of April where the mean temperature is above than 15 °C and always accompanied with a rainy period. (Table 1).

Table 1. *Disease monitoring*

Year	Region	Date of 1st symptomes	Start of epidemic phase	Percentage of infection	observed treatments	1st Treatment	Plantation date
2013	Ain defla	15 march	23 april	90	4	24 march	10 jan.
	Chlef	1 march	20 march	90	4	01 march	08 dec.
	Mostaganem	15 march	26 april	75	4	19 march	15 dec.
2014	Ain defla	18 feb.	30 april	5	7	18 feb.	15 dec.
	Chlef	22 april	/	1	2	16 april	10 jan.
	Mostaganem	18 feb.	/	1	6	20 feb.	10 dec.
2016	Ain defla	09 april	09 april	90	3	10 march	10 jan.
	Chlef	09 april	14 april	75	3	11 april	25 dec.
	Mostaganem	09 april	/	1	5	20 feb.	15 dec.

2. RESULTS OF PREDICTIVE MODEL

After application of the two models according to their descriptions, the results are shown in Table 2. For the smith period model, only full smith period were counted.

The Smith period: Seven full smith periods were observed in 2013 in Mostaganem region, indicating a very favorable weather condition. Only one to two full smith periods were registered in 2014 and 2016, in all regions.

NegFry: This model recommends a maximum of eight treatments in 2013 and 2016 reflecting a favorable condition. A total of four treatments were recommended in Ain Defla and Chlef, in 2014 because of a drought season.

Table 2. *Models prediction results*

	negfry			Smith		
	Ain Defla	chlef	mostaganem	Ain Defla	chlef	mostaganem
2013	/	8	7	/	3	7
2014	4	4	8	1	2	1
2016	6	8	7	2	2	1

Table 3. *Evaluation of the predictive capacity of the two models*

Year	Region	First full Smith period	First treatment recommended by NegFry	First treatment recommended by negfry before epidemic outbreak
2013	Ain defla	/	/	/
	Chlef	+15 days	+ 03 days	+08 days
	Mostaganem	+07 days	+32 days	+06 days
2014	Ain defla	+ 59 days	−08 days	+01 day
	Chlef	/	+ 51 days	/
	Mostaganem	/	+06 days	/
2016	Ain defla	+04 days	+43 days	+1 day
	Chlef	+09 days	+44 days	+ 06 days
	Mostaganem	/	+42 day s	/

The forecasting results for both models showed a good conformity with the observed risk periods (Table 3). The warnings of both models were convenient compared with outbreak of the epidemic. NegFry model gave a warning of one day to eight days before the epidemic outbreak. A full Smith period was observed (04 days to 15 days) before epidemic outbreak (except in Ain Defla region in 2014). The two models are ineffective in predicting first lesions (symptoms). This requires adjustment to include other important parameters such as the vegetative stage, irrigation, evaporation and quality of the inoculums.

CONCLUSION

Late blight epidemics outbreaks are closely related with rainy periods, these periods are characterized by a warm temperature during the day and the night. Favorable temperature and luminosity are insured by clouds.

The two models tested gave variable warnings, but they are complementary. When a full Smith Period occurs and coincides with a treatment recommended by the NegFry model in the same week, there is a high risk that an outbreak of epidemic will occur.

Both models have variables inputs only for climate data and host plant, to generate treatment advice. Further information is needed to increase the accuracy of warnings, such as inoculum quality and primary infection monitoring via maps, vegetative stage, irrigation and evaporation.

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Late Blight Forecasting in Chile

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LATE BLIGHT FORECASTING IN CHILE

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INTRODUCTION

Late blight is the most important disease affecting potatoes in Chile. *Phythphtora infestans* genotype US1 was the responsible of late blight disease until 2006, since then, a new genotype was described. Because of the damage that this new genotype caused, if the weather conditions are favorable, a great amount of pesticides are used to control this disease. Therefore, INIA developed an early warning system based on Blitecast model http://tizon.inia.cl.

However, INIA warning system uses weather information of the last 10 days to obtain the condition of "today". As a consequence, farmers usually are not able to do the chemical control on time using this DSS advice, then, they do not have an optimum control of the disease, so they prefer to use a schedule application.

OBJETIVE

To develop a DSS late blight system using weather forecast data to improve the opportunity of application and efficacy of chemical control using an early warning advice.

MATERIAL and METHODS

During the season 2015-16 one plot was established at INIA Remehue (Los Lagos Region) and in the 2016-17 season, 3 plots were evaluated in different sites: INIA Remehue and INIA Butalcura (Los Lagos Region) and INIA Tranapuente (La Araucanía Region).

A split splot design with 4 replicates were used. The main plots were 3 potato varieties of different susceptibility to late blight: Atlantic, Patagonia and Symfonia. The subplot were: untreated control, application according to every 7 days schedule, INIA warning system and 24 hrs, 48 and 72 hrs forecast model. Chemical treatments were fungicide based on clorothalonil 720 gr/L in dose of 1,35 L/ha and Propamocarb HCl 625 g/L + Fluopicolide 62,5 g/L in dose of 2 l/ha.

Fungicide applications of each treatment were completed according to the alert issued by the model (Figure 1) and residuality of the fungicide. Twelve, 5, 7, 6 and 7 applications were performed in the schedule application, INIA warning system, 24 hrs forecast, 48 hrs forecast and 72 hrs forecast treatments, respectively.

Weather data from the INIA weather network and 24, 48 and 72 hrs weather forecast data were used to calculate the model.

During the season the plants were scored for late blight incidence and severity, estimating the percentage of foliar damage, the Area Under the Disease Progress Curve (AUDPC) and the Relative AUDPC.

Figure1. Condition and favorables days for Late blight by warning system during the growing season 2016-17 at INIA Remehue: A.A= INIA warning system; F 24= 24 hrs forecast model; F 48= 48 hrs forecast model; F 72= 72 hrs forecast model. Green: Conditions not favorable for disease development; Yellow, Orange and Red are conditions low, mediun and high for disease development, respectively. Brown: accumulation of data, Blue: First alert. White: no data.

Figure 2. Late blight symptoms in cultivar Atlantic, under 6 different treatments during the season on January 11, 2017. 1= Untreated control; 2=Scheduled application; 3=INIA warning system; 4= 24 hrs forecast model; 5= 48 hrs forecast model; 6=72 hrs forecast model. 2016-17. INIA Remehue, Chile.

RESULTS

Main results of this study are:

There were few differences between warning system for the day condition and the amount of late blight favorable days detected.

During the season 2016-17, the weather conditions were very favorable for late blight, there by Atlantic untreated control shows symptoms very early during the season with a 90% of foliar damage at 80 days after planting, while Patagonia shows 30% damage and Symfonia a 5% damage (Figure 2 and 3).

Atlantic, a susceptible cultivar, developed a RAUDP significantly higher than Symfonia and Patagonia (Figure 3).

Treatments based on schedule application, INIA warning system and 24 hrs, 48 and 72 hrs forecast model showed no differences between them, but with the untreated control (Figure 4).

The interaction cultivar-treatment during the season was significant, where the cultivars Symfonia and Patagonia did not develop foliar symptoms, but Atlantic had foliar damage since middle season, with 12, 5, 7, 6, 7 sprays respectively, according to the system used as a reference (Figure 5).

Therefore, these results show good efficacy control of Late blight using forecast data, mainly in cultivars with less sensitivity to the disease.
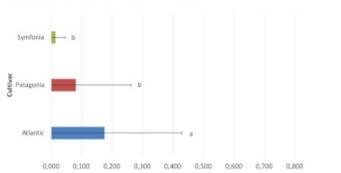
Figure 3. Late blight foliar damage, expressed as relative area under the disease progress curve (RAUDPC) in cultivars Atlantic, Patagonia and Symfonia. Season 2016-17. INIA Remehue, Chile.
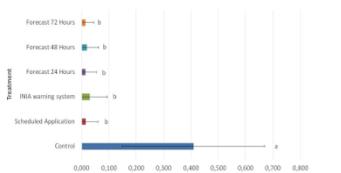
Figure 4. Late blight foliar damage, expressed as relative area under the disease progress curve (RAUDPC), under treatments sprayed according to different warning models. 2016-17. INIA Remehue, Chile.
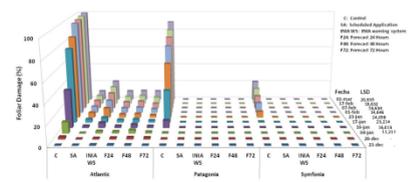
Figure 5. Percentage of Late blight foliar damage in cultivars Atlantic, Patagonia and Symfonia, under treatments sprayed according to different warning models. 2016-17. INIA Remehue, Chile.

CONCLUSIONS

According to the Late blight foliar damage results, differences between varieties were detected, being Atlantic more susceptible than Patagonia and Symfonia.

On the other hand, few differences in late blight control were detected between INIA warning system, 24, 48 and 72 hrs forecast system and scheduled application, however, the last one required almost twice chemical sprays. Similar results were observed in the others sites.

Therefore, warning system using forecast data to control Late blight is a good alternative to improve chemical control efficacy.

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Practical Experiences of Decision Support Systems in the Swedish Potato Field Trials for *Phytophthora infestans* 2011–2016

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Objective

Access to Decision support systems (DSS) can be one useful tool to be able to apply integrated pest management (IPM). Thus, the Swedish Board of Agriculture has tested and evaluated three different DSS to control potato late blight (*Phytophthora infestans*) in the Swedish potato late blight field trials during the last six years, 2011-2016. The programs have also been made available, to some extent, for advisors and farmers to test and evaluate the use of the programs.

Materials and Methods

The three DSS tested were the Dutch program Dacom, tested during 2011-2013, the Norwegian web based system VIPS (Acronym for pest varnings) for late blight, tested during 2013-2015 and the Danish web based Skimmelstyring, tested in 2015-2016. Dacom is commercially available for the Swedish farmers by Grimme and has been used by farmers and advisers to some extent in Sweden. VIPS and Skimmelstyring have been tested and available in cooporation with the Swedish Board of Agriculture and the Norwegian Institute of Bioeconomy Research and Aarhus University, respectively. To get feedback from advisors and farmers about the use of different DSS there has been some financial support from the Swedish Board of Agriculture.

The DSS tested differ in how, and in what degree of freedom, to be used (figure 1-3). All DSS were provided with input of appropriate weather data. For both VIPS and Skimmelstyring special rules for treatment were followed in the trials, linked to eg different chemicals used, spraying intervals and reduced doses. Only in Sweden registered chemicals against potato late blight were used in the DSS treatments. The DSS were tested predominantly in the late blight susceptible variety Bintje at two to three different locations in southern Sweden.
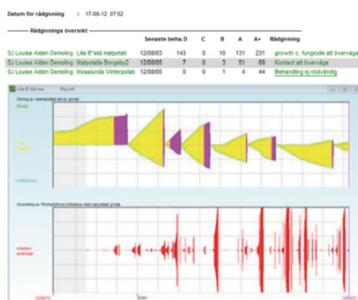
Figure 1. Dacom gives the user a specific advice when to spray and with what kind of substance to spray. However, Dacom also require input of field data from the user, eg different information continuous about the crop, inclusive growth, date of spraying treatments and possibly additional weather data.
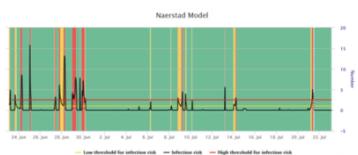
Figure 2. VIPS informs about infection risk, but gives no direct recommendation. It is up to the user to consider the need and time for spraying according to eg cultivar resistance, choice of fungicide and time interval since last application.
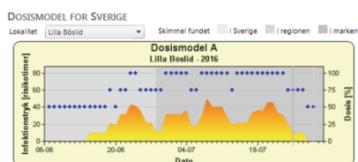
Figure 3. With Skimmelstyring the spraying interval is fixed to seven days and the spraying dose is adjusted to the infection pressure and distance to closest late blight attack.

Results and Discussion

- The different DSS tested have reduced fungicide applications with 20–30 % in field trials with retained effect on late blight, compared to weekly sprayings with full dose.
- Both prolonged intervals and/or reduced dose can be used to reduce fungicide amounts with retained effect.
- Timing is more important than the number of sprayings and dosage.
- Technical problems can obstruct the use of DSS.
- Various DSS attract different growers according to logistic conditions and geographical location.
- Different DSS can give diverse specific information and be more or less labor intensive and easy available.
- Predominantly, advisors and farmers that have used the DSS have been positive, but the extent of future use can be connected to any costs.
- Continued test and evaluation the last year with Skimmelstyring will be performed during 2017 in the late blight trials.

Conclusions

- All three DSS evaluated can be valuable tools to reduce fungicide application when growing potatoes.
- When using DSS it is of outermost importance that the model is used as a support, combined with human experience and common sense.
- Users with different user preferences can be attracted by various DSS.
- Different DSS can be suitable for various conditions in different parts of Sweden.

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Lowering thresholds of qualitative plant risk prediction algorithms: sensitivity versus specificity of Irish Rules for potato blight development

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Lowering thresholds of qualitative plant risk prediction algorithms: sensitivity versus specificity of Irish Rules for potato late blight development

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Introduction

The Irish Meteorological Service (Met Éireann) issues warnings based on an empirical model developed by Burke (1953)(Fig. 2), based on early work by Crosier (1934). Commercial growers do not necessarily rely on blight warnings but do intensify fungicide control when blight warnings are in effect. The model has undergone limited evaluation or refinement; yet significant changes have occurred the potato late blight disease triangle since the rules were first established.

The Aim

… is a qualitative evaluation of:

- The Irish rules in their current form,
- Various environmental thresholds for sporulation and infection
- The duration of risk period as the criteria for blight warning.

Fig. 1. Weather, model output, disease progres and the amount of inoculum for the year 2015 at Oak Park. The duration of „blight period" is quantified as an accumulation of effective blight hours (EBH)
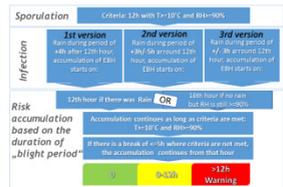
Fig. 2. Simplified algorithm of the Irish Rules used for prediction of potato late blight risk in Ireland.

Methods

A total of 48 versions(2*2*2*3*2) of the model is coded as a five numbers combination representing the variations of model components (Tab. 1).

Code of parameter	RH threshold (%)	Temperature threshold (C°)	Sporulation and germination (hours)	LW window (hours)	LW estimation
1	>=90	>=10	>=12	-3; +3	Only rain
2	>=88	>=8	>=10	-5; +3	RH >= 90% + rain
3				+4	

Table. 1. Set of parameters used in model validation. Example: model coded 11111 is set of parameters currently used in Irish Rules

Assessed is the duration of „blight period"(2,6,12 hours) needed for issuing warning, after the initial criteria for the germination and infection is met.

Disease outbreak information originates from ten years of Teagasc breeding program field trials at Teagasc Carlow. The trial sites were in the radius of up to 500 m from a Met Éireann weather station.

Coding and the analysis is implemented with R (R Core Team, 2013), and packages: ggplot, dplyr, data.table and zoo.

Results timeline
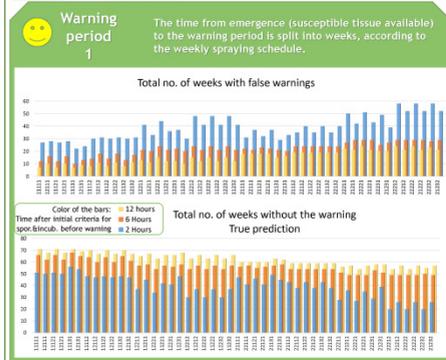
Fig. 3. Model outputs with varying decision thresholds
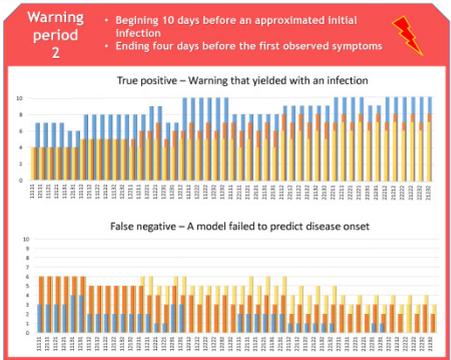
Conclusions/Future Work

- Considering "zero tolerance" to potato late blight, the current version of the Irish Rules did not prove fit for blight risk prediction
- A number of model versions with reduced parameters for the germination and infection successfully predicted start of epidemics. The only consistent threshold parameter in all cases was reduced duration for spor. & germ. to 10 hours.
- Basing the decision to issue a blight warning only on the duration of „bight period" did not prove sufficient
- These results are only indication – small disease observation data set.

- Improve specificity through quantification of the risk during each "blight event"
- Weather station close to trial site – results must be taken with caution from when deploying system on a synoptic level.
- Evaluation of model accuracy on synoptic level with inclusion of weather forecast model outputs.
- Base quantification of risk on algorithms relying on a spatially more consistent variables, i.e., temperature.

The reason why these old empirical rules were successful in predicting outbreaks only when conditions are critical, and failing to do so in the borderline case is a consequence of overcompensations made to accomplish easy application. Major setback of this compromise were challenging situations around crude criteria borderline, and failure to represent soft transition between biological optimum, minimum and detrimental values for targeted organisms. A more detailed, quantitative approach is required to describe pathosystem in a more accurate manner.

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Zellers A and Grothendieck G,(2005). zoo: S3 Infrastructure for Regular and Irregular Time Series. Journal of Statistical Software, 14(6), 1-27. doi:10.18637/jss.v014.i06

Moving forward against potato late blight in Argentina and in the region

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Moving forward against potato late blight in Argentina and in the region

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Argentina is one of the main potato production countries in Latin America.

Potatoes are produced in different climate zones throughout the country, providing the market with fresh potatoes continuously all year round from four potato production areas: early, medium early, medium late, late.

Production of seed is located mainly in 'restricted areas'.

Late blight is the disease causing the most severe yield, quality and economical losses.

Fig. 1. Climatological map of Argentina showing the distribution of the main four potato growing areas in Argentina: early, medium-early, medium-late and late.
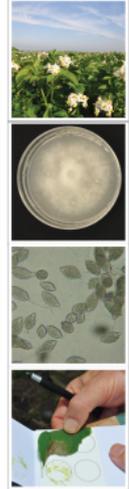
Approaches in Argentina for sustainable management of late blight

Preventive

PhytoAlert DSS

Control efficacy, production costs and environmental were assessed and compared a traditional calendar-based strategy

PhytoAlert DSS reduced:

the use of fungicides **up to 50%**, economic losses **up to 47%** and the environmental impact **up to 48%**
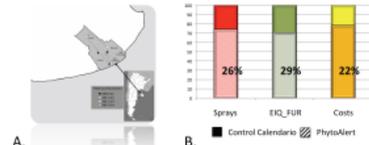
Fig. 2. A. Distribution of PhytoAlert DSS field trials in the South East of the Buenos Aires Province during four potato growing seasons: 2010/11, 2011/12, 2012/13 and 2013/14. **B.** Average (%) of the impact of the implementation of Phytoalert DSS in number of sprays, environmental impact (measured by EIQ-field use rate), and fungicide costs compared to a (weekly) Calendar-based strategy to control late blight.

Molecular epidemiology

Studies were carried out since 2011 in the South East of the Buenos Aires Province and Córdoba Province by sampling and extraction of ADNg in FTA cards and genotyping of populations with an internationally agreed panel of 12 microsatellite markers (Li et al., 2013).

Genetic studies showed that all isolates collected since 2007 to present belong to **genotype 2_A1**, although allelic variants were observed in the material evaluated. They also showed higher levels of resistance to Mefenoxam.
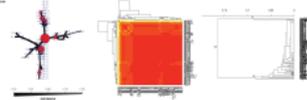
Genotyping in Latin America – Tizón Latino Network

After the launched of the Tizón Latino Network in 2014 in Bogotá, Colombia, we developed a genotyping service to partner countries of the Netwotk. We received samples from Chile, Brazil, Panama and Colombia from different Solanaceae hosts. We analised them using a standardised 12 plex SSR genotyping (Li et al., 2013). The preliminary results had shown diversity of genotypes in Latin American *Phytophthora* populations. Each research group is analyzing in depth those preliminary results obtained to discuss them in the Third Workshop of Tizón Latino 2018 to be held in Cusco, Peru (World Potato Congress 2018 / ALAP 2018).

Chile   **Colombia**   **Chile-Colombia-Argentina-Panama**
Brazil data will be included soon
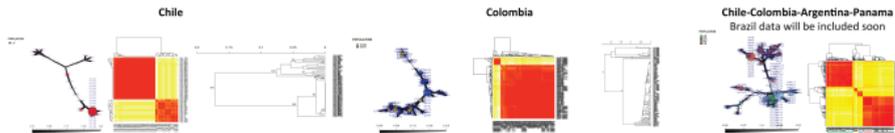
Two main genotypes were described in *P. infestans* populations of Chile: US1 and 2_A1. Recent populations are associated with higher levels of resistance to Metalaxyl.

Phytophthora isolates collected from different Solanaceae species showed diversity of genotypes in the samples analyzed. New SSR profiles were described associated with "new"*Phytophthora* species.

Brazil: first genomic study of *P. infestans* populations covering the main potato producing regions is carrying out. Genotyping is in progress.

Reference: Li, Y., Cooke, D.E., Jacobsen, E., van der Lee, T. 2013. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen Phytophthora infestans. J. Microbiol. Methods 92:316–22

Smartspray
Optimised Detection and Control of Potato Blight: Sensing Pathogens to Inform Smart Spray Decisions

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Smartspray

Optimised Detection and Control of Potato Blight:
Sensing Pathogens to Inform Smart Spray Decisions

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The James **Hutton** Institute

Introduction

Current practice is to apply late blight fungicides prophylactically in a 7-10 day programme throughout the growing season.

Disease risk assessments based on environmental parameters assume the presence of sporangia. Fungicide programmes may therefore be triggered unnecessarily.

This InnovateUK funded project seeks to demonstrate a new prototype device that will sample airborne spores of *P. infestans* and Alternaria in the field, automatically process the sample, quantify DNA by fluorescence and relay results by mobile phone text message.

The aim is to improve current weather-based disease risk models and predictions for late blight, resulting in enhanced decision making ability for growers with respect to fungicide choice and application and therefore more efficient resource use.

Number of confirmed blight outbreaks in potato crops (GB)

Onset of epidemic

AHDB 'Fight Against Blight' Outbreak data

In-field detection of sporangia 2016

There is variation between seasons and geographical locations in the onset of late blight epidemics. Standard 7-10 day fungicide regimes may not take this variation into account

Weather, spore survival and dispersal models in conjunction with evidence of the presence of sporangia of *P. infestans* could help to refine disease control strategies.

- Enhanced disease control and yield
- Reduced or better targeted fungicide applications
- Reduced costs

In-field spore detection and reporting

Test result & met data sent by SMS

Integration with Blight risk model

Risk alert message sent

DNA release and quantification

Grower makes spray decision

Auto-sampler includes DNA release, isothermal amplification & quantification, wireless reporting, integration with infection models & risk prediction sent to end-users.

For further information regarding this project and the device please contact Burkard Manufacturing Ltd., or any of the collaborators.

sales@burkard.co.uk

To Make Cultivar Resistance Durable Spray Fungicide

Kevin Carolan[1], Ruairidh Bain[2], Alison Lees[3], David Cooke[3], Faye Ritchie[4], Neil Paveley[4], Frank van den Bosch[1]

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[3] The James Hutton Institute;
[4] ADAS

TO MAKE CULTIVAR RESISTANCE DURABLE SPRAY FUNGICIDE

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BACKGROUND

Potato blight can evolve insensitivity to the fungicides we deploy against it. At the same time, the effectiveness of cultivar resistance can be downgraded because of selection for virulence. Integrated control is widely believed to be more durable than any one control method alone.

In this project we study the how the integrated use of fungicides and cultivar resistance can be optimized to maximize the durability of disease control. Here we present the potential of using fungicides to reduce the selection for virulence, prolonging the effectiveness of cultivar resistance.

GOVERNING PRINCIPLE

The selection for virulence (S) is a result of the difference in the growth rates of the strains.

$$S = (r_{vir} - r_{avir})$$

Selection can be reduced by either reducing r_{vir} directly, or by reducing r_{vir} and r_{avir} by the same amount.

As both strains are fungicide sensitive, fungicide reduces them both by the same amount.

RESULTS

Increasing the dose of the fungicide decreases the selection for virulence, prolonging the effectiveness of cultivar resistance (Results 1).

After a certain point further increases in dose do not further delay the evolution of virulence.

The effect holds for multiple different fungicides (Results 2) and different levels of cultivar resistance (Results 3).

This provides a useful and simple tool to protect new and existing products, buy appropriately tailoring their use.

CONCLUSION

- If you want to protect your fungicide; grow resistant cultivars.
- If you want to protect your resistant cultivar; spray fungicides.
- Integrated control prolongs durability of crop protection.

Presented in further detail in:
Carolan *et al* Extending The Durability Of Cultivar Resistance By Limiting Epidemic Growth Rates, Proc Roy Soc B (Submitted)

RESULTS 1 – Fungicide can delay virulence.

As we increase the fungicide dose, we slow the growth rate of both the virulent, r_{vir} and avirulent r_{avir} pathogens by the same amount.

This slows selection of the virulent pathogen in the population, delaying the evolution of virulence.

RESULTS 2 – Different fungicides; same effect.

If we increase the efficacy of the fungicide (Figure A to C) we increase the effect. More effective fungicides reduce both r_{vir} and r_{avir} more than less effective fungicides.

RESULTS 3 – Different cultivars; same effect.
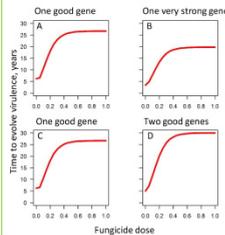
Here we put resistance genes into the cultivar in different ways.

If we increase the strength of a single resistance gene then virulence evolves faster (A & B), but is still delayed by dose.

If we use multiple resistance genes, virulence is further delayed (C & D).

Blightsense
Development of a rapid biosensor system for in-field detection of *Phytophthora infestans*

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Blightsense

Development of a rapid biosensor system for in-field detection of *Phytophthora infestans*

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Soil Essentials Ltd (lead partner)
University of Cambridge,
James Hutton Ltd.
Syngenta UK Ltd.

Introduction

In a typical season, UK potato farmers spray fungicides 10-15 times to reduce losses to late blight.

However, crop losses are not always completely prevented and conversely, some fungicide applications may be unnecessary in a low risk year.

The cost of control in the UK is approximately £350/ha, with costs of up to £72M p.a. during high pressure blight seasons.

Across Europe, total annual potato production is approximately 63,000Mt, representing an average economic value of €6Bn.
The total financial loss associated with late blight is estimated at €1Bn (Haverkort et al, 2008), representing 15% of total farm gate price.

Early pathogen detection in the field is critical for effective implementation of control measures.

Currently, there is no existing method for assessing late blight risk in a field based on the presence of inoculum combined with weather conditions conducive for infection.

This InnovateUK funded project will develop a rapid acoustic biosensor device for in-field identification of air-borne sporangia of *Phytophthora infestans*.

Soil Essentials , a precision-farming SME, together with The James Hutton Institute, University of Cambridge , James Hutton Ltd. & Syngenta will develop an integrated diagnostic tool for early pathogen detection.

Low-cost, antibody-coated acoustic sensors will be coupled with spore-traps.

Disease risk assessments using weather-based models will be enhanced by knowledge of pathogen presence or absence. This information will be used to optimise late blight control.

As a platform technology, the device can be easily adapted to detect other crop & livestock pathogens for wider agricultural impact.

Approach

Biosensor development – detect surface antigens of *P.infestans sporangia using a novel acoustic* immunosensor platform.

Integration with air-sampling device - capture air-borne sporangia and present pre-filtered, concentrated samples to the biosensor element for subsequent detection.

Data Management and Interpretation – incorporate sensor outputs into weather-based disease risk models to provide useful information to growers

Validation - in-field demonstration and validation

For further information regarding the sensor please contact: enquiries@soilessentials.com

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).
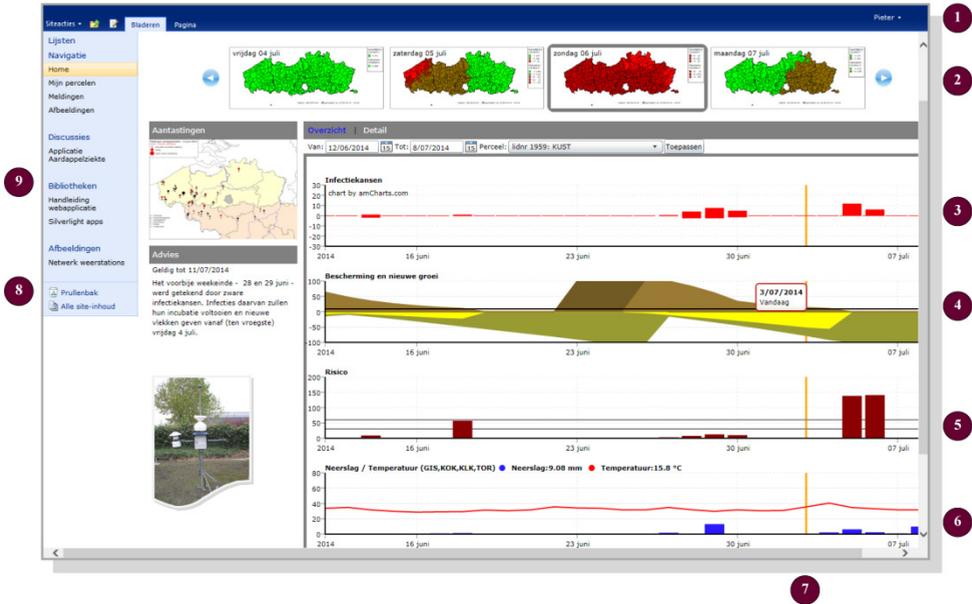
7
All calculations are **continually refreshed** and use detailed hourly weather forecasts 96 hours ahead. The orange-coloured vertical line indicates the **current date** (=today).

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.

Assessment of potato varieties for foliar late blight resistance in Algerian field trials, in relation to *Phytophthora infestans* genotype

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Assessment of potato varieties for foliar late blight resistance in Algerian field trials, in relation to *Phytophthora infestans* genotype

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INTRODUCTION

Late blight caused by *Phytophthora infestans* remains the most serious disease of potato crops in Algeria. The Algerian *P. infestans* populations on potato mainly comprise two clonal lineages, EU_2_A1 and the dominant EU_13_A2 which show complex virulence profiles and metalaxyl resistance [1, 2].

In order to better exploit host genetic resistance and reduce fungicide use, we assessed potato cultivars for foliar late blight resistance under Algerian conditions and for their responses to local *P. infestans* populations.

MATERIALS AND METHODS

❖ **Field trials** :

- A total of 11 registered and new potato cultivars, essentially from France, were evaluated for their resistance to late blight in field conditions; Spunta and Désirée were included as widely grown local standards.

- Experiments were carried out during two years, **2015** and **2016**, at two locations, **Staoueli (ITCMI)** and **Bab-Ezzouar (CNCC)**.

- The trial layout was a randomized Fisher block design with three replications (in each one, 45 tubers per cultivar in 3 rows); each elementary plot was surrounded by Spunta infector plants.

- Percentage of foliar disease severity of individual plants was weekly scored during 1,5 month. The first symptoms occurred in the beginning of April. The scores were converted to the mean defoliation and used for the calculation of the AUDPC (Area Under the Disease Progress Curve).

❖ **Genotyping of *P. infestans* isolates :** Some blight lesions were sampled to obtain live isolates, or squashed onto FTA cards. Isolates were genotyped at 12 microsatellite loci.

RESULTS

View of the trial   *Passion plot*   *Sarpo Mira plot*

➢ Spunta was totally destroyed in both sites at the end of May in 2015 and mid-May in 2016.

➢ Désirée, Ferrari and Eldorado were very susceptible to late blight. However, in 2016, disease progress was slightly slower on Désirée than on the other two cultivars.

➢ On **Eden**, **Florice** and especially **Frivol**, disease progression was slower than on Spunta, but 80-90% of foliar destruction was noticed within 1.5 month in 2015. However, Frivol showed the lowest AUDPC.

➢ In contrast, on **Cephora**, **Fabula** and **Big Rossa**, disease development was weak during the first 20 days of the epidemic. Only 25% of haulms were killed on Fabula (2016) and 50% on Big Rossa (2016) and Cephora (2015), 2-3 weeks later.

➢ **Passion, Coquine** and **Sarpo Mira** showed a high resistance level. On Passion and Coquine, 0.1% of foliage was blighted (some plants with 5%) and on Sarpo Mira, no lesion was visible at the end of the scoring.

➢ **EU_13_A2** *P. infestans* isolates were dominant in both sites. Some **EU_2_A1** isolates were also detected in Bab-Ezzouar.

Percentage of foliar late blight destruction in the two trials : in 2015 at ITCMI (top); in 2016 at CNCC (down)
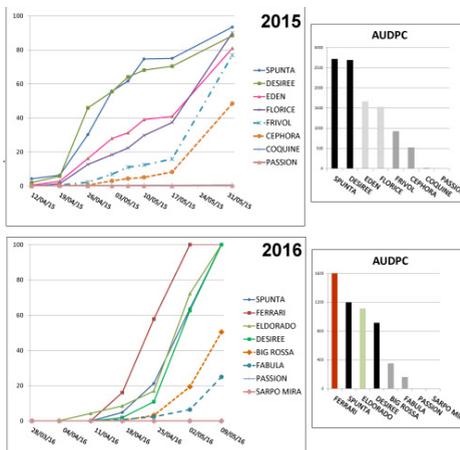
CONCLUSION AND PERSPECTIVES

❖ The cultivars tested under Algerian field conditions differed considerably for late blight resistance.

❖ Three cultivars Cephora, Fabula and Big Rossa were moderately resistant and could represent an alternative to susceptible cultivars as the dominant cv. Spunta.

❖ Three other cultivars Passion, Coquine and Sarpo Mira expressed a highly resistance level in our trials ; their field resistance seems stable. They are then promising for sustainable control strategies against late blight.

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Revisiting late blight resistance genes in complex interspecific potato hybrids

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SUMMARY

Stacking (pyramiding) several late blight (LB) resistance genes (*R* genes) of diverse race specificity in one and the same potato plant provides for high, broad spectrum and durable resistance. Sixty six potato clones under study included the standard potato varieties and interspecific hybrids obtained by introgression breeding and comprising genetic material from up to eight wild *Solanum* species. These genotypes were evaluated for LB resistance in field trials under natural infestation and laboratory tests with detached leaves and screened with sequence characterized amplification region (SCAR) markers for seven genes: *R1*, *R2/Rpi-blb3*, *R3a*, *R3b*, *RB/Rpi-blb1=Rpi-sto1*, *Rpi-blb2* and *Rpi-vnt1.3*. LB resistance of most hybrids was significantly related to the presence of the markers for *R* genes and the number of markers per plant; nevertheless, a considerable portion of resistance apparently depended on unknown or insufficiently researched genes, especially from the species practically new to breeding for LB resistance, such as *S. alandiae*.

KEYWORDS

Phytophthora infestans, Solanum section *Petota*, interspecific hybrids, late blight, stacking resistance genes, SCAR markers.

INTRODUCTION

In traditionally bred potato hybrids, *R* genes for LB resistance introgressed from wild *Solanum* species are combined with polygenic LB resistance and other genes of agronomic significance (Bradshaw, 2017; Gebhardt, 2013). We followed on with our research on pyramiding *R* genes in the complex interspecific potato hybrids using SCAR markers (Fadina et al., 2015). These hybrids have been developed in three Russian breeding centers: Set A in the A.G. Lorkh Institute of Potato Husbandry, Moscow region (PH), Set B in the Institute of Plant Protection, St. Petersburg (PP), and Set C in the N.I. Vavilov Institute of Plant Genetic Resources (VIR), St. Petersburg. The hybrids comprise germplasm from up to eight wild species of *Solanum* L.

section *Petota* and manifest high, broad spectrum and durable late blight resistance comparable to that of var. Sarpo Mira.

Table 1. *R gene markers*

Gene	Chromosome	Prototype	Marker	Reference
R1	5	AF447489	R1-1205	Sokolova et al., 2011
R2/Rpi-blb3	4		R2-2500	Kim et al., 2012
		FJ536325.1	R2-1137	Lenman et al., 2015
			R2-686	Kim et al., 2012
		FJ536346.1	Rpi-blb3-305	Haesaert et al., 2015
R3a	11	AY849382	R3a-1380	Sokolova et al., 2011
R3b	11	JF900492	R3b-378	Rietman et al., 2012
RB/Rpi-blb1 = 8		AY336128	RB-226	Colton et al., 2006
Rpi-sto1			Rpi-blb1-820	Vleeshouwers et al., 2008
		EU884421	Rpi-sto1-890	Haesart et al., 2015
Rpi-blb2	6	DQ122125	Rpi-blb2-976	Van der Vossen et al., 2005
Rpi-vnt1.3	9	FJ423046	Rpi-vnt1.3-612	Pel et al., 2009
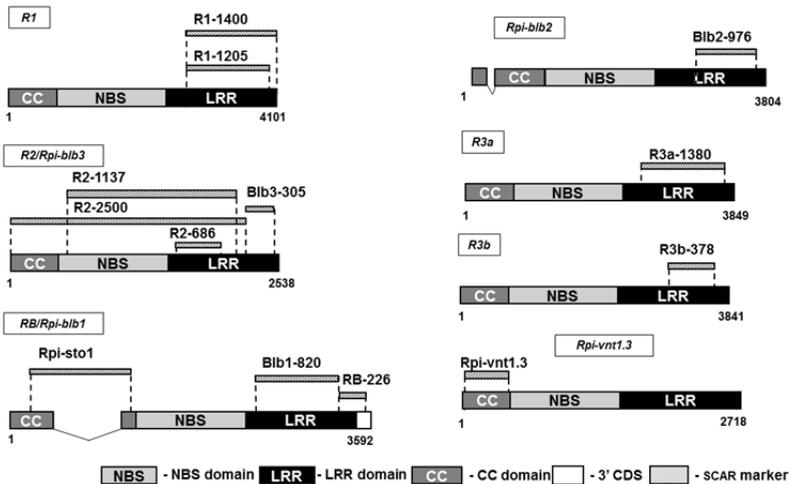
Figure 1. *SCAR markers for R genes for LB resistance*

MATERIALS AND METHODS

Testing potato genotypes for LB resistance

Complex interspecific potato hybrids (Khavkin et al., 2014; Rogozina et al., 2014) were assessed for LB resistance in the IP field trials (Bol'shiye Vyazemy, Moscow region, Russia) under conditions of natural infestation by registering the area under the disease progress curve (AUDPC) against several varieties used as standards (SV). In the laboratory assays, detached

leaves of these genotypes and var. Santé as a control have been infected with the complex race N161 (the IP collection) combining virulence genes 1 to 11. The aggressiveness of N161 in the Lapwood (1965) test with Santé tubers exceeded the indices obtained with all isolates collected in the potato stands under study. The experimental data for LB resistance were transformed to 1-9-point scores.

SCAR markers for R genes

Clones of 49 potato hybrids and 17 varieties were screened with SCAR markers (Table 1, Figure 1) derived from the sequences of already well-characterized *R* genes (prototype genes). In two cases, *R2/Rpi-blb3* and *Rpi-blb1/Rpi-sto1,* more than one marker was used to recognize the particular gene. Wherever possible marker specificity was verified against wild species that were the initial sources of the prototype genes. Previously described methods (Fadina et al., 2015) were employed for PCR amplification of markers from genomic DNA, amplicon cloning and sequencing and phylogenetic analysis of the sequences. The marker sequences were shown to be 98-100% identical to the prototype genes. Agroinfiltration studies (Du and Vleeshouwers, 2014) have been initiated to elucidate whether these markers represent the functional *R* genes rather than their nonfunctional structural homologs.

Agroinfiltration tests

Potato plants were grown from tubers in the IP and VIR glasshouses. Leaves of interspecific hybrids and var. Bintje as a control were infiltrated with *Agrobacterium tumefaciens* strain AGL0 cells transformed with *Avr3a*, *avr3a* and *Avr-blb1= IPI-O1* genes (Armstrong et al., 2005; Vleeshouwers et al., 2008). Plasmids pBI121 comprising the *Avr3a* and *avr3a* genes were constructed in our laboratory (E.A. Sokolova, unpublished), and plasmid pBI121 containing the *IPI-O1* gene was the generous gift from Dr. Dennis Halterman (UW, Madison). Plant hypersensitive response was registered repeatedly for up to 10 days.

RESULTS AND DISCUSSION

LB resistance of interspecific potato hybrids

Laboratory tests in the 2015 and 2016 trials (Table 2) concurred, whereas the field scores in 2016 were usually lower that in 2015 in accord with the weather conditions provoking late blight development.

Marker specificity and the origin of R genes in interspecific hybrids

When thirty-four potato genotypes were assessed with four markers of *R2/Rpi-blb3*, the marker R2-2500 was the least reliable. In three genotypes, the markers R2-1137 and R2-686 were found in the absence of Rpi-blb3-305, while the opposite pattern was never observed (Table 2). Three markers of *R2/Rpi-blb3*: R2-1137, R2-686 and Rpi-blb3-305, produced concurring results, except that ten genotypes positive for this gene contained only R2-1137 marker. The markers Rpi-blb1-820 and Rpi-sto1-890, which recognize two far apart regions of the *Rpi-blb1=Rpi-sto1* gene, produced very consistent results, except for hybrid 2 (194-4t) and var. Priekulsky ranny and Svitanok kievsky. The marker Rpi-blb1-226 is less reliable: it was found in eight hybrids and two varieties in the absence of two other markers of this gene; moreover, in four of these genotypes the Rpi-blb1-226 marker was found in the absence of the most probable sources of the *Rpi-blb1* gene: *S. stoloniferum, S. polytrichon* (conspecific with *S. stoloniferum*, cf. Spooner

et al., 2014) and *S. vallis-mexici* (reportedly a hybrid between *S. stoloniferum* and *S. verrucosum*, cf. Hawkes, 1978).

In set A (Table 2), all hybrids comprise genetic material of *S. demissum*, and this species was the most probable source of the resistance genes *R1, R3a* and *R3b* (Table 2). Apparently in the case of hybrid 97.1.17, high LB resistance depended on genes other than those revealed with our markers.

In set B, potato hybrids already comprising *demissum* germplasm were pollinated with pollen from up to eight wild *Solanum* species. Most notable here are the markers of *R3a* and *R3b* genes seemingly introgressed from *demissum* germplasm, *R2* (or *Rpi-blb3*) gene also transferred from *demissum* (but possibly from *stoloniferum, polytrichon* and *vallis-mexici* germplasm as well), and *Rpi-blb1* gene also from three latter species. Three hybrids comprising the structural homolog(s) of *Rpi-vnt1.3* gene are of special interest: their origin is open to wide speculation. Of special consideration are hybrids 12/1-09 and 13 (50/1 KBA): here we find high LB resistance and just two and one *R* gene markers, respectively.

In set C, the most interesting are hybrids combining the germplasms of *S. andigena* and *S. rybinii* or *S. alandiae* and *S. brevicaule*, two latter species practically new to LB resistance gene studies. Many of these hybrids comprise the markers *R1, R2, R3a, Rpi-blb2* and *Rpi-vnt1.3*. Especially frequent was the marker of *R3b*. Quite unexpected was the marker Rpi-blb1-820 in the hybrid 39-1-2005. Var. Atzimba manifesting high-to-moderate LB resistant was extremely poor in *R* genes markers, and therefore the markers registered in the first-generation progeny of Atzimba x *S. alandiae* crosses must arrive from *S. alandiae*.

Speaking of our collection of interspecific hybrids as a whole, the genes *R1, R2, R3a* and *R3b* were most likely transferred from *S. demissum*; however, other wild species listed in their pedigrees could be the additional or alternative sources of these genes. The presence of the *R1* gene in *S. stoloniferum* was confirmed by detailed studies of this and several other species (Beketova et al., 2017), including cloning of the full-length ortholog *R1*. An independent evidence for the presence of the functional *R1* gene in *S. stoloniferum* was obtained by Rietman (2011) in an agroinfiltration experiment. The germplasm of *S. bulbocastanum* did not participate in breeding the hybrids under study (the hybrid 7 (93-5-30) is the only exception), and wherein we find the marker of the *Rpi-blb1* gene, it was apparently introgressed from *S. stoloniferum*, a tetraploid species, with the genome B most probably coming from *S. bulbocastanum* (Wang et al., 2008). Two other probable sources of this gene are *S. polytrichon* and *S. vallis-mexici.* Three genes, *Rpi-blb1-Rpi-blb3*, present another interesting point. In the natural habitat of *Solanum* species, these *bulbocastanum* genes manifest a characteristic pattern of geographic distribution and are rarely found in one and the same plant (Lokossou et al., 2010). We observed such unique concurrence in hybrid 111 (38 KBA). Markers for genes *Rpi-blb2* and *Rpi-blb3* occur together more often than in combination with those of *Rpi-blb1*. These frequencies differ from the pattern reported by Lokossou et al. (2010); however, one must remember that in our set of data, *Rpi-blb3* is undistinguishable from of *R2*.

Thus, many *R* genes under study are found far beyond the species in which they were first described. Similar results were reported previously when wild *Solanum* species were screened with *Avr* genes (Rietman, 2011) and SCAR markers (Sokolova et al., 2011). In such cases, the interpretation of our marker profiles must wait for further in-depth studies. This concerns the

presence of marker Rpi-blb2-976 in the hybrid 97-13-9 and markers Rpi-blb1-820 and Rpi-sto1-890 in hybrid 39-1-2005 with pedigrees that did not mention *S. stoloniferum*, *S. polytrichon* and *S. vallis-mexici* and primarily the presence of the marker Rpi-vnt1.3 in many hybrids, which are not related to *S. venturii*. Nonfunctional structural homologs (resistant gene analogs) of *R* genes are widely distributed in *Solanum* species (Witek et al., 2016), and therefore it is crucial to prove with independent methods that our SCAR markers reveal the genes that determine LB resistance of particular hybrids.

Agroinfiltration tests
We started verification of *R3a* and *Rpi-blb1* genes by the agroinfiltration technology (Vleeshouwers et al., 2008). Leaves of eleven hybrids each comprising several *R* genes and var. Bintje free from these genes were infiltrated with agrobacteria transformed with *Avr3a, avr3a* and *Avr-blb1=IPIO-1* genes. In five hybrids, the response to *Avr3a* and *avr3a* concurred with the presence or absence of the R3a-1380 marker, and in other six hybrids the evidence from two independent methods did not match. The questionable results could arise because plants were grown from tubers rather than from *in vitro* methods did not correlate. Agroinfiltration with the *IPIO-1* gene produced hypersensitive response in all eleven hybrids although only four of them comprised the Rpi-blb1-820 marker. Variety Bintje responded negatively to all three plantlets (Du and Vleeshouwers, 2014); besides, the discrepancies may indicate the presence of active *R* gene homologs that respond to the *Avr* genes and are not recognized by our SCAR markers.

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 the field trials and in the laboratory tests with detached leaves*

Set	Potato genotypes	R1	R2/Rpi-blb3				R3a	R3b	RB/Rpi-blb1 = Rpi-sto1			Rpi-blb2	Rpi-vnt1	Number of R genes	Resistance, points 2015/2016		Reported wild species in hybrid pedigrees*
		R1-1205	R2-2500	R2-1137	R2-686	Blb3-305	R3a-1380	R3b-378	Rpi-blb1-820	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612		field	lab	
Set A (PH)	2372-60	1	0	1	1	1	1	1	0	0	0	0	4	8/8	6/8	adg, chc, dms, sto
	97.13-9	0	0	1	1	1	1	1	0	0	1	0	4	4/5	3/3	chc, cmm, dms, mga
	97.1.17	0	0	1	1	1	0	0	0	0	0	0	1	7/6	4/4	adg, chc, dms, sem
	2522-173	0	0	0	0	0	1	1	0	0	0	0	2	5/5	3/3	adg, chc, dms
	2584-7	0	0	1	1	1	0	1	0	0	0	0	2	6/5	4/4	adg, chc, cmm, dms, edn, mga, ryb
	2359-13	1	0	1	1	1	1	1	0	0	0	0	4	6/6	4/4	dms
	97.12-18	0	0	0	0	0	1	1	0	0	0	0	2	5/5	4/4	dms, chc, cmm, mga
	25-85-70	0	0	1	1	1	1	1	0	0	0	0	3	5/4	3/4	dms
	2585-80	1	0	1	1	1	0	1	0	0	0	0	3	7/6	6/6	dms
	2585-67	0	0	1	1	1	1	1	0	0	0	0	3	7/6	6/6	dms

R genes and their SCAR markers — grouped as: R1, R2/Rpi-blb3, R3a, R3b, RB/Rpi-blb1 = Rpi-sto1, Rpi-blb2, Rpi-vnt1. **Resistance, points 2015/2016** (field, lab). *Reported wild species in hybrid pedigrees*

Set	Potato genotypes	R1-1205	R2-2500	R2-1137	R2-686	Blb3-305	R3a-1380	R3b-378	Rpi-blb1-820	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612	Number of R genes	field	lab	Reported wild species in hybrid pedigrees*
Set B (PP)	10/5-09	0	1	1	1	1	1	1	0	0	1	0	4	7/6	4/4	dms, mcd, phu, plt=sto, sto, vlm, vrn
	11/6-09	0	nd	1	1	1	1	1	0	0	1	0	4	7/6	3/3	adg, dms, mcd, plt=sto, pnt, sto, vlm
	12/1-09	0	1	1	1	1	0	0	1	1	0	0	2	7/6	6/6	adg, dms, mcd, plt=sto, pnt, sto, vlm
	13/11-09	0	nd	0	0	0	0	1	1	1	0	1	3	7/5	5/5	adg, ber, dms, mcd, plt, pnt, vlm
	14/8-09	0	nd	1	0	0	1	1	0	0	0	1	4	6/4	4/4	adg, ber, dms, mcd, plt=sto, pnt, sto, vlm
	15/13-09	0	1	1	1	0	0	1	1	1	1	0	4	6/6	6/6	adg, dms, mcd, plt=sto, sto, vlm
	16/27-09	1	0	0	0	0	0	0	1	1	1	0	3	7/7	7	adg, ber, dms, mcd, phu, plt=sto, sto, vlm
	18/40-2000	1	0	0	0	0	0	0	0	0	0	1	3	6/6	4/4	adg, dms, mcd, plt=sto, sto
	111 (38 KBA)	0	nd	1	1	1	1	1	1	1	1	0	5	8/6.5	6,5/6	adg, dms, mcd, plt=sto, sto
	113 (50/1 KBA)	0	nd	0	0	0	0	0	0	0	1	0	1	7/6	6/6	adg, dms, phu, sto, vrn and a mixture of the pollen of the hybrids
	128-05-03	0	nd	1	1	1	1	1	0	0	0	0	3	7/6	5/5	adg, dms, phu, ryb, sto, vrn
	97-153-2	0	nd	0	0	0	1	0	0	0	0	0	1	6/6	5/5	adg, dms
	99-4-1	1	nd	0	0	0	0	1	0	0	0	0	2	7/5	6/5	unidentified wild species
	2 (194-4т)	0	0	0	0	0	0	1	1	0	0	0	3	8/6	5/5	adg, dms, phu, sto, vrn
	7 (93-5-30)	0	0	0	0	0	0	1	0	0	1	0	2	7/5	4/4	acl, adg, blb, dms, phu, ryb, sto
	53 (34-5-2003)	0	0	0	0	0	0	0	0	0	1	0	1	6/6	5/5	adg, dms, ryb, sto, vlm
Set C (VIR)	106 (171-3)	0	0	0	0	0	0	1	0	0	0	0	1	7/6	6/6	adg, dms, ryb
	118 (118-5)	0	0	1	0	0	1	1	0	0	0	0	2	8/5	6/6	adg, dms, ryb
	120 (118-6)	0	nd	1	0	0	1	1	0	0	0	0	2	6/5	5/4	nd
	123 (128-6)	1	nd	0	0	0	1	1	0	0	1	0	4	8/6	6/6	nd
	99-6-5	0	nd	1	0	0	0	1	0	0	0	1	2	3/nd	nd/nd	adg, phu, sto,
	99-6-6	1	nd	1	0	0	1	1	0	0	0	1	4	5/nd	nd/nd	adg, phu, sto,
	90-6-2	1	nd	1	0	0	0	0	0	0	0	1	2	7/nd	nd/nd	adg, phu, sto,
	97-155-1	0	nd	1	0	1	1		0	0	0	1	4	8/nd	nd/nd	adg, dms, ryb

Set	Potato genotypes	R1	R2/Rpi-blb3				R3a	R3b	RB/Rpi-blb1 = Rpi-sto1		Rpi-blb2	Rpi-vnt1	Number of R genes	Resistance, points 2015/2016		Reported wild species in hybrid pedigrees*
		R1-1205	R2-2500	R2-1137	R2-686	Blb3-305	R3a-1380	R3b-378	Rpi-blb1-820	Rpi-sto1-890	Rpi-blb2-976	Rpi-vnt1.3-612		field	lab	
	139 (4-1-2012)	1	0	0	0	0	0	0	1	1	1	0	3	9/6	6/6	adg, aln, ber, dms, mcd, plt=sto, pnt, sto, vlm
	117-2	0	0	1	0	1	0	1	0	0	1	1	3	7/5	5/5	Atzimba x aln
	39-1-2005	0	0	0	0	0	0	1	1	1	0	1	3	7/6	6/6	Atzimba x aln
	25-1-2007	0	0	0	0	0	0	1	0	0	1	0	2	5/5	5/5	Atzimba x aln
	134-2-2006	1	nd	0	0	0	0	0	0	0	0	1	2	7/6	6/6	Atzimba x aln
	135-1-2006	0	nd	1	1	1	1	1	0	0	0	1	3	7/5	5/5	Atzimba x aln
	135-2-2006	1	1	1	1	1	1	1	0	0	0	0	3	7/4.5	5/4	Atzimba x aln
	24-1	0	nd	0	0	0	0	1	0	0	1	1	3	8/6	7/6,5	Atzimba x aln
	24-2	0	nd	1	1	1	0	1	0	0	1	1	4	8/6	7/6,5	Atzimba x aln
	8-1-2004	0	nd	0	0	0	0	0	0	0	1	0	1	5/nd	nd/nd	chc, oka
	8-5-2004	0	nd	0	0	0	0	0	0	0	1	0	1	5/nd	nd	chc, oka
	Alpha	0	0	0	0	0	0	0	0	0	0	0	0	4/4	3/3	none
	Atzimba	0	nd	0	0	0	0	0	0	0	1	0	1	5/nd	7/nd	US 133 3 × 52 AT 1
Standard varieties (SV)	Bintje	0	0	0	0	0	0	0	0	0	0	1	1	3/3	3/3	none
	Desiree	0	nd	0	0	0	0	0	0	0	0	0	0	3/nd	2/nd	none
	Eesterling	0	0	0	0	0	0	0	0	0	0	0	0	4/4	3/3	none
	Elizaveta	1	nd	1	0	0	1	1	0	0	0	0	3	4/5	3/4	acl, adg, dms, phu, sto, vrn
	Escort	1	1	1	1	0	1	1	0	0	0	0	4	7/5	6/5	dms
	Gloria	0	0	1	0	0	1	1	0	0	0	0	2	5/4	4/3	adg, dms
	Jubel	1	nd	1	0	0	0	0	0	0	1	1	3	7/nd	nd/nd	Victoria Augusta × 78 92
	Robjjn	0	0	1	0	0	0	0	0	0	0	0	0	5/4	4/4	none
	Sarpo Axona	0	nd	0	0	0	0	1	0	0	0	0	1	8/7	7/7	none
	Sarpo Mira	0	0	0	0	0	1	1	0	0	0	0	2	8/7	7/7	dms and unidentified wild species
	Svitanok kievskij	0	nd	1	1	1	1	1	1	0	0	0	4	5/5	4/4	Adretta x 3774c 71
	Negr	0	nd	1	0	0	0	0	0	0	0	0	0	nd/4	3/3	S. tuberosum L. subsp. chiloense
	Nayada	1	nd	1	1	1	0	0	0	1	1	0	3	nd/6	nd/5	adg, dms, vrn, sto, phu
	Priekulskiy ranny	0	nd	0	0	0	0	0	1	0	0	0	1	5/nd	nd/nd	Irish Cobbler x Jubel
	Zagadka Pitera	0	nd	0	0	0	1	1	0	0	0	0	2	nd/6	nd/5	dms, vrn, sto, phu

*Wild species: acl — S. acaule, adg — S. andigenum, aln — S. alandiae, ber — S. berthaultii, brc — S. brevicaule, chc — S. chacoense, cmm – S. commersonii, dms — S. demissum, edn — S. edinense, mcd — S. microdontum, mga - S. megistacrolobum, oka - S. okadae, phu — S. phureja, plt — S. polytrichon =S. stoloniferum, pnt — S. pinnatisectum, ryb — S. rybinii=S. phureja, sem — S. semidemissum, sto — S. stoloniferum, vlm — S. vallis-mexici, vrn — S. vernei. nd — no data.

Correlation between LB resistance and the number of R genes per plant

While LB resistance of most hybrids is apparently related to the number of the resistance genes per plant, there are nevertheless several genotypes that lack *R* gene markers in spite of high field and laboratory resistance (Table 2). One such case is var. Sarpo Mira: in addition to *R3a* and *R3b* discovered by our screening, this cultivar comprises at least two more NB-LRR genes, *R8* and *R9a* (Jo et al., 2016; Vossen et al., 2016). Several set-C hybrids and var. Atzimba with manifest discrepancy between high LB resistance and low number of recognized *R* gene markers are prospective targets for mining with newly developed markers in hope to reveal new LB resistance genes.

When our sample is purified of several insufficiently researched genotypes that are apparently dominated by as yet unknown *R* genes, we observe statistically significant effect of pyramiding *R* genes on LB resistance of potato hybrids (Figure 2).
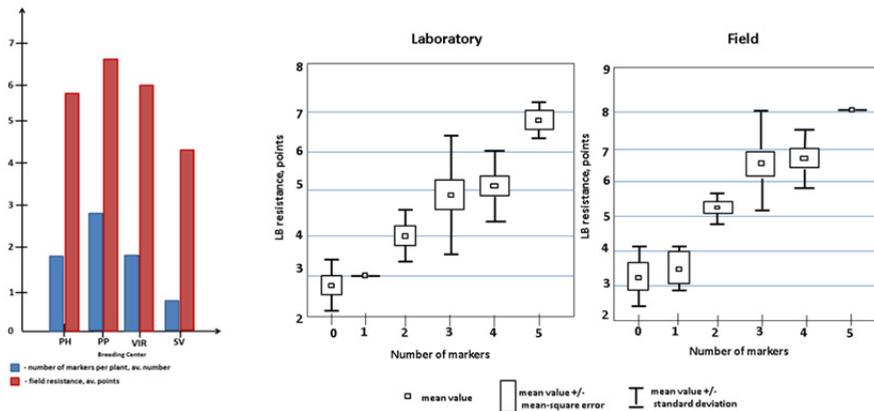
Figure 2. *LB resistance of potato genotypes in field and laboratory trials vs. the number of SCAR markers per plant*

CONCLUSION

The complex interspecific hybrids comprising genetic material from several wild *Solanum* species contain the variety of *R* genes providing the wide range of specific resistance to *P. infestans* strains. Many of these hybrids are apparently a prospective breeding material to be employed as advanced lines to produce new potato varieties with durable LB resistance. These hybrids are also a promising source of new resistance genes or new homologs of already known genes. The advanced potato lines with *R* gene pyramids and superior LB resistance will streamline development of new donors of durable resistance and in this way help anticipate the dramatic changes in pathogen populations.

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Niche-exclusion in the field: the result from competition between generalist and specialist clonal lineages in the Irish Famine pathogen *Phytophthora infestans*?

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Local adaptation in host-parasite systems is common and may apply to the late blight pathogen *P. infestans*. On its major agricultural hosts tomato and potato, different genotype frequencies have been reported for French *P. infestans* populations. This apparent host-related pathogenic specialization in *P. infestans* populations is actually not understood. *In vitro* cross-inoculation experiments suggest that aggressiveness components contribute to population separation by host in the field, at least for potato isolates. The situation seems to less clear-cut for tomato isolates that have been found to be highly aggressive on both hosts. The objective of the present study is to shed light on this discrepancy in order to explain *P. infestans* niche-exclusion in the field. For this, we did cross-inoculation experiments with genetically characterized isolates from both hosts and calculated fitness from individual aggressiveness components.
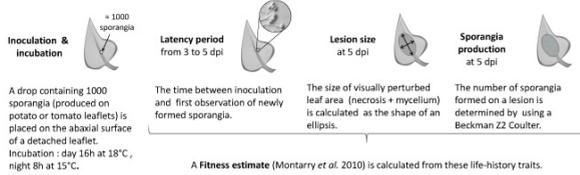
1 Biological material

❖ 21 *P. infestans* isolates from potato and tomato
  → two isolate types

  ▪ 11 potato isolates : 1_A1 (n=2), 6_A1 (n=4), 13_A2 (n=5)
  ▪ 10 tomato isolates : 2_A1 (n=1), 23_A1 (n=5), others (n=4)

Isolates were gentically characterized by 12 microsatellite markers. Genetically similar genotypes are grouped by Multilocus Lineages (MLLs) according to the European nomenclature (shttp://euroblight.net) : Clonal lineage_Mating type (ex. 1_A1).

❖ 2 hosts : potato (cv Bintje) and tomato (cv Marmande)

2 Experimental procedure and studied life history traits

Inoculation & incubation	Latency period from 3 to 5 dpi	Lesion size at 5 dpi	Sporangia production at 5 dpi
A drop containing 1000 sporangia (produced on potato or tomato leaflets) is placed on the abaxial surface of a detached leaflet. Incubation : day 16h at 18°C, night 8h at 15°C.	The time between inoculation and first observation of newly formed sporangia.	The size of visually perturbed leaf area (necrosis + mycelium) is calculated as the shape of an ellipsis.	The number of sporangia formed on a lesion is determined by using a Beckman Z2 Coulter.

A **Fitness estimate** (Montarry *et al.* 2010) is calculated from these life-history traits.

3 *In vitro* symptoms of *P. infestans* 5 days post inoculation

❖ The 21 isolates tested were virulent on both hosts.
❖ On potato, isolate from both hosts caused widespread lesions.
❖ On tomato, tomato isolates induced highly biotropic lesions. Potato isolates caused small necrotic lesions.
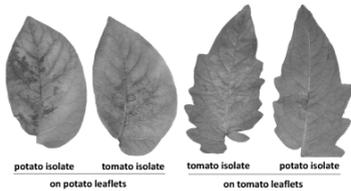
Figure 1 : Lesions caused by potato and tomato isolates on detached leaflets of both hosts. Photographs were taken 5 days post inoculation.

4 Individual life-history traits

❖ There was a clear pattern of host-related local adaptation of *P. infestans* according to the local vs foreign criterion.
❖ Potato and tomato isolates differed by their level of specialization.
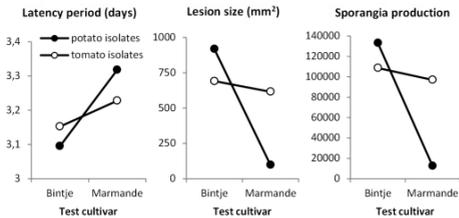
Figure 2 : Local adaptation patterns of life-history traits
11 potato isolates and 10 tomato isolates were tested in two independent experiments.

5 Fitness of both isolate types and within major MLLs

❖ Estimating fitness results in a pattern of local adaptation.
❖ Genotype identity impacts host-related fitness.
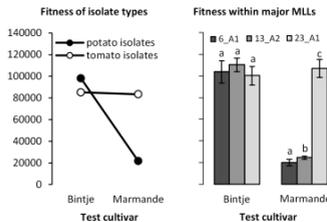
Figure 3 : Fitness of isolate types (left) and within major MLLs (right).
Multilocus lineages (MLLs) group genetically similar *P. infestans* genotypes from potato (6_A1 and 13_A2) and tomato (23_A1). Means were calculated from two independent experiments.

6 General conclusions, discussion and perspectives

❖ Our laboratory tests reveal a pattern of host-related local adaptation and differences in host specificity in *P. infestans* populations.

❖ Local adaptation of *P. infestans* may result from asymmetric fitness differences between generalist and specialist genotypes according to habitats, and end-up in apparent niche exclusion in the field despite shared hosts.

❖ Competitive exclusion or other environmental factors may account for niche exclusion in situations where individual pathogenic fitness is similar.

❖ Our experiments need now to be extended to less artificial conditions, in particular to allow to measure (and possibly predict) competitive rather than individual fitness.

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Pathogenicity of East European strains of *Phytophthora infestans* vs. resistance of colonized potato plants: the profiles of *AVR* genes vs. *R* gene pyramids

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SUMMARY

Phytophthora infestans isolates were collected in the VIR potato plots (Pushkin, St. Petersburg) and in commercial potato stands in several provinces of the European Russia, from Leningrad in the west to Sverdlovsk in the east. Simple sequence repeat (SSR) patterns of these isolates and of monozoospore lines obtained from the Pushkin isolates definitely differed from the SSR profiles reported for *P. infestans* lineages in Western and Central Europe. The analysis of monozoospore lines suggested that the different pathogen strains cohabit one and the same potato leaf. We failed to relate SSR patterns of these strains to plant late blight resistance and the profiles of resistance genes (*R* genes) revealed with specific sequence characterized amplification region. SSR patterns of *P. infestans* genotypes poorly matched the variations in their mating type and such indices of potential and actual pathogenicity as the profiles of virulence factors assessed with the plant differentials, avirulence gene patterns and aggressiveness in the tuber test.

KEYWORDS

Phytophthora infestans, Solanum, potato late blight, SSR genotyping, A1/A2 mating types, aggressiveness, virulence factors, *Avr* genes, *R* genes. SCAR markers

INTRODUCTION

The gene-for-gene paradigm of host-pathogen interaction (Flor, 1971) has provided a workable gateway to investigating the molecular players involved in potato late blight (LB) resistance. These studies encompass the race-specific resistance genes (*R* genes) amply characterized in *Solanum* L. species section *Petota* Dumort. and the avirulence genes (*Avr* genes) of *Phytophthora infestans* (Mont.) de Bary, as well as the major actors of this interaction - the

products of these genes, plant receptor CC-NBS-LRR kinases and pathogen RXLR effectors recognized by these receptor kinases (Rodewald, Trognitz, 2013; Sliwka and Zimnoch-Guzowska, 2013; Vleeshouwers et al., 2011).

P. infestans strains colonizing potato plants on the European territory of Russia have not been sufficiently explored by molecular methods. Previously we reported (Sokolova et al., 2015) that by their SSR profiles, the isolates collected in 2013-2014 in the VIR potato plots, Pushkin (St. Petersburg), considerably differed from the aggressive lineages observed in potato stands in Western and Central Europe (Chmielarz et al., 2014; Cooke et al., 2012). Currently, by screening across a wide span of the European Russia, we confirmed this distinction. The isolates collected from affected leaves can represent mixtures of different strains co-colonizing the same plants. We therefore compared SSR profiles of such mixed isolates to those of monozoospore lines obtained from the former. Finally, we made a tentative attempt to compare the indices of potential pathogenecity, such as virulence factors and *Avr* genes, to actual plant damage in the field and to relate the *R* gene spectra of the colonized plants to their actual LB resistance.

MATERIALS AND METHODS

Phytopathological analyses. Pathogen isolates were collected as described previously (Sokolova et al., 2015) from LB lesions on the leaves of the potato hybrid clones maintained in the Pushkin plots and in commercial potato stands across several provinces of the European Russia, from the Leningrad region in the west to the Sverdlovsk region in the east. The pedigrees of interspecific hybrids list from two to eight wild *Solanum* species. Most hybrids displayed high foliage resistance in the field trials under natural infestation 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 N161 of *Phytophthora infestans* (virulence factors R1 to R11; compatibility type A1) isolated in the Moscow region and scored against the susceptible cultivar Santé as a control (Kuznetsova et al., 2016; Rogozina et al., 2014). The profile of *R* genes in potato hybrid leaves was explored with specific sequence characterized amplification region (SCAR) markers (Fadina et al., this volume).

Isolates of *P. infestans* acquired from lesions on potato leaves were further maintained as clones. Next, monozoospore lines were obtained from the Pushkin isolates by the standard protocol. Conventional methods with some modifications were employed to assess mating type by the oospore development, to estimate metalaxyl resistance, and to evaluate aggressiveness in the Lapwood tuber test; the virulence genes were identified using the plant differentials, and plant LB resistance was assessed in the field and laboratory tests (Kuznetsova et al., 2014; Kuznetsova et al., 2016).

Molecular methods. Genomic DNA was isolated with the AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, United States). All primers were synthesized by Syntol, Moscow (www.syntol.ru). DNA amplification was run in a MJ PTC-200 thermocycler (Bio-Rad, United States). Clones and monozoospore lines were genotyped using the standard protocol for twelve SSR loci (Li et al., 2013; see Sokolova et al., 2015, for particulars). Mating type was identified using a CAPS marker developed by Judelson et al. (1995). *Avr* genes were amplified using the already published protocols (Armstrong et al., 2005; Champouret et al., 2009; Gilroy et al., 2011; van Poppel et al., 2008). Standard procedures were used to clone and sequence the amplicons and to compare the sequences thus obtained to those already published.

RESULTS AND DISCUSSION

SSR profiles of East-European strains of P. infestans
In the Pushkin isolates collected in 2013-2015, we discerned 34-40 alleles in 12 SSR loci, 2 to 6 alleles per locus. The loci SSR11, D13, G11, Pi63, Pi02/SSR3, SSR4 and SSR6 were most polymorphic (Table 1). We collated our data with the allele sizes reported by Li et al. (2013) 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 dendrograms presented on Figures 1 and 2.
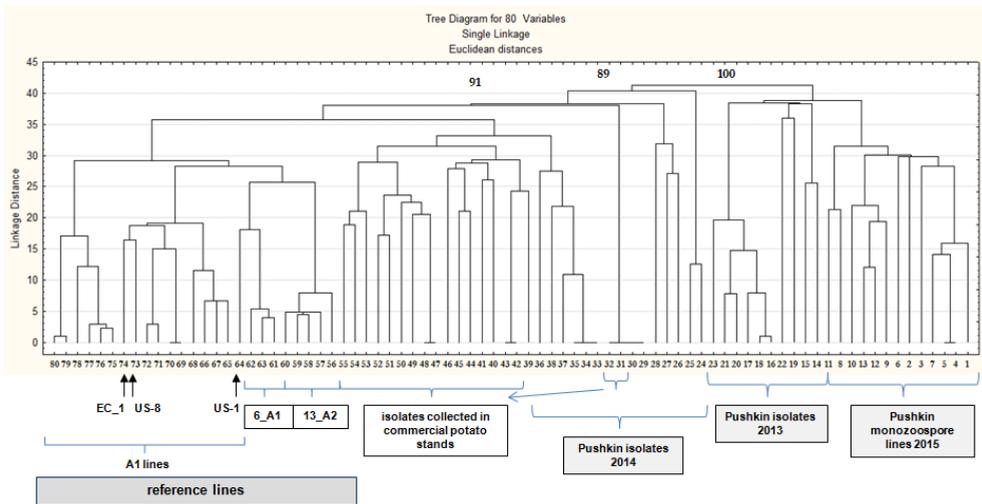
Figure 1. *Phylogenetic analysis of P. infestans isolates collected in the Pushkin plots and in the commercial potato stands in several regions of the European Russia as compared to the reference isolates from the Western Europe and the USA. The tree is built using the Neighbor Joining method; bootstrap values for 1000 repeats are shown where they exceed 0.70. 1-13 – monozoospore lines from Pushkin (2015), 14-23 – isolates from Pushkin (2013); 24-30, 33-38 – isolates from Pushkin (2014); 39 – standard isolate N161; 31, 32, 40-55 – isolates collected in commercial potato stands (see Table 2 for more information); 56-80 – P. infestans lineages from Western Europe and USA (Li et al., 2013), including the standards: 64 - US1_A1; 73 - US8_A2; 74 - EC1_A1.*

SSR profiles of the Pushkin isolates notably changed through 2013-2015 (Table 1, Figure 1); all these isolates mostly differed from those collected in the commercial potato stands beyond the Pushkin plots. Isolates considerably differed by plant genotype and by year. The SSR analysis demonstrated manifest differences between *P. infestans* isolates collected in the European Russia and those from the Western and Central Europe and United States (Figure 1). In particular, SSR genotyping of isolates from the East-European Russia did not reveal the most dangerous lineages 13_A2 and 6_A1.

Table 1. *Year-by-year changes in the allele frequencies at the most variable SSR loci in the Pushkin population and pooled data for the isolates collected in commercial potato stands in European Russia. The major discriminator alleles are highlighted in bold.*

SSR Loci	Pushkin isolates				2014-2015 isolates collected in commercial potato stands
	2013	2014	2015 (cluster I lines)	2015 (cluster II lines)	
	Alleles detected and allele frequencies				
SSR11	**331 (0.15)**	341 (0.73)	**331 (0.30)**	**331 (0.06)**	**331 (0.11)**
	341 (0.55)	355 (0.27)	341 (0.20)	341 (0.47)	341 (0.55)
	355 (0.30)		355 (0.50)	355 (0.47)	355 (0.33)
D 13	**118 (0.65)**	null (0.52)	**118 (0.64)**	**118 (0.16)**	**136 (0.76)**
	136 (0.05)	**118 (0.074)**	**134 (0.18)**	**134 (0.81)**	**138 (0.054)**
	138 (0.10)	**134 (0.11)**	**136 (0.18)**	154 (0.03)	140 (0.03)
	140 (0.15)	**136 (0.15)**			**142 (0.054)**
	154 (0.05)	152 (0.074)			152 (0.054)
		154 (0.074)			154 (0.054)
G11	**154 (0.10)**	null (0.07)	null (0.40)	null (0.06)	null (0.06)
	156 (0.05)	**154 (0.29)**	**154 (0.50)**	**154 (0.06)**	142 (0.08)
	162 (0.20)	**156 (0.29)**	162 (0.10)	**156 (0.38)**	**154 (0.36)**
	168 (0.65)	160 (0.07)		160 (0.44)	**156 (0.11)**
		162 (0.21)		162 (0.03)	160 (0.11)
		168 (0.07)		164 (0.03)	162 (0.25)
					164 (0.03)
Pi63	270 (0.15)	270 (0.27)	270 (0.20)	270 (0.06)	270 (0.32)
	273 (0.05)	**273 (0.23)**	279 (0.80)	**273 (0.47)**	**273 (0.19)**
	276 (0.30)	279 (0.50)		279 (0.47)	**276 (0.03)**
	279 (0.50)				279 (0.46)
Pi02/ SSR3	258 (0.30)	258 (0.23)	258 (0.20)	258 (0.03)	258 (0.17)
	266 (0.05)	**266 (0.15)**	268 (0.80)	268 (0.97)	268 (0.83)
	268 (0.60)	268 (0.62)			
	270 (0.05)				
SSR4	284 (0.40)	284 (0.30)	284 (0.40)	284 (0.375)	284 (0.28)
	288 (0.05)	288 (0.21)	288 (0.10)	288 (0.375)	288 (0.28)
	290 (0.40)	**290 (0.15)**	**290 (0.30)**	**290 (0.13)**	292 (0.14)
	292 (0.05)	292 (0.04)	**299 (0.20)**	292 (0.06)	294 (0.28)
	294 (0.14)	294 (0.30)		**297 (0.03)**	**297 (0.03)**
				299 (0.03)	
SSR6	**240 (0.30)**	**240 (0.15)**	242 (0.50)	242 (0.47)	**240 (0.06)**
	242 (0.20)	242 (0.50)	244 (0.50)	244 (0.53)	242 (0.47)
	244 (0.50)	244 (0.35)			244 (0.47)

The expanded pattern of SSR clusters is shown on Figure 2. Clusters I and II combine 2015 monozoospore lines. The cluster III comprises the A1 lines and reference genotypes US1-A1, EC1_A1 and US8-A2. Lines 13_A2 and 6_A1 are found in the cluster IV. The most important conclusion from the data presented in Figure 2: some strains of *P. infestans* seem to co-occur on one and the same potato leaf. Such differentiation is especially evident in the case of isolates/lines 18/1-1-5 and 103-1-5. Nonetheless most monozoospore lines were subclones of solitary isolates that colonized the particular hybrids. However, is such differentiation of SSR-discriminated genotypes supported by their functional diversity?

Functional characteristics of isolates and monozoospore lines

In 2013 Pushkin isolates, A2 mating type dominated, whereas in 2014, A1 type prevailed (Table 2). A1 type was more frequent in isolates collected in the commercial potato stands. Metalaxyl-resistant isolates were rather uncommon and found only in few commercial potato stands. The profiles of virulence factors greatly differed in these isolates. Monozoospore lines derived from 2015 Pushkin isolates were mostly of A1 mating type and metalaxyl-sensitive (Table 3). They widely varied in the number of virulence factors and aggressiveness (Table 3). All Pushkin isolates and lines were assessed for the presence of *Avr2 и AVR2-like* avirulence genes (Gilroy et. al., 2011). In 2015, considerable changes occurred: the frequency of the dominant *Avr2* allele increased as compared to 2013 and 2014 isolates.

The phytopathological and molecular characteristics of the colonizing strains of P. infestans are not immediately related to LB resistance of colonized potato hybrids

When we compare the monozoospore lines that belong to different SSR clusters (Figure 2), we find that their indices of aggressiveness, virulence gene profiles and metalaxyl resistance are immediately related neither to plant resistance, nor to their *R* gene profiles (Table 3). One should emphasize that the profiles of virulence factors in monozoospore lines do not match the *Avr* genes found in the pathogen. To illustrate, the presence of the *R2* gene rarely matched the *Avr2* gene. Our evidence on virulence factors and *Avr* genes is yet insufficient to prove that one and the same plant is colonized by more than one strain of *P. infestans*.
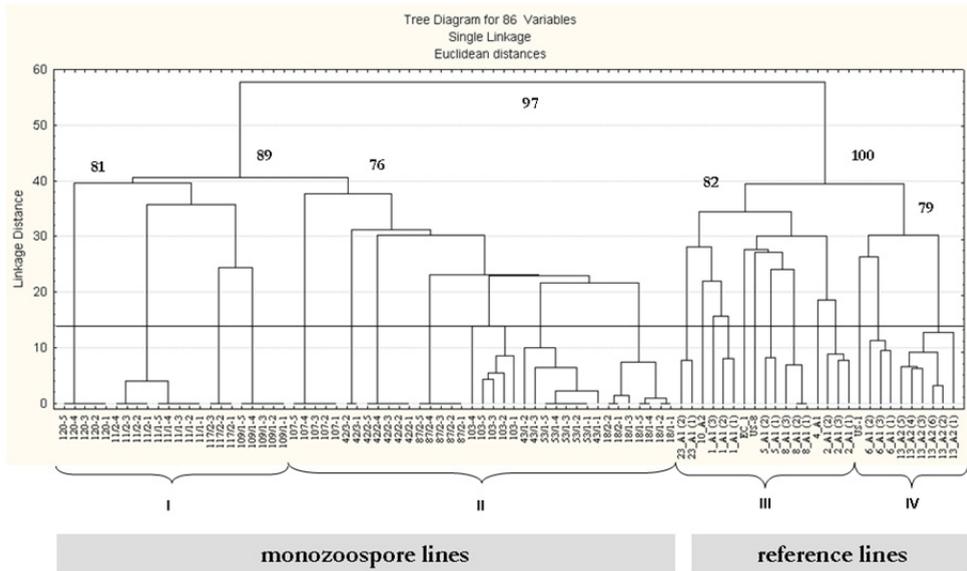

Figure 2. *Phylogenetic analysis of Pushkin P. infestans isolates. The tree is built using the Neighbor Joining method; bootstrap values for 1000 repeats are shown when they exceed 0.70. Distinct SSR clusters of P. infestans genotypes are marked with the Roman figures I – IV: these SSR clusters described in Tables 1 and 3.*

264

Table 2. *Some phytopathological and molecular characteristic of Pushkin isolates (2013-2014) and isolates collected in several regions of European Russia in 2014-2015*

Isolates	Mating type assessment (phytopathol. method /CAPS marker)	Resistance to metaxyl*	Virulence factors	*Avr* genes
2 – 13	A2/A2	S	12341011	*AVR2K N/avr2*
4 – 13	A1/A1	S	234810	*AVR2K*
111-13	A1A2 /A2	S	12347891011	*avr2*
7 – 13	A2/A2	S	124561011	*avr2*
106-13	A1/A1	IR	12345781011	*AVR2K/avr2*
132-13	A1A2 /A2	S	12341011	*avr2*
113-13	A1A2 /A2	IR	1234567891011	*avr2*
131-13	A1/A1	IR	356781011	*AVR2K /avr2, IpiO I,II avr3aEM, avr1*
87-13	A2/A2	IR	1234567891011	*avr2 IpiO I,II; avrSm1(avr9)*
7 – 14	A1/A1	S	124561011	*AVR2K N/avr2*
53-14	nd/A1	S	125671011	*avr2 IpiO I,II AvrSm1(Avr9)*
36-14	A2/A2	S	12345671011	*AVR2/avr2*
43-14	A2/A2	S	12367	*AVR2/avr2*
82-14	A1/A1	S	12345671011	*avr2*
109-14	A1/A1	S	12345671011	*avr2*
119 -14	A1/A1	S	12345671011	*avr2*
132-14	A2/A1	S	12341011	*AVR2K /avr2*
28 -14	A1/A2	S	1234567891011	*AVR2K /avr2*
Standard 161	A1/nd	S	1234567891011	*AVR2K /avr2 avr1*
Len. 17-14	A1/nd	R	34781011	*AVR2*
Len. 28-14	A1/nd	S	125671011	*AVR2/avr2*
Len. 32-14	A1/nd	S	12345671011	*AVR2*
Len. 33-14	A2/nd	S	n.d.	n.d.
Astr. 44-14	A1/nd	R	34711	*AVR2/avr2*
Astr. 45-14	A1/nd	R	34671011	*AVR2/avr2*
Tula 71-14	A2/nd	R	1245671011	*avr2*
Tula 72-14	A2/nd	I R	12345671011	*avr2*
Moscow 29-15	A1/nd	S	12346	*AVR2/avr2*
Moscow 51-15	A1/nd	S	123467810	*AVR2/avr2*
Moscow 56-15	A2/nd	S	12345671011	*avr2*
Moscow 59-15	A2/nd	S	n.d.	*AVR2*
Yar. 65-15	A2/nd	S	31011	*AVR2/avr2*
Yar. 69-15	A1/nd	S	2671011	*avr2*
Sverdlovsk 72-15	A1/nd	R	n.d.	*avr2*
Sverdlovsk 81-15	A2/nd	R	n.d.	*AVR2*
Chuvashia 87-15	A1/nd	S	n.d.	*AVR2/avr2*

*Regions: Astr. - Astrakhan; Len. - Leningrad; Yar. - Yaroslavl. *S – sensitive, IR – intermediate resistant, R – resistant.*

Table 3. Some phytopathological and molecular characteristic of P. infestans monozoospore lines obtained from 2015 Pushkin isolates with different SSR profiles as related to the R gene profiles of the colonized potato hybrids

Plant genotypes	LB resistance*	R genes assayed with SCAR markers	Monozoospore lines and SSR clusters**	Mating type assessment (CAPS marker)	Resistance to metalaxyl ***	AVR genes****	Virulence genes in isolates of P. infestans	Average aggressiveness*****
18/40-2000	6/4	R1	18/1-1 - 18/1-5. II	A1	S	AVR2K/ avr2	1234567891011	WA - HA
			18/2-1; 18/2-2. II	A1	nd	AVR2K/avr2	nd	WA
160-1	nd	**R2/Rpi-blb3**	42/2-1 - 42/2-5. II	A2	S	**AVR2K**	12347891011	MA
			42/3-1; 42/3-2. II	A1	S	**AVR2K**/avr2	12347891011	MA-HA
160-17	7/5	**R2/Rpi-blb3**	43/1-1 - 43/1-3. II	A1	S	**AVR2K**/avr2	12347891011	WA
34-5-2003	6/5	0	53/1-1 II	A1	S	AVR2K/avr2	12345678911	MA
			53/1-2- 53/1-5 II				1234567891011	WA - HA
Robijn	5/4	**R2/Rpi-blb3**	87/2-1, 87/2-3 II	A2	nd	**AVR2K**/avr2 avr3a EM, avr4	nd	nd
			87/2-2 II		S	**AVR2K**/avr2	12378	WA
			87/2-4, 87/2-5. II		nd	avr2	nd	nd
50/1	7/6	RB-226	103-1 - 103-4 II	A1	S	AVR2K/avr2 avr3a EM, avr4	123467891011	WA - MA
			103-5 II		nd		nd	nd
27	8/6	**R2/Rpi-blb3**	107-1 II	A2	S	**AVR2K**	123478910	WA
			107-2 II				12347891011	NA
			107-3-107-5 II		nd		nd	nd
14/8-09	6/4	**R2/Rpi-blb3,** R3a, R3b,	117/2-1- 117/2-2 I	A2	S - IR	**AVR2K**	12345791011	WA - MA
			117/2-3 I		S		1235791011	WA
118-6	7/5	R2/Rpi-blb3, R3a, R3b	109/1-1 I	A1	S	avr2	123578	MA
			109/1-2 I			avr3a EM, avr4	12345791011	WA
			109/1-3 - 109/1-5 I		nd		nd	nd
4-1-2012	8/6	R1, Rpi-blb1/Rpi-sto1	120-1 - 120-5. I	A2	S	AVR2K	12347891011	WA - HA
171-3	7/6	R3b	11/1-1 - 11/1-5. I	A1	S	avr2	47	WA
			11/2-1 I	A1	S	avr2	134781011	
			11/2-2 I				1234781011	
			11/2-3 - 11/2-4. I		nd		nd	

*Field/laboratory trials, points. **See Figure 2. ***S – sensitive; IR – intermediate. ****Amino acid polymorphism N31K in AVR2. K /N is heterozygous. *****Mean values for eight standard varieties: NA -non-aggressive; WA - weakly aggressive; MA - moderately aggressive; HA - highly aggressive. nd – no data.

CONCLUSIONS

Whatever small were the samples of pathogen isolates presented in this communication, this pilot experiment vividly demonstrated wide geographic diversity and considerable year-by-year changes in the *P. infestans* populations. Most hybrids were infested by solitary pathotypes; however, solidary infestation cannot be excluded. Pathogen strains discriminated by SSR analysis were not immediately related to their diversity in aggressiveness and the profiles of virulence factors and *Avr* genes. We failed as yet to demonstrate any link between the phytopathological and molecular characteristics of the colonizing strains of *P. infestans* and such properties of colonized potato plants as capacity for LB resistance (resistance scores and *R* gene profiles).

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268

Foliar late blight development in the UK in relation to EuroBlight fungicide efficacy ratings

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KEYWORDS

Foliar blight, leaf blight, fungicide efficacy, EuroBlight fungicide table, *Phytophthora infestans*

INTRODUCTION

In recent years there have been comments by some agronomists in the UK that foliar blight control is not always as closely related to EuroBlight fungicide efficacy ratings as expected. The decimal leaf blight efficacy ratings are calculated exclusively from results generated in at least six trials, over 2 years, in different European countries (DE, DK, NL and UK). The relationship between the ratings and foliar blight development was checked using results from non-EuroBlight field trials in the UK.

MATERIALS AND METHODS

Five trials that included at least six straight fungicides or mixtures with decimal ratings for leaf blight control were selected. Selection was bias-free; no other trials were suitable for inclusion since they involved too few fungicides with a EuroBlight rating (three or fewer). Foliar blight severities, for the assessment date with peak separation of treatments, were regressed on the cumulative fungicide rating scores for leaf blight, and also the collective ratings for leaf blight plus curative efficacy (Bain and Bardsley, 2009; Bain, 2015). For individual fungicide applications the curative rating was included only if the spray was applied within 0 to 2 days of high risk conditions; defined as a Smith day. The fungicides tested were Diablo (ametoctradin+ mancozeb), Dithane NT (mancozeb), Electis (zoxamide + mancozeb), Infinito (fluopicolide + propamocarb), Laminator Flo (mancozeb), Ranman Top (cyazofamid), Revus (mandipropamid), Shinkon (amisulbrom) + Dithane NT (mancozeb) and Shirlan (fluazinam). Some validation trials included the UK products Invader (dimethomorph + mancozeb) and Valbon + ZinZan (benthiavalicarb + mancozeb). These treatments were excluded from this study because the decimal leaf blight ratings for these co-formulations have been predominantly calculated from EuroBlight trials including substantially different products, i.e. Acrobat and Valbon (NL formulation). In the study product rates in the validation trials matched those used to generate ratings.

RESULTS

In three of the five trials the linear relationship between foliar blight severity and cumulative decimal rating score was significant (Table 1). However, in the 2007 and 2011 trials this was not the case. Incorporating the curative ratings for the treatments, when appropriate, improved the linear relationship in all five experiments (Table 1). For the 2007 and 2011 trials the increase in percentage of variance accounted for was very considerable. An example demonstrating how closely the linear regression line fitted the data is shown in Figure 1.

Table 1 *Significance levels and percentages of variance accounted for from regressions of foliar blight severities on cumulative fungicide rating scores*

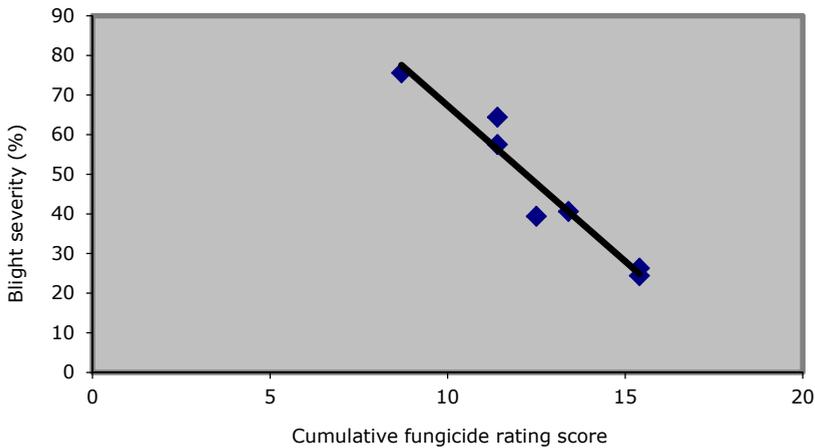| Year of field trial	Number of fungicides tested	Significance level of regression		Percentage variance accounted for	
		Leaf blight rating	Leaf plus curative ratings	Leaf blight rating	Leaf plus curative ratings
2006	6	0.013	0.003	78	89
2007	7	0.159	<0.001	22	93
2009	7	0.021	0.004	62	79
2010	7	0.005	<0.001	79	89
2011	8	0.168	0.011	17	64

Figure 1. *Relationship between foliar blight and cumulative fungicide rating (leaf blight plus curative), 2007 trial*

CONCLUSIONS

The accuracy of the EuroBlight ratings for the fungicides tested in the five validation trials was confirmed, provided curative ratings were also taken into account. One caveat is that care is needed regarding the few ratings that currently cover dual products in Europe, e.g. Acrobat/Invader and Valbon/Valbon + ZinZan. This issue would be alleviated by further harmonisation of products across Europe or the generation of individual ratings. The curative ratings in the EuroBlight table are not yet derived from trials adhering to a common protocol. It would be useful to know the impact of decimal ratings for curative activity on the correlation between cumulative rating score and leaf blight control. Validation of more recent ratings for newer fungicide products requires this exercise to be repeated in a few years time when sufficient results from non-EuroBlight trials are available.

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Revus Top: an efficient tool to control late and early blight of potato

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SUMMARY

The use of a Revus Top fungicide (mandipropamid + difenoconazole), which is characterized by a systemic and translaminar activity and a wide-range preventive and curative effect against *Phytophthora infestans, Alternaria solani*, and *A. alternata*, provides an efficient protection of potato against the early and late blights. The efficiency of the Revus Top fungicide against these diseases was confirmed under both laboratory and field conditions and for both artificial and natural infection background. The obtained results agree with the data of European scientists concerning a high efficiency of Revus Top against the late and early blight of potato.

KEYWORDS

Phytophthora infestans, Alternaria solani, Alternaria alternata, potato, yield, late blight, early blight

INTRODUCTION

Early and late blight are the most devastating potato diseases causing about 4 billion € of global annual losses (Fry et al., 2015). Both diseases are the most harmful in the case of their early manifestation and rapid development during a vegetation season (Anisimov et al., 2009).

Late blight is common in almost all potato-growing regions of Russia. Its causal agent, *Phytophthora infestans*, is able to overwinter in the form of mycelium (infected tubers) and oospores (soil and plant debris). Late blight infects well-developed plants and characterized by the epiphytotic character of development. The most favorable conditions for its development are the cloudy weather, frequent rainfalls, and moderate temperature.

Early blight caused by two *Alternaria* species, *A. solani* and *A. alternata*, is also widespread in Russia and affects leaves, stems, and tubers. Both *Alternaria* species are able to overwinter in tubers, soil, and plant debris in the form of either mycelium, or spores. The pathogens better infect senescent tissues, and the development of the disease is slower than that of the late blight. The preferable weather conditions are high temperatures and long-term dews.

The harmfulness of potato blights may be reduced via the implementation of an integrated potato protection including the use of healthy seed material, disease-resistant cultivars, proper

agrotechnics, and modern fungicides (Kuznetsova, 2010). In spite of significant breeding achievements, chemical protection still remains the most reliable method to control the late and early blights. The use of fungicides delays the start of epiphytoties and decreases the rate of disease development (Filippov et al., 2006). In the case of severe disease development, only chemical treatments may provide any stable yield.

The assortment of fungicides recommended for the control of the late and early blights in Russia is constantly increased. One of the most popular fungicides used to protect potato against the late blight is Revus, a mandipropamid-based translaminar fungicide with prolonged preventive and strong curative effect (Kuznetsova et al., 2011). However, in recent years, a simultaneous development of both late and early blights is observed in many regions of Russia, so potato growers prefer to use fungicides with a wide range of action, such as the Revus Top (mandipropamid, 250 g/L + difenoconazole, 250 g/L), able to control both diseases.

The aim of our study was the laboratory and field assessment of the efficiency of the Revus Top fungicide comparing to the "standard" Acrobat MC (mancoceb, 600 g/kg + dimethomorph, 40 g/kg) fungicide against the late and early blights.

MATERIALS AND METHODS

Field trial arrangement. A small-plot field trial was arranged during two seasons (2012-2013, epiphytotic conditions) on the experimental potato field of the All-Russian Research Institute of Phytopathology (Moscow region) using cv. Red Scarlett. 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.

In total, five fungicide sprayings were applied starting from the canopy closure stage; the time interval between the treatments was 7-10 days. The experimental variants were the following:

 A) Untreated control: no fungicidal treatments.

 B) Treated control: four treatments with Acrobat MC (2 kg/ha) and final treatment with Shirlan (0.4 L/ha).

 C) Revus Top: four treatments with Revus Top (0.6 L/ha) and final treatment with Shirlan (0.4 L/ha).

Land and field treatments. The land treatment of the field included under-winter ploughing, disking, deep ground treatment, pre-planting furrow formation, hilling, application of mineral and organic fertilizers; and a pre-emergence treatment with a Zenkor (2 L/ha) and Fusilade (1 L/ha) herbicides. During a vegetation season, the whole field was once treated with a thiamethoxam-based Aktara insecticide (0.06 kg/hectare).

Evaluation of the duration of the protective effect against *P. infestans*. During a budding stage, potato plants were sprayed with Revus Top, 0.6 L/ha, Acrobat MC, 2 kg/ha (treated control), or distilled water (untreated control). Three, five, seven, ten, and fourteen days after the treatment, potato leaves were detached and inoculated with the spore suspension of *Phytophthora infestans* under laboratory conditions. Four days after inoculation, the number of necroses on the leaves was calculated. Each variant was tested in four replications.

Laboratory evaluation of the protective and curative effect of treatments against *A. solani*. To assess the protective effect of fungicides, potato leaves were collected during the budding stage and sprayed with Revus Top, 0.6 L/ha, Acrobat MC, 2 kg/ha (treated control), or distilled water (untreated control). Next day (24 h after the treatment), leaves were inoculated with an aggressive *A. solani* isolate Mos.p.Ud-10 collected in 2010 in the Moscow region from potato plants (cv. Udacha). The level of leaf infection was assessed 6 days after the inoculation.

 To assess the curative effect of fungicides, potato leaves were collected during the budding stage and inoculated with an aggressive *A. solani* isolate. 48 h after the inoculation, leaves were

sprayed with Revus Top, 0.6 L/ha, Acrobat MC, 2 kg/ha (treated control), or distilled water (untreated control). The level of leaf infection was assessed 6 days after the inoculation.

Assessment of the disease development and crop capacity. Field observations of the early and late blight development were carried out every 7-10 days starting from the first disease manifestations and up to the leaf dying. The level of the early and late blight development was assessed in accordance to the British Mycological Society scale (James, 1972). Based on the obtained data, the AUDPC values were calculated using a "Poteri" software (Filippov, 2012). The crop capacity (t/ha) and the level of tuber infection were determined right after a manual harvesting of plots. A tuber quality assessment including the level of tuber infection and % of marketable tubers was carried out after a one-month storage of harvested potato.

Statistical analysis. The statistical treatment of the obtained data was carried out according to Dospekhov (1985) at the 95% confidence level.

RESULTS

According to the obtained results, both Revus Top and Acrobat MC were highly effective in the potato protection against the late blight. At the same time, after 10 days of exposition, the protective efficiency of Acrobat MC was found to be reduced (Figure 1).

The tested fungicides also demonstrated high protective activity against the early blight. However, curative effect was observed only for the Revus Top application; in this variant, the level of leaf affection was reduced by 79% of the control (Figure 2).
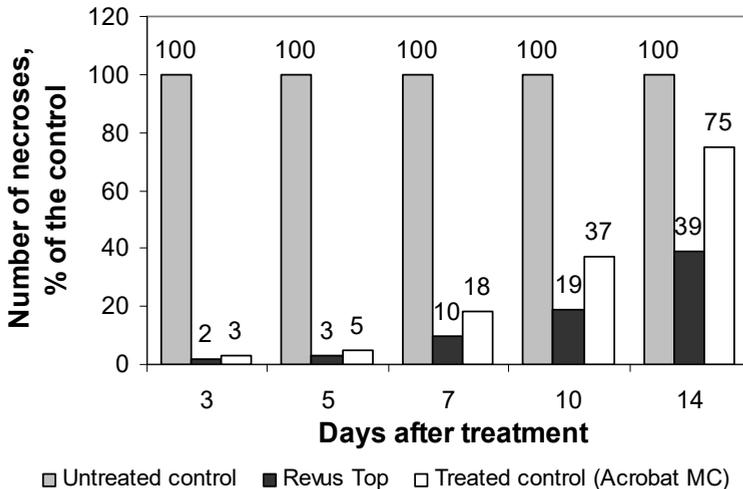

Figure 1. *Duration of the protective effect of the compared variants of potato treatment against the late blight (LSD$_{0.95}$ = 31).*
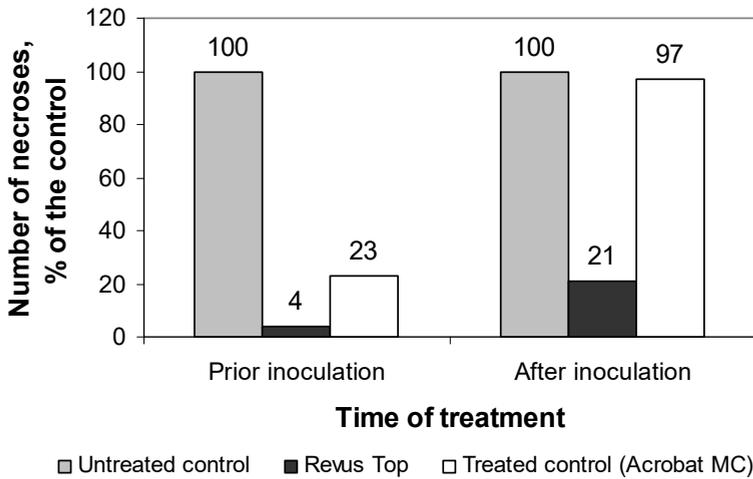
Figure 2*. Protective (left) and curative (right) effect of the compared variants of potato treatment against the early blight. LSD$_{0.95}$ = 15.2 and 19.5 for protective and curative effects, respectively.*

In 2012 and 2013, weather conditions in the Moscow region were favorable for the epiphytotic development of the late blight. In the case of untreated control, the first disease manifestations were observed in the 3rd (2012) and 2nd (2013) decade of June. In the 2nd decade of August, when control plants were completely killed by the late blight, the level of the late blight infection in the "Revus Top" variant did not exceed 15% in 2012 and 20% in 2013; the same values for the "Treated control" variant (Acrobat MC) were 25 and 35%, respectively.

In both years, the first early blight manifestations were observed in the 2nd decade of August. To the date of the last observation (Aug 30), the level of plant infection with both early and late blights in the "Revus Top" variant was 35 and 40%, respectively; in the "Treated control" variant it was 55 and 60%, respectively. The AUDPC values calculated for all experimental variants are shown in Figure 3. In both seasons, the treatment of plants with Revus Top provided better results than the treatment with Acrobat MC. The crop capacity and quality corresponded to the dynamics of the disease development and was comparable for both Revus Top and Acrobat MC treatments (Figure 4).
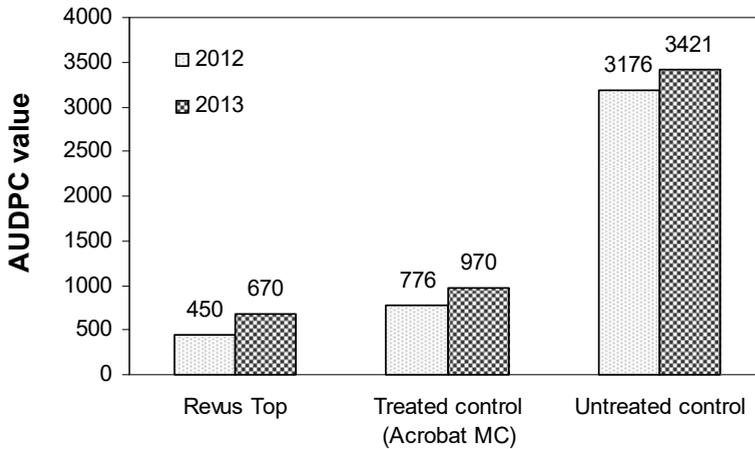
Figure 3. *AUDPC values describing the early and late blight development in the compared variants of potato treatment. $LSD_{0.95}$ = 94 and 86 for 2012 and 2013, respectively.*
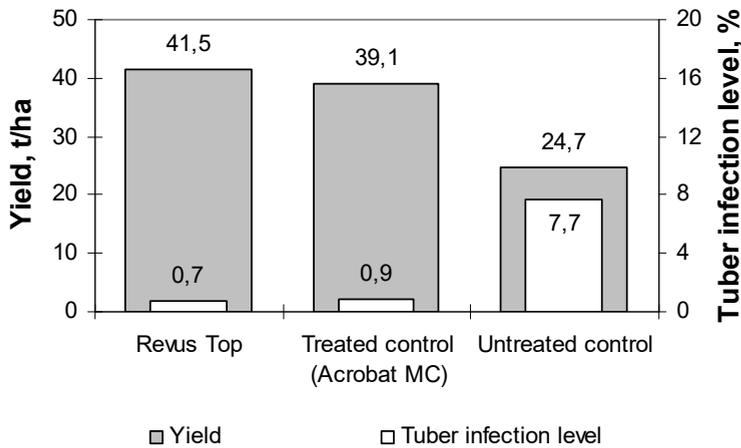
Figure 4. *The average yield ($LSD_{0.95}$= 2.45) and tuber infection level ($LSD_{0.95}$= 2.2) in the compared variants of potato treatment.*

Thus, the use of the Revus Top fungicide, possessing with the preventive and curative activity against the late and early blights, provided efficient protection of potato and the maximum yield increase (+16.8 t/ha). The efficiency of the Revus Top fungicide was confirmed during two seasons under both laboratory and field conditions and for both natural and artificial infection background.

CONCLUSION

1. Compared to the standard fungicide (Acrobat MC), Revus Top provided a similar efficiency of the potato protection against the late blight, but had a prolonged effect (7-14 additional days).

2. Both Revus Top and Acrobat MC demonstrated a high protection effect against the early blight, but the curative effect was observed only for Revus Top (reduction of leaf infection by 79% of the control).

3. Use of the Revus Top fungicide provided a higher yield (+16.8 t/ha) and ~10x less tuber infection level than in the case of the untreated control that was comparable to the "standard" scheme of treatment with Acrobat MC fungicide.

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Fungicide use in tomato by Tanzanian Farmers

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Fungicide use in tomato by Tanzanian Farmers

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Background

SEVIA (Seeds of Expertise for the Vegetable Sector of Africa) is a private sector driven project, funded by two world leaders in vegetable seeds: East-West Seed and Rijk Zwaan, and by the Dutch Ministry of Foreign Affairs. Wageningen University is the third partner.
SEVIA aims to contribute to the development of the vegetable industry in Africa and to food security.

Objective

- SEVIA develops and disseminates best farmer practices for vegetable farmers and it provides accountable and practical information. This research reveals the current practices of the farmers and is the basis of improved farmer advice for the control of late blight
- In seven different regions, a group of 10 vegetable farmers were asked to keep a daily logbook recording their inputs used and labour spent. This was done for a period of 12 to 14 months.

Results

Farmers often confuse late blight in tomato with other diseases. Description of late blight ranges from yellow leaves with black spots to watery spots. Local names for late blight are similar to names for early blight. Spraying is often done without using proper PPE.

Figure 1| Mixing of pesticides to spray tomatoes
Figure 2| Sulphur, copper, mancozeb and mancozeb+metalaxyl based fungicides are most available in agro-shops.

Table 1. Number of active ingredients used in tomato fields by farmers.

Active ingredient	Arumeru	Babati	Bagamoyo	Lushoto	Moshi	Nduruma	Usa	Total
Total fields (no.)	1	4	23	6	13	7	4	
No fungicides used (no.)	0	0	14	0	1	0	0	
azoxystrobin				2				2
carbendazim					2			2
chlorothalonil				1	2	1	1	5
copper oxychloride	1				2			2
hexaconazole		2						2
mancozeb			1	3	5	1	4	14
mancozeb+dimethomorph						1		1
mancozeb+fosetyl-al					1			1
mancozeb+metalaxyl		2	2	5	8	9	4	30
phosetyl-al							1	1
sulphur			3		4			7
thiophanate methyl			5					5
triadimefon		1						1
Total number	**1**	**6**	**11**	**11**	**22**	**12**	**10**	**73**

Farmers generally use contact fungicides and only start using "curative" fungicides when symptoms are clearly visible.
In tomato production costs for all pesticides are 1 – 14% of the total production costs. Yield of tomato is rather low with 4 to 17 t/ha.

Table 2. Economics of tomato cultivation in Tanzanian shilling per ha (x 1,000) (1 Euro = 2400 TZH; 1/5/2017).

	Arumeru	Babati	Bagamoyo	Lushoto	Moshi	Nduruma	Usa	Mean
seeds	79	111	290	381	339	25	64	242
fertilizer	247	205	389	466	437	198	136	344
pesticides	246	232	46	135	211	130	111	134
labour	1,006	811	2,113	557	738	667	759	1,140
others	236	626	629	99	127	49	124	309
Yield (t/ha)	10	8	17	4	14	12	10	13
Income	4,950	3,958	13,126	3,078	5,471	4,598	4,388	7,174
Profit	3,136	1,972	9,658	1,439	3,644	3,528	3,195	5,015

Fungicide use is the highest in the regions close to Arusha. In Bagamoyo not much fungicide is used, less then 1 application per season on average. In all regions the interval between fungicide applications is more than 8 days and can get uo to 16 days.

Table 3. Growing period of tomato, average field size, total use of active ingredient, interval between fungicide sprays (days) and total number of applications in one season per region.

	Arumeru	Babati	Bagmoyo	Lushoto	Moshi	Nduruma	Usa	Mean
Growing days	125	113	135	116	108	127	114	121
Field size (acre)	1	0	0	0	1	1	1	1
A.I. (kg/ha)	16.2	2.6	0.1	6.1	5.1	10.7	13.0	5.1
Interval	15.2	15.2		8.6	12.1	16.1	14.8	9.4
Applications	6.0	3.5	0.6	5.7	5.6	5.6	7.3	4.0

Conclusions

Farmers need training on:
- Safe use of pesticides
- Spray technique in order to apply pesticides in an efficient way
- Proper fungicide strategies to prevent the diseases and avoid resistance of diseases
- Information on the efficacy of fungicides

Farmers should have access to more effective fungicides
- Agro – shop owners need training in this field in order to provide proper advice to the farmers.

Acknowledgements

Efficiency of a Ranman Top use to control potato late blight under epiphytotic conditions in Russia

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SUMMARY

Ranman Top (a.i. cyazofamid) is a contact fungicide, which action is specific to *Oomycetes* including *Phytophthora infestans,* a causal agent of the late blight. Recently this fungicide was approved for the use in Russia. The aim of this study was a field assessment of its efficiency in combination with Ridomil Gold MC under epiphytotic conditions comparing to the untreated control and the reference scheme of treatment (Shirlan + Ridomil Gold MC). The obtained results showed that both treatment schemes provided a similar efficiency of the late blight control and almost equally increased the total yield and marketable fraction of potatoes comparing to the untreated control.

KEYWORDS

Phytophthora infestans, potato, late blight, fungicides

INTRODUCTION

Potato is one of the most economically important crops in the Russian Federation. In recent years, the annual potato production in Russia is about 31-33.5 mln. tons; at the same time, the average crop harvest is only 14.5-15 t/ha (Surinov et al., 2015) that is significantly lower than in Europe and North America, where the average potato harvest exceeds 40 t/ha (FAOSTAT, 2017). Such a major gap may be determined by several reasons, including poor seed quality and infection with various pathogens.

Potato late blight caused by *Phytophthora infestans* (Mont.) de Bary is the most devastating disease in Russia (Anisimov et al., 2009). Under favorable conditions, it may rapidly cause severe leaf infection leading to significant yield losses; the field or storage infection of tubers provides additional losses of edible yield. Until now, fungicides still remain the most reliable tool to delay the development of this disease and to reduce the corresponding losses, especially during epiphytoties. The assortment of fungicides recommended for the late blight control is constantly increased. Ranman Top (a.i. cyazofamid) is a contact fungicide, which was first registered in Europe in 2009 and now is applied in many countries to control fungal diseases of potato, tomato, and some other crops (Desnouck et al., 2012). The activity of this fungicide is

specific to *Oomycetes* at the level of mitochondria: cyazofamid inhibits complex III on the mitochondrial electron transport system. According to the trials performed in North-West Europe, the efficiency of Ranman Top is equal or even better than that of other fungicides; at the same time, the fungicide is completely selective to the treated crops (Desnouck et al., 2012).

Recently Ranman Top was approved for the application in Russia, where almost 80% of the total potato-growing areas are undergone to a high risk of the late blight epiphytoty. In these territories, epiphytoties usually occur every 3-5 years that result in serious yield losses. According to our data, in some years, the total area infected with late blight may reach 1 mln. ha, whereas the total potato-growing area in Russia (both commercial and private fields) in 2015 exceeded 4.2 mln. ha. Prior the recommendations on the choice of fungicides under epiphytotic conditions, each fungicide should be evaluated under field conditions at a severe late blight development. Thus, the purpose of this study was a field assessment of the efficiency of a Ranman Top fungicide under the late blight epiphytoty.

MATERIALS AND METHODS

Field trial arrangement. A small-plot field trial was arranged in 2016 on the experimental potato field of the All-Russian Research Institute of Phytopathology (Moscow region). The area of each experimental plot was 42 m^2; the plots were randomly distributed across the field. Each variant was tested in four replications.

Land and field treatment. Potato (cv. Red Scarlett) was planted on May 13 and harvested on September 1. The land treatment of the field included under-winter ploughing, disking, deep ground treatment, pre-planting furrow formation, hilling, application of mineral and organic fertilizers; and a pre-emergence treatment with a Zenkor (2 L/hectare) and Fusilade (1 L/ha) herbicides. During a vegetation season, the whole field was once treated with a thiamethoxam-based Aktara insecticide (0.06 kg/hectare).

Experimental scheme of treatment. For all experimental variants excepting the untreated control, the number of fungicide treatments was 6. The dates of fungicide treatments were Jun 29, Jul 07, Jul 21, Aug 02, Aug 12, Aug 22. The first spraying was applied when the plant height reached 20 cm; the further treatments were applied every 10-12 days.

The experimental scheme included the following variants:

A) Untreated control: no fungicidal treatments.

B) Treated control: (1) Shirlan, 0.4 L/ha; (2, 3) Ridomil Gold MC, 2.5 kg/ha; (4, 5, 6) Shirlan, 0.4 L/ha.

C) Ranman Top: (1) Ranman Top, 0.5 L/ha; (2, 3) Ridomil Gold MC, 2.5 kg/ha; (4, 5, 6) Ranman Top, 0.5 L/ha.

Evaluation of the disease development and crop capacity. Field observations were carried out on Jun 24, Jun 30, Jul 07, Jul 15, Jul 22, Aug 2, Aug 12, Aug 22, and Aug 30. The level of the early and late blight development was assessed in accordance to the British Mycological Society scale (James, 1972). Based on the obtained data, the AUDPC values were calculated for all experimental variants according to Shaner and Finney (1977). The crop capacity (t/ha) was determined right after a manual harvesting of plots. A tuber quality assessment including the level of tuber infection and % of marketable tubers was carried out after a one-month storage of harvested potato according to Kuznetsova (2007).

Statistical analysis. The statistical treatment of the obtained data was carried out by ANOVA at the 95% confidence level (Dospekhov, 1985).

RESULTS

A high late blight susceptibility of the cultivar used and the weather conditions of 2016 provided the epiphytotic development of the late blight. In the case of the untreated control, the first disease manifestations were observed on June 30. In July and August, air temperature exceeded average annual values by 2.6ºC, and the amount of precipitations was rather significant (Table 1).

Table 1. *Weather data for the vegetation period of 2016 (Moscow region, All-Russian Research Institute of Phytopathology)*

Basic parameters	May	June	Jul	Aug
Average temperature in 2016, ºC	14.3	17.4	20.1	18.5
Average annual temperature, ºC	12.3	16.0	17.4	15.9
Relative humidity in 2016, %	63	66	72	77
Average annual relative humidity, %	68	72	67	72
Average rainfall, mm	66.6	62.4	123.3	73.2
Average annual rainfall, mm	54.6	71.5	83.1	71.3

As a result, in the first decade of August, the level of plant infection exceeded 60%, whereas in the second decade plants were completely killed (Figure 1). Under such conditions, the tested schemes of chemical protection demonstrated a high efficiency. At the same time, a comparison of the calculated AUDPC values showed that Ranman Top provided the maximum efficiency in the late blight control (Figure 2).
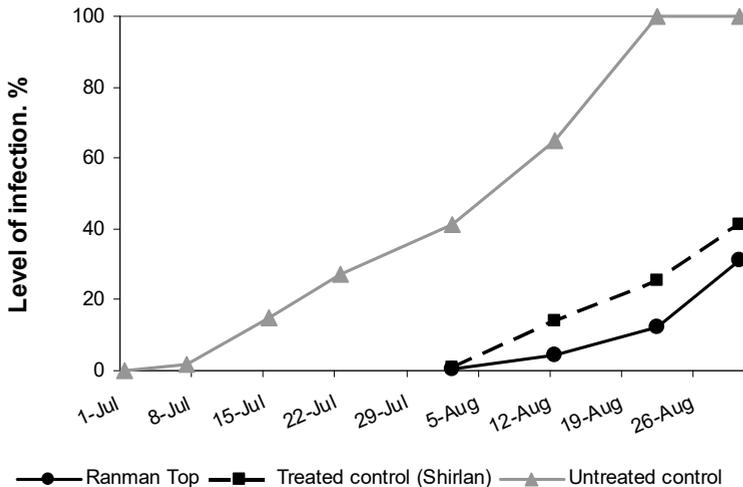

Figure 1. *Dynamics of the late blight development in the compared variants of treatment. The first disease manifestation in the untreated control was observed in Jun 30.*
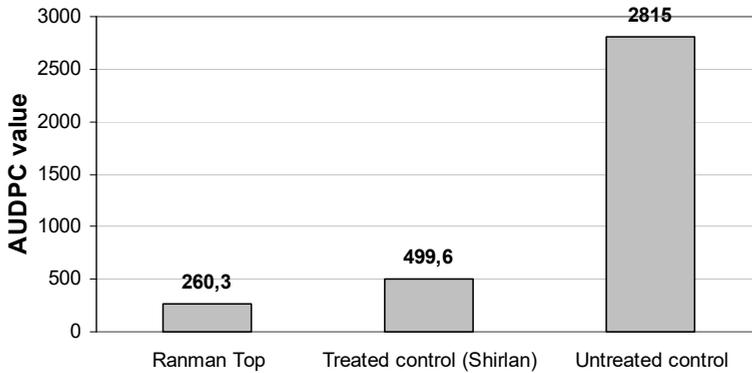
Figure 2. *AUDPC values calculated for the compared variants of treatment (LSD$_{0.95}$ = 75.2).*

The crop capacity corresponded to the late blight development dynamics in the compared variants. In the case of untreated control it was 35.2 t/ha, whereas the treated control and Ranman Top provided 61.9 and 64.2 t/ha, respectively. Therefore, the use of the Ranman Top fungicide at a dosage of 0.5 L/ha provided the maximum yield increase (+29 t/ha) as compared to the untreated control.

The quality of collected potatoes was evaluated after one-month storage. According to the obtained data, the level of tuber infection in both Ranman Top and treated control variants was significantly lower (by 22 and 21.5%) than in the untreated control (Figure 4). The marketable fraction of potato in both treated variants was about 30% higher than in the untreated control (Figure 3).
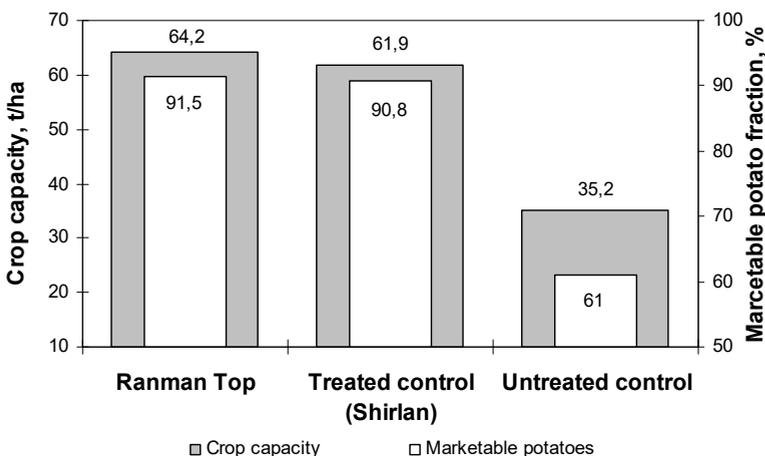

Figure 3. *The total yield (LSD$_{0.95}$ = 6.87) and marketable fraction of potatoes (LSD$_{0.95}$ = 1.5) of the compared variants.*
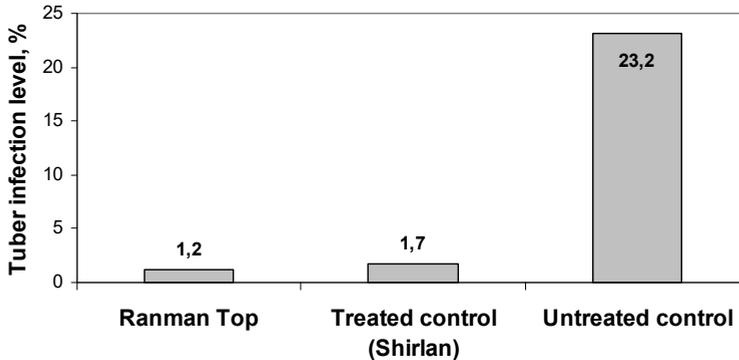
Figure 4. *The level of tuber infection (%) in the compared variants of treatment (LSD$_{0.95}$ = 0.6).*

CONCLUSION

Under the epiphytoty conditions, both Ranman Top and Shirlan (reference fungicide) treatments demonstrated a high efficiency in the late blight control that resulted in a prolonged vegetation period and increased yield and marketability of potatoes comparing to the control. At the same time, treatment with Ranman Top showed a higher suppression of the late blight development and provided the higher yield of potato than the treatment with the reference preparation.

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Using *Pseudomonas* interactions and their potential synergistic effect for sustainable potato protection against *Phytophthora infestans*

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Using *Pseudomonas* interactions and their potential synergistic effect for sustainable potato protection against *Phytophthora infestans*

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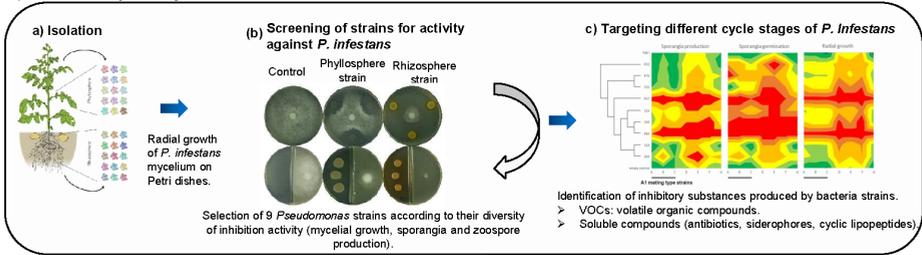[2]Department of Biology, University of Fribourg, Fribourg, Switzerland

Background

Late blight disease caused by *Phytophthora infestans* is a re-emerging problem worldwide and is considered as the most devastating disease of potato. Current protection practices rely on repeated applications of synthetic fungicides or copper-based products, both of which are harmful to the environment. Therefore, researchers have focused their attention on biological control in order to reduce chemical inputs in agriculture.
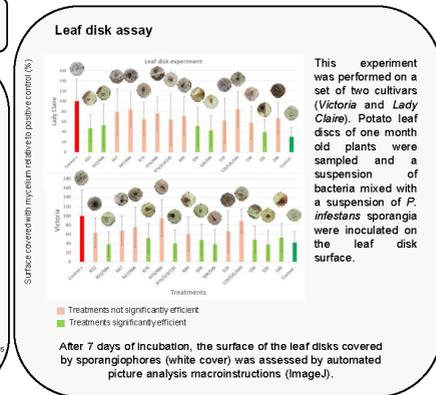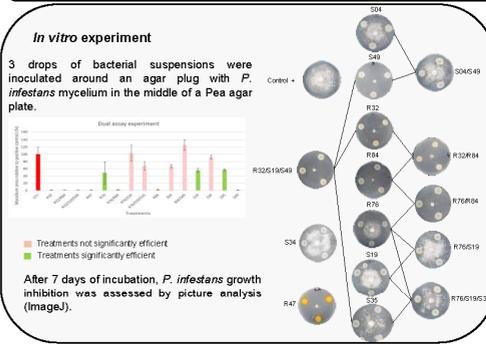
Aim of the study

With the idea that some bacteria can protect plants against disease, potentialy synergistic interactions between native potato-associated *Pseudomonas* have been investigated as potential anti-oomycete agents against *P. infestans*.

Methods and results

9 native potato-associated *Pseudomonas* have been isolated, selected from an extensive strain collection and characterized according to their potential inhibitory effect against *P. infestans*.

a) Isolation

Radial growth of *P. infestans* mycelium on Petri dishes.

(b) Screening of strains for activity against *P. infestans*

Control — Phyllosphere strain — Rhizosphere strain

Selection of 9 *Pseudomonas* strains according to their diversity of inhibition activity (mycelial growth, sporangia and zoospore production).

c) Targeting different cycle stages of *P. Infestans*

Identification of inhibitory substances produced by bacteria strains.
➢ VOCs: volatile organic compounds.
➢ Soluble compounds (antibiotics, siderophores, cyclic lipopeptides).

For the following experiments, all possible single, double and triple combinations between 9 selected strains have been screened for their activity against *P. infestans* by means of *in vitro* experiments and a leaf disk assay.

In vitro experiment

3 drops of bacterial suspensions were inoculated around an agar plug with *P. infestans* mycelium in the middle of a Pea agar plate.

■ Treatments not significantly efficient
■ Treatments significantly efficient

After 7 days of incubation, *P. infestans* growth inhibition was assessed by picture analysis (ImageJ).

Leaf disk assay

■ Treatments not significantly efficient
■ Treatments significantly efficient

After 7 days of incubation, the surface of the leaf disks covered by sporangiophores (white cover) was assessed by automated picture analysis macroinstructions (ImageJ).

This experiment was performed on a set of two cultivars (*Victoria* and *Lady Claire*). Potato leaf discs of one month old plants were sampled and a suspension of bacteria mixed with a suspension of *P. infestans* sporangia were inoculated on the leaf disk surface.

Conclusion

Some of the treatments including *Pseudomonas* combinations showed significant inhibition against *P. Infestans* mycelial *in vitro* and *in vivo*. However, the efficient combinations *in vitro* were generally not the same as the ones *in vivo*. This could be explained by the complexity of the microbiome present natively on the potato leaf disks or the substrates used for bacteria inoculation. Others combinations were not efficient at all compared to single strains which could show antagonistic interactions between some bacterial strains. On the other hand, some combinations in the leaf disc assay showed promising effects which could be due to synergistic interactions.

Outlook

Promising strains combinations will be further investigated in a field trial. The interactions between strain combinations and *P. infestans* could also be tested using simultaneous imaging of fluorescently tagged populations. This approach will allow us to have a better understanding of the interaction of *Pseudomonas* with *P. Infestans* in the phyllosphere, which could lead to the application of new biocontrol strategies in a context of sustainable crop protection.

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The influence of crop rotation and cultivar resistance on the onset of early blight (*Alternaria solani*)

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The influence of crop rotation and cultivar resistance on the onset of early blight (*Alternaria solani*)

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INTRODUCTION

- Early blight (EB) (*Alternaria solani*) is an important disease in potato production worldwide..
- Under favourable conditions EB can cause significant yield losses ranging between 20-50% [1] .
- The influence of the crop rotation and cultivar resistance on the onset EB has not be extensively.

Objectives

- To determine the influence cropping history and cultivar resistance on the occurrence EB.

Hypotheses

1. At least two years without potato will delay the onset of EB.
2. Continuous potato on the same field will lead to earlier attacks as fields that were planted with potato in the previous year. regardless of whether potato was grown continuously or intermittently.
3. Cultivar resistance has no influence on the onset of EB.

Materials & Methods

Experimental design & Cultural practices

- The field experiments were conducted during 2016 growing season at Flakkebjerg Research center, Denmark.
- The experiment was designed RCBD arranged as a split plot design The main plot factor was 6 fields with different cropping histories (Table 1).
- The sub-plot factors was 3 potato cultivars (Agata, Sava and Kuras) with different levels of susceptibility to early blight.
- The cultivars Agata, Sava, and Kuras have been classified as very susceptible, moderately slow blighting and susceptible and slow blighting respectively (Abuley, Unpublished data).
- Certified seed tubers of the cultivars were planted on 11th May, 2016.
- Late blight (*Phytophthora infestans*) was controlled with 150gha-1 mandipropamid (applied as Revus 250g, mandipropamid L-1 SC, Syngenta)

Assessment

- Assessment of the potato plants in the plots for typical early blight symptoms started from 50% emergence of the potatoes.
- Assessments continued every three days until early blight lesions were observed.
- The onset of EB on the potatoes was expressed as days after emergence (DAE).

Statistical analyses

- To test for the effect of cultivar, field and their interaction on the onset of early blight, the onset of early blight expressed in DAE to permutation test.
- The permutation test was performed using the "aovp" function from the "ImPerm" package [2] in R statistical language [3].
- Post-hoc analysis using Tukey honest significance difference (HSD) (p=0.05)

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[2] WHEELER , B. & TORCHIANO, M. 2016. ImPermPermuation test for Linear Models. R package version 2.1.0. ed.
[3] R core team 2016. R: A Language and Environment for Statistical Computing. 3.1.1 ed. Vienna, Austria.

Table 1. Cropping history of field fields used

Field	Cropping history[a]	Previous Crop[b]	Early Blight[c]
P0	No potatoes > 5 years	Fallow	None
P1	2015 (0)	Potato	High
P2	2014 (1)	Barley	High
P3	2013 (2)	Barley	High
P4	2012 (3)	Barley	High
P5	Continuous rotation (0)	Potato	Low

[a]the years represent the last time potato was grown the field and the number of years without potato in parenthesis. [b]the crops that were grown during the potato free years. [c]level of early blight attack at the last time potato was grown on the field: >50% foliar attack =high, 6-49% foliar attach= moderate, ≤5% foliar attack =low; none = no record of early blight.

Results & Discussion

- There was no significant effect of variety on the onset of EB (p=0.347).
- There was no significant effect of Cultivar X field interaction (p=0.867).
- The effect of the rotation field was significant on the onset of EB (p<0.001).
- EB occurred earlier when the interval between subsequent potatoes was less than two years (Figure 1).
- The earliest time for symptoms to show was on the field that was just preceded by potatoes in 2015 (P1) (Figure 1).
- Even though it was expected that EB will occur earlier on the continuous rotation field (P5), the result in Figure 1 showed that EB occurred later on P5 than on the P1 field.
- This could be due the low attack of EB on the P5 fields in previous years (Table 1).
- For the fields that had no potato for two or more years EB occurred at the same time as the fields without potato for long time (figure 1).
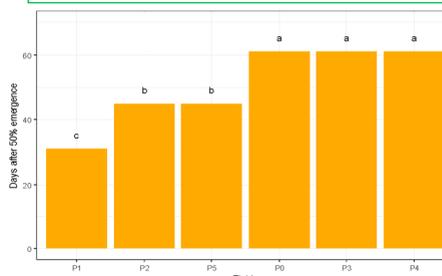

Figure 1. Onset of early blight on fields with different histories with potato. Bars followed by the same letters are not significantly different (Tukey HSD, p=0.05).

Conclusion

- We can conclude the choice variety was not important in delaying the onset of early blight.
- At least 2 years potato free was enough to markedly delay the onset of early blight.
- Planting potato after potato increased the chance of early onset of EB but this also was dependent on the previous severity of EB. Thus for fields with low incidence of early blight, crop rotation may be of little significance.

Monitoring of the SDHI Mutations of *Alternaria solani* in Serbia

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5. 2 Monitoring of the SDHI Mutations of *Alternaria solani* in Serbia

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INTRODUCTION

- **In recent years**, the increasing awareness of the fungicide resistance united many scientists and countries to different strategies to obtain necessary data network required for establishing **a programme to effectively monitor fungicide resistance development** for economically important pathogens

- *Alternaria solani*, causal agent of early blight of potato, has been described as foliar disease which can cause significant yield loses if left uncontrolled (Figure 1)

- **Resistance of *A. solani*** on fungicides from the SDHI group was detected in **2009** in **United States** only four years after registration
- In **2014** reduced sensitivity were detected in Europe: **Netherlands** and **Belgium**
- In **2015**, in **Netherlands**, **Belgium**, **Germany** and **Denmark**
- In **2016**, low frequency of resistant isolates was detected in **Poland, Denmark, Italy, Romania** and **United Kingdom** while moderate frequency was detected in **Belgium, Germany, Netherlands** and **Sweden**

- In **2016**, in Serbia, potato covered around **40.000 ha** with, average production of early potato **30-35 t/ha** and **25 t/ha** for late commercial potato. Potato growing regions are mostly in central and south of Serbia (Leskovac and Čačak)

The objectives of this research were to detect and characterize fungicide sensitivity in populations of *A. solani* in Serbia to the commonly used SDHIs boscalid.

Figure 1. Potato plants showing the symptoms of early blight

MATERIALS AND METHODS

Isolation
- Infected leaf samples showing the typical symptoms were examined under the stereomicroskop. Alternaria cultures were identified on the basis of morphological and cultural characteristics and pathogenicity. Isolates were obtained by single spore method of isolation and transferred to V8 medium for 7 days at 23 °C with a 12 h photoperiod

***In vitro* assay**
- Fungicide sensitivity was evaluated as the concentration of techinical grade boscalid (98%) at which germination was inhibited by 50% (EC$_{50}$) compared with growth of nonamended media. Isolates were classified into categories based on sensitivity (Avenot et al., 2008; Gudmestad et al., 2013)
- All statistic calculations were conducted in program IBM SPSS STATISTICS v20

DNA extraction and PCR Method
- Approximately 20 mg of dry weight mycelium were collected and used for DNA extraction according to the manufacturer's instructions of DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA)
- All isolates were used for molecular detection of SDHI resistant isolates. For monitoring the mutations in the SDH complex which lead to resistance, previosly designed primers and multiplex PCR was conducted (Malik et al. 2013)

RESULTS

- In 2016, **91** monosporial isolates at **7** locations of commercial potato crops were examined:

- **26,4 %** of isolates did **not** carry any known mutations in **AaSDHB, AaSDHC** or **AaSDHD**
- **3,3 %** of isolates carried amino acid substitution in **AaSDHD**
- **63,74 %** of isolates carried amino acid substitution in **AaSDHC**
- **11 %** of isolates carried amino acid substitution in **AaSDHB**

- Based on *in vitro* assay and molecular diagnosis three different phenotypes could be posted (Table 1).
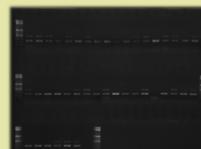

Figure 2. Polymerase chain reaction (PCR) of *A. solani* isolates with primers pairs specific for screening isolates with exchanges in AaSdhB (amplification band of 235 bp, or none), AaSdhC (amplification band of 457 + 235 bp) and AaSdhD (amplification band of 235 +72 bp)

Table 1. EC$_{50}$ values of *in vitro* boscalid assay and assocciated phenotypes of *A. solani* isolates

Class	Phenotype	Mean EC$_{50}$ values (µg/ml)
1	Sensitive	0.2
2	Low level of resistance	8.5
3	Moderate level resistance	17.7

Figure 3. Percentages of *A.solani* isolates carying the mutations in the AaSdhB, AaSdhC and AaSdhD gene

CONCLUSION

As we know, before 2016, monitoring of *A. solani* isolates have not been done in Serbia. The research provided new information about the occurence of SDHI mutations responsible for sensitivity shift of *A. solani* isolates from potato plants in several locations in Serbia. These findings will certantly be valuable for informing and supporting preventive and curative treatments on the emergence of resistance in Serbia.

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Studies about infection of different *Alternaria solani* isolates on *Solanum tuberosum*, *Lycopersicon esculentum* and *Solanum nigrum*

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Studies about infection of different *Alternaria solani* isolates on *Solanum tuberosum*, *Lycopersicon esculentum* and *Solanum nigrum*

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Aim of the work

Early Blight caused by *Alternaria solani* is a highly destructive disease of potatoes and tomatoes. In this study species of the family *Solanaceae* named *Solanum tuberosum* with the cultivars 'Kuras' and 'Maxilla', *Lycopersicon esculentum* cultivars 'Harzfeuer' and 'Bocati' and *Solanum nigrum* were tested on occurred leaf blight. The aim of this work was to study the susceptibility of *Solanum tuberosum*, *Lycopersicon esculentum* and *Solanum nigrum*.

Material und Methods

The plants of the family *Solanaceae* (*Solanum tuberosum* cv 'Kuras' and 'Maxilla'; *Lycopersicon esculentum* cv 'Harzfeuer' and 'Bocati'; *Solanum nigrum*) were cultivated in the greenhouse. 6 weeks old plants were inoculated with a mixture of four different *Alternaria solani* isolates (spray inoculation, spore density: 10 x 10^4 spores/ml). Inoculated plants were kept in a humidity chamber for 48 h at 20 °C and 100% rel. humidity. The disease was rated according to Granowsky und Peterson (1954) in a daily interval.

Results

Two days after inoculation first symptoms on the different plants were visible (fig 1). There was a disease progression untill 8 days post inoculation. Afterwards the progression slowed down. In the greenhouse trial *Solanum tuberosum* showed a higher susceptibility than *Lycopersicon esculentum*. Interestingly the black nightshade (*Solanum nigrum*) was also very susceptible.

The tested genotypes of tomato (Harzfeuer and Bogati) and potato (Maxilla and Kuras) showed different susceptibility to *Alternaria solani*.
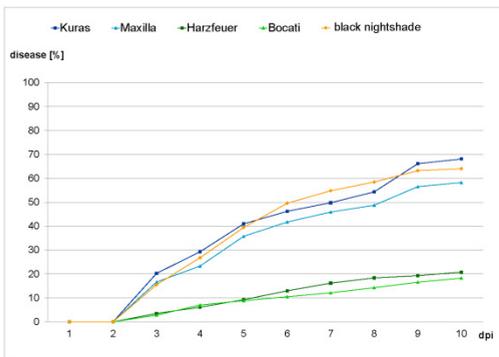

fig. 1: Early blight disease progression of *Solanum tuberosum (cv 'Kuras' und 'Maxilla') Lycopersicon esculentum (cv 'Harzfeuer' and 'Bocati') and Solanum nigrum* in a greenhouse trial

fig. 2: *Alternaria solani* infected leaf of *Solanum nigrum*, natural infection in the field

Summary

The fungus *Alternaria solani* causing early blight on potatoes and tomatoes also infects black nightshade (*Solanum nigrum*). The infection of *Solanum nigrum* is important regarding the biology of the fungus - infected black nightshade plants during the crop rotation increase the soil borne inoculum of *Alternaria solani* and can therefore influence the disease progression on the potato crop.

Incidence of the F129L mutation in Serbian *A. solani* population

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Incidence of the F129L mutation in Serbian *A. solani* population

5.4

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INTRODUCTION

Potato early blight occurs worldwide and strobilurines (QoIs) are frequently used in their control. The presence of the F129L mutations were revealed in *Alternaria solani* populations in different European countries. In 2016, *A. solani* isolates obtained from various commercial potato fields in Serbia, were tested for presence of the F129L mutations.

Genotype II

Genotype I

46%

54%

Fig. 1. Distribution of genotypes I and II in Serbian *A. solani* population

Mutant

Wild type

16%

84%

Fig. 2. Frequency of F129L mutation in *A. solani* genotype I isolates

MATERIAL AND METHODS

During 2016, potato leaves with early blight lesions were collected from different Serbian potato growing areas. Isolates were obtained via single spore isolation directly from diseased tissue and transferred to petri plates containing V8 medium. Genomic DNA of *A. solani* isolates was extracted from mycelia cultivated on V8 medium for 14 days at 21°C. Mycelium were carefully scraped off and ground in liquid nitrogen. Genomic DNA extraction was carried out using the DNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's instructions. Two different primer sets were used for genotypes identification. All isolates were screened for presence of mutations on cytochrome B gene by DNA fragments sequencing.

RESULTS

A. solani isolates obtained from various locations in the Serbia, according to their *cytb* gene structure were identified as two different genotypes (Fig. 1). In the Serbian *A. solani* populations, the F129L mutation was identified in both genotypes. Within the genotype I wild type of strain were dominated with 84% of strains, until the F129L mutation was found in some 16% of strains (Fig. 2). Sequence analysis revealed the F129L mutation also in genotype II isolates, where it occurred in 81% (Fig. 3).
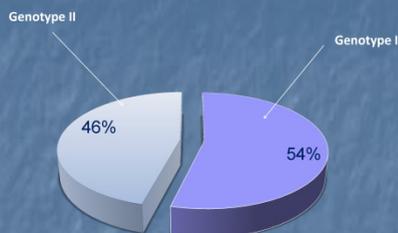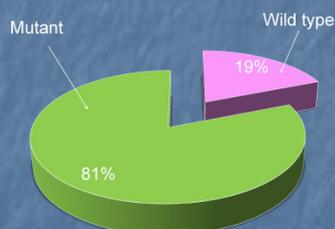
Mutant

Wild type

19%

81%

Fig. 3. Frequency of F129L mutation in *A. solani* genotype II isolates

DISCUSSION AND CONLUSION

Our results suggest that after the survey of *A. solani* in Serbia, two different genotypes were detected among the investigated isolates. After the screening for the presence of the F129L mutation in the cytochrome b gene, mutants are present in both genotypes. The F129L mutation in *A. solani* occured in 46% of the isolates. This indicates on intensive application of QoIs in early blight control, which may contribute to decreasing of the fungicide efficacy. Intensive further monitoring of mutant presence in *Alternaria* population is necessary for improving control strategy for this pathogen.

# Digital diagnostics of potato diseases

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Digital diagnostics of potato diseases

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The need for proper diagnostics

Diagnostics by visual inspection is difficult, leading to many ineffective treatments in the field. Can you name the diseases on the leaflets below? Laboratories may not be available or fast enough to provide the answer. Digital diagnostics using smartphones and analysis software may provide the solution.
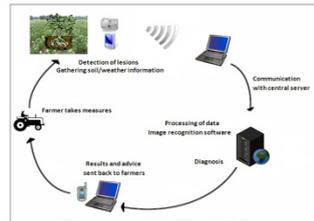
Development & validation of digital diagnostics

- App, hardware and analysis software were developed
- Samples were photographed using hardware & app
- Samples were analyzed in our laboratory to validate
- Based on over 15.000 lesions; algorithms were designed for the analysis software
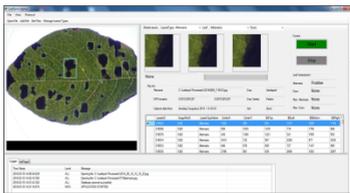

Key observations

- 71% of lesions not caused by microbes (pollution, spray damage etc.), yet many are treated as such.
- Lesion size, colour, shape, number and symmetry are decisive in diagnostics
- Cultivar and soiltype also influence outcome
- 98% similarity between digital and classical diagnostics

The use for digital diagnostics

In the coming season we will test the system with various users and expect to go live in 2018. Expansion to other crops and regions is anticipated.

Diagnostics in a laboratory is good, but when speed is important or when no lab is available, digital diagnostics provides a good alternative. Preventing the use of unnecessary pesticides and only acting when needed allows for better integrated crop management.

Cause of lesions on potato (2009-2016)
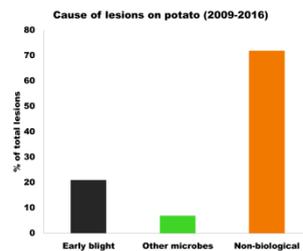
Control of Early and Late Potato Blight: Experiences and views from practice in Denmark

HENRIK PEDERSEN

AKV Langholt, Denmark

In the following, late blight control of potatoes for starch production will be described. Potato varieties for processing and the fresh marked are normally more susceptible to Phytophthora and the preventive treatment is therefore mostly more intensive.

The control strategy is based on one weekly treatment of only two products; Revus (mandipropamid) and Ranman Top (cyazofamid). In Denmark, these fungicides can be used six times each in a season, and this means that Shirlan (fluazinam) will be used 1-2 times, in total 13-14 weekly sprays. In normal or low risk periods, the recommended dose is basically half dose, and in periods with high risk, the dose will be raised to 75 percent of normal dose. Full dose can sometimes be used, but normally farmers will rather mix the lower dose of a preventive product with a full dose of a curative product e.g. Cymoxanil or Proxanil (propamocarb+cymoxanil) in case of visual infections or high infection pressure from the neighboring fields.
To define periods with high or low pressure, the tool "Infection Pressure" in the Danish disease support system "Skimmelstyring" has been developed. It can be used free of charge from the webside: www.landbrugsinfo.dk, where also the registration net for late blight and other tools as daily risk values, weather radar, precipitation, humidity etc. can be found.

At AKV Langholt, we once or twice a week send an e-mail to our potato growers, where we inform about infection incidence and severity, a forecast for infection pressure and our recommended dose and fungicide. More than 90% of the farmers use this information before they make their own decisions.

Infection pressure is calculated as a five-day sum of daily risk hours. However, in the future, we will focus more on the daily risk values. The basic strategy will be: If the risk hours at the day of treatment or the day before are longer than 10 hours, cymoxanil will be added to the basic preventive products, as this curative product has a kickback effect on infections for approximately 24 hours.

Other improvements in the nearest future will be better control of the nitrogen application. We have observed a higher incidence and severity of late blight in fields (and trial plots) with a rapid

new growth of the canopy due to high nitrogen input. Lower application of nitrogen, test for nitrogen content in the petioles, and split application of nitrogen could be one of the solutions.

In the longer term (5-10 years), the change from susceptible to highly resistant varieties will be a way of controlling Phytophthora with less fungicide input. In 2018, five percent of the area of starch potatoes for AKV Langholt will be grown with highly resistant varieties, and onward this area will increase. However, we need to develop a strategy which will delay or protect the single gene resistance in these new varieties.

Early blight is a much bigger challenge. Fungicide resistance and reduced sensitivity against both strobilurins and boscalid (SDHI) is an increasing problem. The occurrence of the mutant type F129L seems now to be the majority all isolates sampled in Danish fields. We know that longer crop rotation and optimal nitrogen application are practical tools which to some degree can be used in the control of early blight but we have still no clear sustainable control strategy both from an economical and efficacy point of view.

A better involvement of the farmers in the decision for both late blight and early blight control is important. They need to be more flexible and willing to deviate from their first choice of variety and fungicide, crop rotation etc. All this to minimize the risk of a breakdown of variety resistances, because this will be our main tool in the prevention of plant diseases and economical loss in European potato production in the future.