EuroBlight
A potato late blight network for Europe

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Preface

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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 17th Workshop was hosted by ADAS in York, United Kingdom.
The Workshop brought together 110 participants from 20 countries from all over the world. Representatives presented the late and early blight epidemics in 2017 and 2018 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 were also included.

The papers and posters presented at the Workshop and discussions in the subgroups are published in these Proceedings, WUR-Special Report no. 19. The current and previous Proceedings are also available on the EuroBlight website www.EuroBlight.net.

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Papers

Epidemics and control of early & late blight, 2017 & 2018 in Europe

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INTRODUCTION

The EuroBlight late blight country profile was launched in 2007 to keep track of the development of early and late blight and its control in Europe in individual countries and over years. This paper reports the development and control of early and late blight in Europe, 2017 and 2018.

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 York special attention was drawn to the collaboration between global networks, and colleagues from North-America, South-America, Africa 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 *Phytophthora infestans,* how these populations evolve, how local strains are spread from one continent to another and how we most effectively can control *P. 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, 2017 and 2018 and thereby describe the foundation for the further insight in the structure and behaviour of the European *P. infestans* (meta) and *Alternaria solani* populations.

LATE BLIGHT COUNTRY REPORTS

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. Information regarding "Date when first infections were reported in more than five conventional, normally planted potato fields" for 2017 and 2018 is shown for all European countries on maps in Figure 1-2.

Estonia

2017: The first outbreaks were recorded in the beginning of July. The first fungicide applications were applied about the same time and mainly systemic fungicides were used. The first outbreaks were recorded when the risk of the development of potato late blight was estimated to be high, the rest of the growing season the risk of late blight was medium.

2018: The late blight risk in 2018 was very low throughout the potato cultivation period. The risk of late blight was higher in the beginning of July and in the beginning of August. In most regions epidemics of late blight did not occur and the crop yield was poor because of the unfavourable weather conditions. The first sprays were conducted in the middle of July, the spraying frequency was very variable according to the region.

Lithuania

2017: Application by fungicides started on the 7th of July. Fungicides were applied with 7 to 11 days intervals. In total, 5 applications were performed. The last application was performed on 11th of August. First disease symptoms were found on 13th of July, therefore first application with fungicide was already sprayed before symptoms appeared. At the beginning, disease development was slow. One week after the first symptoms appearance, disease severity was 0.42% in the untreated control. 15 days after symptoms appearance, disease severity sharply increased and was 26.7% in the untreated control. On 3rd of August, disease severity was 100% in untreated control. Application of fungicides helped to retain significantly less infected plants for a longer period.

2018: Application by fungicides started on the 9th of July. In total, 7 times fungicides were applied. The last application was performed on the 22nd of August. Fungicides were used with around 7-day intervals. At the moment of the first fungicide application, the crop foliage fully covered rows; therefore, suitable conditions prevailed for late blight infection. First late blight symptoms were found on the 25th of July in the trial area. But unfortunately, dry and hot weather conditions did not allow spreading of the disease on much larger scale. In fact, late blight symptoms disappeared in a period of 2 weeks. In August only a few rainy days were recorded, while in September – no significant (>1 mm) rain occurred for three weeks.

Russia

2017: A severe late blight development (yield losses >20%) was observed on potato fields of the Kaliningrad, Leningrad, Vologda, Komi, Tver, Moscow, Murmansk, Kirov, Novgorod, Smolensk, Bryansk, Yaroslavl, Kostroma, Arkhangelsk and Pskov regions. A moderate disease development (yield losses 10-20%) was registered in the Kaluga, Ryazan, Tambov, Belgorod 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, Infinito, Revus Top, Kurzat, and Ridomil Gold MZ. The total number of treatments varied from 3 to 10. Owners of allotment gardens did not use any fungicides. The use of DSSes (Plant-Plus, VNIIFBlight, AGRODOZOR) was rather rare. The most popular potato cultivars were: Red Scarlett, Gala, Udacha, Rosara, Zhukovskiy ranniy, Nevsky, and Impala. The volume of foreign and domestic cultivars used by large agricultural companies was ~80 and 20%, respectively.

2018: A severe late blight development (yield losses >20%) was observed on potato fields of the Kaliningrad, Komi, Tver, Novgorod, Kostroma, Tula, Kaluga, Murmansk regions. A moderate disease development (yield losses 10-20%) was registered in the Leningrad, Vologda, Moscow, Kirov, Smolensk, Bryansk, Yaroslavl, Arkhangelsk, Pskov, Vologda, Ryazan, Ivanovo, Orel, Kursk, and Nizhni Novgorod, Chelabinsc, Sverdlovsk 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, Penncozeb, Infinito, Revus Top, Kurzat, Consento, and Ridomil Gold MZ. The total number of treatments varied from 2 to 7. Owners of allotment gardens did not use any fungicides. The use of DSSes (Plant-Plus, VNIIFBlight, AGRODOZOR) was rather rare. The most popular potato cultivars were: Red Scarlett, Gala, Udacha, Rosara, Nevsky, Impala, and Zhukovskiy ranniy. The volume of foreign and domestic cultivars used by large agricultural companies was ~80 and 20%, respectively.

Poland

2017: The first potato late blight infections in the 2017 season were recorded in the third decade of May and the 1st decade of June. The development of the disease was favored by meteorological conditions prevailing in June (average air temperatures 21°C, rainfall about 60 mm). In July, with a similar amount of average air temperatures, higher amounts of precipitation were observed, which favored a more rapid development of the disease. Higher air temperatures in August and smaller amounts of rainfall created less favorable conditions for the late blight development. The year 2017 can be considered as a year with favorable conditions for the development of the disease. In Bonin (West Pomeranian Voivodeship) in 2017, over 54% higher rainfall was recorded compared to multi-year.

2018: The year 2018 was characterized by high air temperatures and a small amount of rainfall. In Bonin (West Pomeranian Voivodeship) in 2018, the amount of precipitation was reduced by more than half compared to multi-year. Average air temperatures calculated for the growing season (April - September) were higher by around 3°C compared to multi-year. Higher air temperatures and lower amounts of rain were not conducive to the development of late blight in June. More favorable conditions occurred in July when the number of precipitation increased. In August, as in June due to smaller amounts of rainfall and high air temperatures (on average around 23°C) no rapid development of potato blight was observed.

Serbia

2017: Spring of 2017 was warm and wet and due to the favorable conditions, potato plants were planted earlier and the crop development was rapid. The wet conditions were favorable for the disease development and the first warning of late blight occurred at the end of May. From mid of May until the end of June weather conditions were very suitable for *P. infestans* infection. The weather conditions in July and August were very dry, with high temperature and very little rainfall which completely stopped the development of the disease. Potato growers performed from 2 to 5 control treatments during the season.

2018: The potato season 2018 was not easy for potato farmers. High humidity during May and June resulted in early outbreaks of late blight being reported in the potato growing regionsion the south of the country at the end of June. During the summer heavy rainfall and high humidity favored the further development of potato late blight. Due to the prevailing weather conditions, the untreated potato fields were severely infected by late blight at the end of June. First outbreaks were recorded in the fields intensively treated with fungicides on second half of July. The potato growers started with the fungicide treatments at the mid of May. The growers sprayed on average 6-12 times with fungicides during the season.

Romania

2017: The weather conditions were favourable for potato and for foliar late blight development also. Rainfall was close to average during the season in most areas. The first outbreaks in Brasov area was reported in 7 June and subsequently blight was reported in all potato growing areas. A cause of early disease can be the blighted seed tubers due to the high infection pressure of 2016. At the time of the late blight appearance the plants were protected and continued sustained with the treatments, as favorable conditions to the development of the epidemic were met in July. In the majority of crops late blight was well controlled, low levels of tuber blight were reported. Growers used a wide range of active ingredients.

2018: Since March, after exceptional quantities of rain, in April and May was a dry period and potato crops could be established and maintained with difficulty. The first infection in field in Brasov area was found in 3 July. In Harghita area (eastern part of Transylvania in the central part of Romania) in the same period were fields seriously affected by late blight. The disease spread fast in all potato fields and the epidemic pressure was high. In average the potato fields was treated 8-10 times with different fungicides.

Switzerland

2017: During the month of April 2017, it was cool and rainy, even with some snow down to the lowlands. In the first two weeks of May 2017, milder temperatures prevailed and two periods with up to four consecutive main infection and sporulation periods (MISPs) were registered for almost all weather stations considered in the DSS PhyotPRE. The first LB attack was observed on 17th of May in a covered potato field in the central region of Switzerland and late blight epidemic spread over the main potato-growing region. Two longer warm and dry periods in June and beginning of July with temperatures above 30°C reduced sporulation and, therefore, the development of the LB-epidemic. From mid-July, weather conditions were more favourable for *P. infestans* and some late LB-infections occurred. Based on the DSS PhytoPRE and plant protection officers' evaluations, LB-pressure was classified as low in 2017 and farmers could control LB.

2018: In 2018, weather conditions were extraordinary: it was one of the most dry and warm years in Switzerland since the official climate recordings of 1864 (Klimabulletin 2018, MeteoSwiss). Spring started with cool conditions and with some rain. However, from mid-June until November, it was not only exceptionally warm, but also very dry. These exceptionally warm and dry weather conditions also affected the development of the late blight (LB) epidemic: the first LB attacks was observed on 18th May in a covered potato field in the eastern part of Switzerland. At the beginning of June, a LB attack was registered in the central part of Switzerland. Due to several main infection and sporulation periods (MISPs) at the end of May and during the first two weeks of June, the LB epidemic could spread over the potato growing region. However, with the long lasting drought during summer and autumn, LB did not spread further and potatoes suffered more due to the drought or *Alternaria* attack. Overall, LB-pressure was very weak in 2018, and conditions were comparable to 2003 and 2015.

Italy

2017: The potato crop was planted in in mid-April and fully emerged nearly a month later while tomato was transplanted in the first week of May and harvested from August to September depending on the varieties. An unusual mild and dry winter followed a warm spring but rainy events were concentrated in the May. June was dry and July and August were characterized by hot climate with few rainy events (thunderstorms) in mid-July and mid-August. Such dry weather was not favourable for early and late blight infection for most the growing season even when crops have been frequently irrigated in summer. IPI index reached the threshold for the first spray in June and only 1 MISP infection was recorded until July. Then MISP events have been recorded only mid-August and September. Only early blight occurred at the end of the growing season, in July and August, but without causing severe damages on the crops, with disease remaining at low level until harvest. The number of late blight applications were concentrated in May and June on potato, while on tomato they were concentrated from June to the beginning of August. On average, on potato 5 to 7 fungicide applications were carried out.

2018: Climatic conditions, compared with the average, were characterized by the increase of minimum temperature and the decrease of maximum temperature. Climate in April was dry but in May and June were wet. July again was quite dry and rainfalls occurred from mid-August for most of September. The risk of late blight was therefore medium in May and for the first half of June, very low for the rest part of June, July and most of August and turned high during September, when however, most of the tomato crops were harvested. MISP events were concentrated in May and end of August. Late blight occurred on potato in few areas in mid to end of June, while on tomato the first symptom of the disease occurred in August. Early blight occurred at the end of June on potato without causing significant damages and in the second half of August and September due to senescence of the crops. The common disease strategies carried out for late blight and early blight were sufficient to contain the diseases. On average, 6 to 10 fungicide application were carried out.

Finland

2017: The year 2017 was cool and rainy. First outbreaks were detected a little bit later than normal, July 14th and after July 20th several heavy outbreaks were reported also at conventional protected fields. At the end of the season some blight lesions were present at most potato fields. In spite of heavy leaf blight attacks, tuber blight did not cause severe losses. Five to eight fungicide applications were normally used for blight control.

2018: The year 2018 was exceptionally warm and dry. First blight attacks were detected at organic production a little bit later than normal July 14th. However, the blight started to spread as an epidemic only after 10th September at very few locations. In infected fields the blight development was very rapid but most fields remained healthy. At infected fields heavy tuber attacks were observed but mostly also tubers remained healthy. Due to the low infection pressure only few fungicide applications were made but this probably led to heavy late outbreaks in September.

Norway
2017: May and June were a bit wetter than normal, followed by a dry July. Quite a few outbreaks in June, which had above normal number of warnings according to the Nærstad model. August and September were also quite wet, and in many areas a lot of late blight was seen at the end of the season despite a normal, weekly spray schedule. For most of the potato growing regions in Norway, 2017 was a year a bit worse than average concerning late blight.

2018: May, June and July were very warm in most parts of Norway, and in some areas there were no warnings issued at all for the whole of July. The normal number of warnings according to the Nærstad model in eastern Norway was 0-4 for both June and July. On average, the first treatment was applied 2-3 weeks later than normal due to warm and dry conditions. Some growers chose a longer spray interval (14 days) than normal, without having problems with late blight. From the middle of August there was quite a bit of rain, and also September was more normal concerning weather and late blight warnings. Some disease was seen in late August and September.

Sweden
2017: In south of Sweden, the planting was done late and there were reports of frost damage. The late blight reports came in early July. In Mid-Sweden, planting time was normal with a good crop establishment. In late June and early July there were some reports of late blight in this region. In all of Sweden the wet autumn resulted in a difficult harvest, with crops left unharvested due to wet field conditions. In the southern part of Sweden there were some problems in controlling late blight, while a normal or less than usual use of fungicides gave complete control in Mid-Sweden.

2018: A cold spring led in south Sweden to late planting, followed by change to warm weather with a good crop development. The summer was extremely dry and the few reports of late blight came very late in the season, late July and August.

Denmark
2017: Generally, the weather in 2017 during the potato-growing season was favourable (many rainy days, high humidity) for the development of late blight. Some fields with indications of infections from oospores. Early attacks were recorded on 9 June and many fields were infected a week later. There was a severe development of late blight in Denmark, especially during late July and August. During the first week of September, most unsprayed plots were totally defoliated by late blight in Denmark. Unexpectedly, very few tuber infections were seen in 2017. Different strategies with different fungicides such as Ranman Top, Revus, Proxanil, and Cymbal were used in 2017. The best protection, with 95% control, of late blight was when Cymbal (0.25 kg/ha) + Ranman Top (0.5 l/ha) was sprayed three times. Spraying Proxanil (2.5 l/ha) +Ranman Top (0.25 l/ha) twice in the season also gave 95% control of late blight. A weekly spray of Revus (0.25 l/ha) gave 79% control of late blight.

2018: In the Danish surveillance network, the first symptoms of late blight were recorded on leaves and stems on the 8 June. However, high temperatures and low humidity characterized the weather conditions subsequent to the onset of the first symptoms. Widely spread attacks in more than five conventional fields were found a month later. Dry and hot weather stopped the attacks effectively until the second half of August, when the rain started from 11-13 of August. This revived the development of late blight perhaps from inoculum that survived in the stems of the potato crops from previous infections in July. The following fungicides were used singly or in combinations to control late blight: Ranman Top, Revus, Proxanil, Cymbal, Zorvec Enicade and Curzate M WG. Due to the low late blight attack, all fungicide treatments resulted in more than 95% late blight control in 2018. Accordingly, we did not see significant differences in yield (both starch and tuber) of the fungicide strategies.

France
2017: After a very dry and moderately cold winter, the spring climate allowed fairly early planting in good soil structures. The emergence was fast and regular. A very dry and hot period followed after emergence in May up to the beginning of August. In most of France, weather conditions remained dry until early August, generating very high irrigation water requirements. The late blight pressure has been low until this date even though some fields with irrigation and/or thunderstorms may have seen some occasional outbreaks of late blight in June. The late blight pressure was very low and started late in August with a medium or high level according to the regions. 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). The risk of tubers contamination at the end of the season was a medium level. In spite of the dry and hot weather, the Alternaria risk remained at a low level, except at the end of the season, shortly before the haulm killing resulting in very low damages.

2018: After a winter with very mild temperatures and quite high rainfall, potato planting was possible from the middle of April to the middle of May. The emergence was fast and regular. The late blight pressure started late in May or early in June and was extremely high up to middle of June. At the middle of June, many fields showed LB symptoms in most of France. After this period, the LB pressure decreased more or less quickly to low level according to the areas. The weather conditions very hot and dry from the end of June to the middle of August allowed a good control of the LB epidemic. From the middle of August, the weather conditions became wetter and milder allowing a restart of the epidemic, especially in the fields with stem blight symptoms or irrigated fields. A few fields with tuber blight were observed in September. In spite of the dry and hot weather, the Alternaria risk remained at a low level, except at the end of the season, shortly before the haulm killing resulting in very low damages.

Belgium
2017: The month of March was exceptionally warm and dry, marking the beginning of a very dry and also mild spring season. This weather clearly had an impact on the occurrence of late blight – or better, its absence. Moreover, an unexpected late frost period by the end of April not only caused damage to a lot of crops, but also helped to get rid of early volunteer plants (mostly on cull piles). The absence of inoculum sources in combination with extreme and persistent drought led to an exceptional low disease pressure until the beginning of July. Sporadic rainfall from July onwards caused some infection periods, and lesions were observed on volunteer plants towards the end of the month, and in some potato fields from the beginning of August. Regular precipitation periods in August caused almost daily infection opportunities; as a result, disease

was observed here and there in ware potatoes in the second half of this month. The weather was reflected in the advice for growers: spraying interval was 12 days and more until the end of June, 8 days in July and less than 7 days in August.

2018: The year 2018 was exceptional in climatological terms, being the warmest ever, with the warmest summer since the start of observations, a very low number of days with rain, a huge rainfall deficit and extremely high sunshine duration. In the early spring however, this was not yet obvious, on the contrary. A very cold and wet month of March caused a late start of the planting season. From the second week of April, temperatures raised markedly, and together with a very warm month of May it still became a warm spring season. It also became more and more dry, and from June on (and this until August) an exceptional drought period was recorded. Before this drought period however, late blight disease benefited from the presence of inoculum sources (mostly on cull piles, from April 24th) and some conducive weather for infection to develop rather strongly. This caused attacks in some fields early in June, and a strong expansion by mid-June. From then on however, disease pressure dropped to an exceptional low level, allowing for (very) long spray intervals. Only with the return of modest rainfall from August 10, crop growth resumed somewhat, and so did sprayings against both late and early blight. The interval was 6 days in June, 18 days in July, 8 days in August and dropping to 7 days towards the end of the season.

The Netherlands
2017: The last two weeks of February had a short period of frost. The potatoes were planted at a normal time, mostly during the first two weeks of April. The months April, May and June were very sunny and the temperatures in May and June were two or more degrees higher than the 30 year average. The start of the season was dry and in June many growers started irrigating their fields. The second half of the growing season the weather was warm but more humid and changeable weather. Especially during the last weeks of August the conditions for blight were favourable. In some regions we received reports of aggressive attacks which were hard to control.

2018: In the Northern part of the country most growers were able to plant at a normal time (first weeks of April) but to the south most field were planted end of April and the first weeks of May. In this region we also had some heavy rain showers during the first weeks after planting. In many fields the potatoes on lower parts of the field didn't emerge because of flooding. The summer of 2018 was record breaking. High temperatures and much sunshine during the whole summer. In the beginning of August the national average precipitation deficit was 300 mm, significantly greater than the 280 mm experienced in 1976, the year with the previous highest deficit. Where possible farmers were irrigating their fields to keep the potatoes growing. After a few weeks of changeable weather at the end of August attacks of Phytophthora were found in production fields with late regrowth.

Germany
2017: The weather condition and the late blight disease progression were diverse across Germany in 2017. The Northern and Western part had a normal late blight epidemic. In the Southern part it was warm and dry during the summer months therefore early blight was a major problem. The crop emergence was normal (5 -20 May). The first outbreak of late blight was recorded end of May. Attacks in conventional fields were found late May to early June. The further development of late blight was completely different. In the Northern parts of Germany there were favourable weather conditions for the late blight development. The disease pressure was high till end of August. In the Southern part only a few infection periods were observed in

July and August. The use of fungicides was normal in 2017. All kind of products were used; especially mixtures were used in the Northern part of Germany. In the southern part the fungicide treatments mainly focussed to control early blight.

2018: Crops were planted in good conditions but the crop emergence was normal from 3 to 25 May. The first outbreak of late blight in potatoes was recorded by end of May (late in comparison to the previous years). Attacks in different regions and ware potatoes were found in June. Due to the very dry and very hot period between May and August the weather conditions for the development of late blight were very unfavourable. In some regions it was even too dry (only a few hours of leaf wetness during the night was recorded) for the development of early blight (*Alternaria solani*). The number of fungicide treatments was lower than normal. Several farmers focused the fungicide treatments (choice of product and spray interval) on early blight control. Attacks of early blight especially in irrigated fields seemed to be an increasing problem in several potato growing areas.

Scotland
2017: For recent reports up to 2016 the average monthly risk for Scotland was estimated using Met Office Smith Period information from the same seven stations. This was no longer possible from 2017 onwards. Instead the average monthly blight weather risk for Scotland was estimated using BlightWatch Hutton Period information from 10 stations. It's important to note that comparisons of estimated risk from 2017 onwards compared with before 2017 are not valid. In 2017 the average number of Hutton Periods per station was 0.9, 2.5, 4.6, 4.8 and 3.7 in May, June, July, August and September respectively. In 2017 the first late blight outbreaks were both detected on 29 June, in postcode AB30 (crop) and IV8 (outgrade pile). In total there were 40 confirmed outbreaks reported on the AHDB Potatoes-funded blight outbreak maps for Scotland. The progression of crop outbreaks (26 in number) in Scotland was 0% in May, 19% in June, 27% in July, 54% in August and 0% in September There were two confirmed outbreaks on outgrade piles of potatoes (29 June & 30 August) and three outbreaks on volunteers (8 & 9 August and 23 October). There was a high number of confirmed outbreaks in gardens: two in July (7 & 25), four in August (15, 24, 28 & 29) and one in September (6). The last sample was submitted on 23 October.

2018: There were on average 1.1, 2.4, 3.1, 4.6 and 1.0 Hutton Periods per station per month for May to September respectively. The similar risk profile for May to August in both years was not very accurately reflected in the number of confirmed blight outbreaks in crops. The number of confirmed outbreaks of late blight in Scotland was low, the number in crops was very low. The first outbreak (the only crop outbreak) was in a seed crop on 14 August in postcode DD9. Only 10 confirmed outbreaks were reported on the AHDB Potatoes-funded blight outbreak maps, up until 25 September 2018 when the last sample was submitted. The progression of natural infection outbreaks in Scotland was two in August (seed crop and trial plots) and eight in September (four on volunteers [7, 7, 18 and 18 Sep], three in gardens [5, 8 and 25 Sep] and one in trial plots).

England & Wales
2017: One hundred and forty five outbreaks of late blight were reported in 2017 as part of the AHDB Potatoes funded Fight against Blight outbreak maps. One outbreak was reported in April, one in June, 21 in July, 59 in August, 38 in September, 16 in October and one in November. Nine of these outbreaks were from outgrade piles and reported from April to October. General epidemic onset was late, with the earliest outbreak in England and Wales in a commercial crop reported on

5 July in Yorkshire in the north of England. Thirty-three outbreaks were reported on volunteers: 1 in July, 8 in August, 9 in September, 14 in October and 1 in November. Fungicide programmes were well underway by the time the epidemic started so control was generally good. This was the first year that potential issues with late blight control and fluazinam were reported. It was also the year when 37_A2 was more widely reported, particularly in the west and midlands of England. The most frequently applied active ingredients were mancozeb/cymoxanil, fluazinam, cyazofamid, mandipropamid and cymoxanil to both seed and ware crops.

2018: Seventy-eight outbreaks of late blight were reported in 2018 as part of the AHDB Potatoes funded Fight against Blight outbreak maps. Three outbreaks were reported in April, one May, 12 in June, 10 in July, 22 in August, 17 in September, 12 in October and one in November. Three of the outbreaks were on outgrade piles. All of these were reported in April in the South East of England. Few outbreaks were reported until June. The earliest outbreak in England and Wales in a commercial crop reported on 30 May in Pembrokeshire in Wales. The majority of outbreaks were reported in crops. Seven outbreaks were on volunteers and three on outgrade piles. Fungicide programmes were well underway by the time the epidemic started so control was generally good. Weather was less favourable for disease development during July. Fewer issues with poor control of late blight were reported and agronomists reported using less fluazinam in fungicide programmes. 37_A2 was reported in the west of England, Kent and Norfolk and 36_A2, which is not associated with fungicide resistance, was reported in the east of England and Kent. The most frequently applied active ingredients were mancozeb/cymoxanil, fluazinam, cyazofamid, mandipropamid and cymoxanil to both seed and ware crops.

Ireland

2017: Late blight infections were reported from late June in 2017. This followed a period of weather in late May and early June very favourable to the spread of late blight. However, even though infections were reported in most instances in stems when the crop was in canopy expansion, the application of fungicides in most instances was able to halt the disease. Furthermore although weather conditions remained favourable for the spread of the disease throughout the summer, limited outbreaks of the disease was reported. This is most likely due to the fact that intensive fungicide regimes were applied. Limited tuber blight was reported.

2018: Due a prolonged period of dry weather from late May – mid-late July extremely low levels of late blight were reported. In untreated trial plots natural infection was not reported until late August. Coupled with the low disease pressure, in most instances planting of the main crop was considerably later than normal due to unfavourable planting conditions.

EARLY ATTACKS OF LATE BLIGHT

The dates of the first observations of late blight in more than 5 conventional, normally planted potato fields in 2017 and 2018 are presented in Figure 1 and 2. In Figure 3 the Late Blight weather in May, June, July and August 2017 and 2018 is indicated with yellow (low risk), orange (medium risk) and red (high risk).

In Figure 4 and 5 the dates in 2017 and 2018 are presented when attack was recorded for the first time (blue circles) and when attack was observed in 5 or more conventional fields (red triangles).

In Figure 6 the dates are presented when attacks were recorded in 5 or more conventional fields in 2017 (blue triangles) and in 2018 (red triangles).
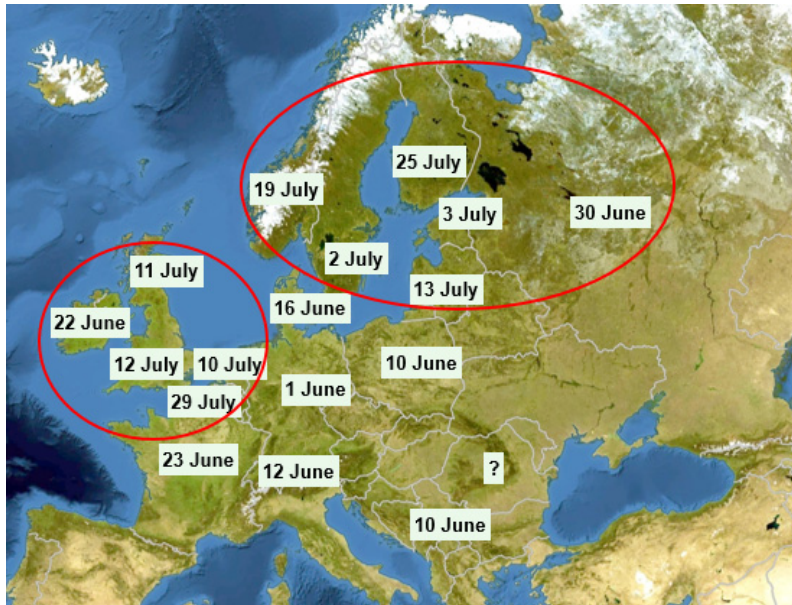
Figure 1. *Date of first observation of late blight in more than 5 conventional, normally planted potato fields, 2017*

Figure 2. *Date of first observation of late blight in more than 5 conventional, normally planted potato fields, 2018*
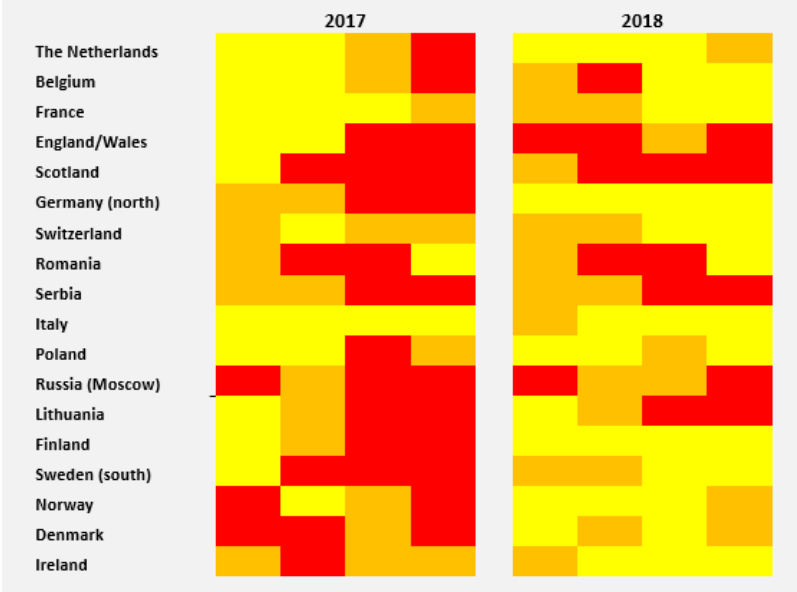
Figure 3. Blight weather in May, June, July and August 2017 and 2018. Low (yellow), medium (orange), high (red) risk
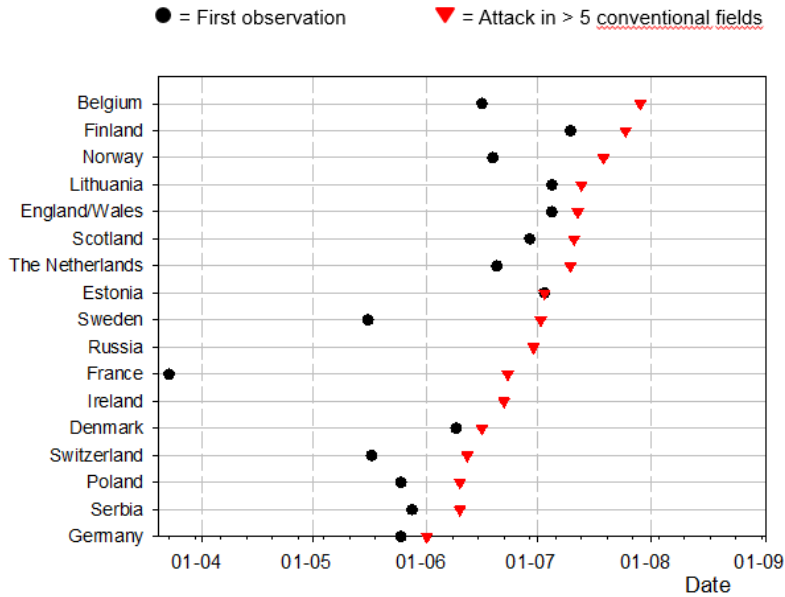
Figure 4. Dates in 2017 when attack was recorded for the first time (blue circles) and when attack was observed in 5 or more conventional fields (red triangles)
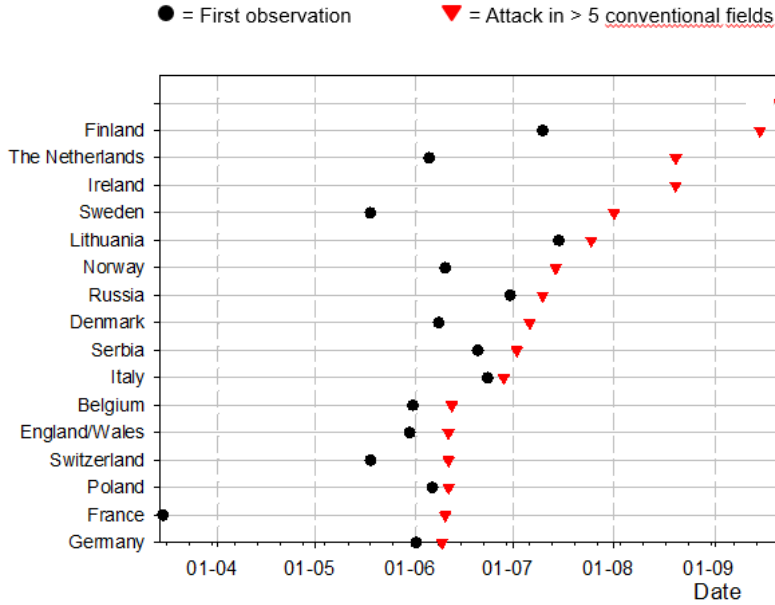
Figure 5. *Dates in 2018 when attack was recorded for the first time (blue circles) and when attack was observed in 5 or more conventional fields (red triangles)*
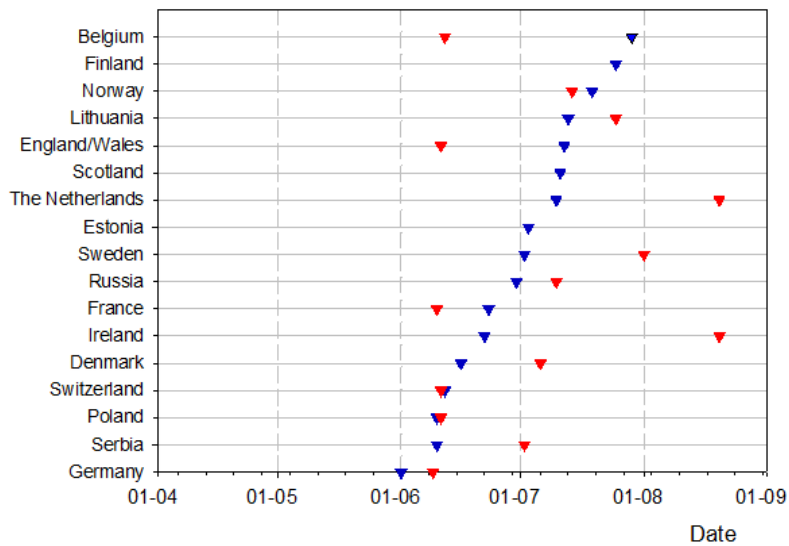
Figure 6. *Dates when attacks were recorded in 5 or more conventional fields in 2017 (blue triangles) and in 2018 (red triangles)*

TUBER BLIGHT

The level of tuber blight in 2017 was reported as low in many countries in Europe, except for some regions in Sweden, Russia, Romania, France, Belgium and Germany where it was reported as medium to high (Figure 7).
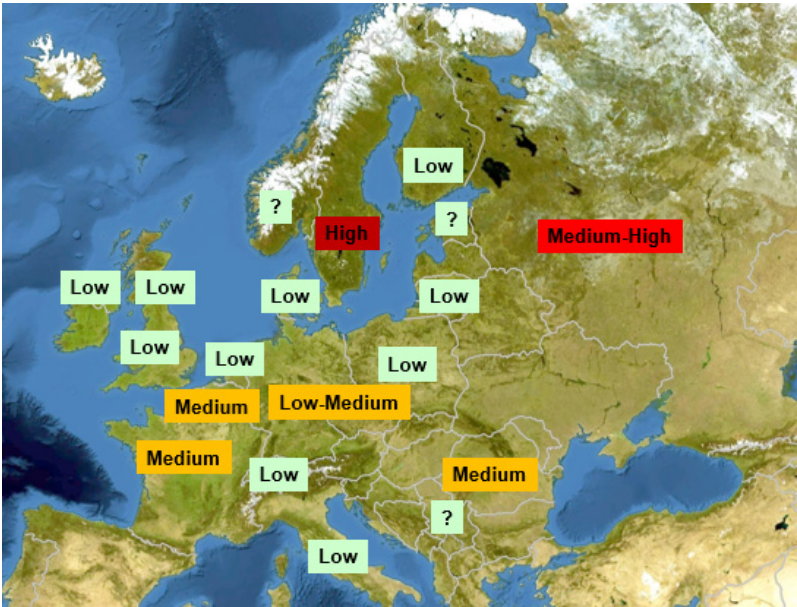
Figure 7. *The level of tuber blight attacks (low, medium or high) in 2017 compared to normal*

INDICATIONS OF OOSPORES

In 2017 infections caused by oospores were reported in Sweden, Finland, Denmark, Estonia and Lithuania (Figure 8).
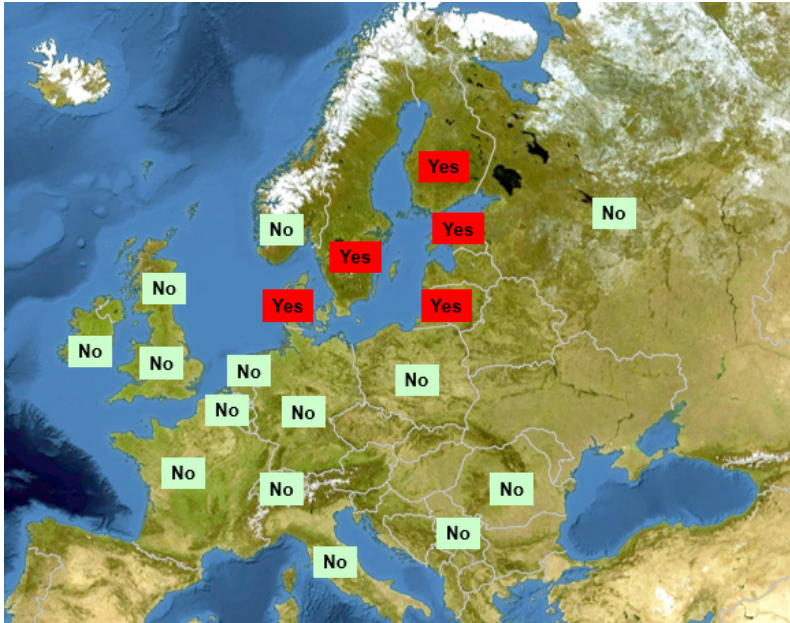
Figure 8. *Indications of oospores in Europe in 2017*

FUNGICIDES AND CONTROL STRATEGIES

In **Estonia** the sprays are mostly conducted based on the weather conditions. Systemic fungicides are used for the first sprays, later contact fungicides are used. The spraying is mostly conducted every 10 days, more often when the weather conditions stay cool and humid. In average the fields are sprayed 5 times per season. In **Lithuania** on average four to six fungicide applications are common practice. First and sometimes second applications are done with contact fungicides. The following two applications are done with systemic fungicides, and followed by one or two applications with contact or translaminar fungicides. In **Russia** in 2017 the average number of fungicide treatments on large agricultural companies was 4-9 for susceptible cultivars and 2-5 for moderate susceptible cultivars. In 2018 the average number of fungicide treatments in large agricultural companies was 3-5 for susceptible cultivars and 2-4 for moderate susceptible cultivars. Farms producing potato for chips used fungicide applications more frequently than other potato-growing farms. The owners of allotment gardens use no fungicides. In **Poland** the number of treatments in 2017 applied against late blight in conventional plantations was up to 5, in seed potato plantations, up to 8 treatments, and in French fries and chips plantations up to 12. In 2018, fewer treatments were applied due to unfavorable conditions for the development of the disease. On fields in the conventional system from 1 to 5, for seed potatoes up to 6. For fries and chips - from 8 to 11. The most common model of controlling late blight was the chemical protection of plants starting at the height of 15-20 centimeters with further continuation. This allowed performing 1-2 preventive treatments. The most commonly applied active ingredients were: propamocarb hydrochloride in mixture with fenamidone, fluopicolide and cymoxanil, metalaxyl + mancozeb, metalaxyl-M + mancozeb, cymoxanil + mancozeb and dimethomorph + mancozeb. Cyazofamid, mandipropamid

+ cymoxanil and cymoxanil + famoxadone were less frequently used. In **Serbia** the most frequently used active ingredients to control late blight were: propineb, propamocarb hydrohloride + fenamidone, mancozeb + cymoxanil, cymoxanil, copper, famoxadone + cymoxanil, metalaxil + mancozeb, cyazofamid, mandipropamid + difenoconazole, dimetomorph + mancozeb, valifenalate + mancozeb, dimetomorph + folpet, ametoctradin+ dimetomorph**,** propamocarb hydrohloride + fluopicolide, difenoconazole. The number of spraying were from two to twelve applications. In **Romania**, an average of 6-10 fungicide applications is a common practice. Usually big farmers do not wait until first symptoms appear and not always the DSS support their decision. The main fungicides applied in ware and seed potatoes are: mefenoxam, propamocarb, fluazinam, cyazofamid, cymoxanil, chlorotalonil.

Also the big farmers, unfortunately and without recommendation make tank mixes of different commercial products. In **Switzerland**, farmers use fungicide applications to control late blight in addition to preventive control measures (certified seeds, less susceptible varieties, ridge quality etc.). At the beginning of the season, systemic fungicides are often applied, and, afterwards, they use protective or translaminar fungicides (or a combination of both) depending on the weather conditions and on the late blight epidemic pressure. Farmers obtain spraying recommendations through their plant protection officers, our DSS PhytoPRE, or the newspaper. In organic potato production, copper products are often used to control late blight (max. 4 kg/ha and 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 the accumulation of waste piles and volunteer plants. In **Italy**, on potato, the most common strategy is to change some actives depending on the risk of infection and the rate of crop growing. At the beginning of the season and with active crop growth, systemic and translaminar fungicide in mixture are preferred. After bloom, when rate of new growth is reduced translaminar and contact fungicides are used. At the end of the season, QoI, SDHI or triazole fungicides are preferred to delay crop senescence and in the meantime to contain early blight. On tomato, copper based product are applied at the beginning of the season to control bacterial spot diseases which normally occur in that period (May-June). In **Finland**, in 2017 in general 5 to 8 fungicide applications were made. Ranman and Infinito were the most commonly used products. Due to heavy blight pressure also metalaxyl containing fungicides were used. In 2018 only 2 to 5 fungicide applications in general were made. In **Norway**, 2017 was a normal season regarding fungicides, but in some areas a lot of late blight was seen at the end of the season despite a normal, weekly spray schedule. In 2018, the first treatment was 2-3 weeks later than normal due to the warm and dry conditions. Some chose a longer spray interval (14 days) than normal. In **Sweden**, contacts or translaminars are the main products. 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 2017 and lower than normal in 2018. In **Denmark**, we mainly spray the protectant fungicides Ranman Top and Revus either based on the standard weekly from the late June spray or according the blight management DSS. We also use curative Proxanil and Cymbal in combination with protectant Ranman Top or Revus when curative action is need. In **France**, 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 remaining very low, growers were able to continue with longer delay between fungicide applications of simple protectant products except in irrigated fields. Very few translaminar and curative products have been used later in the season (after the middle of August) when the blight pressure became medium or high. In **Belgium**, the average number of fungicide applications in 2017 (in susceptible varieties, mainly Fontane and Bintje) was 13, which corresponds with an average interval of 8,3 days for the growing season (from min. 6,8 tot max. 14 days interval). In 2018 the average number of fungicide applications (susceptible varieties,

mainly Fontane and Bintje) was 12, which corresponds with an average interval of 9,5 days for the growing season (from min. 6,7 tot max. 18 days interval). In **the Netherlands, m**ost growers are using three or four different fungicides during the season. Starting with Acrobat, Curzate, Valbon or Revus followed by Infinito, Kunshi, Canvas and Ranman Top. On an average use of about 14 sprays over the years, last year many growers sprayed a few times less. The average acreage per grower is increasing over the years. Many use a DSS to support them in decision-making when to spray. But especially the farmers with many hectares do not vary the spray interval but spray according to the calendar, mostly every 7 days. In **Germany** in 2017, 4 to 10 spays in the South and 6-12 sprays in the North were necessary to control late blight. In 2018, the number of fungicide applications was between 4-10 in the South and 4-8 in the North. 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 271 using 2016 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 fluazinam, cymoxanil/mancozeb, cyazofamid, cymoxanil and mandipropamid. For seed crops, all those surveyed were treated with fungicide and received an average of 10 fungicide applications. The most frequently applied active ingredients were cyazofamid, cymoxanil, cymoxanil/mancozeb, fluazinam and mandipropamid. Most fungicides are applied at a maximum of 7 day intervals. In **Scotland**, official fungicide usage survey data are only collected for every second year. The data for 2018 are not yet available. In **Ireland** in 2017, intensive fungicide programmes, utilising most available chemistries were applied. Fungicides were routinely applied at seven day intervals throughout the season. Fungicides were selected based on the stage of crop growth and their specific properties, e.g. curativity. A reduction in the amount of fluazinam applied occurred amongst the more intensive growers based on concerns surrounding potential emergences of reduced activity in specific *P. infestans* strains. No data is available to support a reduction in efficacy under Irish conditions or the presence of strains with reduced efficacy. In 2018 a reduction in the amount and types of fungicides applied occurred due to the prolonged dry period during the summer months. During the growing season the biggest issue facing growers was the availability of water, and following rainfall sprouting of immature daughter tubers.

POPULATION CHARACTERISTICS

In **Estonia**, *P. infestans* population is diverse with numerous genotypes, which is the result of sexual reproduction. Both mating types are recorded from the same potato fields and oospore-derived infections are initiating the epidemics. *P. infestans* isolates are highly virulent and aggressive. Late blight epidemics usually start from the small allotments, gardens, fields, where rotation is short, fungicides are not applied and various cultivars are grown from self-propagated seed. 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 (65%); the A2 type was reported only within 35% of the total number of isolates. All isolates were identified as of complex races (7-11 virulence genes). The majority of regions were characterized by phenylamide-sensitive isolates, except the Sverdlovsk region (>45% of resistant isolates). In **Poland** in 2017, due to weather conditions, the disease developed rapidly. In Bonin (zachodniopomorskie voivodship) on varieties cultivated without protection, the destruction of plant assimilation area exceeding 75% was found after 20 days from infection for early varieties, 29 days

for medium early ones and 44 days for late ones. Symptoms were mainly observed on the leaves, although symptoms on the stem were also found. Symptoms on the stems appeared on average 14 days later than the symptoms on the leaves. The most common infection site was the middle part of the plant. Only one case had an earlier infection compared to the leaf infection on the stem. Symptoms occurred in the middle part of the plant on the early VINETA variety. In 2018, due to weather conditions, the disease developed more slowly in June. An increase in the severity of the disease was recorded in July when the amount of precipitation increased (on average 63.4 mm). In August, due to the low amount of rainfall and high air temperatures, no development of the disease was observed. Symptoms of blight on the stem most often occurred in the middle part of the plant. There was no previous disease on stems compared to leaves. In **Serbia** in 2018 three genotypes were present with dominance of EU_13_A2 genotype. In **Romania** in 2016, the two isolates that were characterized belonged to the "other" genotypes. 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. More than 200 FTA cards from most of the potato producing areas were collected in 2018. The overall genotypic analysis showed a predominance of the EU_13_A2 and EU_37_A2 clones in the North, and of the EU_13_A2 and EU_6_A1 clones in the West. Some EU_1_A1 isolates were detected in all French areas, while very sporadic EU_36_A2 was found in Northern France. Population composition was similar in 2017. 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 and there are strong indications that oospores function as an important source of primary inoculum. However, during 2017 a clone (EU_41_A2) was found on fields in South and Mid-Sweden. The low incidence of late blight during 2018 resulted in few sampled fields, but in an intensively sampled field trial in Southwest Sweden EU_41_A2 was dominating. In **Denmark**, in 2017 EU_41 was the dominating genotype, but EU_36 (3 isolates) and EU_13 (1 isolate) were also found but at very low levels. In 2018, all isolates sampled were of the EU_41 genotype. In **Norway**, the new genotype EU_41_A2 was discovered for the first time in 2016. This genotype was also found in 2017 and 2018. In **England**, there has been a substantial change in *P. infestans* genotypes. EU_37_A2, which is associated with decreased sensitivity to fluazinam and was first reported in England in 2016, represented 24% of samples collected in 2017. EU_36_A2 was detected for the first time and represented 2% of samples. EU_6_A1 accounted for approximately 49% of samples and EU_13_A2 10% of samples. In 2018, EU_37_A2 was reported in 17% of samples, with a substantial increase in EU_36_A2 to 17% of samples. EU_36_A2 and EU_37_A2 have only been reported in England, there have been no reports in **Wales**. In **Ireland** in 2017 whilst early infections were reported in main crops, timely applications of fungicides prevented their further spread. Although infections were early the subsequent development of epidemics was relatively slow in comparisons to previous season. Limited genotyping confirms the continued dominance of the population by EU_8_A1, EU_6_A1 and EU_13_A2, with no other genotypes selected. Given the extremely low pressures experienced in 2018 limited data is available on the *P. infestans* population composition.

USE OF DSSs

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

Country	DSS
Belgium	PCABlight-DSS
Denmark	Blight Management
England, Wales, Scotland	Blightwatch, Fight against Blight, Plant Plus & BlightCAST
Estonia	Estonian Crop Research Institute
Finland	National Resource Institute: general LB warnings
France	Mileos®
Germany	PhytophthoraModel Weihenstephan, ISIP
Ireland	Met. Service based on Irish rules (Bourke)
Italy	IPI + MISP
Netherlands	ProPhy, Plant Plus, WUR Blight APP
Norway	Negative prognosis, Nærstad model
Romania	Plant Plus
Russia	VNIIFBlight, Agrodozor, Plant Plus
Sweden	Blight Management (DK) & VIPS (NO), Plant Plus
Switzerland	PhytoPRE

ALTERNARIA 2017 & 2018

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. Early blight infections in potato results in several European countries (Sweden, Denmark, Poland, Germany, Netherlands, Lithuania, and Austria) in reduced tuber and starch yield. In Italy EB infection on tomato can reduce the marketable yield. In most of the countries tuber infections are not observed or really rare in the field.

EB DISEASE OBSERVATION AND EB DISEASE PROGRESS

The date of first observation of early blight symptoms in field trials 2017 is shown in Figure 9. The first symptoms occurred mid of June in Serbia, Poland and Germany; mid of July in Italy, France, Denmark and Sweden. In most regions two to five weeks later the disease epidemic started (Figure 10).

In 2018 we had an early outbreak of EB in Poland, Serbia, Italy and Germany (second week in June) (Figure 11). Also some observations of EB attacks are reported from Norway. The disease epidemic started again 2-5 weeks after the first outbreak (first observation) (Figure 12).

In Table 1 the EB specific disease development 2018 from May to September in different European countries is shown. In May and June in all countries with the exception of Serbia and Italy the disease severity in the fields was lower than 20%. The disease severity in July increased in Germany, Poland, Italy, Serbia and Russia up to 50%. In Germany and Serbia the EB specific disease level in August was higher than 50%.

EB: IDENTIFIED *ALTERNARIA* SPECIES

In most countries the *Alternaria* subspecies *Alternaria solani* and *Altenaria alternata* were identified on infected potato leaves (Table 2). As in the previous years in Denmark and Serbia only *Alternaria solani* could be detected. Overall the dominating species during the disease epidemic in 2017 and 2018 was *Alternaria solani* in most European countries.

FUNGICIDE USAGE AND FUNGICIDE RESISTANCE

Different products are registered in Europe for the control of early blight. The following active ingredients were used in different countries to control the disease: mancozeb (multisite), chlorothalonil (multisite), azoxystrobin (QoI), pyraclostrobin (QoI), boscalid (SDHI) and difenoconazole (DMI). According to the regional registration also mixtures of these active ingredients or with oomycete active ingredients (mandipropamid) 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). The monitoring data from 2017 and 2018 confirm that in Germany, Belgium, the Netherlands, Poland and Sweden the F129L mutation in *Alternaria solani* is very dominant. Additionally, in Austria, Denmark and Serbia F129L mutants were found.

SDHI mutants were found in Belgium (Landschoot *et al.* 2017), Germany (Metz *et al.* 2019), the Netherlands, Austria and Serbia.

At the moment different DSS (Decision Support Systems) are available to optimize the use of fungicide applications. In some European countries, disease management is based on computer based systems dealing with calculating favourable weather conditions for an EB infection by *Alternaria solani* or temperature degree-day thresholds. DSS models are existing in Germany (PhytophthoraModel Weihenstephan), the Netherland, Sweden, Poland (DACOM) and Belgium (DSS-Early blight) to optimise the control of EB. Additionally in Germany farmers use threshold values based on the disease progress (Leiminger and Hausladen, 2012) to manage EB.

Figure 9. *First observation of early blight in **2017** in Europe*
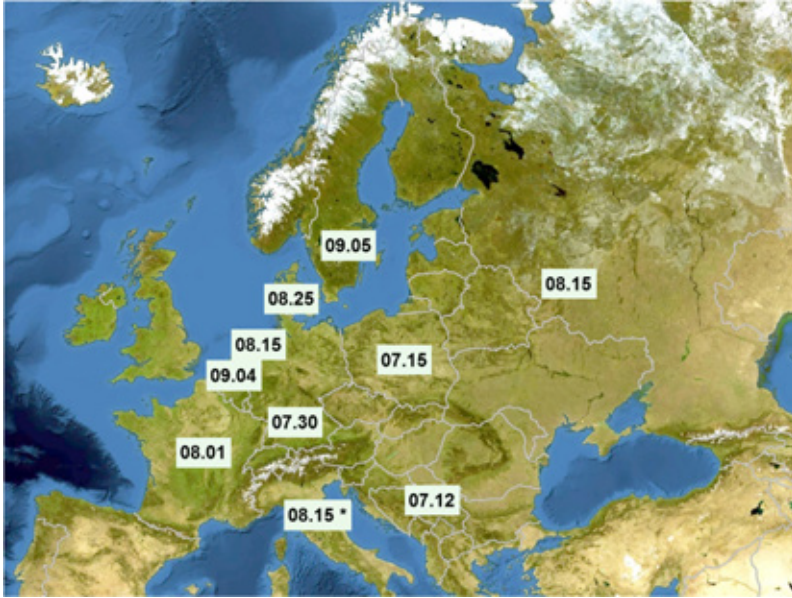
Figure 10. *Start of the early blight epidemic in* **2017** *in Europe*

Figure 11. *First observation of early blight in* **2018** *in Europe*

Figure 12. *Start of the early blight epidemic in **2018** in Europe*

Table 1. *EB specific disease severity 2018 in different European countries*

Legend:
- Yellow: Low <20%
- Orange: Medium 20 - 50%
- Red: High >50%
- White: no data, no eb

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

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											
Italy	√		√	√	√		√	√	√	√	√
France	√			√	√		√		√	√	
Serbia	√	√	√	√	√						
Russia	√		√	√	√		√	√	√	√	√

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The European population of *Phytophthora infestans* in a global context

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SUMMARY

Late blight, caused by *Phytophthora infestans*, causes significant losses to potato and tomato crops on a global scale. The timing and effectiveness of blight management depends on the pathogen's response to the environment, primary inoculum type, virulence to host resistance and sensitivity to fungicide active ingredients. However, the pathogen population is continually evolving and emerging clonal lineages with new traits highlights the need to tailor management to the local pathogen population. This is the rationale behind the EuroBlight consortium's collection of data on the genetic diversity of *P. infestans*, analysed with simple sequence repeat (SSR) genetic markers. Our surveys of late blight infected crops alongside those of partner research institutes and agrochemical companies from 2013–2018 has generated a database of over 8400 geo-tagged European samples. Standardised protocols, SSR allele scoring and a single data format remain critical to the utility of the database and its associated analysis tools and the mapping interface used to view the data.

Currently, much of the pathogen genotyping has been conducted at the James Hutton Institute in Dundee but the effective expansion of this database to other regions depends on sharing the experiences of this lab to ensure the standardised naming of the alleles at each of the 12 SSR loci. We have been working closely with partners in other laboratories within all five networks (EuroBlight, AsiaBlight, Tizon Latino, USABlight and AfricaBlight) to harmonise the methods. Recent updates to the EuroBlight web page include a detailed step-by-step guide to scoring the SSR alleles (fluorescently labelled peaks) with sample images and explanations of the common scoring challenges. A list of the names and expected peak sizes of every allele reported to date is also provided. In addition, a list and the allele combinations of the commonly occurring clonal lineages, examples of minor variants and other known names from the literature are provided. This central data resource should prove a valuable asset for the community of late blight researchers and enable the effective sharing of data across and within networks.

A complex population structure is observed in Europe with around 70% of the sampled population dominated by a few widely disseminated clonal lineages; see maps at www.euroblight.net. The most obvious change in the years 2016-2018 was the overall decline in

the combined frequency of the clones EU_13_A2, EU_6_A1 and EU_1_A1 from 60 to 40% of the population. Over the same time period, the clones EU_36_A2, EU_37_A2 and EU_41_A2 have increased from 10 to 36% of the sampled population and research is underway on the drivers and management implications of this change. The SSR diversity within lineages has also been tracked over time and demonstrates the local spread of some sub-clonal variants. In contrast to the clones, 20–30% of the sampled European population comprises genetically diverse pathogen populations consistent with local, ephemeral oospore-derived sexual populations. These 'Other' diverse populations are more prevalent in some parts of Europe than others. Deeper insights into pathogen diversity at a range of scales in Europe is being investigated via the integration of the R-based population genetics application *poppr* into the Potato Tool Box in the pathogen database.

The flexible structures, systems and tools developed by EuroBlight partners for tracking populations of *P. infestans* in potato and tomato cropping systems across the globe are proving valuable tools. When combined with phenotypic data on the traits, the EuroBlight population tracking approach provides a crucial early warning system that helps mitigate against management failures due to the emergence and spread of new clones. Two recent cases were presented; the dominance of the EU_33_A2 lineage in Plateau State in Nigeria and the appearance of the EU_13_A2 clone in potato crops in Senegal. These are the first reports of these recently emerged European lineages in sub-Saharan Africa and are a cause for concern for crop production in these regions.

AsiaBlight: past, present and future

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SUMMARY

The formation of a late blight network for Asia, AsiaBlight, first proposed in 2014, initially focussed on generating a coarse-scale map of the *Phytophthora infestans* population in Asia to underpin future pathogen studies and disease management strategies. This was progressed during 2016-2019 with the assistance of the Inner Mongolia Potato E & T Center, Hohhot and of the James Hutton Institute, Dundee. FTA cards (funded by Bayer) were distributed from Hohhot to contacts in Asian countries who collected late blight samples and returned them to Hohhot. *P. infestans* DNA was genotyped in Hohhot and at the James Hutton Institute and uploaded to the database hosted by EuroBlight. In addition, other researchers active in Asia also contributed genotyping results to the database. The coarse-scale map shows the aggressive genotype 13_A2 to be widespread across mainland Asia, but the island populations sampled to be distinct and disparate. AsiaBlight began as a network with minimal resources, but has achieved growing recognition, a degree of regional collaboration and limited but successful private-public partnership. Starting in 2018, the CIP–China Center for Asia-Pacific (CCCAP) has taken up the challenge of implementing a self-sustaining AsiaBlight network. A website and workshops have been implemented, and an international meeting has been organized. Progress and future plans for this network are discussed.

KEYWORDS

Phytophthora infestans, potato late blight, China, SSR, population structure

INTRODUCTION

Countries in Asia range from the very wealthy to the very poor. A few have some of the highest malnutrition levels in the world (FAO *et al.*, 2019). Potatoes are highly nutritious, can adapt to marginal conditions and produce more food per unit of water than any other crop, making potatoes a key component of food security. China and India are the two most populous countries in the world and are also the two most important potato producers, generating a third of the world total tonnage (FAOSTAT, 2019). Potato is popular in Asia, with many of its countries ranked within the top 50 potato producers, including Bangladesh (7), Iran (13), Pakistan (20), Kazakhstan (22), Uzbekistan (25), Nepal (26), Japan (31), North Korea (33), Kyrgyzstan (38), Indonesia (43), Azerbaijan (46) and Tajikistan (50) (FAOSTAT, 2019). As in other parts of the

globe, late blight (LB), caused by *Phytophthora infestans*, is the most important disease affecting potato production. An overall picture of *P. infestans* in Asia, including population dynamics, distribution of major genotypes and the most effective control measures, has been lacking. To address this problem, AsiaBlight, an inclusive network of scientists, companies, farmers and other stakeholders working towards reducing the impact of LB in Asia using an integrated approach, was initiated in 2014 under the auspices of the International Potato Center (Forbes, 2015), inspired by the success of EuroBlight and other international late blight networks.

As reported in the 2017 EuroBlight Proceedings (Cooke *et al.,* 2017), AsiaBlight initially focused on establishing a coarse-scale map of *P. infestans* across the region to serve as a baseline for pathogen studies and underpin future endeavours to improve on-farm disease management. Associated objectives of this project 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. For the mapping project, FTA cards were distributed by the Inner Mongolia Potato E & T Center, Hohhot, China to contacts in many Asian countries. This paper provides an update on progress in the mapping project, which will be reported in more detail in a future peer-reviewed paper, and on plans for AsiaBlight, which are being led by the CIP-China Center for Asia-Pacific (CCCAP).

PROGRESS TO DATE

Organisation of sample collection
Collection of late blight samples followed the EuroBlight model with contacts in Asian countries being asked to collect *P. infestans* DNA from late blight lesions onto FTA cards and provide sample details on standard forms. Purchase of 500 FTA cards was funded by Bayer (Regions APAC 1 and APAC 2) and organised with the assistance of the CIP Office, Beijing. Professor Ruofang Zhang volunteered the assistance of her laboratory and staff in the Inner Mongolia Potato E & T Research Center, Hohhot, China to distribute the cards and to genotype the resultant *P. infestans* DNA samples. For more detail on the process, see Cooke *et al.* (2017).

Country contacts and FTA card distribution
Contacts were identified with the assistance of CIP scientists and other researchers. During 2016-2018, efforts were made to contact researchers in Armenia, Bangladesh, Georgia, India, Indonesia, Japan, Kyrgyzstan, the Republic of Korea, Myanmar, Nepal, Pakistan, the Philippines, Taiwan, Tajikistan, Uzbekistan and Vietnam. The late blight population in China was already being investigated in ongoing projects, so this was excluded from the proposed sampling. Researchers contacted in Armenia responded positively, but had to withdraw owing to changed responsibilities.

FTA cards and sampling instructions and forms were therefore sent to:
- Bangladesh (2016)
- Georgia (2016, 2017)
- India (2016)
- Indonesia (2016, 2017)
- Japan (2016)
- Kyrgyzstan (2017)

- Nepal (2016)
- Pakistan (2017, 2019)
- The Philippines (2017)
- Island of Taiwan (2016)
- Tajikistan (2016, 2017)
- Uzbekistan (2016)
- Vietnam (2016)

After FTA cards had been sent out, organisational changes in Tajikistan and Uzbekistan in 2016 resulted in loss of contacts and consequently of the FTA cards sent there. However, other researchers were identified in Kyrgyzstan and Tajikistan and cards were sent to these in 2017. Although FTA cards were sent to India in 2016, it transpired that Indian biosecurity legislation prohibits pathogen DNA from being sent out of the country, so FTA card samples could not be submitted for genotyping outside India. However, contacts were established with researchers engaged in *P. infestans* population studies within that country.

Contacts between several Asian countries and EuroBlight researchers established that sample collection was also currently ongoing in Bangladesh, Indonesia, Japan, the Republic of Korea, Myanmar and the Philippines with, in some cases, samples being submitted to the James Hutton Institute (JHI) or Wageningen University (WUR) for genotyping and mapping.

Sample collection and submission
For the coarse-scale mapping project, late blight was generally sampled during a single season (although sometimes sampling occurred during the winter over two calendar years), except where initial samples yielded insufficient pathogen DNA or where additional contacts were able to collect samples.

FTA cards with sampled *P. infestans* DNA were returned by the collectors to Hohhot along with completed sampling forms (Cooke *et al.*, 2017). Details of samples collected onto FTA cards during 2016-2019 are shown in Table 1.

DNA Extraction and Genotyping
DNA extraction and genotyping were initially carried out in the Inner Mongolia Potato E & T Center, Hohhot. Despite success in achieving implementation of the 12-plex SSR (Li *et al.,* 2013a), it proved difficult to complete the genotyping there because of staffing issues, which resulted in a lack of continuity. In addition, standardisation of allele calling provided a major challenge, despite provision of DNA standards and of a web workshop in October 2018 by David Cooke (JHI). In November 2018, FTA card samples were therefore divided in two, with half being sent to JHI, while genotyping of the remaining halves continued in Hohhot. Samples from eight countries/regions were successfully genotyped and added to the EuroBlight database (Table 1). Data resulting from samples collected by other researchers in Bangladesh, the Republic of Korea, Malaysia, Myanmar and Sri Lanka were also included in the database. Genotyping results from AsiaBlight samples and from other researchers' samples were uploaded to the map hosted on the EuroBlight website (euroblight.net) and also available from the AsiaBlight website (asiablight.org).

Table 1. *AsiaBlight FTA card sampling and genotyping of Phytophthora infestans, 2016-2019*

| Country/region | Year and crops sampled* | | | | Comments on genotyping in JHI | Some samples genotyped | Uploaded to map |
	2016	2017	2018	2019			
Bangladesh	n/a	25 P	n/a	n/a	24 samples genotyped	√	√
Georgia	10 P	n/a	10 P	n/a	Insufficient *P. infestans* DNA in 2016 samples, 8 samples from 2018 genotyped	√	√
Indonesia	10 P	16 P	n/a	n/a	17 samples genotyped, plus 9 samples genotyped in Hohhot	√	√
Japan	10 P	n/a	n/a	n/a	8 samples genotyped	√	√
Kyrgyzstan	n/a	n/a	n/a	n/a	Cards sent 2018, but no samples collected to date as no late blight in 2018 and 2019	✗	✗
Nepal	9 P	15 P, 10 T	n/a	n/a	23 samples genotyped	√	√
Pakistan	n/a	n/a	15 P, 5 T		Insufficient *P. infestans* DNA in 2018 samples, 2019 samples not yet collected.	✗	✗
The Philippines	n/a	10 P	n/a	n/a	10 samples genotyped	√	√
Taiwan	12 T	n/a	n/a	n/a	Plus 8 T (2014-15); DNA from isolates in culture, 4 samples genotyped	√	✗
Tajikistan	n/a	n/a	n/a	n/a	No response from contact since October 2017	✗	✗
Vietnam	n/a	4 P	3 P, 1 T	4 T	11 genotyped	√	√

* P = potato, T = tomato
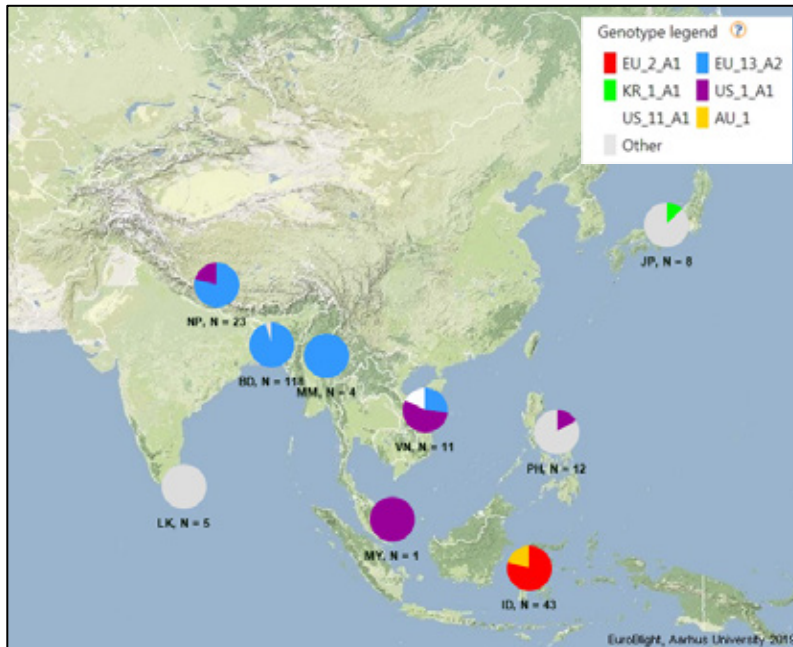
Figure 1. *Phytophthora infestans genotype map for late blight samples collected in Asia (excluding India) from 2016-2019 (includes samples collected by researchers in addition to AsiaBlight FTA card sampling)*

Genotypes identified in Asia included the old US_1_A1 (on tomato) and the aggressive 13_A2 (Blue 13). In particular, 13_A2, previously reported from Asian countries including China and India (Li *et al.*, 2013b; Chowdappa *et al.*, 2015), was found to be widespread across mainland Asia (Figure 1). However, the *P. infestans* populations on the islands of Asia included in the sampling had distinct and disparate genotypes and 13_A2 was not detected.

It is hoped to obtain additional samples from Japan and Taiwan and to collect samples from Pakistan and Kyrgyzstan in 2019-2020, subject to the occurrence of weather conducive to late blight. Full results of the coarse-scale population study are not detailed here, as it is planned to write them up for a peer-reviewed publication.

CHALLENGES

As noted in 2017, several challenges have been associated with the AsiaBlight coarse-scale mapping project:

- Obtaining pathogen samples across a large, politically diverse region was problematic
- It proved difficult to maintain stable communication channels, leading to consequent loss of the contact and the corresponding FTA cards
- National biosecurity legislation restricted opportunities for participation of some countries
- There were technical issues relating to the genotyping protocol and its standardisation

NEXT STEPS

As noted above, it is planned to write up the coarse-scale mapping project for publication in a peer-reviewed journal. If possible, genotyping of additional samples from Japan, Kyrgyzstan, Pakistan and the island of Taiwan is needed. It is necessary to ascertain from collectors/owners of non-AsiaBlight genotyping data from Asian countries whether their results can be included in the proposed coarse-scale map and publication. Questions remain as to how genotyping can best be progressed in Asia and how genotypic data can be integrated with phenotypic data, such as fungicide sensitivity, to allow appropriate management advice.

Starting in September 2018, CCCAP has taken up the challenge of implementing a self-sustaining AsiaBlight network. Since then, CCCAP has had many discussions with LB stakeholders in different Asian countries, to understand better how the new AsiaBlight network can respond to the expectations of the region. Thanks to these conversations, AsiaBlight will focus on training and capacity building, aiming to increase understanding of LB through research and collaboration, and to establish a pan-Asian community of LB stakeholders.

AsiaBlight will hold its third international meeting in October 2019 and three training workshops were also planned for 2019. All AsiaBlight workshops and training will be in Chinese and English. To support communication within the community, in early 2019, an AsiaBlight WeChat group, a Twitter feed and a Facebook page were launched. AsiaBlight´s website (asiablight.org) went live in May 2019 during the EuroBlight Workshop in York.

A broader sampling effort through collaboration within eight different provinces of China has been started as a pilot study. Fungicide efficacy analysis and variety performance studies are currently being established. In parallel, academic researchers are being identified and projects planned for a better understanding of the LB population throughout the region. Thanks to an intensive fundraising effort, CIP-CCCAP hopes AsiaBlight will become financially independent in the next few years.

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USABlight: A Disease Surveillance System for North America

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The late blight disease surveillance system USABlight.org was developed in 2011 with funding from USDA NIFA after severe late blight outbreaks occurred throughout the eastern US from movement of infected tomato transplants (Fry *et al.*, 2013). The disease surveillance system is housed at NC State University and was developed with the assistance of the State Climate Office (Figure 1). The system now contains 8 years of data on late blight disease occurrences on tomato and potato in the US. As outbreaks are reported into the system, they are validated by extension collaborators and then mapped. Outbreaks are recorded via a web GIS system and validators are notified via text or email and contact the reporter to confirm disease. Samples are sent to Cornell University and genotyped by 12-plex microsatellite markers. First outbreaks occur in Florida each year in winter crops and disease occurs later as the season progresses throughout the eastern US.

A clear change in pathogen genotypes has emerged over the past 7 years (Figure 2). The US 22 lineage caused the outbreaks in 2009 on tomato transplant and was more common on tomato than potato. The mefenoxam resistant US-8 lineage has declined in potato over time but still occurs in the western US. The mefenoxan sensitive US-23 lineage is now widespread on both potato and tomato, enabling growers to once again use this compound in the field.

One important goal of USABlight was to improve fungicide use efficiency and reduce the number of fungicide applications for control of late blight. The USABlight disease alert system and the Blight Pro decision support tools developed at Cornell University help growers target when to use fungicides based on weather data. We generated baseline data on the sensitivity of current US lineages of the pathogen to a suite of Oomycete targeted fungicides (fluopicolide, cyazofamid, etridiazole, azoxystrobin, cymoxanil, and mandipropamid) (Saville *et al.*, 2015). Currently most US lineages remain sensitive to all compounds but mefenoxam, while US-8 and US-11 are resistant to mefenoxam. The USABlight team continues to collect, monitor and phenotype *P. infestans* and alert growers of disease outbreaks and lineages nearby so they can better deploy timely fungicide applications to manage this important disease.
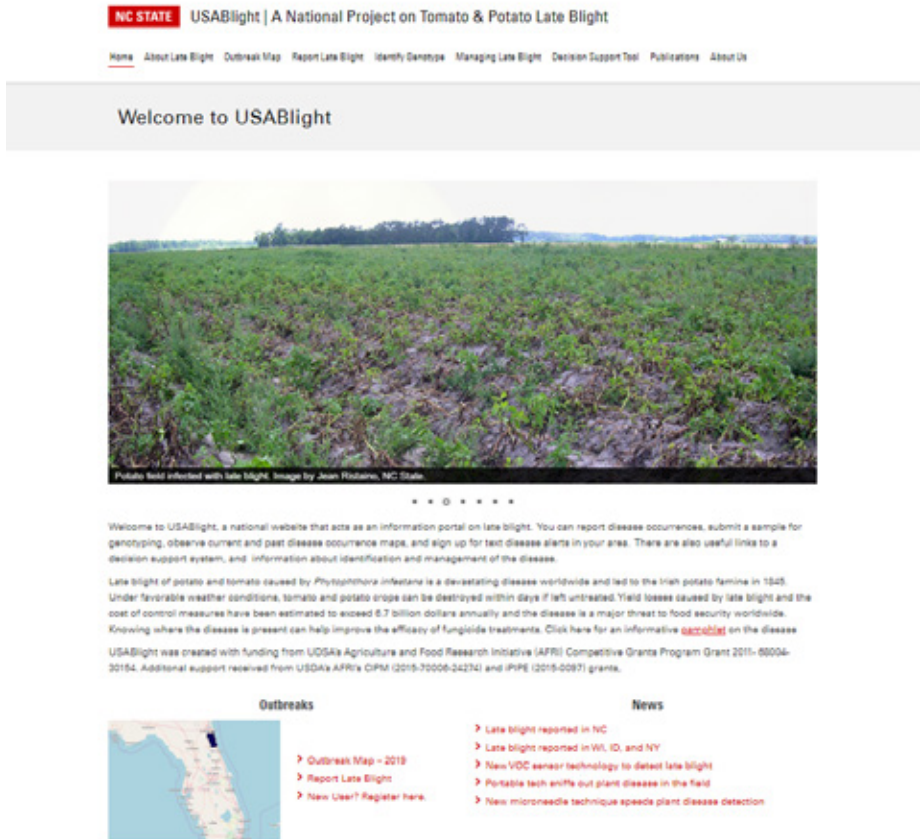
Figure 1. *USABlight web portal. The US national reporting system for late blight*

The USABlight surveillance system is useful since growers can check the map and determine the time to begin fungicide applications if late blight is nearby and weather is conducive. The web statistics on the use of USABlight.org indicates that the outbreak map is the most frequently viewed page after the home page (Ristaino *et al.*, 2019). In fact, 42% of all the sessions on USAblight include viewing the outbreak map. Growers not only view the map but can sign up for text or email alerts when the disease is reported nearby.

We are also studying the populations of the pathogen. We compared the population structure of 18 of the 24 extant US lineages to lineages from Europe, South America, and Mexico (Saville and Ristaino, 2019). Structure analysis, neighbor joining trees, and principal component analyses of 12 microsatellite loci indicated that many recent US genotypes (US-7, US-8, US-11, US-22, US-24) shared significant similarity with lineages from Mexico, while the US-1 lineage clustered with US-1 isolates from Peru. The US-8, US-14, and US-24 lineages, predominantly virulent on potato, and the US-21 and US-22 lineages, predominantly virulent on tomato, formed two distinct and separate monophyletic clades. The US-23 lineage, currently the most prevalent lineage detected in the US, shared allelic diversity primarily with isolates from Bolivia and Brazil

and not the Mexican isolates we tested. Mexican lineages showed evidence of multiple ancestral recombination events. A survey of the presence of RXLR effector *PiAVR2* across all samples revealed the presence of lineages that carried either *PiAVR2*, its resistance-breaking variant *PiAVR2-like*, or both, suggesting lineages have experienced different levels of selection to the *R2* gene in potato. These findings suggest populations of *P. infestans* in the US are the result of introductions from both South America and Mexico.
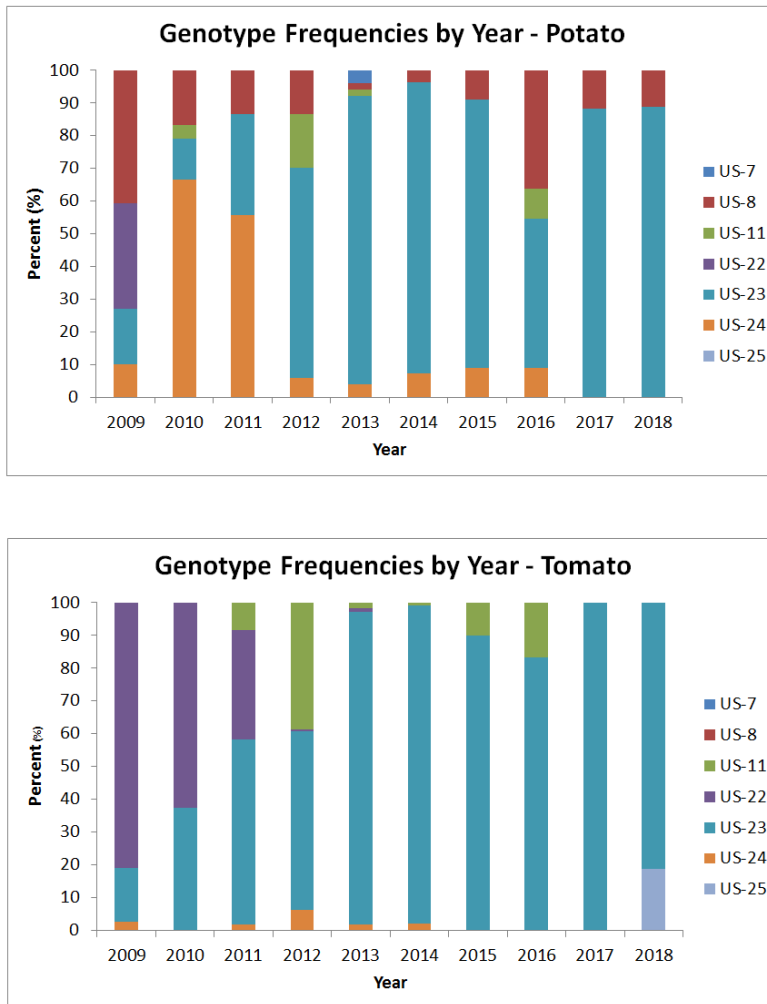
Figure 2. *Lineages of Phytophthora infestans on A) potato and B) tomato, based on SSR genotyping in the US from USABlight.org from 2009-2017*

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AfricaBlight: Present and future

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The establishment of AfricaBlight, an African network coordinating late blight research in the African countries has been proposed. Diverse researchers in the different African countries have been carrying out various activities aimed at either understanding the phenotypic characteristics of *Phytophthora infestans* or, the outcome of the interaction between *P. infestans* and one of its hosts in Aftica (potato, tomato, garden huckleberry). However, very few genetic studies investigating the circulating *P. infestans* strains exists and even the current ones are sometimes fragmented into regions within specific countries in dissimilar years. Consequently, no proper information on the status of the P*. infestans* genotypes in Africa exists. In 2017, a 12-plex microsatellite genotyping approach was validated for the region, at a research hub in Nairobi Kenya, allowing for global comparisons of data from the rest of the world. I propose that harmonization of late blight research through the AfricaBlight network will give researchers a platform to use standard protocols. Proper coordination of the network's activities will however require funding, both nationally and internationally, and hence, the first aim of the proposal is to seek out funding opportunities.

One-class classification for blight risk forecasting

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SUMMARY

Information from crop disease surveillance programs provide real-world data about the drivers of infection events. This study explored the application of machine learning techniques to survey data for potato late blight outbreaks to derive models for forecasting the risk of disease. Five different anomaly-detection algorithms were compared according to their accuracy in forecasting outbreaks: *k*-means, Gaussian mixture model, kernel density estimator, one-class support vector machine, and isolation forest. The results showed that one-class support vector machine had the highest accuracy followed by Gaussian mixture model, with 98% and 97% respectively. There was added value in combining the algorithms in an ensemble to provide a more robust forecasting tool. The techniques used here can easily be applied to outbreak data from other crop pathosystems to derive tools for agricultural decision support.

KEYWORDS
Phytophthora infestans, late blight, machine learning, anomaly detection, decision support

INTRODUCTION
Models for forecasting risk of crop disease are typically derived from experiments in the laboratory, glasshouse or controlled environment chambers, or derived from statistical relationships between environmental data and disease observations in small plot field trials. An argument can be made, however, that experiments conducted under highly standardized conditions or at a small spatial or temporal scale can produce results with little validity beyond the specific environment in which the experiment is conducted. Plant disease epidemics occur at the landscape-scale and are influenced by aspects of the wider environment, such as topography, climate, and variability in host and pathogen populations. Landscape-scale experiments to derive and test models of disease risk are rarely conducted, but surveillance programs for crop diseases can provide useful real-world data about the drivers of infection events at the spatial scale of interest.

Many decision support systems for crop protection are used to make binary decisions about whether to apply a crop protectant (De Wolf and Isard 2007). This approach can be framed as a classification problem, where input data are used to discriminate between risk and no-risk of disease. In a conventional classification problem, data from each class of interest are available,

i.e., healthy and diseased crops, and each object is represented by a vector of features (e.g., date, location, weather data, crop characteristics). Training the classifier involves feeding training examples from both classes to the algorithm so that it can create rules to assign membership based on the individual feature values. When encountering new instances, they can be classified according to the learned rules. In one-class classification, the problem is to classify data when information is available for only one class of observations (Désir *et al.* 2013; Khan and Madden 2010; Mazhelis 2006; Tax 2001). This is an interesting problem because this is typically the case for surveillance programs for crop diseases, where only outbreaks are recorded and data from surrounding healthy crops is omitted. The task, then, is to find the feature values most commonly associated with disease occurrence and those that are anomalous, in order to derive rules that can be used to predict risk/no risk of disease in the future. One-class classification is therefore often considered as outlier or anomaly detection.

In this study we applied anomaly detection algorithms to national late blight survey data to learn the 'normal' weather conditions most commonly associated with outbreaks of disease, and evaluated their ability as tools to forecast risk.

MATERIALS AND METHODS

Input data
The late blight outbreak data spanned a 6-year period (2012–2017) and consisted of the date and coordinates (UK postcode district centroid) of 1049 late blight outbreaks from across GB. These data are collected routinely each year by blight scouts as part of the Agriculture and Horticulture Development Board (AHDB) Potatoes 'Fight Against Blight' campaign. Hourly weather data corresponding to every outbreak location were provided by the UK Met. Office. A 28-day period prior to the date that each outbreak was reported was considered sufficient for relating weather conditions for infection to the dates at which disease was first observed in the crop. On each day in that 28-day period, the minimum daily temperature and the total number of hours of relative humidity ≥ 90% were calculated and used as input features (variables) for the anomaly detection algorithms. These two features were selected as they are historically used to calculate late blight risk alerts in GB. The Smith Period was developed in the 1950's as a DSS to indicate high risk periods for PLB development in GB (Smith 1956). This was replaced by the Hutton Criteria in 2017, which is the current national warning system for late blight in GB via the online service 'Blightwatch'. The Smith Period and the Hutton Criteria will serve as baseline models for comparison with the anomaly detection algorithms.

Machine learning
Among the many anomaly detection algorithms available, five of the most commonly used were selected to compare for forecasting outbreaks: one-class *k*-means, Gaussian mixture model, kernel density estimator, one-class support vector machine, and isolation forest (Goldstein and Uchida 2016). Each algorithm assigns an anomaly score to each data point, describing its outlier tendency. A user-defined decision threshold is applied to the returned scores to create a decision boundary separating nominal samples from anomalies. In the current context, any weather conditions that fall inside the boundary trigger a disease risk alert, otherwise no alert is issued. Each algorithm contains parameters that are learned from the data, and 'hyperparameters' whose value must be set before the learning process begins. In order to fairly evaluate the performance of the algorithms and not introduce biases in the choices of hyperparameters, a

nested cross-validation scheme was implemented. The procedure consisted of two nested *k*-fold cross-validation loops: an outer one to test generalization accuracy, and an inner one for optimization of hyperparameters and the decision threshold used to separate nominal data (producing a risk alert) from anomalies. Model performance was assessed in both the inner and outer loops using an extrinsic performance function, where the models were used to forecast outbreaks in the held-out test set. Any risk alert triggered in the 28-day window of weather data preceding each outbreak was classed as a 'successful' forecast. The decision threshold value was tuned so that approximately 6 in 7 data points were classified as anomalies, producing a risk alert on every 1 in 7 days, on average. Cross-validation accuracy was then measured as the percentage of outbreaks successfully forecasted. The two models used as a baseline for comparison (the Smith Period and the Hutton Criteria) did not require training or tuning of hyperparameters and were tested for accuracy using the same held-out test sets as the anomaly detection algorithms.

RESULTS

All five optimized anomaly detection algorithms produced similar decision boundaries in the temperature and relative humidity data (Figure 1). There were, however, slight differences in the range of values classified as 'normal'. The daily minimum temperature values defining the decision boundaries ranged from: 9.6-12.2, 9.4-12.1, 9.1-12.2, 9.4-12.1, and 8.0-13.0°C for one-class *k*-means, Gaussian mixture model, kernel density estimator, one-class support vector machine, and isolation forest, respectively. The number of hours of RH≥90% defining the decision boundaries ranged from: 6.0-12.3, 4.6-9.6, 5.4-11.0, 3.5-10.0, and 5.6-11.9 for one-class *k*-means, Gaussian mixture model, kernel density estimator, one-class support vector machine, and isolation forest, respectively.
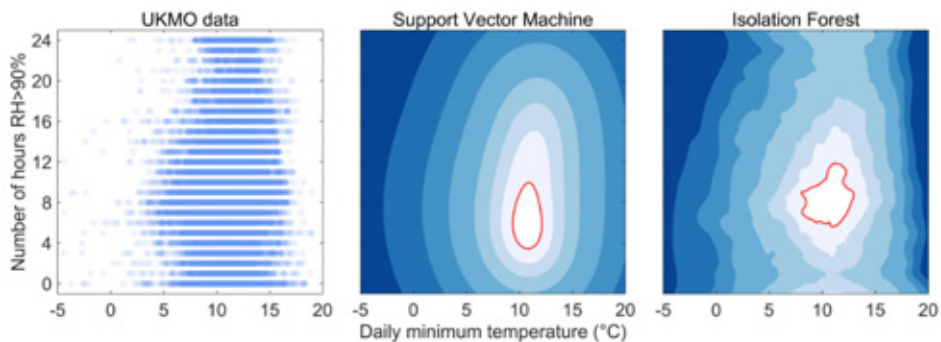
Figure 1. *Comparison of decision boundaries for two of the five optimized anomaly detection algorithms applied to the complete potato late blight outbreak data set: (a) raw weather data in the 28 days preceding each outbreak, (b) one-class support vector machine, and (c) isolation forest. Darker contours correspond to greater outlier tendency, with a bolder line for the decision boundaries*

The Smith Period achieved an accuracy of 81.8% in forecasting outbreaks, with a frequency of alerts of 12.6% (i.e., approximately 1 alert in every 8 days). The Hutton Criteria had a much higher accuracy, at 96.1%, but at the expense of a high frequency of alerts of 31.4% (i.e., 1 in

every 3 days). For the anomaly detection algorithms, predictive accuracy was: 95.7, 97.1, 93.8, 98.1 and 96.2% for one-class k-means, Gaussian mixture model, kernel density estimator, one-class support vector machine, and isolation forest, respectively. Frequency of alerts was consistent at approximately one alert in every 7 days: 14.9, 12.8, 15.0, 14.7, and 13.7% for one-class k-means, Gaussian mixture model, kernel density estimator, one-class support vector machine, and isolation forest, respectively. There was in general good agreement among the anomaly detection algorithms on the dates on which alerts were issued (Figure 2). The Smith Period missed several outbreaks, whereas the Hutton Criteria tended to issue alerts over multiple consecutive days. In order to increase confidence in the robustness of predictions, the five anomaly detection techniques were also combined in an 'ensemble', where each model had a 'vote' on whether an alert should be issued on any given day, and the majority vote won.
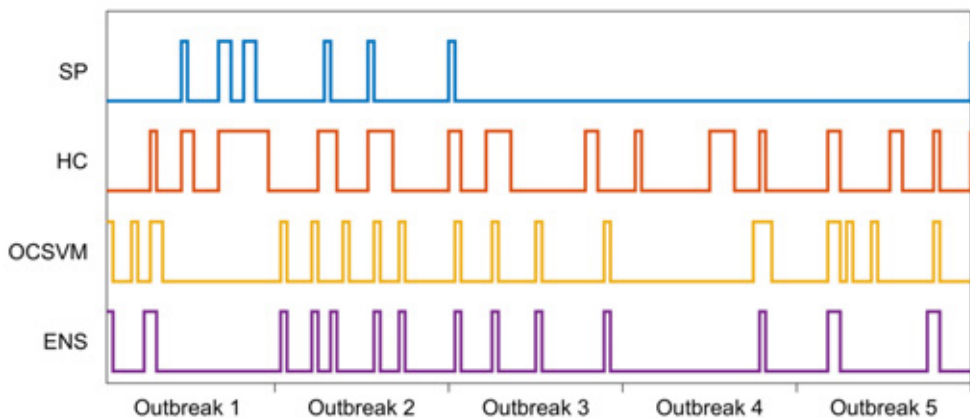
Figure 2. *Comparison of the dates on which alerts were issued in the 28 days leading up to a reported outbreak for a random sample of five outbreaks. Results are provided for the two baseline models (SP = Smith Period, HC = Hutton Criteria), an example anomaly detection algorithm (OCSVM = one-class support vector machine), and an ensemble of the five anomaly detection techniques (ENS)*

CONCLUSIONS

This study outlines a method for deriving crop disease forecasting tools from survey data that contain information on diseased crops only. Anomaly detection algorithms were used to learn the envelope of weather conditions most commonly associated with outbreaks, and then tested for their ability to forecast outbreaks using held-out test data. The algorithms were able to correctly forecast late blight outbreaks with an accuracy of 96.3%, on average, whilst maintaining a low frequency of alerts. The five algorithms used encompassed a range of density, distance, classification, and rule-based techniques to delineate normal/anomalous regions in the input data. Combining these different approaches in an ensemble of anomaly detectors yields a robust tool for forecasting late blight outbreaks that mitigates any weaknesses inherent in the individual models. In future work the ensemble will be made available to the GB potato industry as a cutting-edge 'intelligent decision support system'. The chief advantage of this system over

'conventional' decision support tools will be its ability to continue learning and improving as new outbreak data become available.

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Geodata for the control of potato late blight in Bangladesh

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INTRODUCTION

In Bangladesh, fresh market potatoes are grown as a winter crop on approximately 450.000 ha. Due to frequently occurring fog periods, late blight (caused by *Phytophthora infestans*) is common and highly destructive. Annual yield losses have been estimated at 25-60%. Late blight can be controlled but only by highly frequent, costly fungicide applications. Nevertheless, control failures are common due to challenges presented by the local weather, lack of knowledge and continuous natural pathogen population changes.

The control efficacy achieved primarily depends on the timing of the fungicide applications. Complicating factors are found in crop growth, (absence of) disease pressure and the choice of fungicide. Mancozeb and Metalaxyl are the most commonly used a.i.'s in Bangladesh but the countries *P. infestans* population was found to be 100% EU_13_A2 and therefore metalaxyl resistant. Overall, the general late blight control efficacy can be significantly improved, e.g. by providing farmers with guidance on late blight control. A tailor made near day-to-day decision support system, giving advice on optimal spray timing and choice of fungicides, was developed for this purpose. This GeoPotato system was developed from 2014 – 2019 under the umbrella of the G4AWII program (https://g4aw.spaceoffice.nl/en/).

MATERIALS AND METHODS

The GeoPotato service provides subscribed farmers with preventive spray advice and a suggestion for the type of fungicide (preventive, curative). This advice is communicated through a short text message and/or voice message when a late blight infection event is predicted three

days ahead. In addition, capacity building on integrated control of potato late blight for farmers and extension workers (Training of Trainers for the Agricultural Information Service (AIS) and the Department of Agricultural Extension (DAE)) was carried out to allow for a better understanding of disease development, the GeoPotato advice and disease management. AIS and DAE then train the farmers and encourage and help farmers to subscribe to the free GeoPotato service.

The GeoPotato Service
Technically, the GeoPotato service takes into account and evaluates: the planting date, crop growth, the weather and the most recent spray applied (Figure 1).

Planting date
When growers subscribe for the service, the automated system asks for a planting date. Planting dates in October, November and December are then aggregated in 4 crop cohorts per month each spanning approximately 1 week. These three months cover the complete potato planting season.

Crop cohorts and crop growth data
Satellite data are used to identify potato fields belonging to each of the 12 crop cohorts. Subsequently, the NDVI is measured during each overpass. The LAI is then derived from a time series of (Sentinel 2A and 2B) NDVI measurements for the individual crop cohorts. A simple temperature-based crop growth model is run in the background to fill inevitable gaps in the satellite data occurring e.g. when it is cloudy during the overpass.
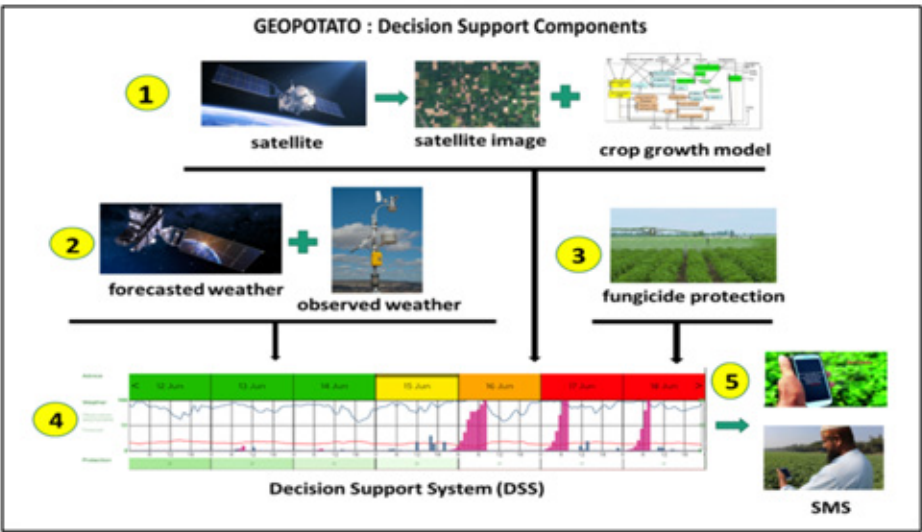
Figure 1. *The various information sources of the GEOPOTATO decision support system to control late blight in potatoes*

Weather data

Weather data serve to identify infection events in the near future (weather forecast) and in the recent past (data from automated weather stations). For this purpose we have assessed the performance of two well-known weather forecasting models, i.e. the Global Forecasting System (GFS) and the Weather Research and Forecasting (WRF) model. Based on a three-days forecasting, the skill of the WRF model was better than the GFS model but both models seriously underestimated the relative humidity, a key parameter driving the biological model simulating the *P. infestans* life cycle. Incorporation of the results (forecasts) of both models in the DSS would therefore underestimate the risk of late blight infection.

The cause of the underestimation of the boundary layer rH lies in the specific geography of Bangladesh in combination with typical regional winter weather conditions. During the winter, northerly winds pass over the Himalaya mountain range rendering the air cold and dry due to the altitude and the Fohn effect. When this cold and dry air comes in contact with "warm" water in Bangladesh's many river systems, dense, late blight conducive, fog often is the result. The lower relative humidity of the higher atmospheric layers is forecasted reasonably well. The high relative humidity of the boundary layer typically is (severely) underestimated.

As a last resort, 10 years of locally measured weather data was analyzed for *P. infestans* infection events. The results were summarized in a "late blight calendar" containing the chance (%) on a late blight infection event for each calendar day. In the final operational version of the GeoPotato system, this calendar replaced the (GFS or WRF) weather forecast.

The DSS

Potato farmers in Bangladesh need approximately three days to discuss, plan and execute a spray application. Thus, the DSS keeps track of the number of infection events since the last spray application plus the infection events predicted for the next three days. From the Euroblight foliar protection score of Mancozeb, the most commonly used fungicide in Bangladesh, it was derived that a Mancozeb application will protect the foliage against approximately five infection events. Thus, when the sum of infection events since the last spray plus the next three days exceeds five, a new spray advice is issued using a short text messaging (SMS) service and a voice message for illiterate farmers.

The user subscription system

Figure 2 shows a schematic of the user subscription system and its components including the DSS. Farmers subscribe to the GEOPOTATO service by dialing a telephone number with an interactive voice response (IVR) system (1 in Figure 2). Extension agents have been trained to support the subscription of those farmers that are not familiar with IVR. The farmers mobile number is automatically recorded while the farmer needs to provide the location of farming, the planting date and the sub-district (*upazilla* in Bangladesh). This information is stored in the aggregator platform that is connected with the DSS (2 in Figure 2). Updated DSS information is shared with the aggregator platform (3 in Figure 2). When the DSS identifies an infection period three days ahead an SMS is prepared for farmers with crops in cohorts and upazilla's at risk (Number 4 in Figure 2). The SMS gateway connects to the mobile networks to send farmers an SMS containing information when to spray and, in general terms, what to spray.
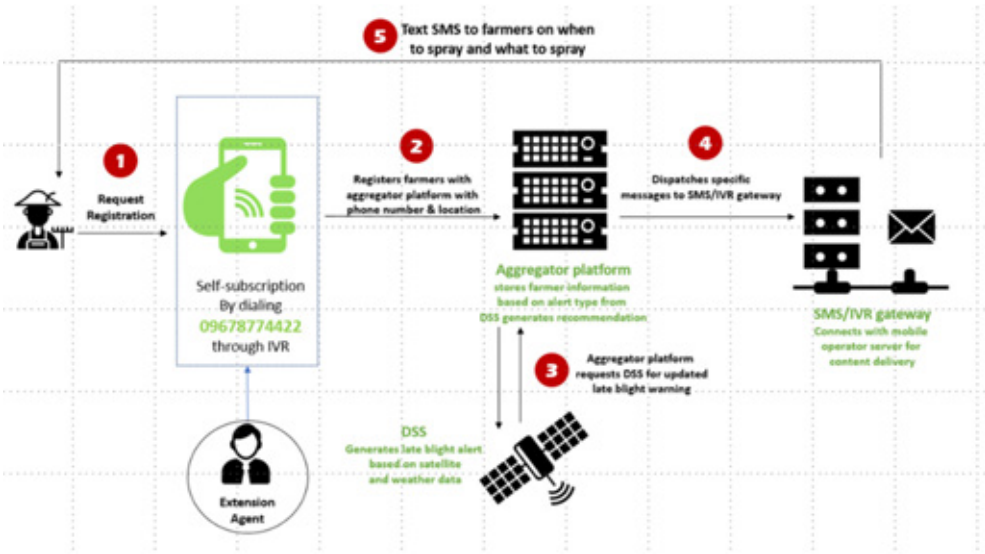
Figure 2. *GEOPOTATO's user subscription system*

The service area's

GEOPOTATO started its services in the 2016-2017 potato season in Munshiganj district just south of the capital Dhaka. Each consecutive year a new district was added, Rangpur for 2017-2018 and Dinajpur for 2018-2019. All districts included are major potato producing areas.

RESULTS

The GEOPOTATO service has run for three consecutive potato growing seasons: 2016/17, 2017/18 and 2018/19. During the first season, the service was only operational in Munshiganj to test the user ICT system and the technical components of the DSS including the GFS weather forecast. In total, 111 farmers received eight SMS alerts during the potato growing season (Pronk *et al.*, 2017). In the post-season evaluation, 94% of the farmers that received SMS alerts were satisfied with the service. A large majority of the participating farmers (92%) faced no problems in understanding the content of the SMS alerts and 87% of the farmers acted upon the alerts received. Based on the favorable farmer response to the GEOPOTATO service, the service was expanded to the Rangpur district in 2017/18 and Dinajpur in 2018/19.

Table 1. *Number of subscriptions to the GeoPotato service in three consecutive growing seasons*

Year	# Subscriptions	Area coverage (districts)
2016/17	111	Munshiganj
2017/18	6,762	Munshiganj + Rangpur
2018/19	42,000	Munshiganj + Rangpur + Dinajpur

The number of subscriptions for all three seasons are given in Table 1. In general, climatic conditions are more favorable for late blight in the Northerly districts of Rangpur and Dinajpur as compared to Munshiganj. In total, 6762 farmers subscribed to the GEOPOTATO service in the 2017/18 season and around 42,000 farmers in the 2018/19 season.

Potato yield was quantified on a limited number of potato fields subscribed to the GeoPotato service and compared to common practice without access to GeoPotato advice. In general, potato yields were positively influenced by the GeoPotato service (Table 2).

Table 2. *Yield change as a result of adhering to GeoPotato late blight spray advice in Munshiganj, Rangpur and Dinajpur*

Potato season	Number of fields	Yield change	District
2016/17	6	+7%	Munshiganj
2017/18	6	-1%	Munshiganj
	14	+22%	Rangpur
2018/19	7	+13%	Rangpur
	6	+20%	Dinajpur

The greater impact of the service on potato yields in Rangpur and Dinajpur is also reflected in the satisfaction of farmers that received SMS alerts in the 2017/18 potato season. In Rangpur, 89% of the SMS farmers was satisfied with the GEOPOTATO service, while a high but significantly lower percentage of farmers (72%) was satisfied with the service in Munshiganj.

DISCUSSION AND CONCLUSIONS

A large part of the rural population of Bangladesh depends on agriculture: crops, livestock, fisheries and forestry. Due to climate change, weather conditions become less predictable and farmers cannot rely on experiences from the past. Additional knowledge and information is needed to upgrade the knowledge and tools used by the farming population. Access to knowledge and information is however a major challenge for farmers.

The GEOPOTATO service is the first operational form of precision agriculture in Bangladesh. SMS alerts aimed at optimized potato late blight control were received and used by >40.000 smallholder farmers in 15 different sub-districts (upazilla's). In its current form, the GeoPotato system is a "one way" decision support system, information is sent to the farmer and hopefully used, but the farmer does not report back. More 'precision' can be achieved by turning the system into a two-way system in which the farmer would report back on the activities carried out. This would however necessitate the use of smart phones or computers instead of the currently widely used GSM phones.

Technical improvements would include a significant improvement of weather forecast models. With a reliable weather forecast, the climate statistic currently used could be replaced by forecasted weather data. That would allow the GeoPotato system to take into account actual forecasted weather instead of a climate statistic based on 10 years of historic weather data.

Despite drawbacks mentioned above, the results achieved with the GEOPOTATO service clearly show the (monetary) value of the service to the farmers subscribed. Significant yield increases are common and common over-spraying is avoided.

Despite the promising results it is not expected that small and resource-poor farmers are willing to pay for the GEOPOTATO service. Farmers in Bangladesh are used to receive public extension services for free, while many inputs (fertilizers, agri machineries, seed, etc.) are also subsidized.

The private agro-advisory service sector in Bangladesh is still in its infancy as in many other less-developed countries. Partners in the GEOPOTATO project are therefore cooperating with various input supply companies and other sector industries to assess whether they are willing to adopt the service for their clients and contract farmers. Agro-input suppliers could e.g. include the GeoPotato service as an add-on service on goods or services they already provide to win more clients and drive market expansion. For potato processing companies it can be part of a strategy to increase farmer production and to reduce potato sourcing costs. Further assessment of the GEOPOTATO service in terms of benefit(s) for farmers, retailors and other private sector companies is part of the strategy to develop a sustainable advisory service for the potato sector in Bangladesh.

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Integrating cultivar resistance into a disease model for controlling early blight (*Alternaria solani*)

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SUMMARY

Field experiments were conducted to integrate cultivar resistance to an early blight-forecasting model (TOMCAST). Three cultivars (cvs. Agata, Sava, and Kuras) that differed for their level of resistance to early blight were either sprayed at every 14 days (standard treatment) or according three different TOMCAST threshold (15, 20 and 25) with Signum WG (Pyraclostrobin + Boscalid). Untreated plots were also included to serve as a check of the disease progression during the season. Results obtained from this study showed that TOMCAST-15 is the best TOMCAST threshold for Sava and Agata. However, for Kuras sufficient control of early blight comparable to the standard treatment was obtained for all TOMCAST thresholds. In conclusion, this study found that fungicide application or model thresholds for recommending fungicide applications could be adjusted according to host resistance to control early blight with fewer numbers of fungicides.

KEYWORDS

Early blight, *Alternaria solani*, Potato, Cultivar resistance

INTRODUCTION

Early blight, caused by *Alternaria solani*, is an increasing problem in Denmark (Abuley and Nielsen, 2017; Nielsen, 2015). The disease can cause substantial yield losses of potatoes if not controlled properly. Generally, the average yield loss due to early blight is 7-20% (Abuley and Nielsen, 2017).

Usually, fungicides, which happens to be the most effective control method available, are used to control the disease at weekly or two-weekly intervals, regardless of the favorability of the environment for the disease development (Abuley and Nielsen, 2017). However, the increasing public concerns about the negative effects of fungicides in the environment, high cost and resistance development associated with frequent use of fungicides have necessitated the use of forecasting models to regulate the application of fungicides (Abuley and Nielsen, 2017; Leimhnger and Hausladen, 2012). Generally, forecasting models predict critical times in the season when the weather is favorable for the pathogen to infect a susceptible host.

Because the development of early blight is dependent on weather variables like temperature and humidity, forecasting models have been developed to forecast when critical times for disease development occurs and thus fungicide application is recommended. For example, models like TOMCAST (Gleason *et al.*, 1995), FAST (Madden *et al.*, 1978), and physiological days (Pscheidt and Stevensen, 1988) have used to time the application of fungicide for controlling early blight.

The TOMCAST model was modified and validated in Denmark recently (Abuley and Nielsen, 2017). Briefly, fungicide application in the modified-TOMCAST model is based on the TOMCAST disease severity value (DSV) and the maturity of the crop. The maturity of the crop is estimated as thermal age (with units P-days) since emergence with the physiological day model (Sands *et al.*, 1979). For example, the first spray is recommended when the plant is 330 P-days and the cumulative TOMCAST DSV is at least 25 since crop emergence (Abuley and Nielsen, 2017). The subsequent spray depends on a predetermined cumulative TOMCAST DSV threshold (e.g. 15, 20). Shorter thresholds denote a shorter interval between subsequent spray and vice versa.

Even though several forecasting models have been used to early blight control successfully, the models omit cultivar resistance. Thus, an implicit assumption in most of the models is that all cultivars are equally susceptible to early blight. However, potato cultivars differ for their level of resistance to early blight and thus the need to include it into models (Abuley *et al.*, 2018). Therefore, the objective of this study was to integrate cultivar resistance into the TOMCAST model to adjust fungicide applications.

MATERIALS AND METHODS
A field experiment was carried out at Flakkebjerg Research Centre in Denmark in 2016. The experiment was designed as a factorial randomized complete block design (RCBD) replicated four times. The factors in the experiment were three levels of cultivar resistance and five levels of fungicide schedules. The cultivars used were cv. Agata (very susceptible, early maturing, ware potato), cv. Sava (moderately slow blighting, medium maturing and ware potato) and cv. Kuras (Slow blighting, late maturing, and starch potato). The order of increase of resistance of the cultivars to early blight is as follows cv. Agata< cv. Sava< cv. Kuras (Abuley *et al.*, 2018). The fungicide schedules were (1) Untreated, here no fungicide was applied; (2) Standard treatment, in which 0.25 kg/ha Signum WG (Pyraclostrobin + Boscalid) was sprayed at a 14-day interval beginning from row closure; (3) Three TOMCAST threshold (15, 20 and 25). For a given TOMCAST threshold treatments, first and subsequent sprayings were recommended based thermal age/physiological age of the crop as described previously (Abuley and Nielsen, 2017). As in the standard treatment, Signum WG (Pyraclostrobin + Boscalid) at a rate of 0.25 kg/ha was sprayed in the TOMCAST treatments.

The potato crops were not inoculated; however, the experimental plots were surrounded by other field experiments that were inoculated with both *A. solani* and *A. alternata*. Thus, an influx of conidia of both *A. solani* and *A. alternata* into the experimental plots was expected.

For running the TOMCAST model, hourly readings of temperature and relative humidity (RH) were taken from the Dalmose weather station (Abuley and Nielsen, 2017). Leaf wetness was estimated as an hour with RH>88%. The thermal age (Physiological age/days); with units P-days, of the potato cultivars were determined from 50% emergence equation described by

Sands *et al.* (1979). The estimated leaf wetness 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

The percentage covered by early blight on each plot weekly. The disease assessment data was fitted to the linearized logistic growth model to estimate the apparent rate of infection for comparisons between the treatments (Madden *et al.*, 2007). The starch yield was assessed for each treatment as described in Abuley and Nielsen (2017).

The rate of infection and starch and tuber were yield analyzed with generalized least squares with the "gls" function in the "nlme" package (Pinheiro *et al.*, 2016) in R (R Core Team, 2016).

RESULTS AND DISCUSSION

The statistical analysis showed that the effect of cultivar, fungicide schedule and the interaction between cultivar and fungicide schedule was statistically significant ($p < 0.001$) for the rates of infection. This suggests that both cultivar resistance and fungicide application are important for controlling early blight. Moreover, the significant interaction between the fungicide schedules and cultivar resistance means that fungicide application could be adjusted according to cultivar the level of host resistance to manage early blight successfully.

The rate of infection or apparent rate of infection is an indication of how fast the disease develops on the cultivars and thus higher rate of infection means faster development of the disease on the cultivar in question. As expected, the rates of infection of early blight on the untreated cultivars were significantly higher compared to the cultivars that were treated with fungicide (Table 1). This is a confirmation of the importance of fungicide in controlling early blight, as it has been shown in previous papers (Abuley and Nielsen 2017; Leiminger and Hausladen, 2012). The rate of infection of the untreated cultivars depicts the natural development of early blight on the different cultivars and thus confirms the fact that the cultivars vary for their level of host resistance to early blight. This is evidenced by the significant differences between the rates of infection of the untreated cultivars (Table 1). As expected, Agata was the most susceptible cultivar to early blight, followed by cv. Sava (Table 1). Kuras was the most resistant cultivar compared to Agata and Sava (Table 1).

The results obtained in this study show that for Kuras no statistically differences were found between the standard treatment and the different TOMCAST thresholds (15, 20 and 25) (Table 1). This means that for Kuras, which is more resistant to early blight compared to the other cultivars, all three TOMCAST thresholds (15, 20 and 25) could be used to recommend fungicide application to control early blight. However, higher TOMCAST thresholds (e.g. TOMCAST 25) could be used for cultivars with higher level of resistance like cv. Kuras to control early blight with fewer numbers of sprays (Table 1).

Contrary to Kuras, the best control of early blight, which is the smallest rate of infection, on cvs. Agata and Sava was found on plots that were treated according to the standard treatment and TOMCAST-15 (Table 1).

Table 1. *Rate of infection of early blight and the total number of sprays Agata, Sava and Kuras treated according to the different fungicide schedules*

	Rate of infection (% day^{-1})			Number of sprays
Fungicide Schedule	**Agata**	**Sava**	**Kuras**	
Untreated	0.30 (0.005) a	0.26 (0.004) b	0.23 (0.008) c	-
Standard treatment	0.12 (0.002) g	0.12 (0.002) g	0.12 (0.004) g	6
TOMCAST-15	0.12 (0.004) g	0.11 (0.004) g	0.12 (0.007) g	4
TOMCAST-20	0.16 (0.002) e	0.15 (0.002) f	0.11 (0.004) g	3
TOMCAST-25	0.18 (0.002) d	0.16 (0.002) e	0.12 (0.004) g	2

For all cultivars, no significant differences were found between TOMCAST-15 (Table 1), which is an indication of the universality of this TOMCAST threshold. However, as the TOMCAST threshold increased (20 and 25), differences were more noticeable between the cultivars for the rate of infection. Whereas no differences were found between the rates of infection on cv. Kuras treated according to the different TOMCAST thresholds, the rate of infection between the TOMCAST threshold increased with TOMCAST thresholds, with the result that the lower the TOMCAST threshold the lower the rate of infection of early blight (Table 1).
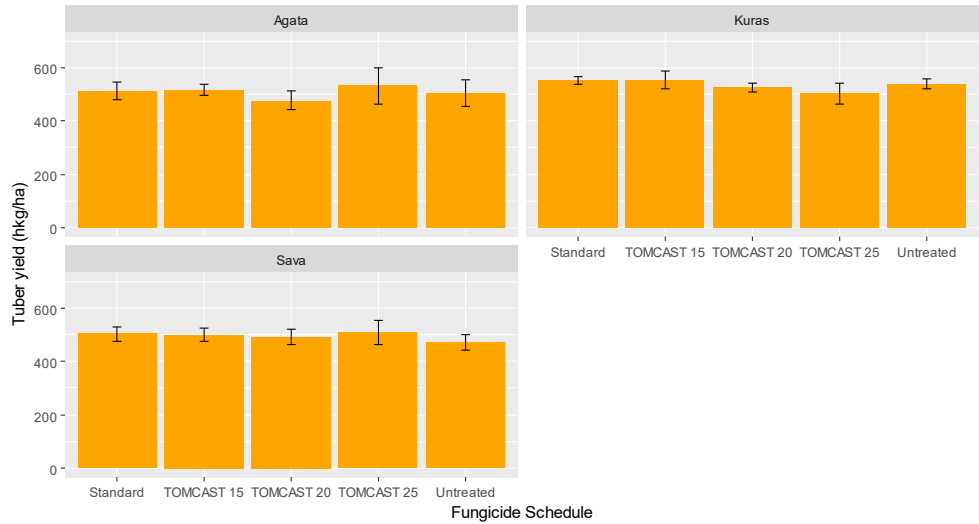
Figure 1. *Mean tuber yield of Agata, Kuras, and Sava sprayed according different fungicide schedules. Means followed by the same letters are not significantly different and vice versa*

Contrary to rates of infection, the statistical analyses showed that neither the effect of cultivar, fungicide nor their interaction was significant ($p > 0.05$) (Figure 1).

CONCLUSION

This study showed that both cultivar resistance and fungicide are important components for controlling early blight. Moreover, fungicide application or forecasting models could be adjusted according to cultivar resistance to reduce fungicide applications. For TOMCAST, this study showed that a longer threshold could be used for resistant cultivars to reduce the number of sprayings.

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Tracking Worldwide Migrations, Evolutionary Relatedness and Reemergence of *Phytophthora infestans*: A threat to Global Food Security

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Phytophthora infestans, an oomycete pathogen, caused the Irish potato famine and is a threat to food security globally. *Phytophthora infestans* was the first species in the genus described and left a path of devastation on potato in its wake in the US, Ireland and Europe in the nineteenth century. Several important research questions of interest in my lab include the identity and source of famine-era lineages, understanding the global distribution of the famine lineage, understanding effector diversity though time and the evolutionary history of the Ic clade of *Phytophthora*. We have identified and tracked the spread of historic *P. infestans* using multilocus genotyping, next generation sequencing, microsatellites, geospatial analytics, and data mining and novel detection methods.

Using historic herbarium specimens, we identified the type Ia mitochondrial lineage caused famine era outbreaks and documented that US-1 (type 1b mitochondrial lineage) migrated later in the mid 20th century globally (May & Ristaino, 2004; Ristaino *et al.*, 2001). We have studied the evolutionary history of the pathogen over the past 150 years and examined differences in historic and modern day genomes (Martin *et al.*, 2013; Martin *et al.*, 2014). Interestingly, genes known to be important for virulence in modern *P. infestans* were absent in historic strains (Martin *et al.*, 2013).

Martin *et al.*, (2013) and Yoshida *et al.*, (2013) sequenced genomes from historical *P. infestans* from infected potato samples in Europe. Both studies corroborated Ristaino *et al.*, (2001) concluding that the historical lineage was not US-1. The famine lineage was distinct from modern *P. infestans* lineages at avirulence loci, and lacked many of the virulent forms of *Avr* genes, suggesting the pathogen genome has expanded over time. Yoshida also suggested that the lineage first emerged in the US from a Mexican source and was rare or possibly extinct (Birch *et al.*, 2013; Yoshida *et al.*, 2013). Martin *et al.*, (2014) refuted this claim by sequencing 45 additional mitochondrial genomes (Avila-Adame *et al.*, 2006) from historic samples, and modern samples and found the famine lineage was not extinct. In addition, the mtDNA haplotype of the famine lineage (HERB-1) (Yoshida *et al.*, 2013) was identified in the Andean hybrid species *P. andina*, suggesting that this mtDNA haplotype was not exclusive to *P. infestans* and that it still exists in South America (Martin *et al.*, 2014; Martin *et al.*, 2016).
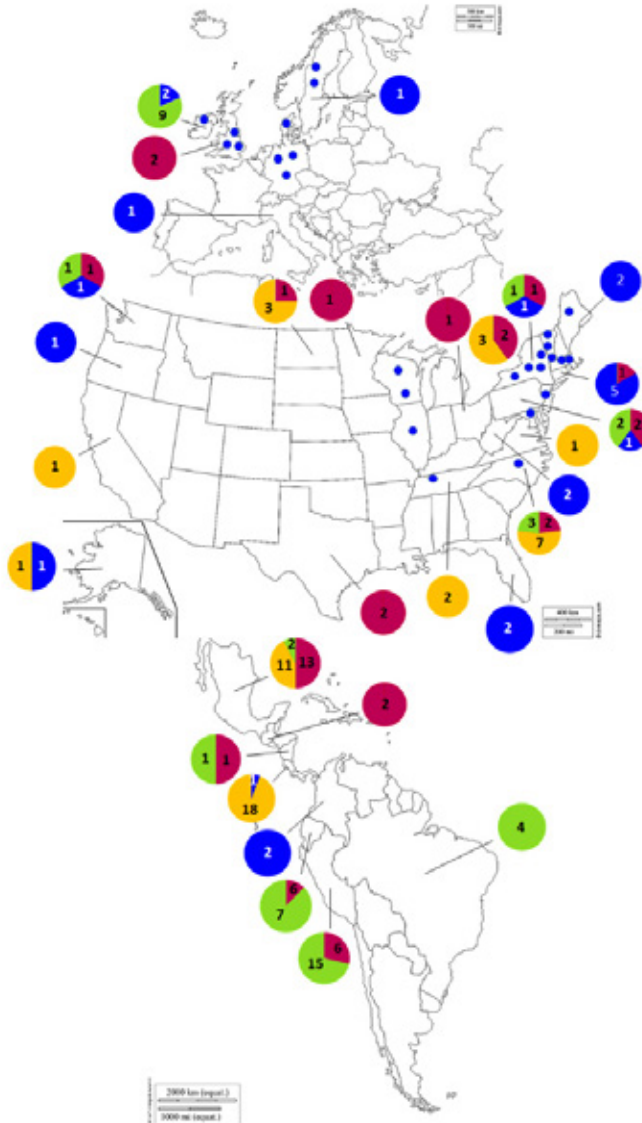
Figure 1. *A) Geographic distribution of multilocus genotypes of Phytophthora infestans. Small blue circles indicate FAM-1 SSR lineage found before 1900, and larger circles indicate samples collected after 1900. Genetic clusters correspond to K = 4 groups by color. Illustration reproduced from Figure 1 Saville et al., 2016 under open access article Creative Commons* https://dx.doi.org/10.1371/journal.pone.0168381

A larger set of historic US and European *P. infestans* from herbaria (1846-1970) and more recent isolates (1992-2014) were genotyped with 12 plex SSR multilocus genotyping (Saville *et al.*, 2016). A single, unique SSR multilocus genotype, named FAM-1, caused outbreaks in both

the US and Europe and was widely distributed (Figure 1). Interestingly, the FAM-1 lineage shared allelic diversity and grouped with the oldest historic specimens collected in Colombia and Costa Rica in the early 20th century. The FAM-1 lineage of *P. infestans* formed a genetic group that was distinct from more recent aggressive lineages found in the US. The US-1 lineage arose later and formed a second, mid-20th-century group with shared allelic diversity with US-1 lineages from Peru. The FAM-1 SSR lineage survived for almost 140 years in the US and was geographically more widespread than originally assumed. This is evidence that FAM-like genotypes once existed in Ecuador, Colombia, and Peru, the known range of *P. andina* (Martin *et al.*, 2016; Saville *et al.*, 2016). Clearly, further studies of the 1c clade of *Phytophthora* are warranted in the region to learn more about the early evolution of *P. infestans*. More recent aggressive lineages formed a genetic group that was distinct from historic lineages (Martin *et al.*, 2016). The FAM-1 spread beyond the US and Europe into Africa, Asia and Australia/ Oceania.

Population genomics data from historic *P. infestans* also links ancestral lineages to *P. andina* in the Andes. Admixture was found between *P. andina* and the historical famine era-European lineage (Martin *et al.* 2016). An important finding from our work is that the famine lineage from historical Europe is one of the parents of the hybrid *P. andina* that is endemic to the Andean highlands of South America. As the *P. andina* lineages are the most basal in a phylogeny that includes much of the known diversity of *P. infestans*, this indicates that it diverged early on in the history of *P. infestans.* Thus, the closest relative of *P. infestans* is likely endemic in South America on a wild host. We are developing new knowledge and using new tools including sensors, population genomics and geospatial surveillance to observe, contain and limit outbreaks by this important plant disease.

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Towards Late Blight IPM in practice: a UK perspective

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SUMMARY

Late blight (*Phytophthora infestans*) in potatoes is a disease which still has the ability to completely destroy a crop or ruin any financial value it may have had. In practice, the usefulness of IPM is limited by several factors, including the need to produce a more-or-less entirely blight-free crop, the selection of cultivars by buyers and not those involved in growing the crop, and the unreliability of weather forecasts looking more than a few days ahead. The emergence of strains of blight which are resistant to, or show reduced sensitivity to existing fungicides is placing increasing reliance on a small number of groups of active ingredients, which is concerning, especially as regards preventing tuber blight infection. With food being abundant, regulatory pressures on the agchem industry will not ease in the foreseeable future. This means that control of blight will have to involve an increased use of resistant cultivars, probably as a mixture of new cultivars, and existing cultivars being given resistance through new genetic technologies.

KEYWORDS

Late blight, *Phytophthora infestans*, IPM, weather forecasts, resistant cultivars

INTRODUCTION

Since late blight first appeared in Europe in the mid 1840's, it has been a disease for growers and their advisors to fear. If left uncontrolled in a season favourable to the disease, and where a cultivar without complete resistance is grown, it still has the ability to completely destroy a crop or render it of no financial value.

IPM THROUGH THE MEASUREMENT OF RISK

For approximately 30 years, blight risk in GB was measured by Smith Periods. A Smith Period occurred when there were two consecutive days where each had a minimum temperature of 10°C for at least eleven hours, and with a relative humidity ≥ 90% (Smith L. P., 1956). Over time, it became clear to those giving fungicide advice to growers, that Smith Periods were underestimating risk, probably because of the agressiveness of newer strains. Following research for a PhD at the James Hutton Institute in Dundee (Dancey S., 2018), the measurement of risk in GB was updated in 2017 to the Hutton Criteria, where the period of relative humidity ≥ 90%

was reduced from 11 hours to 6. To date, anecdotally this change in risk measurement has been a distinct improvement. It is hoped that periodic research, perhaps every decade, will continue to update risk criteria for blight.

IPM, GROWER SIZE AND WEATHER FORECASTS

Larger growers, typically those growing 200 ha's or more of potatoes, are especially vulnerable to the ravages of the disease. These are people whose farming businesses are tilted disproportionally towards the crop, and who therefore cannot afford to suffer a significant financial loss caused by blight. To achieve blight-free crops, they are dependant on a regular planned spray schedule of fungicide application, which requires detailed planning and faultless execution.

Significant improvements in weather forecasting have occurred as a result of the increasing availability of satellite data and increasing computer capacity to run forecasting models (Bauer P. *et al*, 2015). Nevertheless, 7 and 10 day forecasts are not reliable enough to allow large growers to significantly drift from pre-planned fungicide application schedules. Smaller growers, who have the flexibility to change plans at short notice and whose income is less proportionally dependant on the potato crop, can follow changes in forecasts and Hutton Period events to adjust fungicide schedules as required. This sort of flexibility is at the heart of successful IPM.

IPM THROUGH CULTIVAR SELECTION IS NOT IN THE HANDS OF THE GROWER

Because "the customer is always right," cultivar choice is largely driven by buyers and not growers. Growers will normally have a choice of cultivar from a list provided by the buyer, but except in the case of organic production, such choices rarely include cultivars with significant blight resistance. There is very little overlap between cultivars used in conventional and organic production, largely because buyers want the organic segment of the market to be effectively separate from conventional, which means distinct cultivars in the organic sector. With the organic sector being only a tiny fraction of the total potato market, the proportion of the potato area grown with cultivars with good blight resistance is similarly tiny. This needs to change.

AN UNINTENDED CONSEQUENCE OF HERBICIDE LOSS IN OTHER CROPS

Experience suggests that outgrades are still the main source of blight outbreaks in the early part of the growing season. However, the loss of herbicides in minor crops such as field-scale vegetables because of strict EU regulation is resulting in more potato 'volunteers' emerging and surviving in such crops. These can and do act as secondary sources of blight as the season develops. This trend of herbicide loss is only likely to continue and is an increasing barrier to implementing IPM, especially later in the growing season.

THE ROLE OF THE SPRAYER DRIVER IN SUCCESSFULLY IMPLEMENTING IPM

The sprayer driver has a vital (but too often neglected) role in making IPM work in practice. The main principle of blight fungicide application is to properly cover every plant in the field, so that spores blowing into the crop do not find gaps in that cover which allow them to germinate.

Driver errors include:

- Spray quality which is too coarse, resulting in gaps between droplets deposited on the foliage. In the past there was a conflict between correct spray quality for blight prevention and reducing drift, but with the improved nozzle technology developed in the last decade or so, that conflict should no longer be the problem it was.
- Boom height being too low, which is a natural reaction of drivers when wind conditions are marginal. This results in fungicide striping, and therefore strips of crop with less or no fungicide.
- Insufficient overlap at the start of a run when using GPS-controlled sprayers, resulting in poor coverage because of low pressure in the spray lines for the first 1-2m of that run.
- Water in the booms at the start of a spraying session, resulting in triangles of crop with no fungicide at the outer edges of the boom where the sprayer starts off in the field. Unfortunately the same parts of the same field tend to get this treatment with each successive fungicide application, with the inevitable outcome.
- Not using a headland nozzle (a separately-controlled nozzle on the outer edge of the boom) so that crop canopy which flops outwards as it grows into the edge of the field is poorly covered with fungicide, or not covered at all.
- Spraying when the canopy is too wet, resulting in fungicide run-off. This tends to happen when the sprayer is sent a long way from the home base, and the driver does not want to return having not done the job he was sent to do.

In addition, some drivers are much more observant than others in spotting blight (and other problems) in crops, and experience unfortunately suggests that those who pay less attention to the detail in terms of application are also the least observant in spotting and reporting problems.

FUNGICIDE-RESISTANT AND AGGRESSIVE STRAINS ARE A BARRIER TO IMPLEMENTING MORE RIGOROUS IPM POLICIES ON FARMS

The effects of EU-36 and EU-37 on blight fungicide programmes have been both profound and costly. Whereas before these strains appeared, fungicide programmes could be largely based around mixtures of cheaper generics, especially mancozeb and fluazinam, it has proved necessary to broaden the range of fungicides used to include those whose patents mean they are more expensive to purchase. Whereas before the emergence of these strains, programmes could be constructed costing under £200/ha, they now typically cost £350/ha and sometimes more. This increased cost is one which has been carried by growers (that is, not passed onto buyers) and has therefore significantly eroded the net profit (reward for risk) returned to the grower.

RELIANCE ON TOO FEW ACTIVE INGREDIENTS FOR TUBER BLIGHT CONTROL

It can only be hoped that EU-37 will disappear over time, and that the strains which displace it will show normal levels of fluazinam sensitivity. At the moment, the industry is disproportionately dependant on fluopicolide, and especially the QiI fungicides cyazofamid and amisulbrom, for tuber blight prevention. If any of these active ingredients was lost to resistance, then the industry would be extremely vulnerable to a bad blight season. To help prevent this happening, it is important that fungicides are not applied in blocks of say more than two consecutive applications, and that the QiI fungicides, currently sold as single active ingredient

products, are always mixed with a partner with a different mode of action. (Fluopicolide currently comes ready-mixed with propamocarb).

The loss of diquat as a desiccant in 2020 will increase the risk of tuber blight infection at the end of the growing season, because killing potato haulm prior to harvest will take longer than is currently the case, so exposing the crop to potential infection for longer.

IPM THROUGH VARIETAL RESISTANCE

Food is abundant in the EU, and that means the regulatory pressures on pesticides will surely continue. The net result of that, measured over a period of years, is the industry will have to depend on fewer active ingredients from a diminishing number of fungicide classes. Clearly selection pressure on those classes remaining will therefore increase, potentially leading to a cascade of fungicide resistance or reduced sensitivity, resulting in potential failures in controlling the disease. Therefore, from the perspective of IPM, the long term solution will have to come from resistant cultivars. Some of the major European seed houses are now starting to market a significant range of blight-resistant cultivars. How durable those resistant genes will prove to be remains to be seen. Apart from marketing into different sectors of the potato industry (dealt with above) the issues surrounding the widespread adoption of new cultivars are many and complex, but include:

- The ability of the seed house to rapidly multiply-up seed to levels where they gain 'critical mass' in the industry. This can be especially problematic with cultivars which set low numbers of tubers.
- Unknown problems in the cultivars which only become apparent with more widespread adoption. These include susceptibility to other diseases, seasonally-dependant consumer defects such as after-cooking blackening, and physiological problems such growth cracking. Experience suggests it can take 5-10 years of commercial growing to uncover the full range of a new cultivar's 'Achilles heels'.
- Reluctance among buyers to change cultivars. This is entirely understandable, in that once a cultivar has achieved good consumer acceptability, why change to something unknown? In the UK, Maris Piper, which in 2019 was still the most popular cultivar in GB and occupied 13.5% of the GB potato area (despite its introduction in 1963) is the prime example of this reluctance to change (Anon. 2019).

Nevertheless, the increasing pressure on fungicides caused by regulatory pressures, and fungicide resistance or reduced sensitivity, means that resistant cultivars will have to become much more common in the industry. The emergence of newer gene editing technologies such as CRISPR-Cas9 offers hope for the industry in the longer term. This is as long as there is consumer acceptance of such technologies, which most definitely hasn't been the case to date with older gene insertion techniques, as BASF's bruising experience in Europe with the GM blight-resistant cultivar Fortuna clearly demonstrated (Anon. 2011; Anon. 2014). So far, attempts to make CRISPR-Cas9 work in potato blight have not proved successful (van den Hoogen and Govers, 2018), although it must only be a question of time before the technology is mastered. Also, seed houses currently appear to be showing no interest whatsoever in any form of gene editing technologies, but that will surely change.

IMPROVEMENTS IN IPM IN THE FUTURE

Although the potato industry has significantly benefited from the recent introduction of oxathiapiprolin, representing the first of a new class of blight fungicides, current pesticide regulation probably means that the appearance of new fungicide classes cannot be relied on. As a result, big forward steps in IPM are only likely to come from the seed houses in the form of resistant cultivars. In terms of spray application technology, despite the widespread introduction of GPS-controlled sprayers and other aids to the spray operator, the person controlling the sprayer (whether driving it in the field or perhaps in the future remotely controlling it) will always be a key part in making any IPM strategy work in the field. As for weather forecasting, although the easy gains from the use of satellite data collection and enhanced computer modelling appear to have already been made, further refinements will surely increase the reliability of forecasts in the future. But for larger growers, the lack of accuracy of 7 and 10 day forecasts will remain a stumbling block to fully implementing flexible IPM fungicide programmes.

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Integrated control to manage pathogen evolution

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SUMMARY

Previously published mathematical modelling has shown that the selection rate for a new virulent or fungicide insensitive pathogen strain is positively associated with the *per capita* growth rate of the population. In the model simulations (which assumed that virulence and fungicide insensitivity were not genetically linked) fungicide treatment slows selection for virulence, by slowing the growth rates of virulent and avirulent strains, thus reducing the difference in their growth rates that drives selection. By the same principles, host resistance that limits epidemic rate should slow the evolution of fungicide insensitivity. Data are presented here from three field experiments to test the evolutionary principles derived by modelling. Selection for a clonal variant (13_A2), insensitive to phenylamides, was measured on two potato cultivars of contrasting partial host resistance, and not treated or treated with different doses of a phenylamide fungicide. Epidemic growth rates varied substantially between experimental sites and seasons, and the rate of selection was significantly slower where the *per capita* growth rate was lower. Virulence and fungicide insensitivity appeared to be linked in 13_A2. The modelling study made the assumption that these factors were not linked, therefore the pathosystem that was tested differed fundamentally from the assumptions in the modelling study. We conclude that the impact of cultivar and fungicide choice to provide acceptable disease control in the field should be considered alongside the impact of the combination of those control methods on the selection for clonal variants. Where a clonal variant already exists (even at very low frequency) which combines virulence and fungicide insensitivity but is not yet detected then integrating the affected host resistance and the affected fungicide mode of action will not help to reduce selection for the clone.

KEYWORDS

Late blight, *Phytophthora infestans*, fungicides, cultivar resistance, integrated control

INTRODUCTION

A key determinant of durability of host resistance is the rate at which one or more virulent strains are selected for in a pathogen population, thus increasing in frequency until control is eroded. Similarly the effective life (durability) of a fungicide mode of action is determined substantially by the rate of selection for insensitive strains. Aggressive and virulent *P. infestans* genotypes, as well as strains with decreased fungicide sensitivity, have been reported for *P. infestans* populations in the last 10 years. The foliar resistance ratings of several cultivars have been downgraded from resistant to moderately resistant (e.g. Cara with resistance rating of 7 in 2010 and 5 in 2012) when exposed to more aggressive and virulent genotypes such as 13_A2 (Lees *et al.*, 2012). Resistance to phenylamides has been widespread since the 1980s (Carter *et al.*, 1982; Holmes and Channon, 1984), and is a trait also associated with genotype 13_A2. More recently, decreased sensitivity to a widely used fungicide, fluazinam, has been reported for a new genotype, 37_A2 (Schepers *et al.*, 2018). A critical question therefore is how to best manage pathogen evolution of virulence and fungicide insensitivity. It is frequently hypothesised that complete reliance on either host resistance or fungicide is likely to be less durable than an integrated approach. The work reported here tested some principles underlying that hypothesis.

Principles governing the selection of fungicide insensitive strains have been derived and tested extensively against experimental data (van den Bosch *et al.*, 2014). These principles show that reducing the difference in the *per capita* rate of increase of sensitive and insensitive strains, slows selection; $s = r_R - r_S$. Resistance management aims to reduce s, the selection coefficient, by slowing the rate of increase of both the fungicide insensitive (r_R) and fungicide sensitive (r_S) strains, for example, by adding a second fungicide mode of action which is effective against both strains. By extension, it can be inferred from the governing principles that: (i) partial (rate-limiting) host resistance, which is effective against fungicide sensitive and insensitive strains should slow selection for fungicide insensitivity, and (ii) fungicide treatment, which is effective against avirulent and virulent strains, should slow selection for virulence. Modelling has demonstrated that this principle could be used to suggest ways to extend the durability of cultivar resistance (Carolan *et al.*, 2017).

For host resistance to reduce the rate of selection for fungicide insensitivity, however, it would need to affect the growth rate of both fungicide sensitive and insensitive strains. The *P. infestans* population in some countries is predominately clonal, resulting in the greater potential for strong genetic linkage between virulence and fungicide resistance in a set of clonal variants. There is therefore a need to understand how integrated control should be deployed. The aims of the work reported in this paper were: (i) to test whether the rate of selection of strains is associated with epidemic growth rate, as predicted by the governing principle, and (ii) to test the effect of integrated control in a pathosystem where virulence and fungicide insensitivity are not genetically linked.

MATERIALS AND METHODS

Field experimental design
In 2015 and 2016, three experiments were conducted to determine the effects of cultivar and fungicide on selection for the *Phytophthora infestans* clonal variant 13-A2. Two experiments were conducted in Ceredigion, Wales and one experiment in Ayrshire, Scotland. Experiments were laid out in a randomised plot design with four replicates. The cultivars were King Edward

(foliar late blight resistance rating 3) and Cara (5) (British Potato Cultivar Database). Six treatments incorporating three levels of fungicide inputs and cultivar resistance to control foliar late blight were included (Table 1). Plots were four rows wide by c. 8m long, or 8 rows by 5.1 m long. The full dose (1.0 dose) of Ridomil Gold 480 SL (480 g/L metalaxyl-M: Syngenta Crop Protection Ltd) to apply was calculated as 0.155 L/ha. This was taken from the maximum individual dose for metalaxyl-M in Fubol Gold (64% w/w mancozeb + 3.88% metalaxyl-M: Syngenta Crop Protection Ltd).

To minimise the risk of infection by inoculum other than the test isolates the experiments were planted earlier than standard blight fungicide trials (22 April 2015 in Ayrshire and 4 May 2016 for the two experiments in Ceredigion). Experiments were over-sprayed (including to 'untreated' plots) at 7 day intervals with a range of fungicides to prevent ingress of naturally occurring late blight inoculum prior to test treatments being applied. If fungicides were applied after plots were inoculated, the inoculated or infector plants were covered prior to fungicide sprays. Over-sprays were stopped at least 7 days prior to the inoculation date.

Table 1. *Combinations of cultivars and fungicide treatments tested in experiments in 2015 and 2016*

Treatment no.	Product applied	Rate applied [equivalent proportion of the maximum individual dose (where 1.0 = full dose) of metalaxyl-M in Fubol Gold]	Cultivar (foliar blight resistance rating)
1	Untreated	-	King Edward (3)
2	Untreated	-	Cara (5)
3	Ridomil Gold	0.051 [0.33]	King Edward (3)
4	Ridomil Gold	0.103 [0.67]	King Edward (3)
5	Ridomil Gold	0.051 [0.33]	Cara (5)
6	Ridomil Gold	0.103 [0.67]	Cara (5)

Production of inoculum and inoculation procedure for field experiments
Infector plants (cv. King Edward, grown in a polytunnel) were each inoculated with 5ml of a 1 x 10^5 sporangia/ml suspension where the concentration of 13_A2 and 6_A1 had been adjusted to a 5:95 ratio. Plants were maintained at high relative humidity for at least 48 hours to encourage infection. Once 'peppering' symptoms were observed, the plants were ready to be transplanted into plots. Two pot-sized holes were dug in the centre of each plot and a single infector plant was placed in each one.

Leaf sampling for genotyping
Leaf lesion samples for genotyping were taken when sporulating lesions were visible. Twenty-four leaves with sporulating lesions were randomly selected from the central two rows of each plot and incubated in Petri plates containing damp paper tissue prior to pressing onto FTA cards using the EuroBlight protocol method. Once dry, individual FTA cards were placed in separate small Ziploc plastic bags for transport to the James Hutton Institute for genotyping.

DISEASE ASSESSMENT AND EPIDEMIC PROGRESS
Foliar disease was assessed at least weekly during the season (as the percentage of leaf area affected) (Anon, 1976).

Selection coefficient and per capita growth rate

The rate of selection for 13_A2 was quantified for each treatment in each experiment as the selection coefficient (defined in van den Bosch *et al.*, 2014), calculated from the change in the proportion of 13_A2 in the population between inoculation and at least 7 days after the fungicide application). The *per capita* growth rate of the pathogen population (of all strains) was estimated, for each treatment and experiment, by fitting a logistic curve to the foliar late blight severity data. All analysis was done in Genstat 18th Edition (VSN International Ltd, UK).

RESULTS AND DISCUSSION

When comparing the epidemics within each of the experimental sites, the largest differences in foliar blight progress were between the two cultivars rather than fungicide treatments within cultivars (Figure 1). Comparing between sites, the epidemic was relatively slow at the Ayrshire site in 2015, with 58% leaf area affected by 31 August compared to 100% leaf area affected by the 13 August in Ceredigion. The epidemics at Ceredigion in 2015 and 2016 were similar in both years. The largest differences in *per capita* growth rate were between sites and seasons, rather than between treatments. Linear regression analysis showed that fitting separate lines for the two fungicide doses was not justified. Eighty-three percent of the variation was accounted for by regression lines, with a common intercept, fitted to the two cultivars with and without fungicide (Figure 2). Analysis is ongoing to test whether the regression model can be simplified further.

Carolan *et al.* (2017) predicted that any reduction in the growth rate of both the virulent and avirulent strains would decrease selection for virulence. The experimental data support this, with positive slopes in the fitted regression lines for all treatments. The variation in *per capita* growth rate arose predominantly from differences in environment between sites and seasons. Carolan *et al.* also demonstrated that, where virulence and fungicide insensitivity are <u>not</u> genetically linked, fungicide treatment would slow the selection for virulence, by reducing *per capita* growth rate. In the experimental system tested here, virulence and fungicide insensitivity appeared to be linked for 13_A2, demonstrated by the significantly greater slope for the fungicide treatment on Cara relative to the untreated. Hence, cultivar resistance and fungicide treatment slowed the epidemic, but also added to the selection pressure for 13_A2. Overall, selection for 13_A2 was not decreased by fungicide treatment in this scenario: the range of values for the selection coefficients for the more resistant cultivar (Cara) treated with fungicide and all King Edward treatments were similar. It appears that this was due to counteracting selection effects: cultivar resistance and fungicide treatment added to the selection pressure for 13_A2, but also reduced the *per capita* growth rate and therefore slowed the selection for virulence, together resulting in a neutral effect.

There is other evidence that more virulent strains may be selected for on resistant and partially resistant cultivars compared to more susceptible cultivars. When 13_A2 was first tested against cultivars, the foliar blight resistance rating for Cara was downgraded from a resistant (7) to moderately resistant (5) cultivar, whereas King Edward and other susceptible cultivars maintained their original rating (Lees *et al.*, 2012). Similarly, it has been demonstrated previously that 13_A2 was found at a higher proportion on partially resistant compared with susceptible cultivars and that very resistant cultivars, such Sarpo Mira and Bionica, select strongly for 13_A2 (Stellingwerf *et al.*, 2018). This provides strong evidence that where virulence and fungicide insensitivity are genetically linked in clonal variants, integrated control strategies need to consider both the impact of the strategy on the selection for virulence and the

effectiveness of the strategies in the field. Integrated disease management, particularly the use of cultivar resistance in combination with reduced fungicide input, has been proven to decrease the severity of foliar late blight (Fry, 1978; Kirk *et al.*, 2001; Kirk *et al.*, 2005; Nærstad *et al.*, 2007; Bain *et al.*, 2011). In these previous studies, the effectiveness of a cultivar/fungicide combination was tracked by assessing the progress of foliar blight. The impact of the strategies on the proportion of different *P. infestans* strains in these studies, was not determined.

Our findings suggest that where a clonal variant is able to overcome two or more control measures, then integrating those control measures is unlikely to slow selection for that variant. There are, however, scenarios in which integrated control could constrain pathogen evolution. Firstly, where sexual recombination predominates, for example *P. infestans* in Norway and Sweden, then the benefits of integration outlined in Carolan *et al.*, 2017 would apply. In that circumstance, it is still possible that a clonal variant with both host resistance and fungicide insensitivity will arise through sexual recombination and lead to a particularly fit clone, but integrated control may delay emergence of a fit clone. This could be important, as new clones of *P. infestans* have been detected initially in predominately sexual populations (e.g. 13_A2) and have gone on to spread across Europe. Secondly, using a cultural control method that slows the epidemic growth rate, but does not cause selection pressure, should slow selection for both virulence and insensitivity.

As legislation moves towards a hazard based rather than risk based assessment for pesticides, and plant pathogens shift towards more virulent strains and fungicide insensitivity, it is necessary to implement strategies that will be sustainable and cost effective. There is therefore a need to consider both the effectiveness of integrated strategies on disease control, but also the impact of such strategies and legislation on the evolution of future pathogen populations and our ability to maintain control.
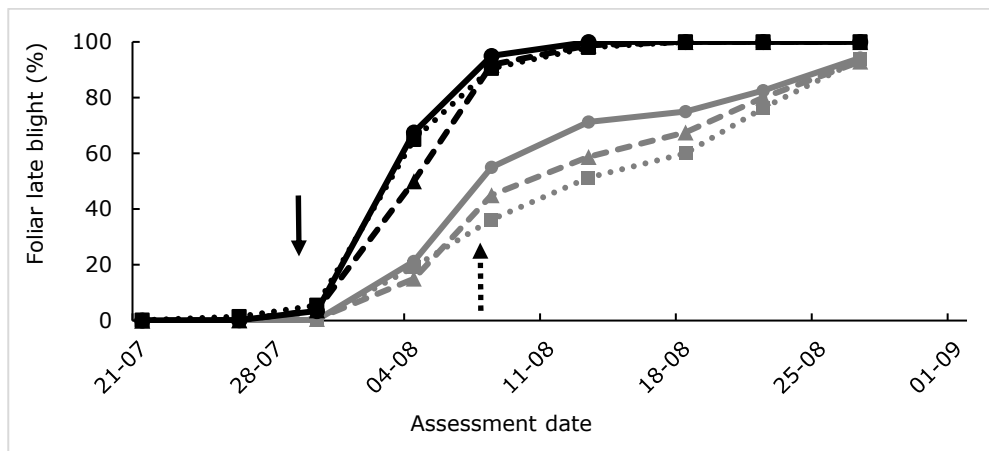
Figure 1. *Disease progress for fungicide dose and cultivar combinations at Ceredigion in 2016. King Edward (black) and Cara (grey). No fungicide (circles), ¹/₃ dose (triangles) and ²/₃ dose (squares). Arrows represent when the fungicide was applied (solid arrow: 30 July) and when the lesion samples were taken (dotted arrow: 8 August)*
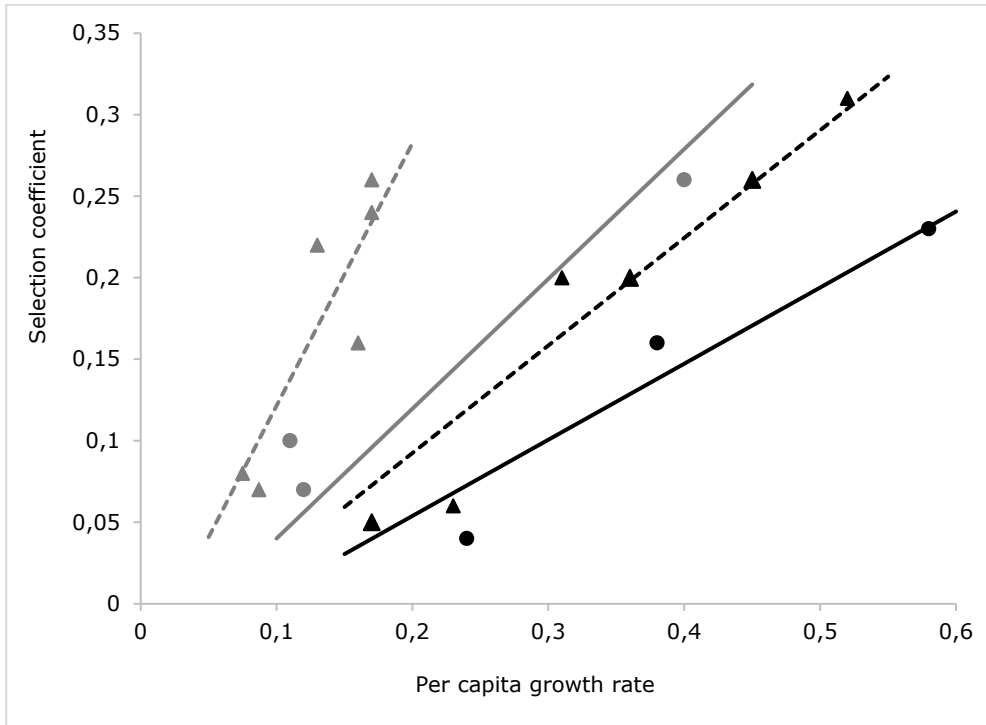
Figure 2. *Selection coefficient plotted on per capita growth rate of the epidemic for each fungicide dose and cultivar combination from three field experiments (R^2 = 0.83). King Edward (black) and Cara (grey). No fungicide (circles) and with fungicide (triangle). The lines represent the fitted model for with (dotted lines) and without (solid lines) fungicide for each cultivar. The difference between fungicide doses did not justify separate regression lines*

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SDHI cross-resistance pattern of *Alternaria solani* field mutants and consequences for Early Blight control

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SUMMARY

Alternaria solani is a fungal pathogen, which can infect commercially important *Solanaceae* crops such as potato and tomato. After first infection, dark-brown leaf spot symptoms with characteristic concentric rings appear on leaves. From there, asexually produced conidia are dispersed leading to further infections in the canopy, in case weather conditions are favorable. To stop such infections, fungicides are widely used. However, *A. solani* strains, which are adapted to selection pressures from commonly used respiration inhibitors such as quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs), were reported by various researchers over the last years.

To monitor the spread of such phenotypes, samples were collected in important potato growing regions in Netherlands, Belgium, Germany and Great Britain from 2016 to 2018. Molecular tools were applied to identify the mutations responsible for detected phenotypes. A cross-resistance study was conducted to demonstrate the impact of detected mutations on selected compounds from the group of SDHIs. Finally, greenhouse experiments were conducted to elucidate the impact of the detected mutations on commercially available solutions and its' implication on fungicide resistance management is discussed.

KEYWORDS

Alternaria solani, early blight, fungicide resistance, SDHI, QoI, DMI, efficacy

INTRODUCTION

Alternaria solani is a highly destructive pathogen causing the Early Blight disease of potatoes and tomatoes, which leads worldwide to significant yield losses. Different factors, such as environmental conditions and plant physiology seem to have an influence on the disease progress of Early Blight disease. Epidemics primarily caused by *Alternaria solani* mainly occur when the weather is warm and dry with short periods of high moisture (Chaerani and Voorrips, 2006). First symptoms occur on lower leaflets at favourable weather conditions. Subsequently, the pathogen spreads onto higher leaf levels within the crop. At high disease severity, whole

plants can get defoliated. Thus, heavy infections can cause considerable quantitative as well as qualitative yield losses (Chaerani and Voorrips, 2006).

The control of this polycyclic diseases requires multiple, targeted applications of fungicides (Leiminger and Hausladen, 2009). Amongst the fungicides registered, respiration inhibitors such as quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs) play a key role in the management of Early Blight disease. Also, demethylation inhibitors (DMIs) have been registered to control Early Blight disease for several years. Up until now, no cases of field resistance were reported in *A. solani* for DMI fungicides (FRAC, 2018). Even though both sub-groups of respiration inhibitors show no cross-resistance, in recent years resistance cases were reported for SDHI as well as QoI fungicides from the US and Europe (FRAC, 2018; Landschoot *et al.*, 2017; Baukse *et al.*, 2018a). Reduction in QoI fungicide sensitivity can be attributed to point mutations in the mitochondrial target gene, cytochrome b (*cyt-b*). In *A. solani*, the substitution of phenylalanine (F) to leucine (L) at position 129 (F129L) has been observed. This F129L-genotypes leads to low resistance factors towards different QoI fungicides (Pasche *et al.*, 2005). In contrast to QoIs, reduction of SDHI-sensitivity is caused by several mutations in the four subunits of the succinate dehydrogenase (*sdh*) genes leading to a more complex cross-resistance pattern within the group of SDHIs. Sequencing of boscalid-resistant field isolates of *A. solani* showed in subunit *sdh*B an amino acid exchange from histidine (H) to trypsine (Y) at amino acid position 278, which lead to the *sdh*B-H278Y-genotype. Another mutation in the same base-triplet conferred an amino acid exchange from H to arginine (R), resulting in the *sdh*B-H278Y-genotype (Mallik *et al.*, 2014). In subunit *sdh*C a mutation at amino acid position 134 caused an exchange from H to R leading to the *sdh*C-H134R-genotype. A similar amino acid substitution was found in subunit *sdh*D at position 133 resulting in *sdh*D-H133R-genotype. In field isolates originating from the US, another amino acid substitution was found in subunit *sdh*D. At amino acid position 123 aspartic acid (D) was exchanged to glutamic acid (E) leading to the *sdh*D-D123E-genotype (Mallik *et al.*, 2014), which was to the authors knowledge up until now not reported to be present in Europe (FRAC, 2018).

Cross-resistance patterns across SDHI fungicides are complex due to the fact that the impact of reported genotypes on SDHI-sensitivity varies between the mutation and SDHI tested. Field isolates of the *sdh*B genotypes displayed *in-vitro* low resistance factors towards penthiopyrad, whereas *sdh*C or *sdh*D-genotypes showed high resistance factors to the same fungicide. Boscalid showed for all genotypes high to very high resistance factors. In contrast, both *sdh*B mutants were sensitive to fluopyram, while *sdh*C and *sdh*D mutants showed low to moderate resistance to the fungicide (Gudmestad *et al.*, 2013). Such an incomplete cross-resistance pattern was also observed by conducting *in-vivo* greenhouse (Gudmestad *et al.*, 2013; Bauske *et al.*, 2018b). To elucidate the consequences of the reported genotypes on commercially available SDHI containing products, following experiments were conducted:

- Sensitivity testing and molecular analysis of European field isolates collected from 2016 to 2018
- *In-vitro* SDHI cross-resistance study testing identified genotypes
- *In-vivo* study to evaluate impact of genotypes on efficacy of solo SDHIs as well as SDHI containing products

MATERIALS AND METHODS

Sampling, cultivation and sensitivity testing of Alternaria solani
Infected leaves were collected randomly from commercial fields. Isolation was done similar to the methodology described by FRAC (2006). In most cases, five isolates per sample were generated. Fungicide sensitivity was determined for selected DMI, QoI and SDHI-fungicides in a microtiter assay similar to that described by Hu et al (2011), but using a conidial suspension adjusted to 10^4 conidia per milliliter. Two replicates were tested per isolate. EC_{50} values were calculated from the blank-corrected extinction values using the ABASE software package.

DNA isolation and pyrosequencing
Isolates with an EC_{50} value of more than 1 ppm of boscalid and/or fluopyram or more than 0.1 ppm of azoxystrobin were selected for further molecular analysis. For each of the isolates, DNA isolation and pyrosequencing were performed similar to that described by Weber *et al.* (2015).

SDHI cross-resistance study
When possible, twelve isolates from each genotype were tested together with twelve isolates showing a wild-type sensitivity in a microtiter assay as described in paragraph "Sampling, cultivation and sensitivity testing". In total seven active ingredients belonging to the mode of action group of succinate dehydrogenase inhibitors (SDHIs) were tested in this cross-resistance study. Two replicates were tested per isolate. The experiment was repeated twice.

Greenhouse experiment
Potato plants (*Solanum tuberosum*, var. Rambo) were grown until the growth stage of four fully expanded leaves in a greenhouse at 18°C. Subsequently, plants were treated with commercial fungicidal products as listed in Table 1 at a water rate of 400 L/ha in a calibrated spray cabin. After drying, treated plants were brought back into the greenhouse.

Table 1. *SDHI containing products used for protective treatment of potato plants one day prior to infection at 400 L/ha in a calibrated spray cabin*

Product name	Full Product label rate	Fungicide(s)	Fungicide rate(s)
Cantus WG 500 g/kg	0.134* kg/ha	Boscalid	67 g/ha
Sercadis SC 300 g/L	0.25 L/ha	Fluxapyroxad	75 g/ha
Luna Privilege SC 500 g/L	0.125 L/ha	Fluopyram	62.5 g/ha
Perseus SC 75+50 g/L	0.75 L/ha	Fluoxapyroxad + Difenoconazole	56 g/ha + 38 g/ha
Propulse SE 125+125 g/L	0.5 L/ha	Fluopyram + Prothioconazole	62.5 g/ha + 62.5 g/ha

*Adjusted boscalid dose-rate to match the full label rate of 0.25 L/ha Signum SC (containing 267g of boscalid)

One isolate of *A. solani* per detected SNPs from paragraph "DNA isolation and pyrosequencing" was selected for the greenhouse experiment. Spore suspensions were generated for the selected five isolates (four genotypes and a wildtype isolate) and adjusted to 10^4 spores per milliliter.

Plants were inoculated with *A. solani* by spraying of treated plants with spore suspension until plants were wet. After drying, treated plants were brought back into the greenhouse and placed under plastic covers to create 100% relative humidity for the first three days. Subsequently, covers were removed and plants were incubated at 18°C in the greenhouse.

Disease severity was scored from 0-100% according to Duarte *et al.* (2013). Analysis for difference in treatments was done for each genotype by performing an one-factorial ANOVA and a posteriori Tukey's HSD test ($p \leq 0.05$). Analysis for difference in each genotype to the genotype a Students' *t*-test ($p \leq 0.05$) for independent samples was performed. Efficacy expressed as percent ABBOTT was calculated for each product by using means of treatments and comparing it to the untreated control. Two experiments were conducted testing solo SDHIs and mixture concepts (see Table 1).

RESULTS AND DISCUSSION

Sensitivity status of Alternaria solani towards DMIs, QoIs and SDHIs
In 2016, in total 203 isolates of *A. solani* were generated from Germany, France, Netherlands, Belgium, Denmark and Sweden. The monitoring was initiated in 2016, continued in 2017 with a smaller number of samples (n=9) and was expanded in 2018 to include also Nordic countries (n=20). Even though the number of isolates was moderate compared to monitoring procedures for other pathogens, results were in line with those reported by other companies in those years (FRAC, 2018).

The mEC_{50} values for the DMI fungicides prothioconazole and difenoconazole ranged from 0.28 to 0.98ppm and from 0.004 to 0.009ppm, respectively (see Table 2) in 2018. This was in an acceptable range compared to the mEC_{50} values of the reference isolates for prothioconazole (0.17 – 0.35ppm) and difenoconazole (0.003 – 0.006ppm). The highest individual EC_{50} value were 1.38 and 1.43ppm of prothioconazole for two isolates detected in the same German sample in 2017. However, a mix of both isolates could be controlled on the same level as an isolate showing wild-type sensitivity by a foliar dose of 62.5 g/ha prothioconazole in an *in-vivo* experiment (data not shown). In general, shifting of DMI fungicides leading to reduced field efficacy was reported for other plant pathogens. Therefore, *A. solani* needs to be monitored continuously towards prothioconazole in order to follow the evolution of the pathogen in the future.

Table 2. *Results of Alternaria solani sensitivity monitoring conducted from 2016 to 2018 testing the DMI-fungicides prothioconazole and difenoconazole in an in-vitro microtiter assay*

	Year	2016		2017			2018			
	Country	BE	NL	BE	DE	NL	BE	DE	NL	DK	SE
no. of	sites	2	7	2	4	3	6	5	4	2	3
	isolates	13	34	14	18	21	26	26	24	12	15
Prothio-conazole	mean	0.36	0.33	0.36	0.43	0.35	0.72	0.45	0.35	0.52	0.31
	site-range	0.35-0.36	0.24-0.41	0.30-0.43	0.27-1.12	0.29-0.43	0.56-0.98	0.39-0.51	0.32-0.40	0.48-0.54	0.28-0.41
Difeno-conazole	mean						0.006	0.004	0.007	0.005	0.007
	site-range	n.t.*	n.t.	n.t.	n.t.	n.t.	0.004-0.009	0.004-0.004	0.006-0.009	0004-0.005	0.005-0.007

(mEC50 [mg/L] label at left axis)

*n.t.: difenoconazole was not tested in 2016 and 2017

With exception of Denmark, moderate to high frequencies of isolates showing the F192L substitution in the *cyt-b*-gene were detected (36% – 100%). The F129L-genotype causing moderate resistance factors for QoI-fungicides, such as azoxystrobin. F129L-genotypes were reported to be controlled by a protective application at full dose rate (Pasche *et al.*, 2005; Hausladen *et al.* 2015). However, dependency on long term efficacy or curative application timing lead to the erosion of efficacy of QoI-fungicides in artificially infected field trials or greenhouse trials (Adolf *et al.*, 2017; Liljeroth *et al.*, 2015). Most isolates detected carrying the F129L-mutation also carried a mutation in one of the *sdh*-genes.

The mEC$_{50}$ values of reference isolates for the SDHI fungicides boscalid and fluopyram ranged from 0.03 – 0.06ppm and 0.06 – 0.1ppm, respectively, showing only a slight difference in intrinsic activity of the two SDHI fungicides. In contrast, EC$_{50}$ values of suspicious isolates increased to 1.18 – >30ppm of boscalid or 0.007 – 1.55ppm of fluopyram. Therefore, pyrosequencing assays were performed in order to identify mutations in the *sdh*-genes. With exception of Denmark, moderate to high frequencies of *sdh*B-H278Y and *sdh*C-H134R genotypes were detected (9%-38% and 17%-80%, respectively). Only few isolates showed the *sdh*B-H278R or *sdh*D-H133R genotype (in total 5% and one single isolate, respectively). All SNPs reported in this study were reported previously at similar frequencies (Hausladen *et al.*, 2017; Landschoot *et al.*, 2017; FRAC, 2018). The *sdh*D-D123E mutation reported by Baukse *et al.* (2018b) originating from the US was not detected in any of the European isolates tested in this study, which was also not mentioned by other European researchers up until now (Hausladen *et al.*, 2017; Landschoot *et al.*, 2017; FRAC, 2018). Also, in agreement with most other researchers, multiple mutations in *sdh*-genes did not occur simultaneously in one isolate, but in every case one mutation in one *sdh*-gene was responsible for each of the respective phenotypes of suspicious isolates. Isolates showing none of the mutations tested showed a sensitivity comparable to the mEC$_{50}$ value of sensitive reference strains.
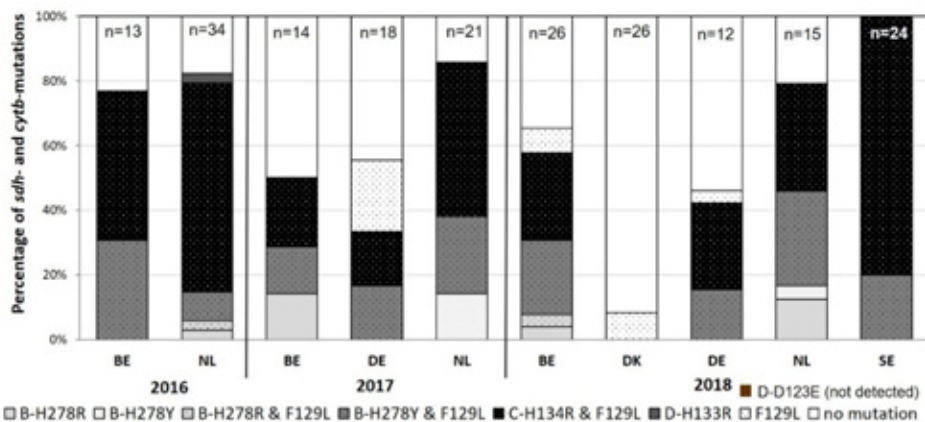
Figure 1. *Percentage of sdh- and cyt-b-genotypes in populations of Alternaria solani collected in different countries from 2016 to 2018*

SDHI cross-resistance study

To characterize the impact of detected genotypes on the activity of SDHI fungicides, in total seven SDHI fungicides from different chemical classes were tested in an *in-vitro* cross-resistance study.

Boscalid clearly showed high resistance factors of more than 100 for all genotypes tested indicating a full *in-vitro* resistance (see Table 3). Interestingly, genotype *sdh*B-H278Y as well as *sdh*B-H278R showed a hypersensitivity to fluopyram. All other SDHIs from the pyrazole-4-carboxamide class, such as bixafen and fluxapyroxad showed low resistance factors. For genotype *sdh*C-H134R as well as *sdh*D-H133R genotypes moderate resistance factors were measured, whereas moderate to high resistance factors were observed for all other SDHI fungicides. This difference is especially evident for the frequently occurring *sdh*C-H134R genotype. Such an incomplete cross-resistance between *in-vitro* tested SDHIs was reported for *A. solani* previously by Gudmestad *et al.* (2013) and for other plant pathogens by Sierotzki and Scalliet (2013).

Table 3.   *Mean resistance factors caused by four genotypes of Alternaria solani towards seven different fungicides of the SDHI group determined in an in-vitro cross-resistance study in relation to the mEC_{50} value of wildtype isolates*

		mean Resistance Factor [mEC_{50} mutation / mEC_{50} wildtype]						
		pyridine-carboxamide	pyridinyl-ethyl-benzamide	pyrazole-4-carboxamides				
Genotypes	n	Boscalid	Fluopyram	Bixafen	Fluxa-pyroxad	Iso-pyrazam	Penthio-pyrad	Benzovindi-flupyr
B-H278R	9	137	0.8	2	2	2	4	2
B-H278Y	12	169	0.7	13	19	14	62	13
C-H134R	12	>1200*	32	95	99	608	>229	406
D-H133R	1*	>283	39	48	51	384	>173	229
mEC50 value of wildtype	12	0.025	0.033	<0.004	0.009	0.008	0.131	0.005

The highest concentration tested in the microtiter assay was 30ppm of active ingredient. In case the EC_{50} value of at least one isolates was higher than 30ppm, the mean Resistance Factor caused by the genotype is marked by the ">"-sign and printed in bold-face

In-vivo efficacy of SDHI-containing products

However, detection of mutations or *in-vitro* measurement of increased EC_{50} values do not necessarily lead to field resistance. Therefore, an *in-vivo* greenhouse study was conducted to determine the impact of the described genotypes on the efficacy of a protective application of commercially available product concepts at their recommended field rates at realistic spray conditions one day prior to inoculation. Under such more realistic test conditions, a significant loss of efficacy of a solo applied boscalid as present in the full label rate of 0.25 L/ha Signum® was observed for all genotypes tested (7% – 63%) compared to an efficacy of 86% of boscalid in controlling the wildtype isolate (see Figure 1). Disease severity on untreated plants was significantly lower at seven days after inoculation for the selected *sdh*B-H278Y and *sdh*B-H278R

isolates compared to the wildtype isolate and significantly higher compared to the *sdh*C-H134R, *sdh*D-H133R isolates. An interaction between aggressiveness of isolates and efficacy of products cannot be excluded, but the less aggressive *sdh*B-H278Y and *sdh*B-H278R isolates showed after ten days similar losses of boscalid-efficacy as for the other genotypes after four to seven dates (data not shown). These results show the impact of high resistance factors caused by the detected genotypes as observed in the *in-vitro* cross resistance study (see Table 3) and leads to a complete loss of efficacy under field conditions as reported in by Metz and Hausladen (2019) in an artificially infected field trial.
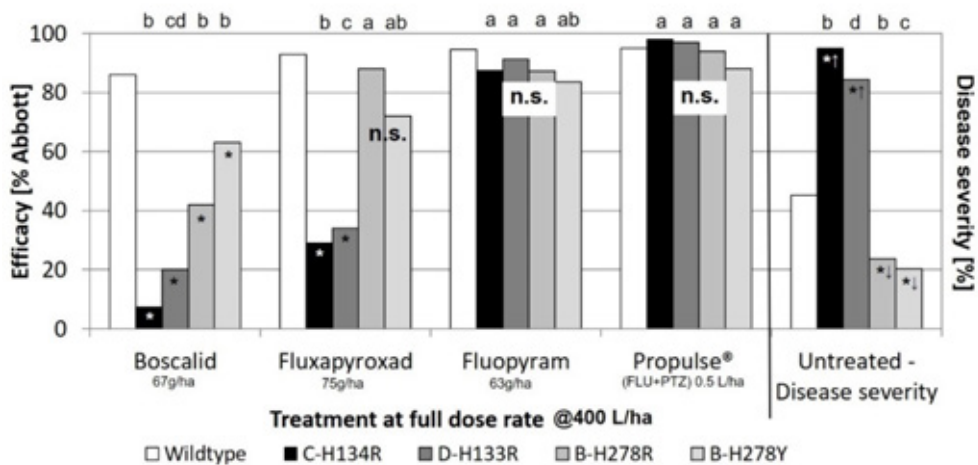
Figure 2. *Efficacy expressed as %ABBOTT of different solo SDHIs and SDHI containing products in controlling four genotypes of Alternaria solani carrying different mutations in sdh-genes as well as the wildtype tested in greenhouse experiments. Disease severity of the tested isolates is given for the untreated control. Different letters indicate significant differences in disease severity between treatments of one genotype according to Tukey's HSD test. An asterisks indicates a significant difference in disease severity between a given genotype and the wildtype isolates within the given treatment according to Student's t-test*

Also, the solo application of fluxapyroxad showed a significant decrease in efficacy from 93% for the wild-type isolate to 29 – 34% for *sdh*C-H134R and *sdh*D-H133R genotypes, respectively (see Figure 1). Such results show the impact of moderate to high resistance factors observed in the *in-vitro* cross resistance study (see Table 3) and would probably lead to recognizable loss of efficacy under field conditions. But for *sdh*B-H278Y and *sdh*B-H278R isolates, the efficacy was nearly on the level of the wild-type isolate (72 – 88%), not only seven but also after ten days of incubation (data not shown). This reveals that no or only a minor impact on field efficacy of a protective application of fluxapyroxad is to be expected for control of *sdh*B-H278Y and *sdh*B-H278R mutations with a low *in-vitro* measure resistance factors of 2-19 (see Table 3) In contrast, more severe losses in efficacy are to be expected for *sdh*C-H134R and *sdh*D-H133R genotypes under field conditions, thus a strong mixing partner is needed. Difenoconazole is used at rate of 125-150 g a.i. per hectare, e.g. in the product Revus Top[®]. However, in the product

Perseus® at full label rate of 0.75 liter per hectare the difenoconazole rate is reduced to 38 g a.i. per hectare. At such a low rate of difenoconazole, the aggressive *sdh*C-H134R isolate cannot be fully controlled (65%) on the level of the wildtype isolate (89%), since fluxapyroxad is not contributing in controlling of that genotype (see Figure 2). This lack of efficacy gets even more obvious in case the Perseus® dose-rate is reduced to ½ or ¼ of the recommended dose rate (to 70% or 43% efficacy, respectively), whereas the level of control of the wildtype as well as the *sdh*B-H278R-genotype stays on a high level (80% or 93%, see Figure 2). This result shows that the high intrinsic activity leading to a difenoconazole mEC_{50} value for the sensitive reference strains of 0.004ppm as observed in the *in-vitro* test-system (see Table 2) does not translate directly into a high *in-vivo* control efficacy of a reduced rate of difenoconazole in case the mixing partner fluxapyroxad is compromised.

The solo application of fluopyram showed a control efficacy of 84 and 87% for the genotypes *sdh*B-H278Y and *sdh*B-H278R on the same level as the control efficacy for the wildtype (95%, see Figure 1). This observation in this *in-vivo* study was in line with the observation of resistance factors lower than 1 in the *in-vitro* study (see Table 3). Genotypes *sdh*C-H134R and *sdh*D-H133R showed for a one day protective application a similarly high efficacy of 88 and 91%. However, first small spots of chlorosis were visible on the leaves, especially for the *sdh*C-H134R isolate. However, increase of incubation time to 10 days under constant optimal conditions for the fungus did not lead to formations of lesions, whereas the untreated and boscalid-treated plants were completely defoliated (data not shown). In general, it cannot be excluded, that the long-term or curative efficacy of a solo fluopyram application to control *sdh*C-H134R and *sdh*D-H133R genotypes could be reduced. Gudmestad *et al.* (2013) Bauske *et al.* (2018b) showed, that a reduction of dose-rate of 100ppm of fluopyram to 10ppm resulted in small reduction of mean efficacy for *sdh*C-H134R (from 99% to 83%) and for *sdh*D-H133R (from 100% to 86%) as well as a stronger reduction for *sdh*D-D123E (from 95% to 66%). A further reduction by a factor of 10 to 1ppm of fluopyram does not only lead to further reduction of efficacy for the genotypes tested (63%, 37%, 33%), but also to reduction of efficacy to control wildtype isolates (from 100% over 99% to 84%). Metz and Hausladen (2019) reported, that efficacy of a spray program testing only solo fluopyram-applications in a 14 day spray interval was reduced from about 70% in plots artificially inoculated with a wildtype isolate to about 40% in plots inoculated with the *sdh*C-H134R-genotype. This level of control efficacy was on the level of that of solo boscalid-treatments in plots artificially inoculated with the wildtype isolate (about 45%). In contrast, the efficacy of boscalid was reduced to less than 10% in the artificially inoculated plots with the *sdh*C-H134R-genotype of the same field trial. In the present *in-vivo* greenhouse study, the mixture concept Propulse®, containing additionally the DMI prothioconazole, showed a high efficacy to control all tested genotypes of 88 – 98% (see Figure 1 and 2). This was on the same level as that observed for the wildtype isolate (95 – 98%). Also, no spots of chlorosis were visible even after 10 days indicating, that all genotypes were fully controlled. Even at a not recommended reduction to ½ or ¼ of the full Propulse® label rate, the protective efficacy to control the *sdh*C-H134R-genotype stays on the same level (98% or 85%) as that of the wildtype (96% or 90%, see Figure 2). These results are in agreement with reports of very good field efficacy of Propulse® also in regions from which previously the *sdh*C-H134R genotype was reported (Evenhuis *et al.*, 2018).

The results of the *in-vitro* and *in-vivo* studies reported here as well as reports from experiments by other researchers highlight the need for product concepts containing SDHIs to have a mixing partner, which is able to fully control the disease on its' own, as recommended in the resistance

management guidelines annually published by the FRAC SDHI Working group (FRAC, 2018). Together with a better knowledge on disease identification, disease prediction models as well as utilization of biocontrol agents, such as *Bacillus* spp. or *Trichoderma* spp. (Metz et al, 2019), all available tools could be combined in an integrated Early Blight disease management including an effective fungicide resistance management to keep a sufficient number of effective disease management tools available for farmers in the future.
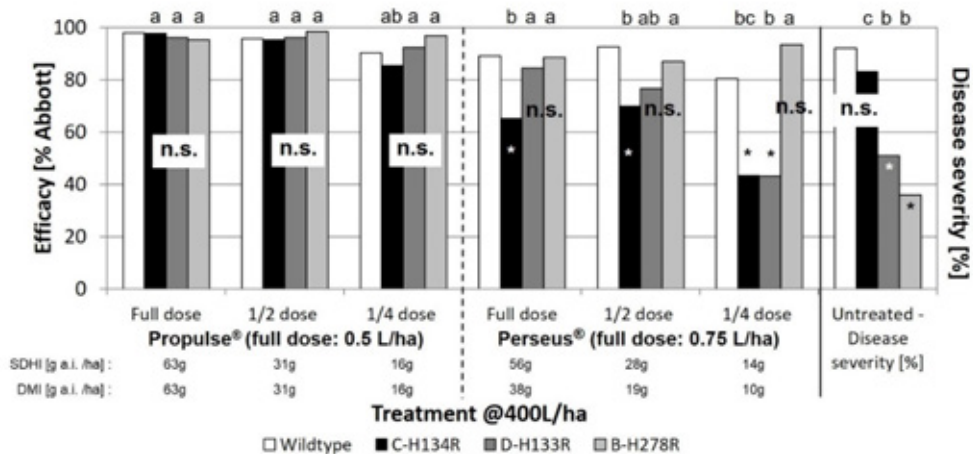
Figure 3. *Efficacy expressed as %ABBOTT of different solo SDHIs and SDHI containing products in controlling four genotypes of Alternaria solani carrying different mutations in sdh-genes as well as the wildtype tested in greenhouse experiments. Disease severity of the tested isolates is given for the untreated control. Different letters indicate significant differences in disease severity between treatments of one genotype according to Tukey's HSD test. An asterisks indicates a significant difference in disease severity between a given genotype and the wildtype isolates within the given treatment according to Student's t-test*

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Treatment strategies against early blight and fungicide resistance in *Alternaria solani* in Sweden

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SUMMARY

Several years field trials have revealed changes in the efficacy of fungicides used for early blight control in starch potato. The field efficacy of both the QoI fungicide azoxystrobin and the SDHI fungicide boscalid decreased substantially during recent years in the starch potato region in South Sweden. A new product based on fluopyram and prothioconazole gave by far the best control in field trials 2018. The onset of the epidemiological phase of the early blight disease varied among years in our trials with almost a month difference in onset time. Therefore, development of prognosis models is very important for correct timing of fungicide treatments (Abuley, 2017). The trials also showed that there are significant differences in resistance against early blight among starch potato cultivars available for growers and that optimal fertilization is important for limiting the infection by the early blight pathogen, *Alternaria solani*. Leaf fertilization with nutrients that according to leaf nutrient analysis were deficient in the mid-season resulted in less infection.

We also investigated the presence of mutations in *A. solani* associated with loss of sensitivity to fungicides. Several hundred isolates collected between 2009 and 2016 were investigated for the presence of the F129L mutation, associated with loss of sensitivity to azoxystrobin. While only single isolates with mutation was found 2009 and 2010 a quick shift was observed during the following years and the populations at several sites was dominated by isolates harbouring the F129L mutation from 2014 and onwards (Edin et al, 2019). Laboratory studies indicated that F129L isolates had EC50 values about 10-15 times higher than wild type isolates (Odilbekov *et al.*, 2016). Comparing F129L isolates collected 2014 and 2017 indicated that the EC50-values had increased further with time (Odilbekov *et al.*, 2019).

We also investigated the presence of mutations associated with reduced sensitivity to boscalid. 2014-2016 30-40 isolates were investigated each year. The first year only a few isolates with the H134R mutation was found but during the following years the frequency of isolates with this mutation increased. In 2017 around hundred isolates were investigated and 65% of the isolates harboured the H134R and 25% harboured the H278Y mutations. Using the pure chemical boscalid wildtype isolates had an EC50 value of around 0.1 µg ml^{-1}. However, we could not

analyse the isolates with mutations since the spore germination was almost 100% at 1 µg ml^{-1} and higher concentrations could not be obtained due to the low solubility. However, we also tested spore germination at higher concentrations using the commercial product Cantus® (only a.i. boscalid) and that indicated that most of the isolates with mutation had an EC50 value above 100. Analysis of the growth rate at artificial agar media also showed that wild type isolates grew significantly slower at concentrations above 0.1 µg ml^{-1} while isolates with either H134R or H278Y mutation continued to grow at higher concentrations.

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Towards a knowledge-based approach for an integrated control of *Alternaria* spp. in potatoes

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SUMMARY

Field trials in different seasons and multiple locations were performed in order to establish the timing for the first treatment against early blight (*Alternaria solani*) that proved to be effective and necessary for a good control of the disease. The results of the trials were used to fit a simulation model for a better prediction of disease pressure and to obtain an improved advice for a better timing of applications, and especially the first application. Besides weather parameters, the disease model also takes crop stage and physiological resistance of the crop into account. As both seasons 2017 and 2018 were markedly hot and dry, an attempt was made to account for increased susceptibility of the potato crop caused by drought stress. However, even if there was increased susceptibility of the crop caused by drought stress, the pathogen failed to profit from this due to a lack of sufficient leaf wetness periods. In both years, a clear distinction could be made between treatments that were performed too early – all with no or very low levels of disease - and treatments that were performed too late – with disease levels approaching that of the untreated control – and a critical date for the first necessary application could be established. The disease model was able to simulate the observations in the field.

KEYWORDS

Potato early blight, *Alternaria solani*, control strategies, disease model, simulation models, physiological resistance, senescence

INTRODUCTION

As in many other potato areas around the world, the potato early blight disease (*A. solani*) is viewed as a growing concern for potato growers in Flanders, Belgium. Factors such as more favourable weather conditions or stricter fertilisation guidelines with lower nitrogen dosage could be contributing to this increase. However, the shift in available late blight fungicides towards more oomycete-specific fungicides, without an effect on early blight, plays an important role. As specific early blight fungicides are made available for the control of the disease, potato growers are facing the question: "How and when should I use these fungicides to achieve an optimal, cost-efficient crop protection?"

MATERIALS AND METHODS

Field trials were carried out in cooperation between PCA (Kruishoutem), Inagro (Rumbeke-Beitem) and University of Ghent (UGent) at three locations. Results were consistent for the three locations. Trials from the location in Kruishoutem (B) in 2017 and Elsegem (B) in 2018 will be discussed in this paper. No artificial inoculation was done in the trials, although it has to be mentioned that in both years in an adjacent trial (<25 m), autoclaved barley seeds inoculated with *A. solani* were applied between the rows in the month of June. In both years, the ware potatoes cv. Fontane were planted at the end of April and emerged towards the end of May. All plots were left untreated with regard to early blight until the last week of July. From then on, treatments were started for an increasing number of variants, each one with a start date one week later than the previous one. Once started, a good disease control against early blight was maintained with weekly applications of a mixture of Shirlan + Tanos, alternated with Narita (a.i. difenoconazole). Besides an untreated control, one other variant was sprayed weekly with mancozeb (Dithane WG 2 kg/ha), beginning at the time of the first specific treatment against early blight.

Table 1. *Trial setup and protocol for 2017 and 2018*

| Start date | **Applications** | | | | | | | |
	t1	t2	t3	t4	t5	t6	t7	t8
t1	T+S	N	T+S	N	T+S	N	T+S	N
t2	-	T+S	N	T+S	N	T+S	N	T+S
t3	-	-	T+S	N	T+S	N	T+S	N
t4	-	-	-	T+S	N	T+S	N	T+S
t5	-	-	-	-	T+S	N	T+S	N
t6	-	-	-	-	-	T+S	N	T+S
t7	-	-	-	-	-	-	T+S	N
t8	-	-	-	-	-	-	-	T+S
UTC	-	-	-	-	-	-	-	-
mancozeb7d	mcz	mcz	mcz	mcz	mcz	mcz	mcz	mcz

T+S	Tanos + Shirlan
N	Narita
mcz	Dithane WG

The same setup and design – a randomized complete block design with 4 replicates – was used in 2017 and 2018. Disease assessments were performed weekly, and severity was expressed as percentage diseased leaf area for the two central rows of each plot.

Since 2013, a weather based disease model derived from the FAST model (Madden *et al.*, 1978) has been used to simulate the development of early blight and to predict the date for a first application. Age-dependent susceptibility of the potato crop has been added to the calculations in the model, in function of the degree of crop senescence.

RESULTS AND DISCUSSION

Growing season 2017
The first lesions of early blight in the trial were found on August 21th, with start of epidemic development from August 28th onwards in the untreated control. Disease severity reached 37,5% in the untreated control on September 11th. All treatments with a start date on or before August 21 had a very low disease rating at the end of the season (<0.03%). Starting with an early blight fungicide on August 28th led to a disease level of just below 1%. A weekly application of mancozeb from July 25 onwards gave sufficient protection, leading to a very low disease level (0.03%) at the end of the season.

Based on these observations, August 28th seems to be a turning point or deadline for a protection against early blight. Best result from a preventive application was obtained with a spraying performed on August 21th. Adding earlier treatments did not contribute to the level of protection at the end of the season: no difference in effectiveness was observed between the start date of August 21th and earlier treatments, i.e. adding one, two, three or four (starting July 25th) previous treatments.
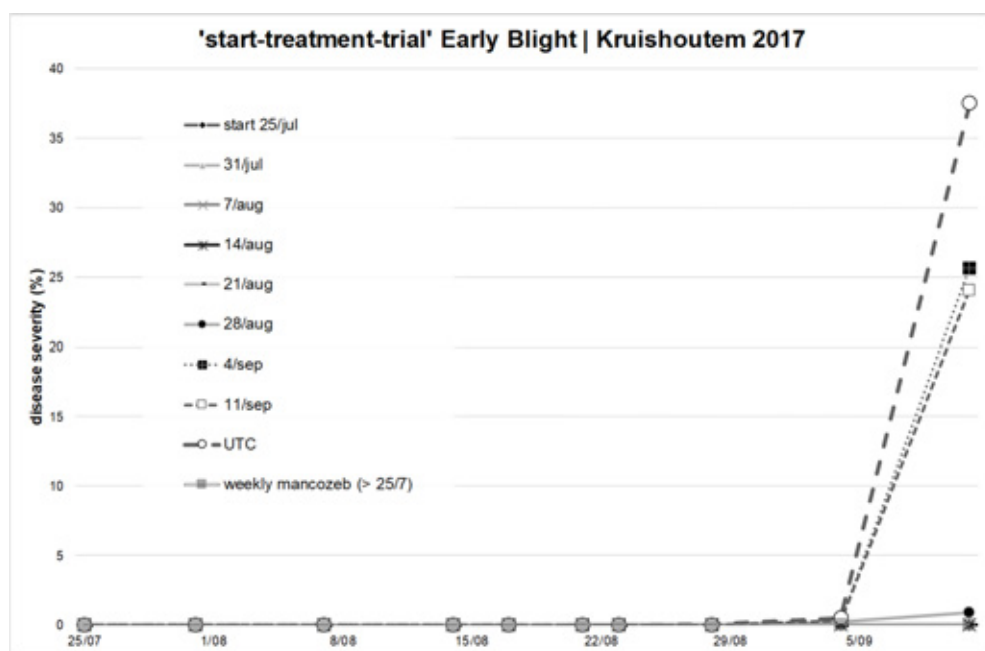
Figure 1. *Development of early blight disease severity (%) in 2017, for different dates of first treatment*

Simulation model

According to the disease model, a first application against early blight in 2017 was advised on August 23th – five days ahead of the critical date of August 28th, but a few days after the first observations of early blight lesions in the trial.

As the growing conditions in 2017 were hot and especially dry, it was obvious that the potato crop was suffering from drought and the associated stress. It could also be assumed that this factor led to an increase of crop susceptibility for the disease. On the other hand, the very dry weather conditions, and more specifically the lack of dew periods during the night, were by no means conducive to development of the disease. This was reflected in the many observations in the field, where attacks of early blight remained absent.

In order to assess the supposed increased susceptibility of the potato crop, an additional factor for stress was added to the disease model. The size of this factor was chosen in order to fit the model with the observations in the field trials. With an increase of susceptibility with 30%, the model predicted a first application date of August 17th.

Growing season 2018

The first lesions of early blight in the trial were found on August 16th, with start of epidemic development from August 29th onwards in the untreated control. Disease severity reached 12,5% in the untreated control on September 27th. All treatments with a start date on or before August 16th had a very low disease rating at the end of the season (<0.1%). Starting with an early blight fungicide on August 23th led to a somewhat higher disease level of 1.8%. A weekly application of mancozeb from July 26 onwards gave sufficient protection, leading to a very low disease level (0.03%) at the end of the season.

Based on these observations, August 23th seems to be a turning point or deadline for a protection against early blight. Best result from a preventive protection was obtained with an application performed on August 16th. Adding earlier treatments did not contribute to the level of protection at the end of the season: no difference in effectiveness was observed between the start date of August 16th and earlier treatments, i.e. adding one, two or three (starting July 26th) previous treatments.
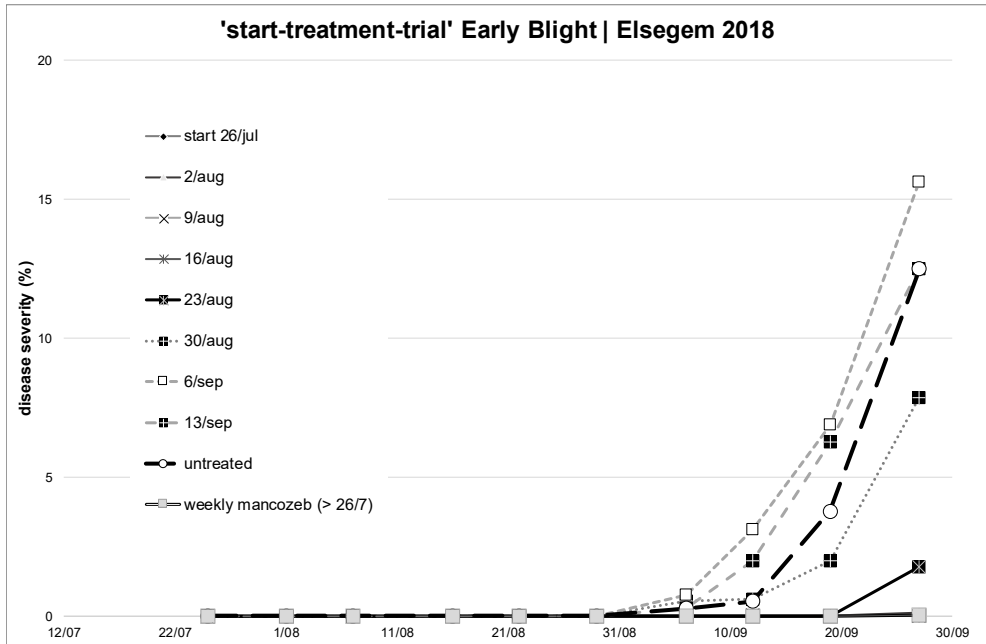
Figure 2. *Development of early blight disease severity (%) in 2018, for different dates of first treatment*

Simulation model

According to the model, a first application against early blight in 2018 was advised on August 19th – four days ahead of the critical date of August 23rd, but again a few days after the first observations of early blight lesions in the trial.

As the growing conditions in 2018 were very hot and exceptionally dry, it was obvious that the potato crop was suffering from drought stress, and it could be assumed that this led to an increase of crop susceptibility for the disease. On the other hand, the very dry weather conditions, and more specifically the lack of dew periods during the night, were by no means conducive to development of the disease. This was reflected in the many observations in the field, where attacks of early blight remained absent.

In order to assess the supposed increased susceptibility of the potato crop, an additional factor for stress was added to the disease model. The size of this factor was chosen in order to fit the model with the observations in the field trials. With an increase of susceptibility with 50%, the model predicted a first application date of August 16th.

CONCLUSIONS

In the prevailing growing conditions in Flanders, early blight has to be regarded as a disease of the senescing potato crop. A certain level of senescence seems to be a condition for epidemic development of *A. solani*. Preventive applications with specific fungicides against early blight can be necessary to safeguard the potato crop against undesirable disease levels.

These trials are designed to answer the most important question in this regard: when do we have to start treating for a cost-effective and efficient protection? In both seasons, the trials managed to distinguish a clear and critical deadline for a first treatment. It also became clear that additional applications, carried out earlier, did not add to the level of protection of the crop.

The disease model, used to simulate development of the disease and more specifically calculate a critical time for first treatment, gave satisfying results in both seasons. The model uses both weather data and crop data – i.e. physiological resistance derived from crop senescence – as input. Using an additional factor for stress, which could lead to increased crop susceptibility, could improve the results of the model.

In both years, the potato crop suffered from severe drought stress. Although this could well cause an increased crop susceptibility, it was determined that the pathogen could not fully benefit from this, mostly due to the lack of dew periods, and hence leaf wetness, during the night.

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IPM2.0: Test of a DSS including information from a trap nursery

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INTRODUCTION

For Danish conditions, conventional farmers need information on when to start chemical control, choice of fungicide type and dosage, and how to adjust control tactics (mainly fungicide) during the season. After a decade of projects in the mid-2000, involving Aarhus University, the Danish extension service and the potato industry, it was concluded that a more practical approach for a robust DSS was needed reflecting the structural development of the potato sector. The number of growers was generally decreasing, whiles the size and number of fields per farm were increasing. From a logistical point of view, the advisors and farmers told us that it was not possible to make a field-specific treatment for late blight control.

The second component that was important for decisions on the concepts and structure of the DSS was the success of using reduced dosages. This was documented in several field experiments (Hansen *et al.* 2002; Nielsen *et al.* 2008). Based on experiences from several years of field experiments in the 1990s and early 2000s, a general approach was decided to develop a system that recommended applying fungicide on a weekly interval (as conventional). However, the actual amount or dosage of fungicide (mostly Revus/Ranman Top has been used in the trials) was varied according to the infection risk, the amount of inoculum in the region and the type of potato (Starch or Ware) and the level of cultivar resistance. We developed the calculation of infection pressure and the dose model to recommend the type and dosage of fungicide to use during the season (Nielsen *et al.*, 2008; Nielsen *et al.* 2010; Nielsen *et al.* 2015).

The decision about when to start is another story. The rule of thumb for decades was to start spraying fungicide preventatively before row closure. From 1985-1997 we used the German negative prognosis to initiate fungicide sprayings (Hansen, 1992). After 1997 when we experienced the first indications of infections from oospore, this model became invalid or unreliable for predicting when to start fungicide application, albeit we still use the negative prognosis to forecast when the first attack from infected tubers is expected. We do not identify infections from oospores every year. Moreover, we do not have a working model to predict infections from oospores, but from experience, we know that:
- Narrow crop rotation increases the risk of oospore-driven early infections
- Heavy rainfall together with a moderate to high infection pressure at crop emergence increases the risk of early infections from oospores.

Description of the operational Danish Blight Management system

1. During crop emergence, "risk areas" are identified and inspected for attacks from oospores. Risk areas are areas with narrow crop rotations where oospores might be present. If it rains and the infection pressure is moderate to high then the risk is higher for oospore-driven infections. Interactive GIS maps for precipitation and infection pressure is available as well as local calculations of rain and infection pressure.

2. Early attacks of late blight are reported via the Nordic Surveillance system that has been operational since 2010. Recently, all Nordic countries have started using the BlightTracker smartphone APP for uploading recordings of early attacks of late blight (Hansen *et al.*, 2019).

3. A network of potato advisors and scientists (7-8) meet every Monday morning for a telephone meeting to inform each other and exchange ideas and views of the regional and local situation of blight risk, when to and how control actions should start. In this situation the regional maps are very useful, to indicate regions with a high risk of late blight. We also include information from other national surveillance systems used in our neighbouring countries – ISIP for Germany, Fight against Blight for the UK and information from the Netherlands. If late blight is recorded early in these countries, this is a warning to us in Denmark. The regional potato advisors are a very important dissemination channel for late blight advice because not every grower looks at the internet about alerts of late blight.

4. When blight has been found in a region, it is evaluated if this is a local attack in a special field or due to oospores. Additionally, the advisors evaluate the weather-based blight risk in the forthcoming week. If the forecast says, the weather favourable for late blight a majority of the farmers in this region (distance 25-50 KM) will start fungicide application with reduced dosages in more resistant cultivars.

5. When late blight is established in the region (>5 conventional fields reported in the surveillance system) then a majority of farmers spray according to the Blight Management System – Dose Model. As a rule of thumb, spraying is done on a weekly basis with low/reduced dosage contact fungicide when the weather is unfavourable for late blight and full dosage during periods with high infection pressure (weather favourable for late blight). When late blight is widespread and epidemics are observed in organic potatoes (or untreated plots), this will indicate that the spore loads will increase; thus, the next time a high infection pressure is indicated in the forecast then many farmers will use a stronger (expensive) compound with systemic effect. Stronger compounds are also used if the spraying is done too late compared to an important infection risk.

6. With climate change, we have seen a tendency to extreme weather including some periods with very heavy new growth as well as long periods with dry weather. When the rains resume after a drought/dry spell, heavy new growth can happen. During these periods farmers' experience has shown that a 7-day spraying interval is rather long and thus a 5-day interval is needed to control late blight. This kind of decision-making is not yet included in the Blight Management system, and thus this is a decision to be taken together by the advisor and the farmer.

7. Reduced dosages are not recommended if there are more than a few lesions (>0.5% severity) of late blight in the field. In such situation, Blight Management is of minor use because new infections could arise from rain splash via rainfall events alone. This is not included in the system. Very often, however, a dry weather spell and intensive chemical control can eradicate a minor attack.

The approach of the Blight Management DSS, where fungicide is sprayed weekly, but the actual dose is varied depending on infection risk is also appealing for many farmers in Sweden (Louise

Alden personal communication). Therefore, the Blight Management system was tested in Sweden during the 2015 and 2016 growing seasons.

When to start with preventive fungicide applications
The DSS components used for making decisions on when to start the control strategy is given in Figure 1.

Surveillance network

Rain during crop emergence: Risk of oospores

Regional infection pressure

Infection pressure + Rain (postal code)

Infection risk from infected tubers
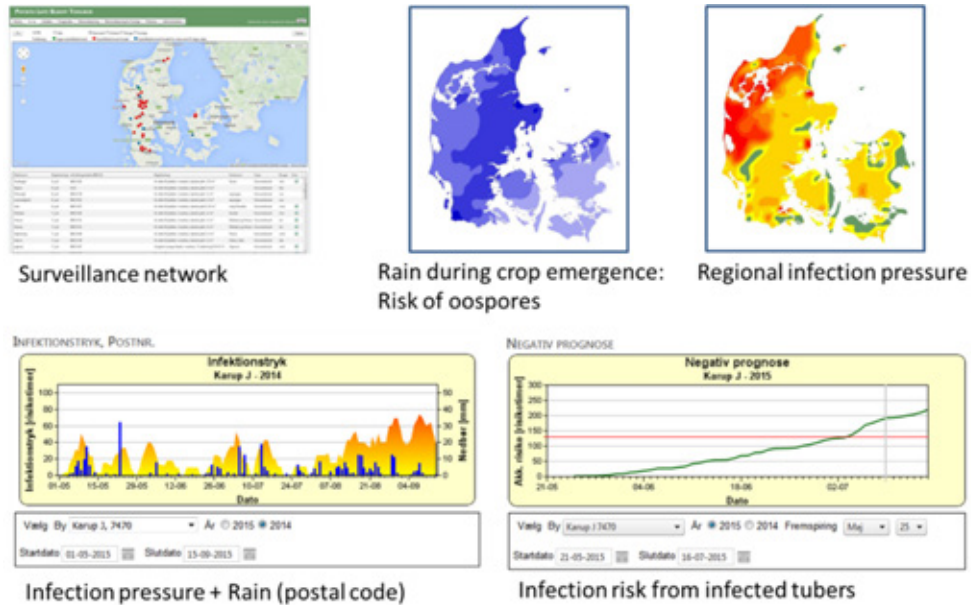
Figure 1. *DSS components used to decide how to start control of potato late blight with fungicide*

The surveillance network was developed in collaboration with the other Nordic countries based on experiences from a similar system called WebBlight, which includes the Baltic countries and Poland. The current version is an integrated part of the Potato Late Blight toolbox and it includes the use of the BlightTracker smartphone APP for uploading of results directly from fields (Hansen *et al.*, 2019).

Rainfall during crop emergence is a prerequisite for activating oospores in the soil. The user can select the start and end for a time-period to show on the map.

The regional infection pressure is based on calculation of the infection pressure in more than 600 grids (interpolated using approximately 80 weather stations with relative humidity (RH) in Denmark). The daily calculations are stored in a database with daily weather variables and model calculations. The dynamic map is generated using Visual studio/Telerek tools and not dedicated GIS systems.

The infection pressure is calculated on postal code level using all available weather stations in Denmark- weighted distance interpolation. The user can select Postal code and the start and end dates. To visualize what happened last year or previous year the user can select a similar overview of infection pressure one or two seasons back in time.

The negative prognosis is used to calculate the risk of primary attacks from infected tubers. The user can select a postal code, the start date and end date, and the date of crop emergence. To visualize what happened last year or previous year the user can select a similar overview of the Negative prognosis one or two seasons back in time.

The starch industry in Denmark asked how to deploy cultivars that are more resistant in the most effective way based on IPM. One way of deploying resistance cultivars would be to implement the IPM2.0 approach (Hansen *et al.*, 2018; Kessel, 2017). For the 2018 growing season, this approach was tested in a field trial at Flakkebjerg with three cultivars that varied their level of resistance to late blight. The objective was to test the Danish DSS in more resistant cultivars including the IPM2.0 approach as an add-on component.

METHODS

Trap Nursery
In 2018, a trap nursery, which included all the Black 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 was established in Denmark. The three cultivars tested – Kuras, Novano and Nofy and other cultivars were added to the trap nursery. The second goal for adding new and more resistant cultivars to the experiment was to i) evaluate the level and type of host resistance in the additional cultivars tested using the EucaBlight approach (Hansen *et al.*, 2007) and ii) use the data as input to the modelling work.

The trap nursery was infected naturally and disease scorings were carried out weekly during the season as severity (%). Yield was measured as hkg/ha and tuber blight as percentage infected tubers after harvest. The IPM2.0 strategy was compared with weekly sprays with ½ dosage of Ranman Top. Cymbal or Proxanil was used if a spray was too late or actively sporulating late blight lesions are seen on the potatoes in the field.
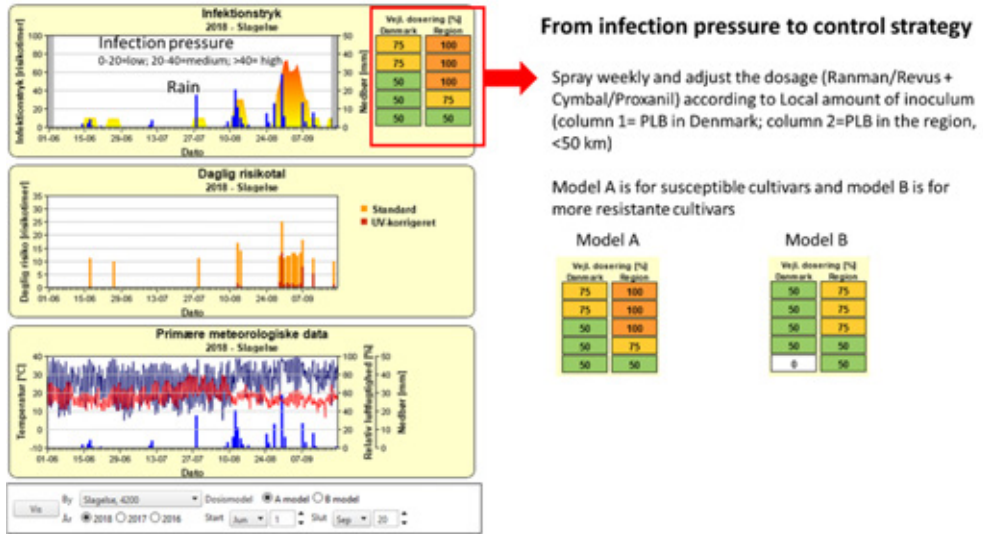
Figure 2. *Calculation of infection pressure at AU, Flakkebjerg, 2018. The dosage using contact fungicides is calculated based on infection pressure with parameters, Model A and B. Model A = susceptible cultivars and Model B = more resistant cultivars. The upper panel (Infektionstryk) is the infection pressure and the blue bars in the upper panel is the amount (mm) of rainfall. The middle panel (Daglig risikotal) is the daily risk values. The red colored-bars within daily risk values is the proportion of new inoculum that survive during the day. The lower panel (Primære meteorologiske data) shows the hourly relative humidity (RH), temperature and daily amount (mm) of rainfall*

The model A and B above is used to simplify the system for the farmers. The original model consists of four phases and for the current experiment we reduced the dosages more than used in the Model A and B (Modal A and B in Figure 2 corresponds to phase 2 and 3 in Figure 3, respectively).

Dosis	Fase 1	Fase 2	Fase 3	Fase 4
Infektionstryk > 60	0	0	50	100
Infektionstryk 41 - 60	0	0	50	75
Infektionstryk 21 - 40	0	0	50	50
Infektionstryk 1 - 20	0	0	0	50
Infektionstryk 0	0	0	0	50

Figure 3. *Adjusted parameters for the dose model*

The adjusted model for use of dosages is then combined with resistant cultivars, disease observations from the trap nursery information and pathogen information defined as:

- Phase 1: No attack in the country (do not spray at all)
- Phase 2: Attack in the country (do not spray at all)
- Phase 3: Attack in the region <50 km from trial (spray ½ dosage (50%) if the infection pressure >20)
- Phase 4: Attack in the differentials with complementary resistance as the cultivar tested (spray half dosage for infection pressure 0-40, spray 75% dosage if infection pressure is 41-60 and full dosage if the infection pressure is >60.

The complementary resistance was evaluated from official knowledge about resistance in the differential cultivars, cultivars tested and the trap nursery results from the previous year (Figure 4).
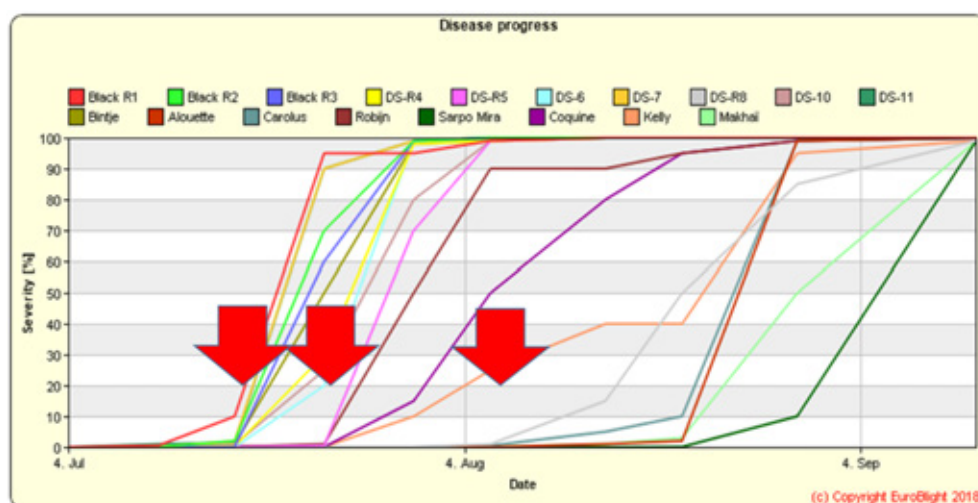
Figure 4. *Disease progress curves from trap nursery, the previous year, 2017) at Dronninglund, Denmark. The red arrows indicate three distinct periods for start of epidemic development. First arrow (from the left) is the very early maturing and susceptible group, the second arrow is the medium maturing and more resistant cultivars than the first group and the third arrow is the late maturing and more resistant cultivars*

Complementary cultivars for Kuras – first red arrow: When first blight is found in one of Group 1 Differentials: Black R1; Black-R2; Black-R3; Black-R4; Bintje; DS-6; DS-7; DS-10 DS-11.

Complementary cultivars for Novano - second red arrow: When first blight is found in one of Group 2 Differentials: DS-R5; Robijn; Coquine; Kelly.

Complementary cultivars for Nofy – third arrow : When first blight is found in one of Group 3 Differentials: DS-R8; Carolus; Alouette; Makhäi; Sarpo Mira.

When one of the cultivars in each of the three groups were attacked, then the dosage parameters shifted from phase 3 to phase 4 (Figure 3).

RESULTS AND DISCUSSION

The weather in 2018 was extremely dry and warm. The weather-based risk for disease development as calculated by the infection pressure was low until 10 August (Figure 2). Late blight was observed in the trap nursery on the 8 August, probably initiated by the rain and low infection pressure 8-10 days before. In the IPM2.0 trial, late blight was observed in the untreated plots on the 20 and 21 August. The last observations were scored on the potato cultivars on 14-17 September. First treatment according to the routine treatment was carried out on the 20 June after late blight was observed in the country.

Late blight was observed on Black R3, Black R6, Black R7, Alouette and Coquine on the 8 August and the IPM2.0 treatments was carried out on the 13 August. As this was later than recommended date, a stop treatment (i.e. 2l/ha proxanil) was applied. A stop treatment was also carried out on Kuras on 12 September because actively sporulating lesions was found in this cultivar (not in Novano and Nofy).

The level of disease in the untreated plots at the end of the season (17 September) was 85% on Kuras, 91% on Novano and approximately 1% on Nofy. In the treated plots, the attacks were 4-6% in Kuras and below 1% in Novano and Nofy (Figure 6). There were no significant differences between the disease level of the routine and IPM2.0 treatments (Figure 6). 13 treatments with primarily half dosage of Revus and Ranman Top, one stop treatment (proxanil) were carried out for Kuras according to the routine treatment, which translated to a total treatment frequency index (TFI) of 8 for Kuras. For the IPMBlight2.0, 6 similar treatments were carried out including one stop treatment (2 l/ha proxanil) for Kuras. The dosage of the other preventive treatments varied according to the infection pressure for Kuras. The total TFI for the IPMBlight 2.0 treatment for Kuras was 5.75. In Kuras, which is susceptible under Danish conditions, it was possible to reduce the fungicide use by 28% for following the BlghtManager+IPM2.0 approach compared to the routine/weekly spray with half dose Ranman Top or Revus.

The TFI for the routine and the IPMBlight 2.0 treatments for Novano and Nofy is shown in Figure 5. Following the IPMBlight 2.0 approach resulted in 35% reduction in fungicide use (TFI) compared the routine treatment for Novano and Nofy.

Although Danish growers use reduced dosages during low-risk periods, it is uncommon for growers to use ½ dosage of preventive compounds in weekly intervals. However, results for resistant cultivar like Nofy, in which the disease severity on the untreated plot was less than 1% at the end of the season, lower/reduced dosages of preventative fungicides could offer effective control of late blight. However, one key question is whether it is worth spraying or protecting such resistant cultivars with fungicides, considering the insignificant difference between the disease level between the untreated and the fungicide treatments? In the IPMBlight 2.0/Euroblight concept, we rely on concepts of "Gene Stewardship" and "active ingredient Stewardship". By "Gene Stewardship", we mean protecting the R-genes in resistant cultivars from being defeated by the pathogen. By "Active ingredient Stewardship", we mean protecting the active that we must protect the R-genes in the cultivars from being defeated by the pathogen and the active ingredients in the fungicides to being ineffective due to resistance by the pathogens to the active ingredients in the fungicides from being

ineffective due to resistance development by the pathogen. Therefore, for sustainable use of resistant cultivars, it is recommended to protect (spray fungicide) the most resistant cultivars when they are under pressure due to age (age-dependent resistance) or high infection pressure. Similarly, it is recommended that one should only use the active ingredients/fungicides only when it is needed to protect R-genes to keep the inoculum pressure on the cultivar low. In this way, the risk of *P. infestans* developing less sensitivity to certain active ingredients and adapt to certain cultivars (resistances) will be minimized. The active ingredients and the resistance go hand in hand so to say.
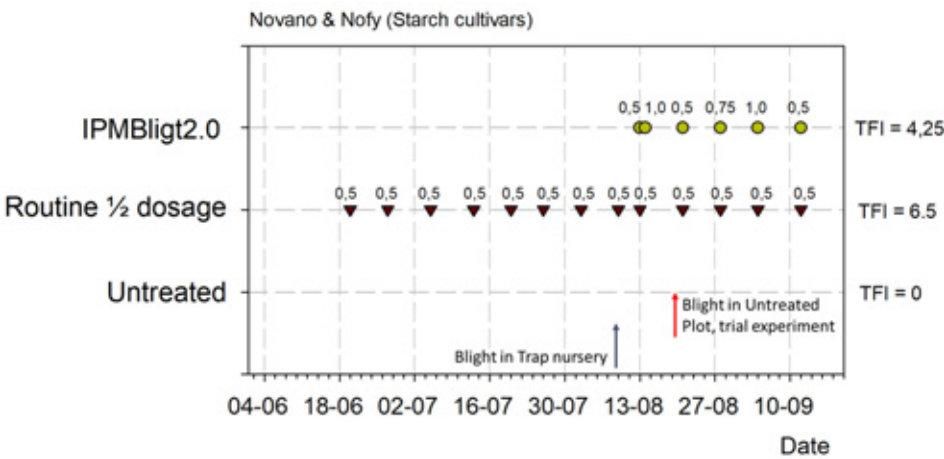
Figure 5. *Treatments according to Blightmanager+IPM2.0 approach compared to routine treatment as weekly sprays with ½ dosage of Ranman Top and Revus in the Novano and Nofy experiment*
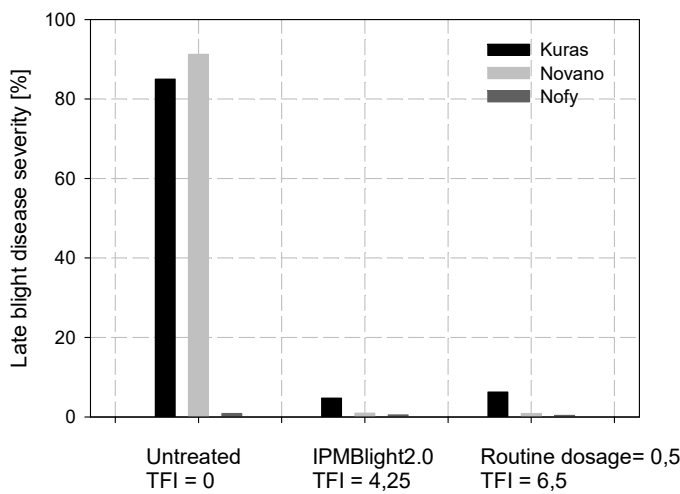
Figure 6. *Disease level end of season in a DSS experiment using three different cultivars and three different treatments: untreated, weekly sprays with ½ dosage Ranman/Revus and according to DSS + IPMBlight2.0 approach that includes use of data from a local trap nursery*

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Late blight (*Phytophthora infestans*) control in Tomato (*Solanum lycopersicon*) in Tanzania

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ABSTRACT

Between 2011 and 2018 surveys and tests have been carried out to determine current practices in tomato cultivation and to find out how late blight control in tomato can be improved in Tanzania. Surveys were organized and implemented by Wageningen University and Research together with Rijk Zwaan Afrisem in the framework of the Sevia (Seeds for Expertise and Vegetables in Africa) project. The tests and some other surveys have been done in commission of Sevia by Sokoine University of Agriculture and TARI Tengeru. In this article the results of these activities are described.

INTRODUCTION

Tomato originated from South America and was introduced into Europe in the 16th Century and later to East Africa by colonial settlers in the early 1900s (Wamache, 2005). In Tanzania tomato is under a total of almost 25,000 hectare one of the most important vegetable crops in terms of acreage (Table 1). In the period between 2012 and 2017 data was collected from 50 tomato fields located in Babati, Arusha, Moshi, Bagamoyo and Lushoto (Table 2). It revealed that with a profit of over 5 million shilling (approximately 2,000 euro) per hectare tomato is quite profitable. Roughly half of the costs are material costs while the other half is spent on labour. In terms of cost share only 6% is spent on crop protection while trellis costs are high with over 12% of the total cost.

Table 1. *Acreage of vegetable crops in Tanzania*

Crop	Regional zone					Mean
	Central	North East	North West	South East	South West	
Amaranths	736	856	1054	848	317	3811
Bitter Aubergine	1009	858	870	165	265	3167
Cabbage	1495	1681	1836	320	324	5656
Carrot	281	326	82	89	31	809
Chillies	601	880	726	732	287	3226
Cucumber	96	431	282	887	7	1703
Egg Plant	60	142	139	84		425
Ginger	93	1261	108	71	102	1635
Okra	1027	1383	1495	3204	987	8096
Onion	2528	1747	1046	1072	2381	8774
Pumpkins	580	38	607	480	56	1761
Radish	713	175	146	73	118	1225
Spinach	955	685	349	1105	325	3419
Tomatoes	7491	5047	6526	3751	1879	24694
Turmeric	478	133	313	41	175	1140
Watermelon	200	132	1167	1861	72	3432
Mean	18343	15775	16746	14783	7326	72973

Source: Compiled data from Regional reports from the NATIONAL SAMPLE CENSUS OF AGRICULTURE 2007/2008

Table 2. *Crop balance sheet for tomato*

Parameter	Quantity	Unit	Unit price	Unit	Total	Share in total costs
Yield	13,648	kg/ha	566	TSh/kg	7,729,668	
Land ploughing / bed making					22,477	0.9
Seeds	450	g	519	TSh/g	233,664	9.4
Fertilizers					351,392	14.2
Crop protection					143,467	5.8
Herbicides					1,488	0.1
Irrigation fuel					121,609	4.9
Trellis					313,629	12.7
Transport costs					4,864	0.2
Other costs					865	0.0
Total material costs					1,193,455	48.3
Hired labour	1030	hr	1243	TSh/hr	1,279,950	51.7
Family labour	783	hr				
Profit					5,256,263	

REGISTERED FUNGICIDES AND AVAILABILITY OF FUNGICIDES TO CONTROL LATE BLIGHT

In Tanzania the Tropical Pesticides Registration Institute (TPRI) under the ministry of Agriculture is responsible for the registration of pesticides. They publish the registered, either full with a renewable registration for five years or provisional with a two year non-renewable registration, pesticides in the Gazette. According to the latest Registered plant protection substances for use in the United Republic of Tanzania (URT), Issue of October, 2018 a total of 425 products are registered of which 275 products representing 80 single or combinations of active ingredients (A.I.) are allowed for use in tomato. To control late blight in tomato a total of 188 products with 38 different single or combinations of A.I. are registered (Table 3). In Tanzania mainly contact

fungicides (e.g. chlorothalonil, copper, mancozeb, propineb and sulphur) are registered providing 51% of the total registered fungicides with efficacy against late blight. Next to those products almost 20% of the registered late blight fungicides contain metalaxyl or a related active ingredient belonging to FRAC code group 4. In principle it can therefore be concluded that more than sufficient fungicides are registered to control late blight and at the same time prevent resistance build-up of the pathogen. However, based on surveys conducted to agro dealers only a limited range of active ingredients are available to farmers to buy (Everaarts *et al.*, 2011 and Everaarts *et al.*, 2014). The reason for this is that farmers are not asking for other products since they are not aware of them, while agro shop owners are not putting more expensive fungicides on the shelf as farmers are mostly asking for cheaper products mainly those containing metalaxyl.

Table 3. *Number of products per active ingredient or combination of active ingredients of formulated products registered in 2018*

Active ingredient(s)	Full registration	Provisional registration	Mode of action(s) FRAC code
carbendazim	9		1
carbendazim + tebuconazole	1		1+3
carbendazim + chlorothalonil	0	1	1
chlorothalonil	28	1	M5
copper based	14	1	M1
cymoxanil + mancozeb	4		27+M3
cymoxanil + copper oxychloride	1		27+M1
cymoxanil+ cyazofamid	1		27+21
cymoxanil + mancozeb + dimethomorph	1		27+M3+40
dimethomorph	1		40
dimethomorph + mancozeb	3		40+M3
dimethomorph + chlorothalonil	2		40+M5
dimethomorph + prochloraz	1		40+3
ethaboxam	1		22
famoxadone + cymoxanil	1		11+27
folpet	2		M4
fosetyl-al	5		P7
fosetyl-al + mancozeb	1		P7+M3
fosetyl-al + fenamidone	1		P7+11
mancozeb	33		M3
benalaxyl + mancozeb	0	1	4+M3
iprovalicarb +mancozeb	1		40+M3
mancozeb + sulphur	1		M3+M2
mancozeb + copper sulphate	1		M3+M1
mandipropamid + difenoconazole	1		40+3
metalaxyl + mancozeb	34		4+M3
metalaxyl+ triadimefon	0	1	4+3
probineb	3		M3
cymoxanil + propineb	3		27+M3
propineb + fluopicolide	1		M3+43
propamocarb	4		28
propamocarb + fosetyl -al	1		28+P7
propamocarb + fluopicolide	1		28+43
propamocarb + fenamidone	1		28+11
pyrimethanil	3		9
sulphur	14		M2
tetraconazole + chlorothalonil	1		3+M5
thiophanate methyl	3		1

CURRENT FUNGICIDE APPLICATION PRACTICES IN TOMATO

Tomato production in the United Republic of Tanzania is hampered by several factors and average yield is between 10 to 20 ton/ha. One of the major constraint is Late Blight caused by the oomycete *Phytophthora infestans*. In order to prevent losses caused by this pathogen farmers mainly rely on the use of fungicides. Common fungicides used are mancozeb and metalaxyl based ones (De Putter *et al.*, 2017). In the dry season when less rainfall is expected farmers tend to apply just a couple of mancozeb sprays. In the rainy season farmers spray more frequently with mancozeb/metalaxyl based fungicides (Table 4). Since farmers are afraid that fungicides will be washed off the crop, they avoid spraying before expected rain but spray immediately after rain even when using products with contact fungicides only. The fungicides are applied by using knapsack sprayers with 15- 17 l content. Nozzles are not maintained and while spraying applicators are not wearing proper PPE. In the best case they wear wellingtons with some makeshift rain coat (Figure 1).

Table 4. *Typical spray schedules of tomato farmers in Moshi Region, 2015*

Variety	Kipato F1		Kipato F1		Rio Grande (OP)	
Yield	37 t/ha		28 t/ha		11 t/ha	
Season	date	activity/spray	Season date	Activity/spray	Season date	Activity / spray
					wet	3-apr mancozeb + metalaxyl
					wet	13-apr mancozeb + metalaxyl
dry	7-jul	transplanting	wet	25-dec transplanting	wet	27-apr transplanting
dry	16-jul	mancozeb + metalaxyl	wet	2-jan sulphur	dry	2-mei mancozeb
dry	4-aug	mancozeb + metalaxyl	wet	16-jan sulphur	dry	8-mei mancozeb
dry	15-aug	mancozeb + metalaxyl	wet	27-jan sulphur	dry	24-mei mancozeb + fosetyl-al
dry	19-aug	mancozeb + metalaxyl	wet	20-feb 1st harvest	dry	31-mei mancozeb + fosetyl-al
dry	25-aug	mancozeb + metalaxyl	wet	28-feb sulphur	dry	14-jun mancozeb
dry	8-sep	1st harvest	wet	9-mrt sulphur	dry	22-jun mancozeb
dry	10-sep	mancozeb	wet	16-mrt last harvest	dry	28-jun mancozeb
dry	20-okt	last harvest			dry	5-jul mancozeb
					dry	19-jul mancozeb + metalaxyl
					dry	26-jul mancozeb + metalaxyl
					dry	28-jul 1st harvest
					dry	7-aug mancozeb + metalaxyl
					dry	24-aug mancozeb
					dry	18-sep last harvest
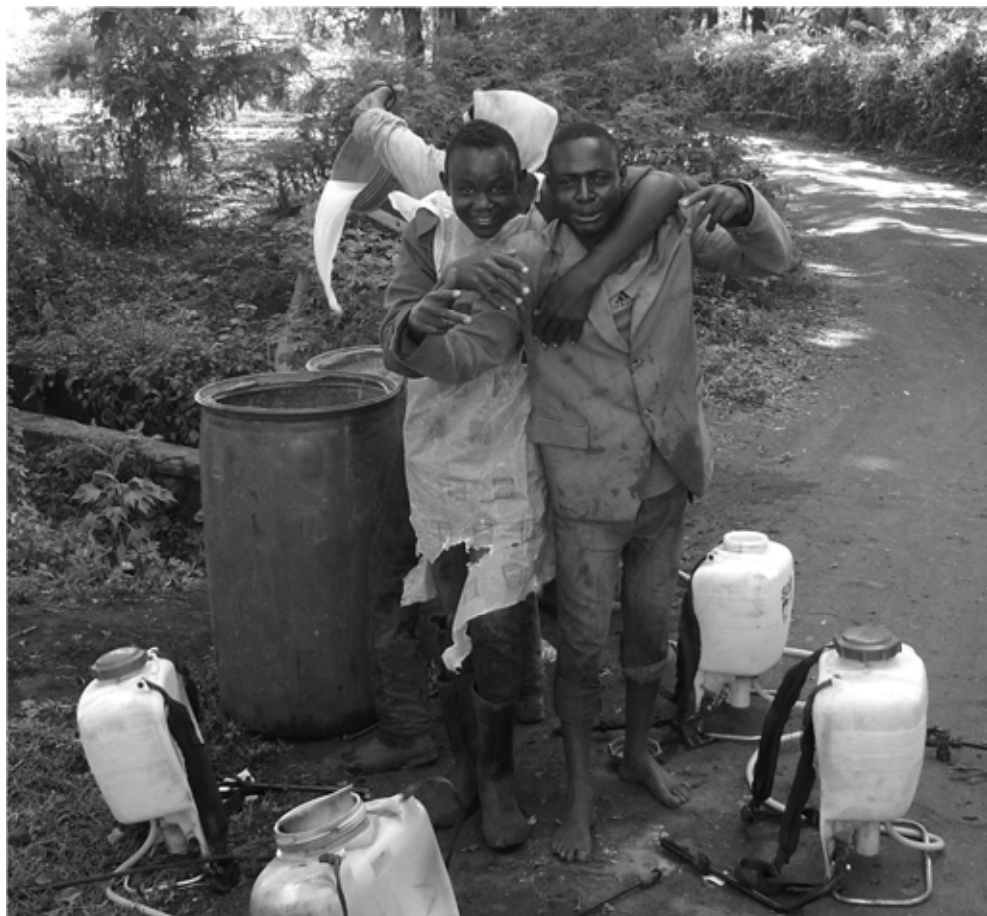
Figure 1. *Pesticide applicators in Tanzania, the person left has some plastic cover and wears boots, while the person right has no foot wear at all*

Since farmers frequently use products containing the phenylamide metalaxyl or the related active ingredient mefenoxam, more than the recommended 2 times a season, resistance of *P. infestans* against this active ingredient is suspected (FRAC pathogen risk list). The current recommendation is to limit phenylamide use to 2 to 4 consecutive sprays and to apply only in the early or the active growing stage of the crop (FRAC phenylamide general recommendation).

RESISTANCE OF *P. INFESTANS* TO METALAXYL OR METALAXYL-M

To find out if resistance of *P. infestans* against metalaxyl/mefenoxam is present a test was done by Sokoine Agriculture University in Morogoro. Tomato and potato fields from six regions (Mbeya, Njombe, Iringa, Morogoro, Tanga and Arusha) were inspected for late blight disease in April to June 2017. From the infected fields, leaves with infected fresh, nicely sporulating lesions on the leaflets were sampled. A total of 63 leaf samples were collected for processing, culturing and identification at the Sokoine University of Agriculture (SUA) Plant Pathology laboratory and

for *Phytophthora infestans* DNA analysis by using FTA cards. The FTA card analysis was carried out by James Hutton Ltd., Dundee, United Kingdom. From each sample four leaf discs taken at the edge from the infected part were placed between potato slices of the *P. infestans* susceptible potato variety Akira. After the potato slices surfaces were sterilized a leaf disc was placed underneath a slice in a sterile petri dish. After mycelia became visible on the upper side of the potato slice mycelium was transferred to a petri dish with a Pea V8 ampicillin agar medium. Tests were performed with the fungicides Ridomil Gold MZ 68 WG (Metalaxyl-m 40 g/kg + Mancozeb 640 g/kg) (Syngenta Crop Protection, Switzerland) and Ivory M72 (Metalaxyl-m 80 g/kg + Mancozeb 640 g/kg) (Arysta Life Science, France). The test was done in three replicates per fungicide and concentration for each *P. infestans* isolate. Petri dishes were filled with 25 ml double-distilled water containing fungicide with a calculated concentration of 0, 0.001, 0.01, 0.1, 1, 10 and 100 mg/l metalaxyl-m. Per isolate leaf discs of either tomato (var. Tanya) or potato (var. Arika) were taken from greenhouse reared plants. The discs were placed upside down in the petri dish with a given metalaxyl-m concentration. Three hours after placing the discs in the petri dish 30 ul droplets with 20,000 spores/ml were put in the center on the floating leaf discs. The petri dishes where then placed for six days at 20ºC in the light on a bench. Mycelium growth was visually observed in percentage of affected leaf area (Figure 2 and 3). On all tested isolates in tomato the affected leaf area was more than 50%. Samples collected from fields in Arusha showed a dose response effect. The overall conclusion is that resistance is present since even at the highest concentration tested more than 50% of the leaf disk surface was affected by mycelium growth.

Analysis of the FTA cards showed that most of the isolates were either US-1 or 2-A1 and one possible 13-A2 isolate, but doubtful since the DNA material collected was not conclusive enough for an accurate analyze.
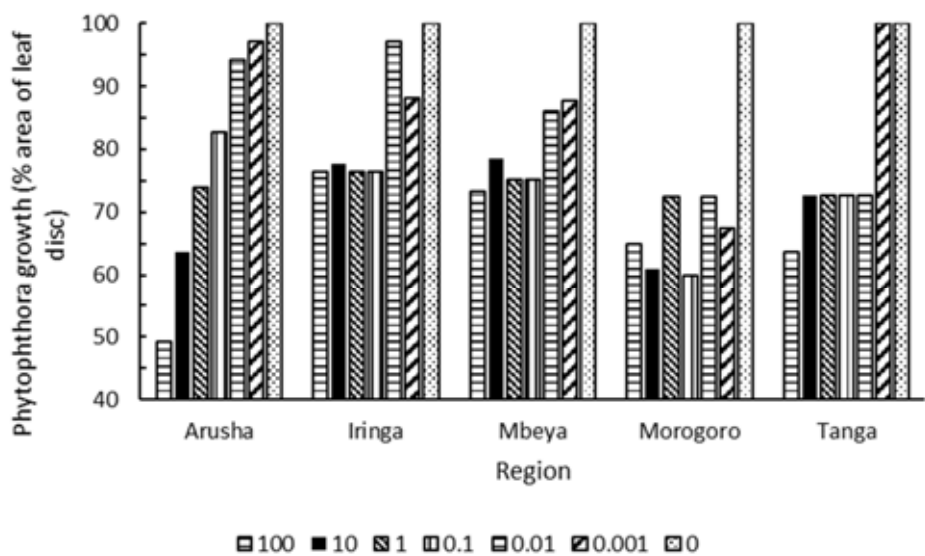
Figure 2.   *Effect of Ivory M72 on P. infestans growth on tomato leaf disks*
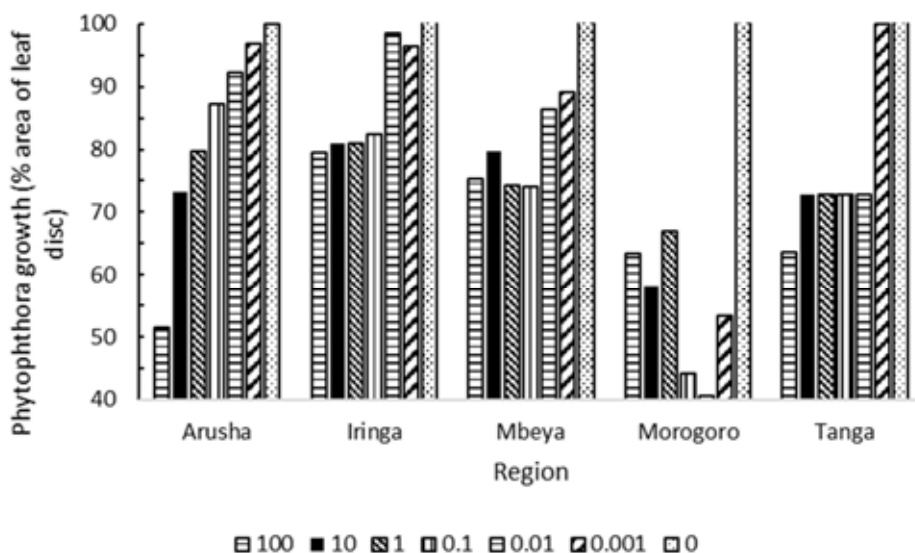
Figure 3. *Effect of Ridomil Gold on P. infestans growth on tomato leaf disks*

EFFECT OF FUNGICIDES AND ADJUVANTS ON THE INCIDENCE OF *P. INFESTANS* IN TOMATO

To prevent resistance proper use of fungicides is essential but also application technique is an important factor to achieve an optimal control *of P. infestans*. Currently farmers are not adding adjuvants to their fungicide sprays. In 2017 a test was carried out by Tanzania Agricultural Research Institute (TARI) Tengeru, Arusha to determine the effect of two different adjuvants: Aquawet 15 SL (nonylphenol ethoxylate) (Osho Chemical Ltd.) and Silwet Gold (Heptamethyl Trisilozane 84% And Polyakylene Oxide 16%) (Arysta Life Science) with three different fungicides: Ebony 80 WP (mancozeb 80%) (Balton Tanzania Ltd.), Milraz 76 WP (propineb 70% + cymoxanil 6%) (Bayer) and Victory 72 WP (mancozeb 64% + metalaxyl 8%) (Sineria Industries Tanzania).

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each plot measured 2 m wide by 4 m long. Planting of tomato var. Tanya was done at a spacing of 60 cm x 50 cm. Fungicides were sprayed at the label recommended rates every 14 days. Tomato was grown in line with good agricultural practices.

During the experiment disease incidence was observed in week 9 after planting. Disease was rated on a scale of 1 to 9 as described by Gwary and Nahunnaro (1998) in which 0 = 0% (no disease), 1 = when 10% of leaf area is affected; 3 = 10 - 20% of leaf area affected; 5 = 20 – 30% of leaf area affected; 7 = 30 – 60% of leaf area affected; 9 = over 60% lesion area of the whole leaf affected. Then the rating scales were converted into percentage severity index (PSI) for analysis of disease severity using the formula: PSI = (Sum of individual numerical rating/Total number of assessed x maximum score in scale) x 100.

Marketable yield was recorded for all harvest periods based on local criteria. Based on yield and an estimated market price of 500 Tsh and input costs the net benefit and benefit cost ratio was calculated.

With adjuvants it seems the disease rating is lower as compared to the applications with the respective fungicides without adjuvants applied (Table 5). The highest yield was present with Milraz 76WP, where the yield increased to approximately 30 t/ha when adding an adjuvant to the spray solution. Difference in yield between Ebony, only mancozeb, and Victory, mancozeb plus metalaxyl, is negligible. When adding Aquawet to those fungicides yield increased to 18 t/ha with Ebony but only to 5 t/ha with Victory. The reason for a more or less similar results between Victory and Ebony might be related to possible metalaxyl resistance. This would imply that only the mancozeb component in Victory was effective in preventing late blight just like with Ebony. The reason that adjuvants didn't improve the efficacy of Victory might be due to an already good formulation of this product.

Table 5. *Effect of adjuvant sprayed with fungicides of tomato yield*

Treatment	Disease rating	Marketable yield (tons/ha)	Net benefit (Tsh x 1,000/ha)	Benefit/cost ratio
Ebony 80	19.3 a	72.0 b	33,828	3.1
Ebony 80+Aquawett	5.7 a	90.2bc	36,884	3.5
Ebony 80+Silwet	4.3 a	75.8 b	29,513	2.5
Milraz 76	19.0 a	80.8 b	37,334	3.1
Milraz 76+Aquawet	3.3 a	109.7 c	45,709	4.0
Milraz 76+Silwet	3.7 a	110.7 c	46,064	4.0
Victory 72	20.3 a	77.3 b	36,163	3.3
Victory 72 +Aquawet	4.3 a	81.8 b	32,333	2.8
Victory 72 + Silwet	8.3 a	81.7 b	32,168	2.7
Control	100.0 b	10.0 a	-2,653	0.7
CV (%)	51.6	26.62		
LSD	19.4	20.66		
F-test	***	**		

Means followed by same letter(s) in the column do not differ (P>0.05)

EFFECT OF SPRAY STRATEGIES ON THE INCIDENCE OF P. INFESTANS IN TOMATO

In the period of April till July 2018 control of late blight in tomato (open pollinated var. Tanya) with three different commonly used fungicides combined with fixed or flexible (depending on rain conditions) spray intervals of the fungicides against the tomato late blight was tested. The test in a complete randomized block design with three blocks was done at TARI Tengeru near Arusha, Tanzania. Each plot contained 32 plants planted at 50 x 60 cm. Ebony 80WP (Mancozeb 80%), Victory 72WP (Mancozeb 64% + Metalaxyl 6%) and Milraz 76 WP (Propineb 70% + Cymoxanil 6%) were sprayed at their recommended label rates. Ebony was sprayed in a fixed calendar spray frequency of 7 or 14 days to resemble farmer's practice. The timing of the applications of the other treatments depended on the climatic conditions and crop stage (Table 6) Ebony was sprayed at a set interval per crop stage, 7 or 14 days at the long interval or 3 and 7 days at the short interval

strategy. In case of moist (rainy) conditions the interval was interrupted with a stronger product. In one strategy Milraz was chosen while in the other strategy Victory was chosen.

Table 6. *Strategies in the experiment*

Strategy	Crop stage	Spray frequency (rain = when precipitation exceeds 5 mm/day)
7	0 – end	standard every 7 days spray with Mancozeb
14	0 – end	standard every 14 days spray with Mancozeb
flex long	0-30 days	Normal 14 day interval but if it rains between 7 – 14 days, spray the next day after the rain day and start again with a 14 day interval.
	31-60 days	Normal 7 days interval but If it rains after 4 days spray next day and start again with 7 day interval
	60-90 days	Normal 14 days interval If it rains after 7 days spray a day after rain and start with 14 days interval
flex short	0-30 days	Start with 7 days interval. If it rains after 4 days spray a day after rain and start with 7 days interval
	31-60 days	Start with 4 days interval. If it rains after 2 days spray a day after rain and start with 4 days interval
	61-90 days	Start with 7 days interval. If it rains after 4 days spray a day after rain and continue with 7 days interval

Based on the climatic conditions and crop growth the final applied number of fungicide applications are presented in Table 7.

Table 7. *Actual applied fungicides per strategy*

Treatment code	Strategy	Mancozeb	Milraz	Victory
T1	Control	0	0	0
T2	14 day Mancozeb	7	0	0
T3	7 day Mancozeb	14	0	0
T4	Flex long Mancozeb only	11	0	0
T5	Flex short Mancozeb only	18	0	0
T6	Flex long Mancozeb/Milraz	8	2	0
T7	Flex short Mancozeb/Milraz	14	4	0
T8	Flex long Mancozeb/Victory	7	0	3
T9	Flex short Mancozeb/Victory	15	0	3

Severity of late blight was recorded on the basis of 1-6 rating scales as described by Gwary and Nahunnaro (1998). where scale 1=trace to 20% leaf infection, 2=21-40% leaf infection, 3=41-60% infection, 4=61-80 infection, 5=81-99% infection, 6=100% leaf infection or the entire plant defoliation and then the rating scales were converted into percentage severity index (PSI) for the analysis of disease severity using the formula: Percentage Severity index = sum of individual numerical rating/Total number of assessed x maximum score in scale x 100.

Based on total marketable yield of all harvest periods, market price of 400 Tsh/kg, costs of inputs and labour, the net benefit of each treatment was calculated as well as the benefit cost ratio.

Disease severity with the calendar spray strategy was higher than the ones from the flexible strategies (Table 8). No significant differences were observed in disease severity between the flexible strategies. In terms of marketable yield it is clear, especially for the flexible strategies that shortening the interval gave the highest yield and best economic performance. Between the fungicides selected in the strategy no big differences were seen, although it seems that a more stronger active ingredient, e.g. cymoxanil or metalaxyl, gave slightly better results than applying mancozeb only as in treatment T4 and T5.

Table 8. *Disease severity, yield and net benefit of the different strategies tested in 2018*

Treatment	Disease Severity week 10	Weight of marketable yield (ton/ha)	Weight of non marketable yield (ton/ha)	Net benefit (Tsh x 1,000/ha)	Benefit cost ratio
T1 Control	5.0 a	0.8 b	0.8 d	320	-0.9
T2	2.7 ab	20.4 b	7.0 abc	8,160	0.3
T3	4.3 a	5.9 b	2.9 cd	2,360	-0.6
T4	1.7 b	23.7 b	5.3 abcd	9,480	0.5
T5	1.0 b	58.0 a	9.4 ab	23,200	2.6
T6	1.3 b	20.8 b	4.0 bcd	8,320	0.2
T7	1.0 b	67.0 a	7.3 abc	26,960	3.0
T8	0.3 b	27.8 b	4.0 bcd	11,120	0.7
T9	0.3 b	75.3 a	10.2 a	30,120	3.4
CV (%)	68.4	49.7	52.7		
LSD	2.3	28.6	5.2		
F-test	**	***	*		

Means in the same column followed by the same letter (s) are not significantly different according to Duncan's multiple range test at 0.05 level of significance

CONCLUSIONS

Tomato production in Tanzania faces a lot of challenges of which a proper late blight control is one. In principle more than a sufficient number and range of fungicides are registered for use in tomato to control late blight. However, in agro shops only a few products are available mainly mancozeb and metalaxyl based products. As a result farmers also use a lot of those products only with the risk of causing resistance among the *Phytophthora infestans* pathogens present in Tanzania. A test conducted by Sokoine University of Agriculture showed that this is highly likely the case already. From the most common available fungicides it also seems that products containing metalaxyl are not so effective. When adding adjuvants to fungicides their efficacy is improved. Finally it appears that a more interactive spray strategy considering crop stage and climate is more effective than a standard 7 or 14 days interval. A shorter interval in this case is more economic than maintaining longer intervals even though two times more applications are used.

In order to improve late blight control, farmers should receive more training on proper use of fungicides where attention is paid to strategy and application technologies. Moreover, agro shop owners should also receive information on efficacy of fungicides and resistance risks when no

proper control strategy is implemented by farmers. In this way a wider range of products can become available at outlets.

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Pathogenicity – a driver for the epidemic potential of the clonal lineage EU_41_A2 of *Phytophthora infestans* inside sexually reproducing populations of Nordic European countries?

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SUMMARY

In Nordic European countries, *Phytophthora infestans* populations appear sexually reproducing, with unique SSR genotypes. Nevertheless, an extensive survey of European populations of the late blight pathogen*,* carried out within the EuroBlight network*,* highlighted the recent emergence of a new clonal lineage, named EU_41_A2, which was first detected in Denmark in 2013. This raises the question of the epidemic potential of the success and the persistence over time of this asexual newcomer inside sexual populations, and of its impact on late blight management strategies. The aim of this study was to analyse, using bioassays, the aggressiveness and the virulence in the EU_41_A2 lineage. Its phenotypic traits were then compared with those of samples from sexual populations of *P. infestans,* collected in 2016 and 2017 from three Nordic partner countries of the IPMBlight2.0 project (Denmark, Norway and Estonia). Among the unique genotypes, a large response variability was observed for aggressiveness traits as well as for virulence. The sporulation mean values showed that the Danish unique genotypes had the greatest sporangial production, and isolates from Norway the lowest one. Danish unique genotypes were also more virulent than those from the two other countries. Isolates of the EU_41_A2 clonal lineage showed a high aggressiveness level, similar to that of Danish unique genotypes on Bintje detached leaflets; most of them were also virulent to 9 to 11 *R* genes. Clonal and sexual Danish isolates were therefore not clearly distinguished based on the phenotypic traits explored in this study. The dispersal and expansion of the EU_41_A2 clonal lineage inside sexual populations could possibly be explained by some other ecological processes, such as its overwinter survival in the context of climate change; this would facilitate the co-existence of clonally and sexually reproducing *P. infestans* genotypes.

KEYWORDS

Late blight, *Solanum tuberosum*, aggressiveness, virulence, clonal lineage, sexual reproduction, genotypic diversity

INTRODUCTION

Phytophthora infestans is renowned for fast and dramatic changes in genotype occurrence, as well as for its capacity to adapt rapidly to changes in its environment, with strong impacts for a durable management of late blight. To examine in real time the ongoing evolution of these populations, an extensive survey of European populations of the late blight pathogen has been undertaken by the EuroBlight network since 2013 (Cooke *et al.*, 2019).

In Nordic and Baltic European countries, *P. infestans* populations appear sexually reproducing, with unique SSR genotypes (Sjöholm *et al.*, 2013; Runno-Paurson *et al.*, 2016; Kiiker *et al.*, 2019). These populations are composed of ephemeral, genetically diverse isolates, probably originating from oospore inoculum. These oospores can survive in the soil for several years, between potato growing seasons, whereas, because of cold winters, survival of *P. infestans* clones in infected potatoes and weed hosts was so far significantly be impaired. Nevertheless, the EuroBlight survey highlighted the recent emergence in northern Europe of a new clonal lineage, named EU_41_A2, first detected in Denmark in 2013. This asexual lineage remained local in 2014, but spread throughout Denmark in 2015 and then to Sweden and Norway in 2016; its expansion continued to the North/ North-East (Poland) in 2017 (EuroBlight data, maps visible online on https://agro.au.dk/forskning/internationale-platforme/euroblight/). This raises the question of the epidemic potential of the success and the persistence over time of this asexual newcomer inside sexual populations, and of its impact on late blight management strategies.

Little is known about the new *P. infestans* emerging EU_41_A2 lineage. Therefore, we intended to further characterize, using bioassays, important pathogenicity traits in the EU_41_A2 lineage, such as aggressiveness (disease severity on detached leaflets of the susceptible host, cv. Bintje) and virulence (ability to overcome host resistance genes, *R1* to *R11* from an international differential set and to four resistant cultivars in the fields, Carolus, Alouette, Kelly and Sarpo-Mira). These phenotypic traits were then compared with those of samples from sexual populations of *P. infestans,* collected in 2016 and 2017 from three Nordic partner countries of the IPMBlight2.0 project; Denmark, Norway and Estonia (Andrivon *et al.*, 2017).

MATERIALS AND METHODS

Isolates sampling

Isolates of *P. infestans* were collected from potato plants in three Nordic and Baltic countries, Denmark, Norway and Estonia. Infected potato leaves from several cultivars were predominantly sampled in conventional and organic production fields, but also in some trials. A total of 199 isolates was sampled in 2016 and 2017 during the growing season, from July to September (around 40 alive isolates per year and country, except in Denmark in 2016 where only 6 isolates were collected). In addition, 25 EU_6_A1 and 26_EU_13_A2 isolates obtained from Great-Britain and France during the same period were added for phenotypic comparison. Isolates were stored in darkness at 15°C by serial transfers on pea agar medium, until they were tested (around six months).

Microsatellite genotyping

Isolates were genotyped at 12 microsatellite loci at INRA (France) and at the James Hutton Institute (D.E.L. Cooke, UK). Amplification of the single sequence repeat (SSR) markers was carried out in multiplexed PCR assays; PCR products were then capillary electrophoresed and the microsatellite data were used to define multilocus genotypes (MLGs).

Phenotypic characterization

Foliar aggressiveness and virulence of the isolates were analysed in detached leaflet bioassays. Plants from potato genotypes were grown from seed tubers in pots filled with 1:1:1 sand-peat-compost mixture, in a glasshouse regulated at 15-20°C. Leaflets were collected for experiments on 7-8 week-old plants. For inoculum production, as pathogenicity could be affected during axenic culture, isolates were multiplied separately onto detached leaflets of cultivar Bintje to prepare sporangia suspensions adjusted to 3 x 10^4 spores. mL^{-1} and chilled at 4°C for two hours before inoculation on plant material.

Aggressiveness was measured on susceptible cultivar Bintje leaflets. Each leaflet was placed abaxial face up on the lids of inverted Petri dishes containing 10 g L^{-1} water agar (two leaflets per dish), and inoculated by depositing a 20 µL drop of sporangial suspension (around 600 sporangia) at the leaflet center. Ten technical replicates per isolate were performed. Incubation temperatures were 15°C night - 18°C light (with a 16 hours light period). Two components of aggressiveness, lesion area and number of sporangia per lesion (quantified with a particule counter), were scored five days post inoculation.

Virulence tests were carried out on detached leaflets of the Black's differential set of 11 potato clones with *R* specific genes and on Bintje as susceptible cultivar, according to the protocol detailed by Andrivon *et al.* (2011). In addition, virulence of Nordic and Baltic isolates was assessed on leaflets of four resistant cultivars, Carolus, Alouette, Kelly and Sarpo-Mira.

RESULTS

Genotypic structure of the populations

A high genotypic diversity was found inside *P. infestans* populations of the three countries, using the 12 simple sequence repeat markers. Most of the isolates had unique MLGs and appeared only once during the two sampling years; they were named "other" isolates according to EuroBlight network. However, 18 isolates (8 from Denmark and 10 from Norway on both years) had the clonal lineage EU_41_A2 profile; on the opposite, in Estonia, among the 80 sampled isolates, no EU_41_A2 MLG was detected (Table 1).

Table 1. *Number of analysed P. infestans isolates according to their multilocus genotype (MLG), the sampling year and the country*

Genotype (MLG)	Year	Denmark	Norway	Estonia	Total
EU_41_A2	2016	-	6	-	6
	2017	8	4	-	12
"other"	2016	6	29	40	75
	2017	31	35	40	106
Total		45	74	80	199

Aggressiveness characterization

No significant differences in lesion areas (around 850 mm^2) were found between EU_41_A2 and "other" isolates from the three countries. In the same way, sporulation did not significantly differ between EU_41_A2, Danish and Estonian "other" isolates (Figure 1). However, Norwegian "other" isolates had a significantly lower sporangia production compared to any of the other groups. Inside the EU_41_A2 samples, isolates did not show significant differences in sporulation according to the country (Denmark and Norway), nor to the year with Norwegian isolates sampled in 2016 and 2017 (Figure 1).
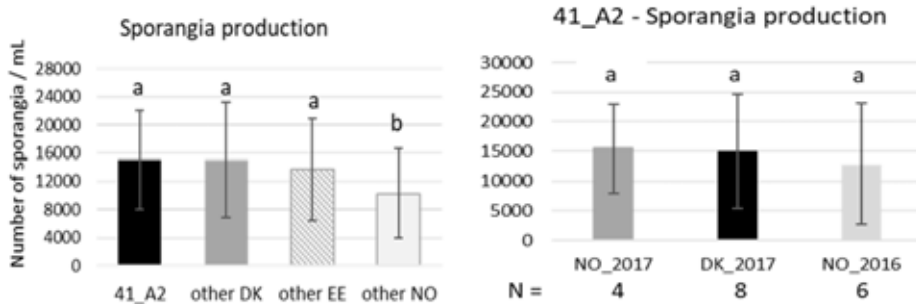
Figure 1. *Spore production (number of sporangia per lesion) of 2017 P. infestans isolates on potato cv. Bintje detached-leaflets (mean of 10 replicates). Measurements made after five incubation days, at 15°C night / 18°C light (16 h light period)*
Left: comparison between isolates EU_41_A2 in black (n = 12), "other" from Denmark in dark grey (n = 31), "other" from Estonia in shaded (n = 40) and "other" from Norway in clear grey (n = 35). Right: comparison between EU_41_A2 isolates collected from Norway in 2017 (in dark grey), in 2016 (in clear grey) and from Denmark in 2017 (in black). Columns with the same letter do not differ significantly (P<0.05). Vertical bars indicate standard deviation

The Nordic and Baltic isolates were then compared to some dominant clonal lineages, EU_6_A1 and EU_13_A2, sampled in western Europe (Figure 2). For lesion size, only EU_6_A1 isolates were significantly different from all the isolates with the largest lesion area. For this trait, EU_41_A2 and "other" isolates from the three countries caused lesions similar in size to EU_13_A2 isolates. For sporulation component, the greatest sporangial production was obtained with the EU_41_A2 and Danish "other" isolates which were statistically similar to EU_6_A1 isolates. Estonian "other" isolates were close to EU_13_A2 isolates which sporulation was significantly smaller than that of EU_6_A1 isolates. Finally, Norwegian "other" isolates produced the lowest number of sporangia.
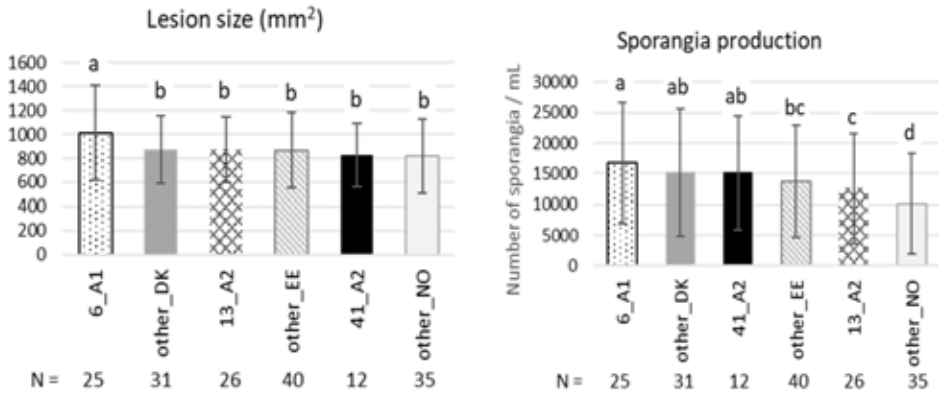
Figure 2. *Aggressiveness traits of Nordic and Baltic P. infestans isolates (EU_41_A2, "other" from Denmark, Estonia and Norway) compared to EU_6_A1 and EU_13_A2 clonal lineage isolates from France and Great Britain. Bioassay performed on cv. Bintje detached leaflets*
Left: mean lesion size and right: mean number of sporangia per lesion within each genotype group of isolates. Different letters above the bars indicate significant difference

Virulence

We examined the ability of 2016-2017 EU_41_A2 and "other" isolates from the three countries to overcome foliar late blight resistance on eleven potato *R* genes differential plants, in a laboratory test (Figure 3). Among the 199 sampled isolates, 15 (2 isolates EU_41_A2 and 13 "other") gave no or weak lesions on cv. Bintje; they were removed from the analysis.

Virulence profiles of EU_41_A2 were highly complex; 44% of the isolates overcame 11 to 10 *R* resistance genes (except *R9*)*,* and 50% were virulent against 8 to 9 *R* genes. Danish "other" isolates also presented highly complex patterns: 30% overcame 10 to 11 *R* genes of the differential set and 50%, 8 to 9 specific *R* genes. A great diversity of pathotypes was noticed inside "other" isolates from Estonia and especially from Norway. Estonian "other" isolates were less virulent than EU_41_A2 and Danish "other" isolates: 43% were virulent to 8 to 11 *R* genes, 50% to 6 to 7 *R* genes and 7% to 4 to 5 *R* genes. Finally, Norwegian "other" isolates showed the least complex pathotypes. No isolates were virulent to the 11 *R* genes and only 3% to 10 *R* genes. The majority of these isolates (56%) overcome 6 to 7 *R* genes and 16% only 2 to 5 *R* genes.

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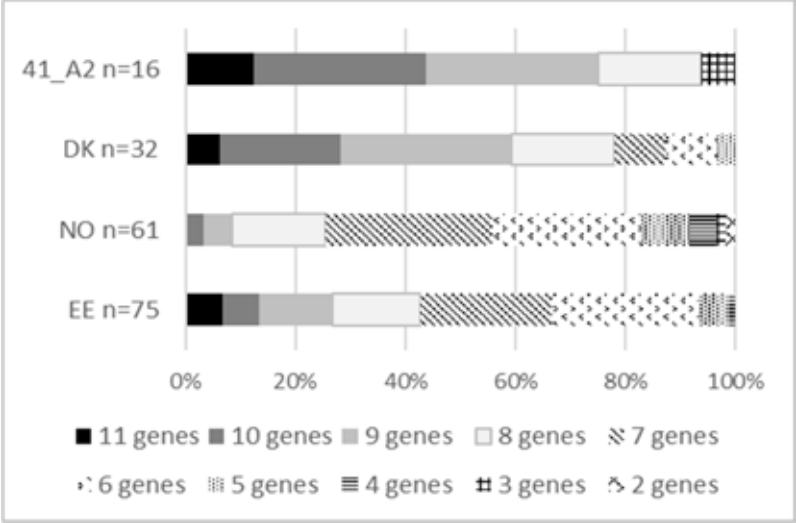
Figure 3. *Mean number of overcome R genes (R1 to R11 from the Black's differential set) by the isolates sampled in 2016-2017 (in percent). First line: EU_41_A2 isolates, 2nd line: Danish "other" isolates, 3rd line: Norwegian "other" isolates; 4th line: Estonian "other" isolates. Each color presented the number of overcome R genes by the isolates; in black, 11 genes, in dark grey, 10 genes; in clear grey, 9 genes, etc.*

The six *R* specific genes, *R1*, *R3*, *R4*, *R7*, *R10*, *R11* were generally overcome by the isolates. Interestingly, EU_41_A2 and Danish "other" isolates were clearly distinct from Estonian and Norwegian "other" isolates according to their behaviour to the five *R* genes, *R2*, *R5*, *R6*, *R8* and *R9* (Figure 4). Most of EU_41_A2 and Danish "other" isolates (more than 80%) were virulent to *R2* and *R6*, 50% of them to *R5* and *R8* and 10% to *R9*. On the opposite, only 20 to 40% (or less) of the Estonian "other" isolates and especially of the Norwegian "other" isolates overcome these five *R* genes.
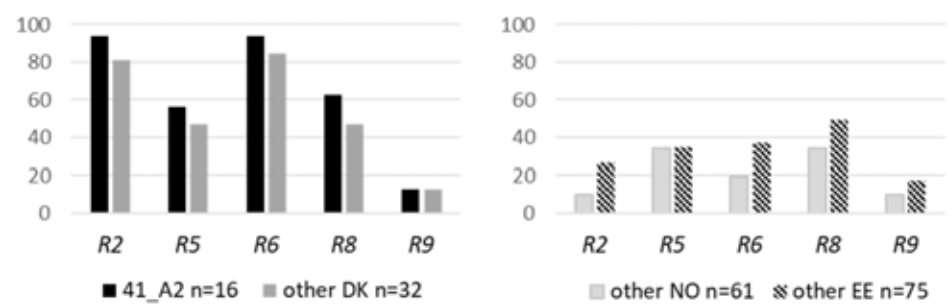
Figure 4. *Percentage of virulent 2016-2017 P. infestans isolates against five R specific genes (R2, R5, R6, R8, R9) from the Black's differential set, based on a detached leaflet bioassay*
Right: virulence of EU_41_A2 isolates (in black) and Danish "other" isolates (in dark grey). Left: virulence of "other" isolates from Norway (in clear grey) and from Estonia (shaded columns)

The virulence of the isolates was also tested in the laboratory, on four potato cultivars which are resistant in the field, cvs Carolus, Alouette, Kelly and Sarpo-Mira (Figure 5). The majority of the isolates did not overcome the resistance of Carolus and Alouette. Moreover, only a few isolates were virulent against Sarpo-Mira; but 40% of the Estonian "other" isolates overcame its resistance. However, on cv. Kelly, virulence of the EU_41_A2 and Danish "other" isolates differed strongly from that of Norwegian and Estonian "other" isolates; more than 60% of the isolates from the first group were virulent against Kelly resistance, whereas 5% and 24% of Norwegian and Estonian "other" isolates, respectively, were not virulent on cv. Kelly.
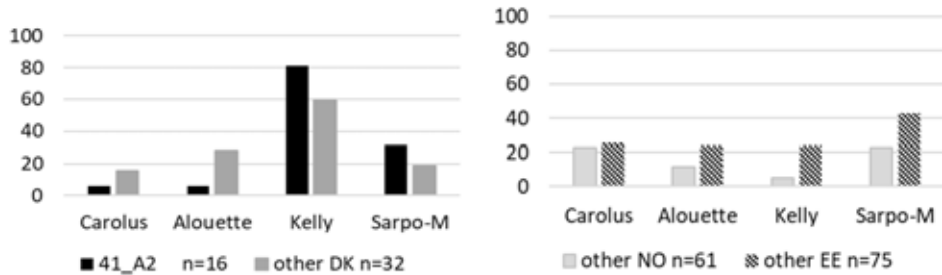
Figure 5. *Frequency of virulent 2016-2017 P. infestans isolates against four potato cultivars, Carolus, Alouette, Kelly and Sarpo-Mira. Bioassay performed on detached leaflets*
Right: virulence of EU_41_A2 isolates (in black) and Danish "other" isolates (in dark grey). Left: virulence of "other" isolates from Norway (in clear grey) and from Estonia (shaded columns)

DISCUSSION AND CONCLUSION

To understand the potential of the asexual EU_41_A2 emerging lineage for invasive and persistence success inside *P. infestans* sexual populations, we compared, in using detached leaflets bioassays, its pathogenicity traits (aggressiveness and virulence) with those of samples from sexual populations collected in 2016-2017 in three Nordic and Baltic countries.

This study, the first to describe phenotypic characteristics of EU_41_A2, revealed that it is a highly aggressive and virulent lineage. Nevertheless, the Danish sexual population presented large phenotypic similarities with EU_41_A2 isolates. Clonal and sexual Danish isolates were therefore not clearly distinguished based on the phenotyping explored in this study. By contrast, large differences were noticed between unique genotypes from the three countries, with Norwegian isolates showing the lowest sporangia production and the simplest virulence patterns. This result is consistent with previous reports on 2003 Nordic populations where Norwegian *P. infestans* isolates seemed less aggressive and appeared with simpler pathotypes than Danish ones (Lehtinen *et al.*, 2008, 2009). In this work, aggressiveness bioassays were performed on the susceptible cv. Bintje which is not cultivated in some Nordic and Baltic countries, as Norway and Estonia. Further studies, using some other dominant susceptible potato cultivars in these countries, such as cv. Mandel in Norway, would be useful to analyse aggressiveness level of these populations to local susceptible hosts, and to investigate their potential adaptation to major potato cultivars.

Investigating pathotype composition provides information of utmost importance for breeding for crop resistance. In the current study, virulence patterns of EU-_41_A2 and Danish "other" isolates, on Black's differential set, were similar and highly complex. Moreover, the mean number of virulence factors of Danish isolates increased from 2003 to 2016-2017; virulences to R5, R8 and especially to R2 and R6 were more common in the current Danish isolates than in the 2003 population (Lehtinen et al., 2008). By contrast, Estonian and especially Norwegian "other" isolates showed a great diversity of virulence profiles, but with more simple pathotypes than those of Danish populations. This result confirms that sexual populations do not spread widely within a growing season and that these local populations seemed well adapted to their regional conditions (Sjöholm et al., 2013; Kiiker et al., 2019).

Only few isolates were able to overcome the resistance of Carolus (with Rpi-chc1 gene), Alouette (with Rpi-vnt1.3 gene) or Sarpo-Mira (with R3a, R3b, R4, Rpi-smira1, Rpi-smira2). This emphasizes the need to incorporate diverse resistance sources into breeding programs. to avoid selection, expansion and dominance of specific P. infestans genotypes. Indeed, specific R genes, e. g. R9, are overcome by some isolates from each population, although it has never been introduced into commercial cultivars. The plasticity of P. infestans genome would reduce the efficacy of breeding resistance based simply on the accumulation of R-genes. In contrast, cv. Sarpo Mira has proved to possess high partial blight-resistance, with generally no apparent changes in resistance level in recent cultivar field trials (Abuley and Hansen, 2019). Although it is not known whether EU-41-A2 was or not present in these trials, a promising way for a complete IPM strategy is to combine different types or levels of host resistance with other control practices.

This study suggests that the EU_41_A2 lineage has a high invasive potential in Norway, but more limited in Denmark where EU_41_A2 isolates would be in competition with highly aggressive and virulent sexual isolates. Due to an effective clonal propagation and spread, this highly pathogenic P. infestans genotype would have the ability to respond quickly to selective pressure, and successful isolates could, over short periods, become dominant in the Norwegian population. However, these predictions are not entirely consistent with current observations. This discrepancy therefore suggests that, while phenotypic measurements under controlled conditions are important, they may not completely reflect P. infestans fitness in the field. Additional factors, such as environmental conditions, fungicide insensitivity, may then be involved in the selection of P. infestans genotypes. The dispersal and expansion of the EU_41_A2 clonal lineage inside sexual populations could then be explained by some ecological processes, as its overwinter survival in the context of climate change or a thermal adaptation during epidemics (Mariette et al., 2016). The capacity to adapt locally to changing environmental and climatic conditions may allow EU_41_A2 isolates to better cope with global changes and would facilitate the co-existence of clonally and sexually reproducing genotypes. Interactions analyses between aggressiveness, virulence and some other traits such as the fungicide insensitivity or temperature response may help to predict invasive traits of EU_41_A2 lineage. Further investigations are then crucial to explain the invasive success of this EU_41_A2 clonal lineage and its epidemic and fitness potential inside sexual populations, in order to promote sustainable control strategies against late blight.

In conclusion, this study provides important data to understand the pathogenicity fitness of P. infestans in Nordic countries. However, a limited number of EU_41_A2 isolates was available in this study and further work needs to be performed to obtain a more comprehensive sampling,

and thus to better evaluate the fitness of this clonal lineage. Continuous blight monitoring in potato production regions must therefore be carried on to examine the ongoing evolution of the European *P. infestans* populations, especially in the North-Eastern regions. Indeed, the changes in *P. infestans* populations directly influence the development and deployment of resistant cultivars and the performance of disease warning systems. Such data might be helpful in supplying information to breeders and in developing more sustainable cropping systems for late blight management strategies.

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Monitoring of *Phytophthora* spp on potato crops and Solanaceous host in Latin America

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INTRODUCTION

Late blight caused by the plant pathogen *Phytophthora infestans* (Mont.) de Bary is globally the most devastating disease that affects potato (Fry 2008; Grünwald and Flier 2005). In Latin America, late blight is considered one of the most limiting diseases on potato and tomato production (Adler *et al.* 2002, 2004; Forbes *et al.* 1997,1998; Vargas *et al.* 2009; Lucca *et al.*, 2017; Chaves, *et al.* 2019). In tropical countries, over the past few years, the pathogen has been associated with large outbreaks on semi-domesticated plants such as tree tomato (*Solanum betaceum*) and other Solanaceous plants (Forbes *et al.* 2011; Oliva *et al.* 2010; Mideros *et al.* 2018).

Despite the intensive effort to control the disease in potato and tomato crops, to date, this pathogen remains a major threat to crop production (Fry 2008). Nearly $ 4 billion is invested in the control of this pathogen (Judelson and Blanco, 2005; Haldar *et al.*, 2006), and around € 9 billion per year is associated with production losses of these crops (Haverkort *et al.* 2016).

Knowledge of the population genetic variation within and among populations is crucial for understanding the main outcomes of the evolutionary processes of pathogens (Hartl and Clark 1997; Hedrick 1985).

Molecular markers provide important information regarding genetic and population diversity, and different types of markers have been used to understand the population genetics of several plant pathogens (Drenth *et al.* 1994; Fry *et al.* 1991; Lees *et al.* 2006; Li *et al.* 2010, 2012, 2013; Cooke *et al.* 2012; Lees *et al.* 2009).

Genetic changes have been reported in *P. infestans* populations globally and in particular in Latin America, that can reduce the effectiveness of disease management practices.

The last years, TizonLatino (https://tizonlatino.github.io/), a consortium of Latin American late blight researchers, is using a standardised tool based on twelve simple-sequence repeat (SSR) markers (Li *et al.*, 2013) for genotyping studies, to provide useful information regarding the levels of genetic diversity and the population structure to study *P. infestans* populations. To reach this goal, the Mycology and Bacteriology Lab of INTA Balcarce is working collaboratively with the Genomic Unit of the Biotechnology Institute (INTA) to carrying out SSR genotyping and new available genomic strategies that can be applied to the study *Phytophthora* populations in Latin America. The Genomic Unit of INTA is a node of the 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.

Results from SSR markers showed that population of *P. infestans* is mostly composed of clonal lineages with distinct genotypes characterized by SSR and confirmed by another analyses. In addition, recently, a new *Phytophthora* species, *P. betacei*, was described infecting tree tomato crops in Southern Colombia (Nariño State).

These data will provide important insights into the genetic population dynamics of *P. infestans* and provide comprehensive information for developing an appropriate disease management strategy against late blight disease in Latin America.

STUDIES IN ARGENTINA, CHILE AND BRAZIL

To determine whether the population is composed of one or several clonal lineages, and if there are differences in fungicide sensitivity and host preference, we carried out a genotypic and phenotypic characterization of *P. infestans* populations in Latin America funded by a Fontagro Project ATN/RF 16678-RG.

The population reported for Argentina, Chile and Brazil by Forbes *et al.* in 1998 and by Adler *et al.* in 2002, has changed in the present. Clonal lineages were determined using Poppr software (Kamvar *et al.*, 2004) and comparing the results with *Phytophthora* global databases (David Cooke, personal communication). The first report of this change was detected in Argentina (Lucca and Huarte, 2012), where the population present in the late 90s, constituted by clonal lineages belonging to mating A2 (AR1 to AR5), was displaced by 2_A1, a genotype reported in Europe in early 80s (Cooke *et al.*, 2012). Later, when we analyzed samples from Chile and Brazil, the results showed that the dominant clonal lineage was also 2_A1, displacing US-1 (Chile) and BR-1 (Brazil) clonal lineages. This old European clonal lineage was also recently reported in Africa (Njoroge *et al.* 2019).

Furthermore, sensitivity to the most commonly used systemic fungicides in Latin America is being assessed in the framework of Fontagro Project ATN/RF 16678-RG to help to adapt management decisions to control late blight.

Training and outreach activities between partner countries, in particular for Late blight studies, with Tizon 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.

Tizon Latino is planning to include more Latin America countries in their genotyping studies. The results of this broad regional genotyping will be presented and discussed in the next meeting of Tizon Latino Network to be held in 2020 in León, México (in the framework of Latinamerican Potato Association Meeting ALAP 2020).

These data will provide important insights into the genetic population dynamics of *P. infestans* populations and provide comprehensive information for developing an appropriate disease management strategy against late blight disease in Latin America.

STUDIES IN COLOMBIA

The studies carried out by Chaves *et al.*, 2019, shown that neither geographic location nor the potato genotype significantly shaped the population structure of the *P. infestans* in central Colombia. The populations of central Colombia are composed of a high number of genotypes that exhibit a high similarity among themselves. For the first time, the subclonal variation was thoroughly described in the most important potato production region in Colombia, including the number of MLG and their geographic distribution.

The results could have a significant impact on designing new strategies to control the disease in Colombia and to deploy new potato genotypes.

Since the information on the population structure of *P. infestans* on tomato crops in Colombia is limited, a phenotypic and genotypic characterization of isolates of *P. infestans* on commercial tomato (*Solanum lycopersicum*) crops in the Andean Region of Colombia is carried out by Giovanna Danies's group. The genotypic diversity for these isolates was assessed using microsatellite markers and mitochondrial haplotyping. Furthermore, sensitivity to three systemic fungicides (mefenoxam, cymoxanil, and fluopicolide) and tomato-potato host preference was also evaluated. This work, currently under review, will establish the actual state of *P. infestans* associated to commercial hybrid tomato varieties in Colombia and the sensitivity of the isolates to the mentioned fungicides. Furthermore, it is important to assess aggressiveness and virulence parameters different varieties of potato and tomato to determine the presence of resistance genes in these varieties as part of control of *P. infestans*.

A new *Phytophthora* species, *P. betacei*, was described infecting tree tomato crops in Southern Colombia (Nariño State) (Mideros *et al.*, 2018) by Restrepo's group. The latest results also permitted to reinforce and support the description of the new species and describe the expanded geographical range of *P. betacei* in the central region of Colombia (Cundinamarca State).

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Results from the trap nursery network in Europe

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SUMMARY

Trap nurseries with potato cultivars with one or more known resistant genes (R-genes) were established in different countries in Europe. The objectives of the trap nursery were to monitor the stability of R-genes in potato cultivars in Europe in space and time, hunt for new genotypes of *Phytophthora infestans* and to test mega cultivars. Late blight severity was regularly monitored and assessed on the differentials throughout the growing seasons in 2017-2019. A system for data collection and analysis was established. In this paper, we discuss the methodology used for analyzing the disease assessment data from trap nurseries. Additionally, we show some results of the trap nursery from Droninglund in Denmark in 2019.

KEYWORDS

Late blight, *Phytophthora infestans,* Trap nursery, Disease progress curves, Apparent infection rate (AIR), area under the disease progress curve (AUDPC)

INTRODUCTION

Late blight, caused by *Phytophthora infestans*, is the most important disease on potato and tomato in the world. The disease has the potential of causing substantial yield loss when not controlled. Fungicides are still one of the major means of controlling late blight. However, the frequent application of fungicides can be expensive, environmentally unfriendly and could result in the development of *P. infestans* isolates that are resistant or insensitive to fungicides. Resistant cultivars provide a more environmentally friendly means of preventing or reducing the impact of late blight. However, R-genes deployed in potato cultivars are easily defeated by new *P. infestans* races, due to the dynamic nature of the pathogen. The best strategy to control late blight is to integrate both host resistance and fungicide within the integrated pest management (IPM) context.

The population of *P. infestans* in Europe is rapidly changing, with the emergence of new clones like EU_36_A2, EU_37_A2 and EU_41_A2. The emergence of these new genotypes of *P. infestans* raises the question of "what is their impact on late blight epidemic, virulence on cultivars and control of late blight in Europe?" These questions were answered in the IPMBlight2.0 project (Corbiere *et al.* this proceeding). As part of the IPMBlight2.0 project, trap nurseries were established from 2017 to 2019 in different countries in Europe (Denmark, Sweden, France, Scotland and Estonia) to serve the following purposes:
1. To monitor the temporal and spatial stability of resistance (R) genes in potato cultivars to *Phytophthora infestans* populations in Europe.

2. To hunt for new genotypes of *P. infestans* as well as phenotyping these new *P. infestans* genotypes in Europe.
3. Test of mega cultivars; that is how to control late blight on cultivars with high level of genetic resistance to late blight with less fungicide.
4. As an input to the IPM Blight 2.0-decision support system (DSS).

The trap nurseries consisted of potato cultivars or breeding lines with one or more known R-genes (See Table 1).

Figure 1. *Example of a trap nursery in Denmark. The trap nurseries are used to do live monitoring of the stability of resistance genes against Phytophthora infestans genotypes in the field*

As stated earlier in this paper, the trap nurseries serve several purposes; however, we shall restrict the focus of this paper to the methodology for analyzing the disease data generated from the trap nurseries and some results from some countries.

Table 1. *Details of the differential set and their resistance gene content (R-gene)[1]*

Differential sets	Genotype for 2018	R-gene content (Zhu *et al.*, 2015)
Black R1	**Craigs Snow White**	R1
Black R2	**Black 1512c (16)**	Unknown
Black R3	**Pentland Ace**	Unknown
DS-R4 / Black R4	**MaR4: CEBECO 4431-5**	R4
DS-5 / Black R5	**MaR5: Black 3053-18**	R1, R2, R3b,
DS-6	**MaR6: Black XD2-21**	R1, R2, R3a
DS-7	**MaR7: Black 2182 ef(7)**	R3a, R4
DS-8	**MaR8: Black 2424 a (5)**	R3a, R3b, R4, R8
DS-R8	3020-018	R8 = Rpi-smra2
DS-9	**MaR9: Black 2573 (2)**	R1, Rpi-abpt1, R3a, R3b, R4, R8, R9
DS-R9	3151-04	R9
DS-10	**MaR10: Black 3681 ad (1)**	R3b, R10
DS-11	**MaR11: Black 5008 ab (6)**	R3b, R10
DS-r½<<<<<	**BINTJE**	None
Sarp Mira	**SARPO MIRA**	R3a, R3b, Rpi-smra1, R4, R8
DS-smira1	3079-08	Rpi-smira1
Alouette	**ALOUETTE**	Unknown
Carolus	**CAROLUS**	Rpi-chc1
INRA 92T118.36	**COQUINE_INRA 92T118.36**	Rpi-*iiii* + QTL's
INRA 95T118.2	**KELLY_INRA 95T118.2**	
INRA 95T141.12	**MAKHAÏ_INRA 95T141.12**	

Monitoring the occurrence and development of late blight on the differentials
The cultivars or differentials in the traps nurseries were monitored regularly for typical late blight lesions or symptoms until total defoliation or the end of the season. The severity of late blight on each cultivar was assessed as the percentage leaf area covered by late blight lesions. Frequent assessment, as will be discussed later, is key for proper estimation of the epidemiological parameters for comparing the disease development on the differentials. For example, for a reliable estimate of the apparent infection rate (AIR) of late blight on a cultivar, more than two assessment data is required.

Data management and analysis of the disease data
An online data management system was established for handling data generated from the trap nurseries based on the former Eucablight methods (Hansen *et al.* 2007). Country managers of the trap nurseries can upload the disease assessment and other information about the trial (e.g. location, differentials) into the system. Among several others, the system generates a trial site map that shows the location of the trap nurseries (Figure 2) (2), disease progress curves of selected differentials (Figure 3) and calculations of different epidemiological parameters (e.g. area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC) apparent infection rate (AIR)) (See Table 2).
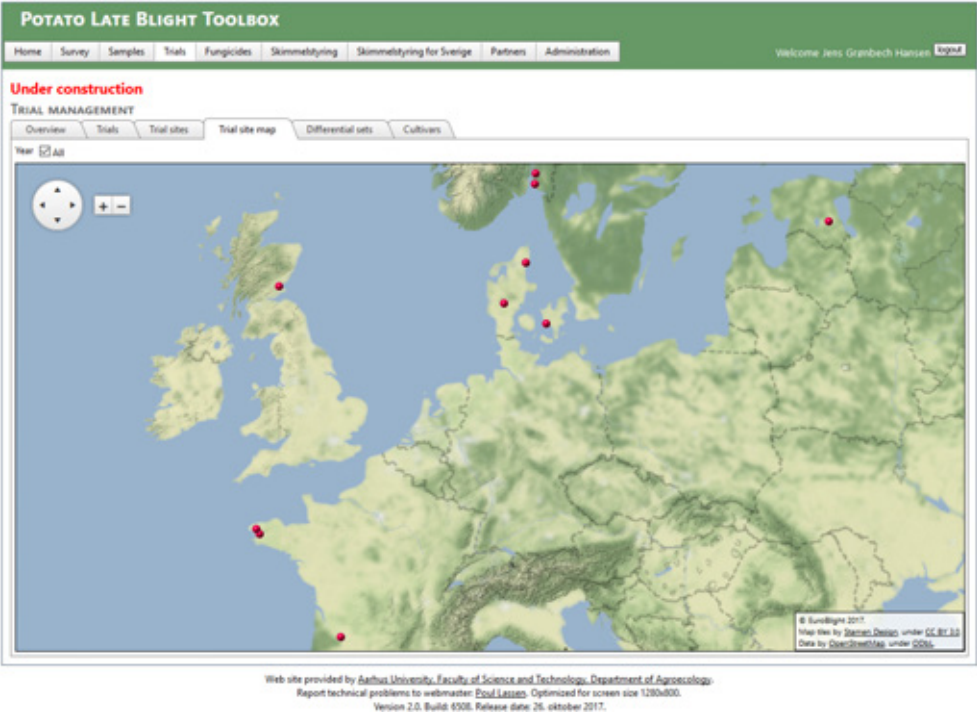
Figure 2. *Trial site map showing where the trap nurseries were established (red dots) in 2017*
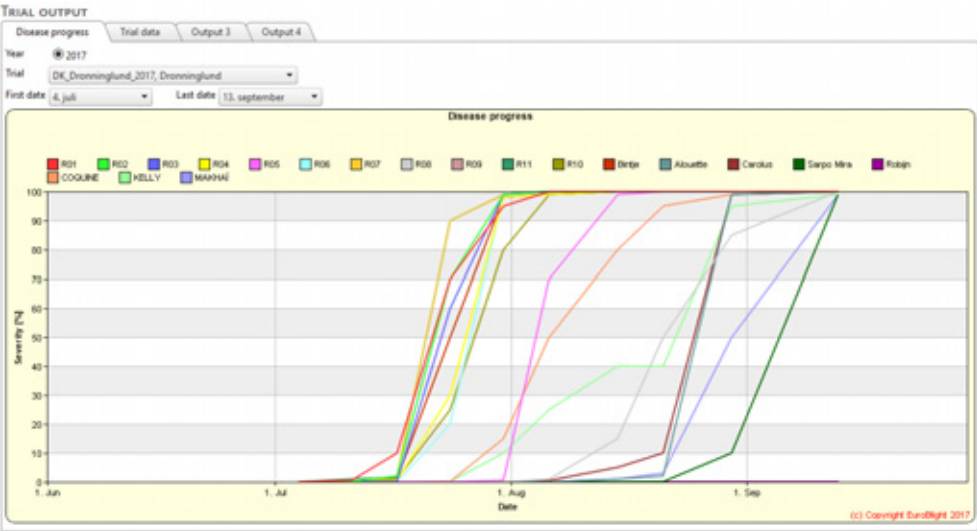
Figure 3. *The development of late blight (Phytophthora infestans) on differential sets in Droninglund in Denmark in 2017*

METHODS AND ANALYSES APPLIED TO THE DISEASE DATA FROM THE TRAP NURSERIES

The disease assessment data taken over time in the season is expressed graphically as disease progress curves (DPC) (Figure 3). DPCs shows the development of late blight on cultivar/differentials during the season and thus provides good grounds for comparison between the differentials. The system estimates or calculates several epidemiological parameters (or curve elements) from DPC of the differentials. For comparing the differentials, we calculate the following curve elements.

1. AUDPC and rAUDPC

Area under the disease progress curve (AUDPC) is an estimation of the total diseased tissues or leaf area covered by the disease for the entire duration of the epidemic. We define the duration of the epidemic as the time elapsed from the onset of late blight until the entire plant is defoliated (100% severity) or the last assessment in cases where 100% severity is not reached.

We calculate AUDPC with the mid-point method (Shanner and Finney, 1977) with the formula below.

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) \left(t_{i+1} - t_i \right)$$

Where "y" is the percentage of leaf area covered with late blight at t^{th} assessment date and "n" is the number of assessments.

Generally, AUDPC is not comparable across experiments, especially in cases where the duration of the epidemic differ between experiments or treatments. Therefore, we calculate a standardized version of AUDPC, called relative area under the disease progress curve (rAUDPC). rAUDPC is calculated by first dividing AUDPC by duration of the epidemic, then finally dividing by the "maximum potential AUDPC". The maximum potential AUDPC is the AUDPC a cultivar or differential would have if it had 100% severity at all assessments times.

2. Apparent infection rate (AIR)

The apparent infection rate (AIR), or, the slope of the DPC, represents the rate of disease increase during the epidemic phase of the disease development. To estimate the AIR, the disease assessment data is transformed to the linearized logistic and gompertz models (Campbell and Madden, 2007). A linear regression of the transformed disease assessment (as dependent variable) and time in days (as independent variable) is then fitted. AIR is estimated as the slopes of the regression lines for the logistic (AIR_L) and gompertz (AIR_G) models. The coefficient of determination (R^2 or R-squared) are given for the goodness of fit of each regression is also calculated to allow for model selection. The slope from model with the best fit (high R^2) is chosen. Usually, the logistic model gives the best fit; thus, we use the logistic model estimate AIR.

The following are the steps that are taken to estimate AIR of each differential.
1. The disease severity data are converted from percentage (%) to a 0-1 scale by dividing the severity data in percentage by 100.
2. Only disease severity data from 0.005 to 0.99 (or 0.5%-99%) are used.

3. Only the first observation in 95-99% is included in the calculation of AIR.
4. The disease severity data in 0-1 scale are transformed to ln(y/1-y) and –ln(-ln(y)) for the logistic and gompertz transformations, respectively
5. If the severity value just prior to the first assessment of 100% is below 90%, then the first assessment of 100% is lowered to 99%. For example, disease assessment data 0, 0.1, 5, 40, 100, 100, 100, will be changed to 0, 0.1, 5, 40, 99, 100, and 100. In this example, the original data have only 2 assessments between 0.5 and 99% (5 and 40%) and AIR will not be calculated. When a situation like this happen, it will be indicated by a star in the result table. After change of the first assessments of 100% to 99%, the total result for AIR will be calculated.

As a rule of thumb, the total result for AIR for a cultivar is excluded if there are less than three observations between 0.005-0.99 of any of the replicates, the slope (AIR) of the fitted regression line is less than zero, or the coefficient of determination of the regression fit is less than 0.5 in any of the replications.

3. Days until 1% and 5% disease
Based on calculations of AIR (slope) and the intercept from regression of the logistic linearized equations we calculate the number of days until 1% disease and 5% disease from first observation in the trial. AIR and the days until 1% and 5% disease severity for a cultivar are excluded if there are less than three observations between 0.005-0.99 in any of the replicates or the slope (AIR) of the fitted regression line is less than 0 or the coefficient of determination of the regression fit is less than 0.5 in any of the replications.

Determination of the type of resistance via the Delta method
The observed field resistance of potato cultivars to late blight could either race-specific (RS), race-non-specific (RNS) or both RNS and RS (Vander plank, 1968). The type of resistance has a huge epidemiological significance and disease control for that matter. RS is governed by the gene for gene relationship and controlled by single or few genes. Usually RS provides a complete resistance against avirulent races of the pathogen. However, upon the arrival of virulent races of the pathogen, RS breaks down and the plant becomes very susceptible. In other words, RS type of resistance delays the onset of epidemic until the arrival or emergence of virulent races. In contrast, RNS does not delay onset of epidemic, but rather slows down the development of the disease. The performance of RNS depends on the aggressiveness a trait that is generally postulated to be polygenic and hence evolve slowly. Consequently, RS is normally highly efficient but short lived, whereas RNS is providing partial, but more lasting protection.

To determine the type resistance in the cultivars or differentials, we use the Delta method. Succinctly, the delta method calculates the difference between the AIR (Delta a) or time to reach 1% (Delta t) of a test cultivar and that of a reference susceptible cultivar. **Delta t** is as the difference between Days until 1% for the cultivar tested and Days until 1% for Bintje. **Delta a** is the difference between AIR for the cultivar tested and AIR for Bintje.

A graph is generated with Delta a on the x-axis and Delta t on the y-axis (See Figure 4). Type of resistance identified via Delta plot are as follows:
- **Susceptible cultivars:** Cultivars with the same or a higher AIR and less number of Days until 1% disease than Bintje. These cultivars will be located in the lower right corner in the graph

- **Race-specific cultivars (RS):** Same or a higher Air and more Days until 1% disease than Bintje. Upper right corner in the graph
- **Race-non-specific cultivars (RNS):** Lower Air and the same or less number of Days until 1% disease than Bintje. These cultivars will be located in the lower left corner in the graph
- **RS + RNS (or RS not overcome):** Lower AIR and more Days until 1% disease than Bintje. These cultivars will be located in the upper left corner in the graph
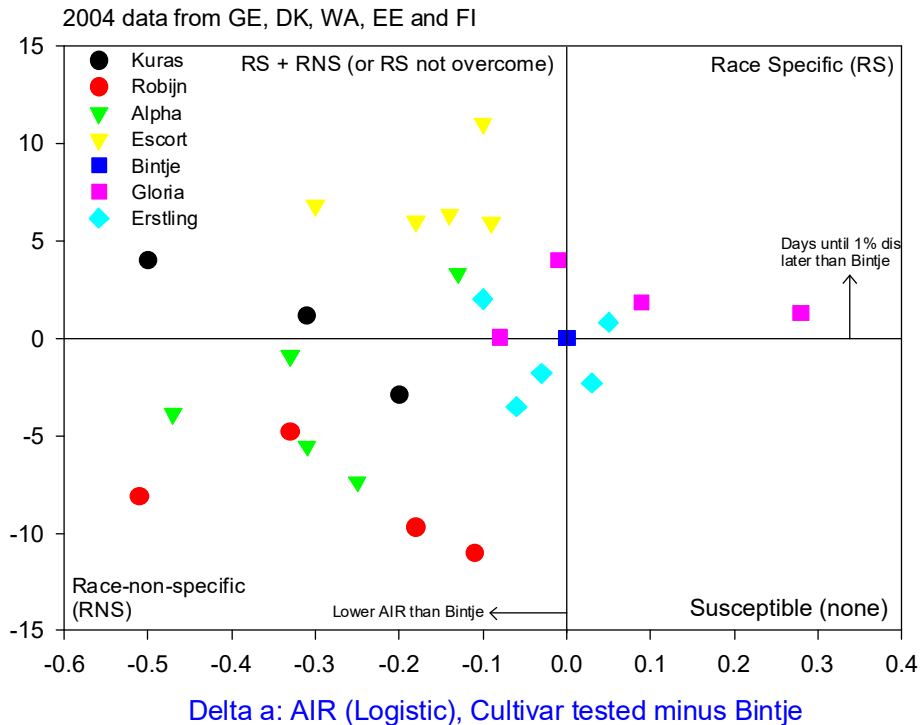
Figure 4. *Example of Delta plot showing the type of resistance of different cultivars*

RESULTS FROM A TRAP NURSERY AT DRONINGLUND IN DENMARK IN 2019

Here, we present some results from the trap nurseries from Droninglund in Denmark in 2019.

The disease progress curves showing the development of late blight on the differential sets and some cultivars are shown in Figure 6. Except for Sarpo Mira, PL11-0111, Nofy, Avito and Ardeche, late blight developed successfully to reach 100% severity on the other cultivars/differentials (Figure 6).

A summary of the estimated or calculated curve elements (AUDPC, rAUDPC and AIR) of the differentials from the trap nursery at Droninglund in 2019 are also shown in Table 2. Except DS-9, which had AUDPC and rAUDPC of zero, all the differentials/cultivars had AUDPC and rAUDPC

greater than zero (Table 2). The zero AUDPC and rAUDPC of DS-9 was because no late blight was observed on this differential at all assessment dates (Figure 5).

As stated earlier in this paper, the AIR are shown for cultivars with more than two assessment values between 0.5 and 99%, and AIR greater or equal to zero and R-squared of at least 0.5 (see Table 2).
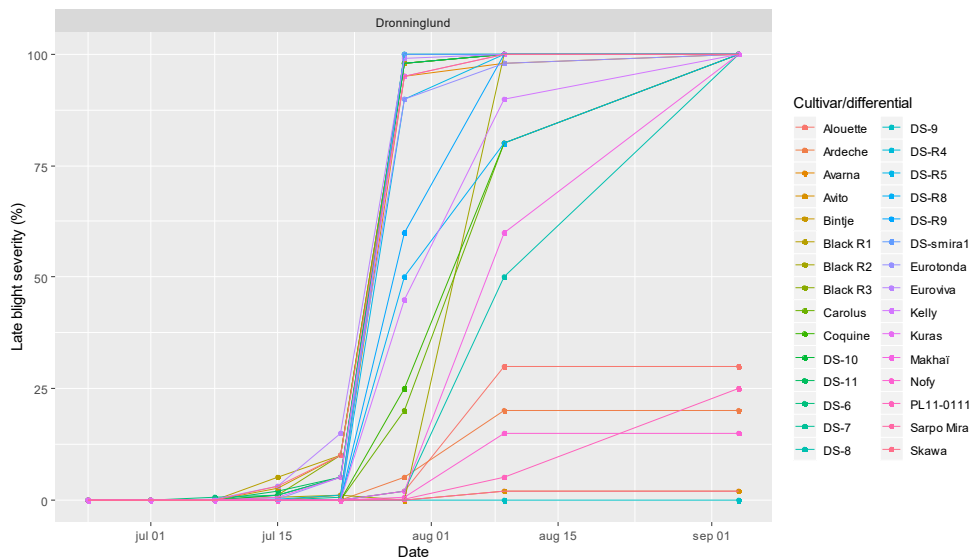
Figure 5. *Disease progress curve (DPC) of the differential sets from the trap nurseries in Denmark (Droninglund) in 2019*

Table 2. *The area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), apparent infection rate (AIR), the number of observations between 0.5 and 99%, coefficient of determination (R^2)*

Differential/Cultivar	Observation	AUDPC	rAUDPC	AIR	R-squared
DS-9	0	0	0	-	-
Sarpo Mira*	2	63	0.009	-	-
Avito*	2	63	0.009	-	-
PL11-0111*	2	419.3	0.058	-	-
Nofy	3	477	0.066	0.08	0.53
Ardeche	3	675	0.094	0.03	0.53
Alouette	3	963	0.134	0.07	0.53
DS-8	3	2243	0.312	0.22	0.97
Makhaï	3	2428	0.337	0.22	0.94
Carolus	3	2960	0.411	0.15	0.97
Coquine	3	3005	0.417	0.15	0.98
Black R2	3	3160.5	0.439	0.42	0.96
DS-R8	3	3230	0.449	0.12	1.00
Kelly	3	3370	0.468	0.12	0.95
DS-R9*	2	3690	0.513	-	-
Eurotonda	3	3959.75	0.550	0.36	0.84
DS-R5*	2	3960	0.550	-	-
Avarna*	2	3975	0.552	-	-
Kuras	3	4043.5	0.562	0.59	0.94
DS-smira1*	1	4050	0.563	-	-
DS-7*	2	4053.5	0.563	-	-
DS-R4*	2	4058.4	0.564	-	-
DS-10	3	4074	0.566	0.61	0.89
DS-11	3	4081	0.567	0.56	0.84
DS-6	4	4095.5	0.569	0.45	0.79
Skawa	3	4096	0.569	0.46	0.89
Black R3	3	4109	0.571	0.61	0.94
Bintje	3	4119.5	0.572	0.54	0.89
Black R1	3	4137	0.575	0.49	0.83
Euroviva	3	4167.42	0.579	0.58	0.90

*on a differential/cultivar indicates that for that differential/cultivar, the apparent infection rate (AIR) value was not calculated for one or more of the following reasons. (1) Less than three observations between 0.5 and 99%. (2) AIR was less than zero. (3) Coefficient of determination (R-squared) was less than 0.5

CONCLUDING REMARKS

We have given a review of the methodology employed to analyze the trap nursery data. The dynamic nature and the ability of *P. infestans* to overcome deployed R-genes makes the establishment of trap nurseries to monitor the stability of R-genes an attractive exercise. Even though R-genes are an important component of integrated pest management (IPM) of late blight, the ability of *P. infestans* to defeat or overcome R-gene is of huge concern. Therefore, it is important regularly to establish trap nurseries to monitor the evolution of the pathogen within and between growing seasons. This would aid scientists to know when and which R-gene has been defeated quickly and growers or advisors to adapt their fungicide application plan.

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Report of the Control Strategies Subgroup meeting on 15 May 2019

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1. *ALTERNARIA* BLIGHT

Initially there was a combined meeting of the Alternaria Subgroup and the Control Strategies Subgroup to resolve an issue with the early blight fungicide efficacy ratings protocol. After a prolonged discussion the following changes to the protocol were agreed. In the efficacy ratings trials the early blight test fungicides should be applied only until the percentage of early blight reaches 10 to 15% in the untreated control (Agreed). Weekly disease assessments should continue until desiccation but only those made up to 4 weeks after the last application of the test fungicides will be included in the calculation of ratings (Agreed). The strategies programmes and their efficacy ratings should be removed from the early blight fungicide table (Agreed). Alternaria Subgroup members departed at this point to hold a separate meeting, leaving 34 to take part in the specific meeting of the Control Stategies Subgroup.

2. PHYTOPHTHORA BLIGHT

2.1 Changes to the late blight fungicide efficacy table
Prior to the York workshop Corteva asked that the EuroBlight fungicide experts give non-decimal ratings (zero to three yellow dots) for the co-formulation of oxathiapiprolin + famoxadone (Zorvec Encantia) based on the experts' experience with this product and also information provided by Corteva. The product is now registered in Ukraine as a co-formulation. These ratings were added to the table.

The following changes were made to the table between September 2017 and May 2019.

Fungicide	Dose rate (kg or l/ha)	Change to table
fluazinam + azoxystrobin	0.5	Leaf rating of 3.6 added
(zoxamide + mancozeb) + cymoxanil	1.8 + 0.2	Leaf rating of 3.4 added
benthiavalicarb	0.5	Leaf rating of 4.2 added
(zoxamide + cymoxanil) + fuazinam	0.45 + 0.4	Leaf rating changed from 4.3 to 4.0 (additional trial data)
(zoxamide + dimethomorph) + fluazinam	1.0 + 0.4	Provisional leaf rating (4.6; 5 trials) changed to full rating (4.2; 6 trials)
(pyraclostrobin + dimethomorph) + adjuvant	2.5 + 1.0	Leaf rating of 4.0 added
oxathiapiprolin + famoxadone	0.5	Leaf rating 4.9 added Tuber rating 4.1 added Non-decimal ratings added
oxathiapiprolin + amisulbrom	0.15 + 0.3	Leaf rating 4.9 added
propamocarb + fluopicolide	1.6	Mobility changed from S+T to S+C/T

At the 16th EuroBlight workshop (Aarhus) a request to include tank mixes of fungicide plus adjuvant in the late blight fungicide efficacy table was not agreed. The recent inclusion of (pyraclostrobin + dimethomorph) + adjuvant (2.5 + 1.0) in the table was questioned at the York workshop. This is the only listing that includes a tank-mixed adjuvant. It was agreed in York that for a tank mix of fungicide product plus adjuvant to be included the adjuvant should be specified on the product label. The regulations for adjuvants should be consistent with those previously agreed for tank-mix fungicide partners (Agreed). At the 13th EuroBlight Workshop (St Petersburg) the following was stated 'To be included in the EuroBlight table the tank mix has to be registered in at least one country in Europe, i.e. the tank mix is included on the product label (Agreed). The product label has to mention the specific tank mix partner (Agreed).'

The column of non-decimal ratings for protectant activity should be removed from the table because the highest rating (three yellow dots) now includes too many fungicides (Agreed).

In the fungicide table co-formulations are distinguished from tank mixes by the stated fungicide dose information only. It was agreed that in the table clearer distinction was now required. Jens Grønbech Hansen should be consulted on the most appropriate way to display this information in the website table (Agreed).

2.2 Ratings trials
Up to the time of the York workshop there were no reports made by advisers to EuroBlight of reduced efficacy for specific fungicides in relation to their EuroBlight ratings. As intimated previously, any future reports should be addressed to Huub Schepers and include supporting evidence.

The suggestion was made that because of new dominant genotypes in Europe, i.e. 36_A2, 37_A2 and 41_A2, fungicides with existing decimal leaf blight ratings should be retested (Not agreed). The three main reasons for rejection of this suggestion were: 1) the relative ratings for fungicide efficacy are unlikely to change (except of course if there is resistance to a target site), 2) more new genotypes are likely in the future and it's impractical to retest efficacy for every new genotype, 3) 36_A2, 37_A2 and 41_A2 are not yet in all European countries.

Concern was expressed over the possible long-term approval status of mancozeb given that it is has until now been the reference treatment in the EuroBlight trials systems to generate leaf blight and tuber blight decimal ratings. It was agreed that if mancozeb does lose EU approval in the future then it would remain the reference treatment through the use of trial permits and product imported into the EU as necessary.

2.3 Action points still outstanding from the Aarhus meeting
The Best Practice guides have still to be revised, for Europe initially. It was agreed in York that the guides should be revised. In May 2017 Ruairidh Bain and Faye Ritchie agreed to assist Huub. There is the possibility that the 2013 document 'Managing the risk of late blight' could be useful as a starting point. The completed European-centred guides, containing more detail and better quality information, will be put on the EuroBlight website.

All of the protocols previously available on the two older European websites still need to be updated if necessary and transferred onto the new EuroBlight website. This is required not only for members of EuroBlight but to facilitate the sharing of protocols with researchers in other blight networks.

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

2.4 New initiatives and developments
EuroBlight has proposed the co-ordinated testing of bio-rational control agents to generate leaf blight efficacy ratings. The starting point for this initiative is one trial in the Netherlands in 2019. The efficacy ratings generated will be placed in a separate table from the late blight fungicide efficacy one. The reference treatment will be completely untreated. One copper-based fungicide will be included as a treatment. The protocol should be placed on the EuroBlight website (Agreed).

3. 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 fungicide tables in this paper have been copied from the website on 1 Nov 19 to provide a record of previous versions.

Background information about the tables ('General comments about the ratings table for late blight fungicides', 'General comments about the ratings table for *Alternaria* fungicides' and 'Definitions' is available in the workshop proceedings section of the EuroBlight website (see Bain & Kennedy, 2017).

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Early blight fungicide table_NEW

Efficacy of fungicides for the control of early blight caused by *Alternaria solani* and *Alternaria alternata*. Updated 31 October 2017.

Report: Fungicide evaluation to rate the efficacy to control earlyblight for the EuroBlight table January 2018

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 (see technical report to be uploaded soon). [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). Rating 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 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.

Late blight fungicide table

Updated 6 May 2019 (added tuber blight score for oxathiapiprolin + famoxadone (0.5))

The effectiveness of fungicide products/co-formulations for the control of *P. infestans* based on the **highest** rate registered in Europe. These ratings are the opinion of the Control strategies Sub-Group at the EuroBlight workshop, May 2017 and are based on field experiments and experience of the products performance when used in commercial conditions.

Use **Mouse Over** on header titles to find **explanation** on variables e.g. mobility mode of actions C, T and S. You can click on the header texts to **multiple sort the table** (1. click= Descending, 2nd click=ascending, 3rd click=unsort). Use this PNG version for mobile phones

Product (Dose rate [litre or kg/ha])	Leaf blight	Tuber blight	New growth	Stem blight	Protec-tant	Curative	Anti sporulant	Rain-fastness	Mobility	Year
copper				●	●●	0	0	●	C	1900
dithiocarbamates (2.0)[1]	2.0	0.0		●	●●	0	0	●●	C	1961
chlorothalonil				●	●●	0	0	●●●	C	1964
cyazofamid (0.5)	3.8	3.8	●●	●	●●●	0	0	●●●	C	2001
fluazinam (0.4)	2.9			●	●●●	0	0	●●●	C	1992
zoxamide + mancozeb (1.8)	2.8			●[5]	●●●	0	0	●●●	C + C	2001
amisulbrom + mancozeb (0.5+2.0)	4.5	3.7		●	●●●	0	?	●●●	C + C	2007
ametoctradin + mancozeb (2.5)	3.7		]6	]6	●●●	0	0	●●●	C + C	2011
fluazinam + azoxystrobin (0.5)	3.6								C + C	2016
famoxadone + cymoxanil				●●	●●	●●	●	●●●	C + T	1996
(zoxamide + mancozeb) + cymoxanil (1.8+0.2)	3.4								C + T	2001
mandipropamid (0.6)	4.0		●●	●●	●●●	●[4]	●●	●●●	C/T	2005
mandipropamid + difenoconazole (0.6)	4.0		●●	●●	●●●	●[4]	●●	●●●	C/T + C	2005
benthiavalicarb (0.5)	4.2								T	2018
benthiavalicarb + mancozeb (2.0)	3.7			●●[5]	●●●	●●	●	●●●	T + C	2003
cymoxanil + metiram				●●	●●	●●	●	●●	T + C	1976
cymoxanil + copper				●●	●●	●●	●	●●	T + C	1976
cymoxanil + mancozeb				●●	●●	●●	●	●●	T + C	1976
dimethomorph + mancozeb (2.4)	3.0			●●	●●●	●	●●	●●●	T + C	1988
dimethomorph + fluazinam (1.0)	3.7	3.3	●	●	●●●	●	●●	●●●	T + C	2012
fenamidone + mancozeb (1.5)	2.6			●●[5]	●●●	0	●●[5]	●●	T + C	1998
(zoxamide + cymoxanil) + fluazinam (0.45+0.4)	4.0								C/T + C	2013
(zoxamide + dimethomorph) + fluazinam (1.0+0.4)	4.2								C/T + C	2015
mandipropamid + cymoxanil (0.6)	4.4		●●	●●	●●●	●●	●●	●●●	C/T + T	2013
(pyraclostrobin + dimethomorph) + adjuvant (2.5+1.0)	4.0[7]								C/T + T	2012
benalaxyl-M + mancozeb[2]	3.0		●●	●●	●●●	●●●	●●●	●●●	S + C	1981
metalaxyl-M + mancozeb[2]			●●	●●	●●●	●●●	●●●	●●●	S + C	1977
metalaxyl-M + fluazinam[2]			●●	●●	●●●	●●●	●●●	●●●	S + C	
propamocarb + cymoxanil + cyazofamid ([2.0]+0.5)		4.6							S + T + C	2012
propamocarb + cymoxanil (2.0)				●●	●●●[9]	●●●			S + T	2011
propamocarb-HCl + fenamidone (2.0)	2.5		●●	●●	●●●	●●	●●	●●●	S + T	1998
propamocarb-HCl + fluopicolide (1.6)	3.8	3.9	●●	●●	●●●	●●	●●●	●●●	S + C/T	2006
oxathiapiprolin (0.15)			●●●	●●●	●●●	●●	●●●	●●●	S	2017
oxathiapiprolin + famoxadone (0.5)	4.9	4.1	●●●	●●●	●●●	●●	●●●	●●●	S + C	2018
oxathiapiprolin + amisulbrom (0.15+0.3)	4.9								S + C	2018

[1] Includes maneb, mancozeb, propineb and metiram. [2] See proceedings 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 1%. [7] A provisional rating based on 5 EuroBlight experiments. [8] Observations from several trials indicated that both New growth and Stem blight were ++. [9] In some trials the curative activity was +++.

EuroBlight
A potato late blight network for Europe

Posters

Practical Experiences of Integrated Control of *Phytophthora infestans* in the Swedish Potato Field Trials in 2011-2018

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Practical Experiences of Integrated Control of *Phytophtora infestans* in the Swedish Potato Field Trials in 2011-2018

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Objective

In order to develop the implementation and knowledge of integrated pest management (IPM) regarding control of potato late blight (*Phytophtora infestans*) the role of fungicide applications have been studied, mainly within the use of different decision support systems (DSS), as well as the importance of cultivars. Another part of this work has been to make DSS for potato late blight available to Swedish farmers and advisors.

Results and Discussion

- The three DSS tested reduced fungicide applications with up to 20-30% within the field trials with retained effect on late blight, compared to weekly full dose sprayings (Fig. 1-3)

- Timing of fungicide applications, both at high- and low late blight risk turned out to be a very important factor on late blight attacks, more important than the number of sprayings and dosage

- Delaying the first spray of the season was one way to reduce fungicide applications

- Prolonged spraying intervals and/or reduced doses can be used successfully to reduce fungicide applications, yet with retained effect on late blight

- Using DSS there is a risk of technical problems that can obstruct and devastate the use of the systems

- The three DSS tested differ, e.g. due to the labour intensity needed, availability and specificity they can attract different growers in different areas

- The late blight resistance of different cultivars varies, which can be used to reduce fungicide amendments

- A combination of reduced doses and more resistant cultivars has turned out to be favourable in reducing the total amount of fungicides with retained effects on late blight
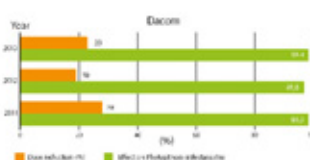
Figure 1. Fungicide dose reduction and effect on *Phytophtora infestans* infections using Dacom in the Swedish potato field trials 2011-2013, in total nine trials.
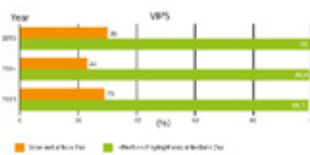
Figure 2. Fungicide dose reduction and effect on *Phytophtora infestans* infections using VIPS in the Swedish potato field trials 2013-2015, in total eight trials.
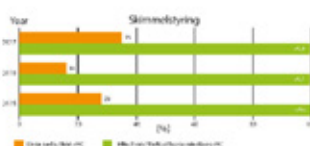
Figure 3. Fungicide dose reduction and effect on *Phytophtora infestans* infections using Skimmelstyring in the Swedish potato field trials 2015-2017, in total six trials.

Anna Gerdtsson   Louise Aldén

Materials and methods

Fungicide doses, fungicide strategies and timing are important parts of integrated control and significant within the use of DSS. This have been studied in the Swedish potato field trials in 2011-2018, in total 20 trials. Three different DSS, Dacom, VIPS and Skimmelstyring, have been tested and evaluated. Within these studies the importance of resistance to late blight of different cultivars were also studied. Skimmelstyring and VIPS have been tested and made available for Swedish farmers and advisors by the Swedish Board of Agriculture in collaboration with Aarhus University and the Norwegian Institute of Bioeconomy Research, respectively.

Conclusions

- Well-functioning DSS to control potato late blight has shown to be a valuable IPM tool with both environmental and economic benefits

- Cultivars, fungicide doses, fungicide strategies and not least timing can affect late blight attacks significantly. In combination they can be used within a DSS to create a robust way to handle potato late blight

- Due to the risk of technical problems using DSS, it is of outermost importance that the user handles the DSS as a support in combination with the users own experience and observations

- A user friendly DSS is very important for the extent of use

- Various DSS work in diverse ways and they can attract different users based on pre-requisites and other individual preferences

- During the last years the DSS Skimmelstyring and VIPS have been made available to Swedish farmers and advisors by the Swedish Board of Agriculture, to test and evaluate the use of the programs

- In southern Sweden about 50% of the starch potato growers now use either Skimmelstyring and/or VIPS with the aim to reduce fungicide use with 50%

Trends in potato late blight epidemics in Switzerland since 1990

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Trends in potato late blight epidemics in Switzerland since 1990

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Introduction

In the framework of PhytoPRE, the decision support system for potato late blight (PLB), the development of PLB-epidemics were evaluated using small, untreated potato plots distributed over the potato growing region in Switzerland. Since 1990, plots were planted with a highly susceptible potato variety, primarily Bintje, comprising the Agroscope Late Blight Observation (LBO) Network. LBO disease monitoring was conducted in collaboration with the cantonal plant protection offices and advisors. Epidemic trends in all of Switzerland (between 1990 and 2016) and regionally (between 2000 and 2016) were assessed.

Results

- During the 27-year period, no change in the start of the epidemic was detected over time (Fig. 1A), but half of the monitored plots in the LBO Network appeared to be infected marginally earlier over time (Fig 1B)
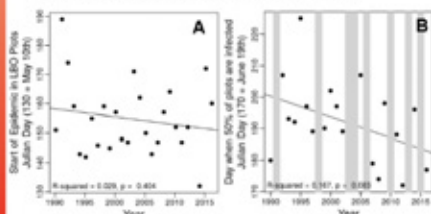
Figure 1: Analysis of A) the start of the epidemic in the LBO Network and B) Julian day when 50% of LBO Network plots became infected (vertical grey lines show years when infection did not reach 50%).

- When all plots are included in the linear regression (not only the first outbreak), infections appear occur earlier over time (Fig. 2A).

- Data from LBO plots since 2000 were used to assess regional trends over time (Figs. 2B and 3) and to assess the accuracy of the DSS, PhytoPRE (Fig. 4).
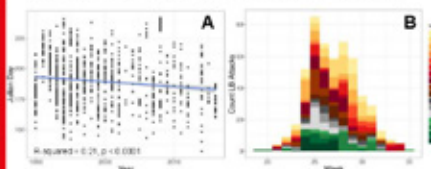
Figure 2: A) When all outbreaks in LBO are considered, they occur on average earlier over time. B) Histogram showing LBO infections per week since 2000 that are colored according to year.

- We used LBO plots associated with different weather stations to evaluate trends within smaller geographic areas.
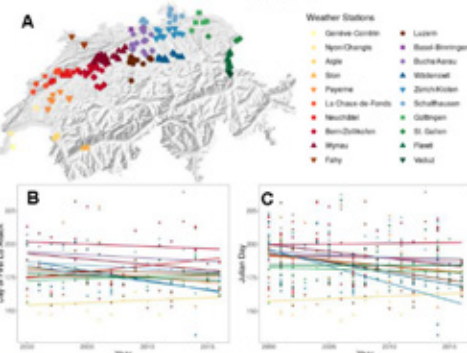
Figure 3: LBO plots since 2000 colored according to closest weather station. Only weather station with 10 or more years of data are included in B and C. A) location of LBO plots across Switzerland, B) First day of PLB attack in LBO plots across years, showing a significant effect of weather station (p < 0.001) but no significant effect of either year (p= 0.395) or year:weather station interaction (p= 0.956), C) Occurrence of all PLB attacks in LBO plots across years, showing significant effects of year (p < 0.001) and weather station (p < 0.001), but not their interaction (p = 0.239).

- To evaluate the predictive power of the DSS, PhytoPRE, risk prediction from 4-7 days ahead of the infection were assessed (medium, high and no risk warnings). We found no significant changes in false negatives over time (p = 0.192).

Figure 4: DSS PhytoPRE output (medium, high or no risk) for all infected plots in LBO system 4-7 days prior to the occurrence of an infection. Numbers above bars show number of plots included in each year. Risk predictions for plots that showed no infection were not evaluated.
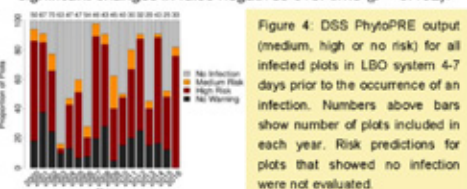
Conclusions

The first LBO infections on a country-wide and regional basis are not occurring earlier with time. On average, all infections during the season seem to occur earlier, perhaps suggesting that outbreak may be spreading faster. These results may be confounded by a reduction of LBO plots with time. No temporal trends in PhytoPRE's predictive ability were found.

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Improving the quality of late blight DSS through airborne inoculum quantification

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UCLouvain

Improving the quality of late blight DSS through airborne inoculum quantification

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Context

Decision Support Systems (DSS) are key tools for the integrated management of potato late blight (PLB) caused by *Phytophthora infestans*. Weather data are used to predict infection risks as well as incubation curves. However, DSSs are generally based on the assumption that there is no inoculum limitation. Therefore, we investigate the relationship between airborne inoculum and disease outbreaks. By means of spore traps and quantitative PCR assays, we draw up profiles of airborne inoculum during potato cropping seasons. Results of the 2018 season are presented below and compared to the spray warnings given by the Walloon DSS (operated by CARAH ASBL, Ath, Belgium) (Fig. 1).

Weather data

Fig. 1 Integration of the airborne inoculum of *P. infestans* in order to improve late blight DSSs.

Material & Methods

Field trial

The experimental field (500m²), located in Louvain-la-Neuve (Wallonia, Belgium), was planted with Bintje potato cultivar (4.6 plants/m²) and was initially set up for a fungicide trial. Four subplots of 10m² remained untreated during the 2018 cropping season. On the other plots, fungicides were applied following the Walloon Decision Support System (DSS). PLB disease intensity was evaluated periodically by visual observation.

Spore sampling

A 7-days recorder Burkard spore trap was placed in the field near the experimental plots. The orifice of the spore trap was 1 meter above the ground and the throughput was set at 10 liters per minute, which corresponds to a daily volume of 14.4m³. The spore trap adhesive band exposed to the airflow was collected every week and cut into daily segments following the method of Dedeurwaerder et al. (2013). Spore trapping was effective from the 10th of August 2018 to the end of December 2018.

Airborne inoculum quantification

Total DNA was extracted from each daily segment using a protocol based on Lee et al. (1990). The quantification of the airborne inoculum of *P. infestans* was assessed by quantitative PCR using a Taqman probe and specific primers amplifying a 167 bp product into ITS1 region (Lees et al., 2012).

Relationship between quantification cycles (Cq) and sporangial air density expressed in sporangia per cubic meter was determined by means of a calibration curve (see Fig 2).

Results

Fig. 3 Assessment of *P. infestans* airborne inoculum during 2018 potato season by means of a Burkard spore trap. Spray warnings were computed using the Walloon DSS. Disease intensity was periodically evaluated through the estimation of the percentage of the leaf area covered by symptoms (visual observations).

Fig. 2 Relationship between real-time PCR quantification cycles (Cq) and number of *P. infestans* sporangia (log transformed). This calibration curve was obtained by means of three independent assays consisting of DNA extraction followed by qPCR of a known number of sporangia.

- DNA from *P. infestans* airborne inoculum was amplified by quantitative PCR in an accurate and specific way. From the calibration curve presented in Fig. 2, a single sporangium is detected at 35.36 Cq.

- Late blight airborne inoculum was detected before PLB outbreak in the experimental field (Fig.3).

- Periods without inoculum detection occurred during the 2018 potato growing season. Sporangial densities in the air ranged from 0 to 12 sporangia/m³ as a maximum during the sampling period (Fig. 3).

- Data of airborne inoculum could improve PLB management by limiting pesticide application to periods with positive detections (Fig.3).

Next steps: 2019 potato cropping season

In order to evaluate whether the quantification of airborne inoculum can improve PLB DSSs, a network of spore traps is set up throughout the Wallonia potato-growing region during the 2019 potato cropping season.

Four Burkard spore traps are placed in four reference experimental fields, each of them supplied with a weather station (CRA-W/Pameseb network) and a homemade passive spore trap (Fig. 4). Eight other passive spore traps are also set up in potato growers fields. Data on airborne inoculum will be compared to PLB disease intensity as well as the infection risks predicted by the Walloon DSS.

Fig. 4 Homemade, conical passive spore trap

Fig. 5 Spatial distribution of the spore traps network across the Wallloon potato-growing region for the 2019 cropping season. Each reference field is supplied with a Burkard, a passive spore trap as well as a weather station while growers fields are supplied with passive spore traps.

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The re-launch of the AsiaBlight network

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EuroBlight workshop
York, England
May 23-25, 2019

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The re-launch of the AsiaBlight network
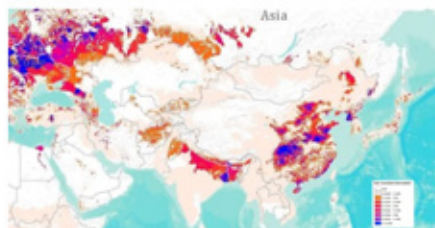
Figure 1. Distribution of potato cultivation in the world. Colors indicate the % crop land dedicated to potato, 2017 (Source RTB map).

Abstract

Countries in Asia range from the very wealthy to the very poor. A few of these show some of the highest malnutrition levels in the world. Potatoes are highly nutritious, can adapt to marginal conditions, and produce more food per unit of water than any other crop, making potatoes a key component of food security.

China and India are the two most populated countries in the world. They also are the two most important potato producers, with a third of the world total. Potato is popular in Asia, having 12 countries ranked in the top-50 potato. As in other parts of the globe, Phytophtora infestans (Late blight–LB) is the most important pathogen affecting potato production.

AsiaBlight got started in 2015 with the objective of establishing a coarse-scale map of LB in Asia. Since 2018, the CIP–China Center for Asia-Pacific (CCCAP) has been working to implement an AsiaBlight network that is self-sustaining, and that is able to respond to the expectations of LB stakeholders in different Asian countries, including capacity building regarding LB, and improving LB management through information, communication, collaborations, and research.

Potato and LB in Asia

In terms of human consumption, potato is the third most important food crop in the world, after rice and wheat. Asia, a continent that has about half the total world population, produces about half of the total global potato production.

The potato disease late blight (due to oomycete Phytophtora infestans), is one of the biggest threats to food security in Asia and in the world. This disease destroys entire potato crops, and not only is able to develop resistance to fungicides, but also overcomes the natural genetic resistance that new potato varieties may have against it.

Figure 2. World potato production (in %). Adapted from FAOSTAT 2017, mt=million metric tons

A CIP survey of potato leaders and stakeholders, by region, ranked the most important potato issues that could trigger increased risk towards food security, that could result in an increase of global poverty, as could make potato production less sustainable. From this study, LB and drought show up as the two most important issues for Asian potato stakeholders.

Breeding goal	Priority Top 12	Priority Global	Priority ESEA	Priority SWCA
Drought tolerance	1	2	2	1
LB resistance	2	1	1	✓
Improved nutritional quality	3	✓	✓	✓
Resistance to soil diseases	4	✓	✓	✓
Improved cropping systems	5	6	✓	✓
Earliness	6	3	4	1
Yield	✓	4	3	4
Improved seed storage	✓	5	6	5
Virus tolerance/resistance	✓	✓	5	1

Table 1 = Survey of top 12 potato issues in CASE (2018). Priority Global = Global survey. ESEA = East and Southeast Asia (cases shown in the global survey). SWCA = Southwest and Central Asia (cases shown in the global survey).

In Asia, there is a strong prevalence of limited information regarding LB. This lack of knowledge could result in lower potato yields, higher production costs and higher use of inputs due to their misuse. A key strategy to succeed in fighting potato late blight could be for LB stakeholders in Asia to join forces together through the AsiaBlight network.

AsiaBlight – Who are we?

AsiaBlight has been established as an inclusive network of scientists, companies, farmers and other stakeholders working on potato late blight, in Asia.

Together the network will build knowledge, skills, technology, promote collaborations, share information, and perform research on LB.

The main objective is to better understand and manage late blight, and through that, to accelerate the alleviation of rural poverty in Asia, while enhancing potato productivity.

AsiaBlight – 2019 activities

AsiaBlight Activity	What/Where/When	Date
International Workshops	Workshop for 100 participants from Asia.	Q4 2019
Trainings	2 to 3 workshops/year on LB management, in English and Chinese.	Q2-Q4 2019
Website	New AsiaBlight website, in Chinese, English then extend to other languages.	Q2 2019
P infestans genotyping/ phenotyping	CCCAP is establishing the protocols in Yanqing, China to study LB population and its resistance to fungicides.	All 2019
Expand the network	Expansion from China to Georgia, Vietnam, Tajikistan, Kyrgyzstan, Vietnam, Bangladesh etc.	All 2019
Fundraising	Sponsors will be found all through Asia. AsiaBlight will create a corporate sponsorship program.	All 2019

AsiaBlight – Logo contest

CIP-CCCAP organized an AsiaBlight logo contest. Thanks to a generous first price of $800, we obtained multiple entries from all over China and Asia. After a rigorous selection process, the winning logo was declared the winner. The winning logo depicts potatoes, LB damage, and the geographical diversity present in the Asian continent. Congratulations to Buddhi Prakash Sharma Adhikari, won the contest for AsiaBlight logo. Buddhi is a PhD retired Scientist from Nepal Agricultural Research Council (NARC), National Potato Research Program, Nepal.

Figure 3. Winning logo from the AsiaBlight Logo Contest.

The AsiaBlight network is active!

Asian representation and expansion:

AsiaBlight's goals are to become successfully implemented in Asia, starting with China and the extend it to the rest of Asia. In parallel, AsiaBlight will represent Asia in the global LB network, together with EuroBlight, Tizon Latino, USABlight, and others.

Communication platforms

A WeChat group, a tweeter account, and a website: www.asiablight.org will be launched.

Yearly international conference

Asian LB stakeholders will have the opportunity to meet at AsiaBlight's main conference, which will be focused exclusively to the science of LB. This yearly conference will be held in China this year.

Increase capacity building: training workshops

The workshops will be targeted to Asian potato stakeholders. Thanks to them, they will be better able to manage LB potato thanks to becoming aware of new information and LB-tools.

Research

LB genotyping for population dynamics, host resistance, and fungicide efficacy studies are being set up at CIP-CCCAP's facilities in Yanqing (China). Collaborations with leading Chinese potato scientists are being set up.

Fund raising

Grant application and our special financial and in-kind sponsorship program will allow AsiaBlight to become financially independent.

Applications of machine learning to identify drivers of *Phytophthora infestans* population diversity

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SUMMARY

Phytophthora infestans, the causal organism of late blight, is a devastating pathogen of potato and tomato crops that has been increasing in incidence and complexity worldwide. The objectives of this study were to analyse the genetic diversity of *P. infestans* populations in Great Britain (GB) in order to identify areas with a disproportionately high occurrence of non-clonal lineages. Analysis of >2000 late blight outbreaks during 2006-2017 revealed 163 non-clonal outbreaks in different postcode districts across GB. Spatial autocorrelation and space-time analyses revealed areas of positive and negative local spatial autocorrelation of non-clonal outbreaks (clusters and outliers). An Emerging Hot Spot Analysis identified postcode districts with increasing values of non-clonal outbreaks through time. These analyses provide the first quantitative evidence of space-time clustering of non-clonal outbreaks of late blight in GB. The results of this study will be used to guide future efforts to identify the drivers associated with increased diversity of the late blight pathogen in GB.

KEYWORDS

Hotspot analysis, oospore, *Phytophthora infestans,* point pattern analysis, spatial statistics

INTRODUCTION

Sexual reproduction of the potato late blight pathogen, *Phytophthora infestans*, directly affects control strategies of the disease. This is because oospores formed through sexual recombination can act as primary inoculum, surviving in the soil in the absence of the host (Smart and Fry, 2001; Montarry *et al.*, 2007). The sexual cycle also enhances fitness by spawning recombinant genotypes that may be more aggressive or resistant to fungicides (Cooke *et al.*, 2003; Andersson *et al.*, 2009; Leesutthiphonchai *et al.*, 2018). Additionally, the presence of oospores in soils can lead to an early epidemic during the growing season (Flier *et al.*, 2001; Hannukkala *et al.*, 2007; Andersson *et al.*, 2009; Cooke *et al.*, 2018).

In Great Britain (GB) the population of the pathogen remains dominated by clonal lineages reproducing asexually. However, differences in genotypic diversity have been found between populations in the northern and southern regions of potato production. Populations of

P. infestans in the north east of Scotland show a higher diversity of genotypes (Cooke *et al.*, 2016, 2018) and although there is not a direct evidence of soil-borne oospore as primary inoculum in British crops, evidence of sexual recombination in this area is accumulating.

A better understanding of the nature and causes of geographic variation in pathogen diversity would provide crucial information for improved management of the disease. In this study we applied the space-time pattern mining tools of ArcGIS to potato late blight outbreak data in GB during 2006-2017. The main objective was to explore the data for patterns and trends in the occurrence of non-clonal outbreaks in the northeast of Scotland and other areas of Great Britain.

MATERIALS AND METHODS

Outbreak data acquisition
Historical potato late blight outbreak data from the AHDB Potatoes 'Fight Against Blight' (FAB) campaign were obtained. These data are collected on a voluntary basis by Blight Scouts drawn from members of the industry who routinely walk potato fields during the season. Information on >2000 outbreaks from across GB during 2006 to 2017 were available: outbreak coordinates, date of observation, variety of potato cultivated, source and stage of the disease, the name of the scout who collected the sample, and the genotype of the pathogen. For confidentiality reasons the coordinates recorded for each outbreak are for a postcode district and not for a potato field.

Spatiotemporal analysis
ArcGIS ® software was used to analyse patterns and trends in the distribution of non-clonal outbreaks and to generate maps for visualisation purposes.

Spatial Cluster And Outlier Analysis
In order to identify regional anomalies in the distribution of non-clonal outbreaks between postcodes districts, a local autocorrelation index (Anselin Local Moran's I) was computed for the total number of non-clonal outbreaks in each postcode district. The Cluster And Outlier Analysis identifies statistically significant spatial clusters of high values (High-High), cluster of low values (Low-Low), outliers in which a high value is surrounded primarily by low values (High-Low), and outliers in which a low value is surrounded primarily by high values (Low-High). The analysis calculates a local Moran's I statistic (Anselin, 1995), a z-score (standard deviation) and a p-value (statistical probability). A positive value of the local Moran's I statistic indicates that a feature is a part of a cluster. A negative value of the statistic indicates that a feature is a part of an outlier. A 95% significance level (p-value<0.05) was used to indicate significant clusters or outliers of local autocorrelation.

Emerging Hot Spot Analysis
Before running an Emerging Hot Spot Analysis, a netCDF (Network Common Data Form) space-time cube was generated by aggregating non-clonal outbreaks into bins defined by the postcode district and year. The netCDF file stores data in space (as latitude and longitude coordinates) and time. By assigning a count of non-clonal outbreaks to each bin in each postcode district a trend in count over time can be evaluated. The netCDF space-time cube was used as an input for an Emerging Hot Spot Analysis, to visualize and identify statistically significant increasing or decreasing trends in the occurrence of non-clonal outbreaks through the period 2006-2017.

The Emerging Hot Spot Analysis evaluates spatiotemporal patterns using two statistical measures: (1) the Getis-Ord Gi* statistic (Ord and Getis, 1995) to identify the spatial and temporal clustering of non-clonal outbreaks, and (2) the Mann-Kendall trend test (Mann, 1945; Kendall and Gibbons, 1990) to evaluate temporal trends across the time period. The Getis-Ord Gi* (space-time hot spot analysis) returns a z-score and p-value for each bin by comparing the number of non-clonal outbreaks in a given bin with the number of non-clonal outbreaks in the neighbouring bins. Then the hot and cold spots trends are evaluated using the Mann-Kendall trend test. The resultant trend z-score and p-value for each location with data and the space-time hot spot z-score and p-value for each bin are used to categorise significant (p-value<0.05) hot and cold spots. The analysis returns a map with different categories of increasing trend (hot spots) or decreasing trend (cold spots). Categories include new, consecutive, intensifying, persistent, diminishing, sporadic, oscillating and historical hot and cold spots. For the analysis, the 4 closest postcode districts were used to define neighbourhood size in space and temporal neighbours were defined using one prior time interval (1 year).
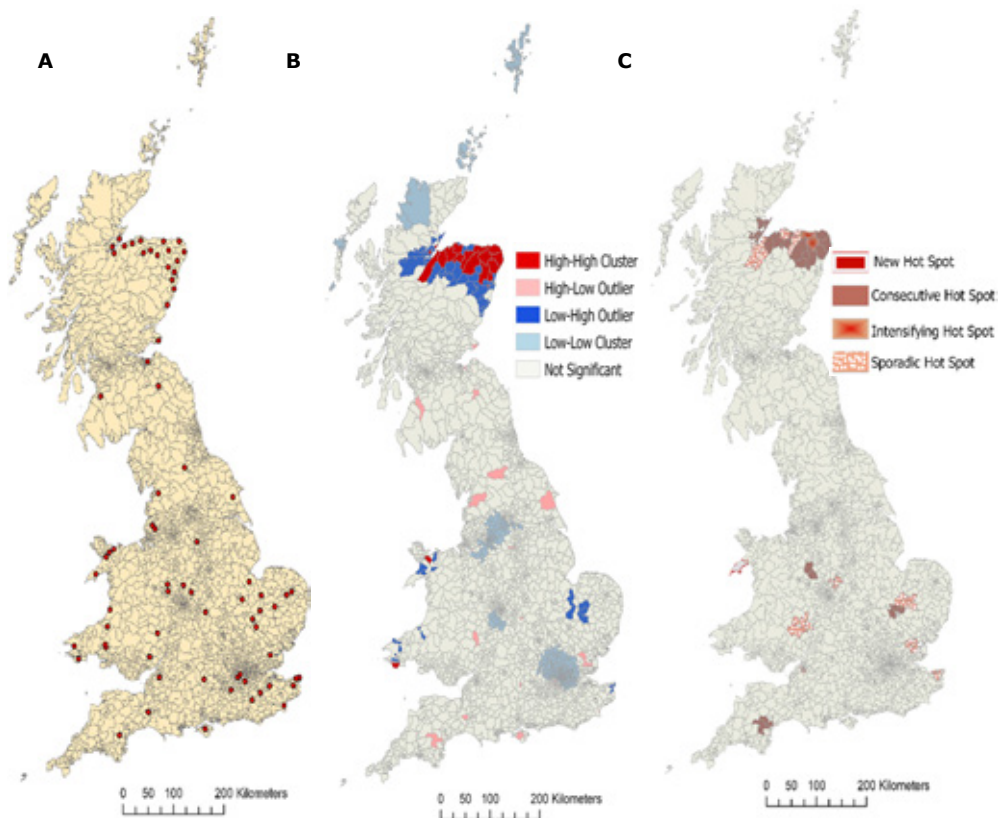
Figure 1. **A.** *Postcode districts and distribution of non-clonal outbreaks in Great Britain (2006-2017).* **B.** *Cluster And Outlier Analysis (COA) results from non-clonal outbreak data (2006-2017).* **C.** *Emerging Hot Spot Analysis (EHSA) results from the space-time cube created using the non-clonal outbreak data*

RESULTS

Data summaries

For the period 2006-2017, a total of 95 non-clonal outbreaks were registered in Scotland and 68 in England and Wales. In terms of the distribution of non-clonal outbreaks in Scotland, a large cluster of postcode districts with a high number of non-clonal outbreaks was identified in the north-eastern region (Figure 1A). In this region (Aberdeenshire, Moray and Inverness) a total of 90 outbreaks were recorded for the period. In contrast, in the south of Scotland (Fife, Angus, Edinburgh, Ayr and Scottish Borders) only 5 non-clonal outbreaks were registered.

Spatial Cluster And Outlier Analysis

From the Cluster And Outlier Analysis, statistically significant clusters of high or low values as well as outliers with values that are statistically different than their neighbouring postcode districts were identified. Interestingly, a large cluster of postcode districts with a high number of non-clonal outbreaks surrounded by postcode districts with a high incidence of non-clonal outbreaks (high-high cluster) was found in the north east of Scotland, in Aberdeenshire, Moray and Inverness (Figure 1B).

Emerging Hot Spot Analysis

The Emerging Hot Spot Analysis revealed postcode districts with increasing values of non-clonal outbreaks through time. Particularly, a large cluster of consecutive, intensifying and sporadic hot spots was found in the northeast of Scotland. The results revealed two intensifying postcode districts in Aberdeenshire (AB45 and AB53 postcode districts). A new hot spot was identified in Wales, and a few isolated consecutive and sporadic hotspots were revealed in the south of England (Figure 1C).

CONCLUSIONS

This study provides the first quantitative evidence of space-time clustering of non-clonal outbreaks of potato late blight in GB. The causes and consequences of the presence of statistically significant clusters of postcode districts with high and increasing incidence of non-clonal outbreaks in the north east of Scotland are not yet certain. According to Cooke (2018), the introduction of new genotypes to this northern Scottish region from areas with higher genotypic diversity is limited, because this area is a seed potato producing region and the import of new genotypes from other countries is restricted. Soil-borne oospores as primary inoculum is therefore the most likely source of genotypic variation in this area. Several isolated non-clonal hot spots were also identified in the south (England and Wales), and a more intensive investigation regarding the source of this diversity is required.

This study provided an overview of the space-time pattern of the genotypic diversity of the pathogen. A more exhaustive analysis of the causes and consequences of these hotspots or clusters of non-clonal outbreaks is the subject of a future study. The areas of intensifying diversity identified in this study have been intensively sampled, and a wide variety of machine learning techniques will be applied to these data, together with potato crop distribution and meteorological data, to identify the drivers of pathogen diversity in GB.

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Screening for resistance to late blight in wild potato species and landraces in greenhouse conditions in Peru

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Screening for resistance to late blight in wild potato species and landraces in greenhouse conditions in Peru

Summary

Fifty-seven accessions of wild potato species and 352 accessions of potato landraces hold in the International Potato Center (CIP) were tested for resistance to late blight (LB) in a series of greenhouse experiments from May to December 2018, in Lima, Peru. Wide phenotypic variation for resistance was found within and among species. Six accessions of wild species (Solanum albornozii, S. andreanum, S. lesteri, S. longiconicum, S. morelliforme, and S. stenophyllidium) had more than 60% of genotypes with susceptibility values between 0 to 3. Likewise, 46 of 352 accessions of potato landraces presented similar susceptibility values than those of resistant varieties used as controls. With this study we identified source of resistance to LB to be used by breeders or by farmers directly, in the case of potato landraces. The results confirm the value of conserving potato genetic resources.

Introduction

Late Blight (LB), caused by Phytophthora infestans, is the primary biotic constraint affecting potato in humid areas or seasons. The disease frequently kills the plants if no fungicides are used. The most efficient method to control LB is the use of resistant varieties. In CIP's pre-breeding program LB resistance from wild species Solanum paucisectum, S. chiquidenum, S. cajamarquense, and S. sogarondinum have already been introduced to elite breeding lines to broaden the current base of resistance. These efforts have resulted in promising candidate varieties, which have been evaluated by smallholder farmers in the Peruvian highlands in participatory varietal selection trials. These new candidate varieties are highly resistant to LB, and the genetic basis of this resistance is expected to be different from what is already present in our breeding populations. The potato LB pathogen, however, is capable of rapidly developing strains that can overcome this resistance. Therefore, it is important to have broad source of resistance genes available for their use in potato breeding.

Methods

Fifty-seven accessions from 30 wild potato species and 352 accessions of potato landraces were selected according to the following criteria: a) being part of the core collection of CIP's Genebank; b) not been tested for LB resistance; and c) availability of planting material, especially true seed of wild potato species. Four local varieties with known levels of resistance to LB: 'Yungay' (susceptible), 'Kawsay', 'Chugmarina', and 'Pallay Poncho' (resistant), were used in the experiments as controls.

True potato seed of wild potato species, was propagated in incubation chambers and then in a greenhouse. Depending on seed viability, the number of plants tested varied among 30 to 100 genotypes per accession. In the case of potato landraces, in vitro plantlets were propagated under greenhouse conditions. Between 8 and 15 plantlets per accession were tested for LB resistance.

Plants were inoculated before flowering with P. infestans isolate POX67 (Perez et al. 2014) using a sporangial suspension (3 x 10³ sporangia per ml) and maintained in a humid chamber at 18–20°C and relative humidity >90% for 5 days. Each plant was sprayed until run-off with 30–60 ml of sporangial suspension with a hand-held sprayer.

Visual evaluation of LB severity was done at 3 and 5 days after inoculation according to Forbes et al. (2014) (Fig. 1). AUDPC, relative AUDPC, and susceptibility scale values were calculated using severity values according to Forbes and Perez (2014) and Yuen and Forbes (2009).

Figure 1. Reaction to Phytophthora infestans in potato plants: resistant (left) and susceptible (right).

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Results

Accessions of S. andreanum, S. albornozoii, S. lesteri, S. longiconicum, S. morelliforme, and S. stenophyllidium have high frequency of resistant genotypes (susceptibility scale values < 3); S. lesteri (accession CIP 764265) and S. albornozoii (CIP761164) had 94 and 84 % of genotypes with susceptibility values ranging from 0 to 3, respectively (Fig. 2). In the case of potato landraces, we found wide phenotypic variation for resistance to LB (Fig. 3). Forty-six accessions had high resistance levels (susceptibility values < 3) among them 27 accessions of S. tuberosum subsp. andigena, 5 of S. phureja, and 3 of S. stenotomum. Two hundred accessions from 352 accessions tested for resistance to late blight had susceptibility scale values more than 6.
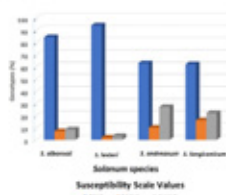
Figure 2. Distribution of resistance to LB on genotypes of Solanum albornozii (CIP761164), S. lesteri (CIP764265), S. andreanum (CIP763108), and S. longiconicum (CIP763966) according to susceptibility scale values proposed by Yuen and Forbes (2009).
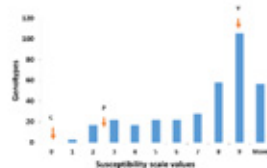
Figure 3. Histogram for distribution of resistance to LB on genotypes of Peruvian potato landraces compared with Peruvian varieties "Yungay" (Y, susceptible), Pallay Poncho" (P, moderate resistant) and "Chucmarina" (C, resistant).

Conclusions and next steps

These results confirm the value of conserving rare populations of potato genetic resources and provide evidence of resistance to late blight in endemic Solanaceae species found in the center of origin of potatoes. These results also complement those obtained by Perez et al. (2001 and 2014). Next steps include the evaluation of resistant genotypes with more isolates collected recently (Lindqvist-Kreuze et al, 2019), and the creation of a catalogue for resistant genotypes available for breeders, farmers and public in general.

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Acknowledgments

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The influence of weather conditions on potato late blight during the growing season in Barsa land, Brasov, Romania

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The influence of weather conditions on potato late blight during the growing season in Barsa land, Brasov, Romania

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INTRODUCTION

Potato late blight development is favored by high humidity and moderate temperature. Because the fungus is very sensitive to the climatic conditions the attack intensity is different a year to other.
To control the diseases multiple fungicide applications are necessary.
In average 10-12 treatments during the season were used to control potato late blight.
Based on the data of main weather conditions and late blight infection between 2016-2018 to NIRDPSB Brasov, the authors analyzed the influence of temperature and humidity on potato late blight in the summer months.

MATERIAL AND METHODS

In 2016-2018, different fungicides were used in controlling late blight attack on16 Romanian potato varieties. Trials were laid out according a randomized block design with three replicates.
Planting was made in 25 March 2016, 30 March 2017 and in 11 April 2018.
In all cases, cultivation and maintenace was in line with current good agricultural practice.
Late blight infection was assessed according to the 0.01-100% scale, where 0.01 means the absence of any visible lesion and 100 means a 100% necrotic tissue.

RESULTS

In 2016 the first late blight symptoms were recorded on 31 May, the earliest appearance in the last 23 years. The whole season had favourable conditions for epidemic development of late blight, followed in August by drought, which have strongly reduced the foliage to the early varieties.
In 2017 the first late blight symptoms were founded in 7 June. The attack intensity was high due to the favourable weather conditions (high number of rainy days).
In 2018 the first late blight symptoms were recorded on 3 July. With lot rainfalls in June and July, blight developed in outbreaks and a defoliation process (depending on variety) started in mid-August. Based on three years observation data were identified some varieties which are less sensitive (Castrum, Marvis) or relative resistant (Rustic, Cezarina). A medium resistance manifested other 9 varieties (Kronstad, Zamolxis, Braşovia, Cumidava, Cosiana, Braşovia from tissue culture, Ruxandra, Sarmis and Ervant) and the last 3 varieties (Roclas, Christian and Tâmpa) lost their resistance. Observations regarding varieties resistance to late blight attack are in view the infection pressure at the plot level starting from the time of epidemic everywhere. In this time the resistance to infection progression can be modified even on less sensitive varieties due to the interrelationship between adjacent planted varieties. To reduce the number of spray applications, it may actually be better to choose early maturing varieties than late maturing ones. Early or medium early varieties bulk up before the foliage is affected by blight.

CONCLUSIONS

Potato late blight is a factor which decrease the yield.
Not all the farmers have resources for effective control, with top products, which are generally more expensive.
Therefore, the cultivation of some varieties with good resistance to disease represents a solution to improve the crop, but also to have a healthier potato from the point of view of pesticide residues. The number of sprays to control potato late blight could be reduced when a weather-based disease model was used.

Acknowledgement

Late blight on foliage 2016

Late blight on foliage 2017

Late blight on foliage 2018

Efficacy of Zorvec™ Encantia® fungicide for the potato late blight control: Russian experience

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SUMMARY

Efficiency of the Zorvec™ Encantia® (oxathiapiprolin + famoxadone) fungicide against potato late blight has been evaluated under epiphytotic conditions comparing to the standard Ridomil Gold MC (mancoceb + mefenoxam) fungicide. Field trials demonstrated the best protection was provided by Zorvec Encantia: the corresponding AUDPC value was equal to 90, while that for Ridomil Gold MC and untreated control reached 232 and 3244 ($LSD_{95} = 67.8$). Biological efficiency of the tested and standard fungicides was 97 and 93%, respectively. Potato yield for the compared variants reached 8.6, 31.4, and 33.4 t/ha for the untreated control, Ridomil Gold MC, and Zorvec Encantia, respectively. Thus, application of Zorvec Encantia provided the maximum yield increase (24.8 t/ha), while that for Ridomil Gold was 22.8 t/ha. The quality of harvested tubers assessed after a one-month storage showed that both Zorvec Encantia and Ridomil Gold MC treatments significantly reduced the tuber blight level (25.5 and 25.1%, respectively) and increased the marketable fraction of potatoes by 50 and 48%, respectively.

KEYWORDS

Phytophthora infestans, potato, late blight, oxathiapiprolin-based fungicides

INTRODUCTION

Fungi and oomycetes are the most economically important microbial pathogens of agricultural plants including such important crop as potato. Damage caused by this group of pathogens can reach 70-80% of the total global economic losses associated with microbial diseases of agricultural plants (Moore *et al.*, 2011). For potato, the most harmful pathogen from this group is *Phytophthora infestans* (Mont.) De Bary; yield and storage losses associated with this oomycete may reach up to 70–100% in the absence of any protective measures (Haverkort *et al.*, 2009). Application of fungicides still remains to be the most adopted late blight control method worldwide. The ability of the pathogen to develop resistance to the actively used fungicides necessitates the search for novel compounds able to control the late blight development via new biochemical targets.

Oxathiapiprolin (OXPT) is a novel fungicide discovered by DuPont and the first member of a new class of piperidinyl-thiazole-isoxazoline fungicides. The new fungicide showed a high activity towards a range of plant pathogenic oomycetes including different *Phytophthora* species, such as *P. capsici* (Ji and Csinos, 2015), *P. infestans* (Cohen *et al.*, 2018a), *P. citrophthora*, *P. syringae*, *P. nicotianae*, and *P. hibernalis* (Gray *et al.*, 2018). It acts at multiple stages of the pathogen's life cycle inhibiting zoospore release and zoospore and sporangia germination, blocking mycelial growth within the host plant before visible lesions occur, and preventing lesion expansion and spore production (Cohen *et al.*, 2018b). OXPT targets an oxysterol binding protein (OSBP), a member of the OSBP-related proteins family of lipid transfer proteins (Pasteris *et al.*, 2016). Lipid-binding proteins are involved in many cellular processes related with oxysterol, including signaling, vesicular trafficking, lipid metabolism, and non-vesicular sterol transport (Weber-Boyvat *et al.*, 2013); however, the exact function of OSBP in *Phytophthora* and other oomycetes still remains unknown (Miao *et al.*, 2018).

Due to a unique site of action in oomycete pathogens, OXPT did not show any cross-resistance to other fungicides. At the same time, since the compound is a single-site inhibitor, the corresponding resistance risk is assumed to be medium to high (Cohen *et al.*, 2018b). Therefore, some OXPT-based fungicides have been developed, which represent mixtures with chlorothalonil (Orondis-Opti), mandipropamid (Orondis-Ultra), mefenoxam (Orondis-Gold), or famoxadone (Zorvec-Encantia). Until recent time, only a few studies have been conducted to evaluate the efficacy of such mixtures against foliar oomycete plant pathogens; in the case of late blight, the only published study was arranged on tomato plants and included combinations of OXPT with chlorothalonil, azoxystrobin, mandipropamid, and mefenoxam (Cohen *et al.*, 2018a). At the same time, characterizing and comparing features of fungicides is critical for understanding how to use them for effective late blight disease management.

The objective of the present study was a comparative field evaluation of the efficiency of Zorvec Encantia (oxathiapiprolin + famoxadone) and Ridomil Gold MC (mancozeb + mefenoxam) fungicides against potato late blight under epiphytotic conditions in Central Russia.

MATERIALS AND METHODS

Field trial arrangement
A small-plot field trial was arranged in 2017 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. Arizona) was planted on May 30 (late planting because of excess soil moisture) and harvested on September 15. The land treatment 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 herbicides. During a vegetation season, the whole field was once treated with a prosulfocarb-based herbicide (2 L/ha, 18.07.2019) and 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 Jul 11, Jul 20, Jul 31, Aug 08, Aug 16, Aug 23.

The experimental scheme included the following variants:

A) Untreated control: no fungicidal treatments.
B) Treated control: (1) Tanos, 0.6 kg/ha, (2-3) Ridomil Gold MC, 2.5 kg/ha, (4) Tanos, 0.6 kg/ha, (5-6) Shirlan, 0.4 L/ha.
C) Zorvec Encantia: (1) Tanos, 0.6 kg/ha, (2-3) Zorvec Encantia, 0.5 L/ha, (4) Tanos, 0.6 kg/ha, (5-6) Shirlan, 0.4 L/ha.

Evaluation of the disease development and crop capacity

Field observations were carried out on Jul 11, Jul 25, Aug 2, Aug 16, Aug 23, Aug 30, Sep 08, and Sep 15. The level of the early and late blight development was assessed according 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. 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.

RESULTS

A high late blight susceptibility of the cultivar used and the weather conditions of 2017 (Table 1) provided the early development of the late blight. During June and first two decades of July, air temperature was lower than average annual values, and the amount of precipitation was significant. Under such conditions, the first disease manifestations on the untreated control were observed on July 10. The further disease development was epiphytotic: in August the level of plant affection exceeded 60%, and in the beginning of the third decade plants were completely killed (Figure 1). In relation to the early blight, single leaf lesions were observed only at the end of the vegetation season (September 8), so no significant influence on the yield was registered. Under such conditions, the best protection was provided by the scheme included Zorvec Encantia. A comparison of the calculated AUDPC values showed that this variant of protection provided the maximum efficiency in the late blight control (Figure 2); the biological efficiency of this scheme and the protected control variant was 97.2 and 92.8%, respectively.

Table 1. *Weather data for the vegetation period of 2017 (Moscow region, All-Russian Research Institute of Phytopathology)*

Basic parameters	May	June	Jul	Aug
Average temperature in 2017, ºC	10.4	13.9	17.2	18.0
Average annual temperature, ºC	12.3	16.0	17.4	15.9
Relative humidity in 2017 %	61	71	74	72
Average annual relative humidity, %	68	72	67	72
Average rainfall in 2017, mm	51.0	115.1	137.6	9.6
Average annual rainfall, mm	48.7	71.5	83.1	71.3
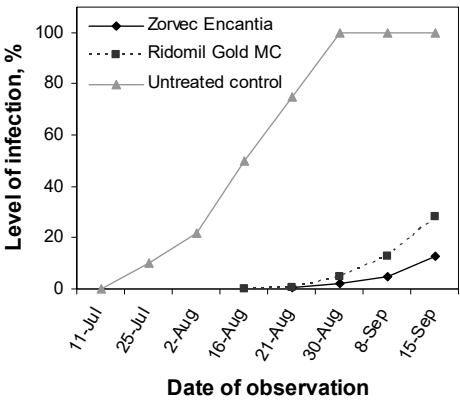
Figure 1. *Dynamics of the late blight development in the compared variants of treatment. The first disease manifestation in the untreated control was observed in the first decade of July*
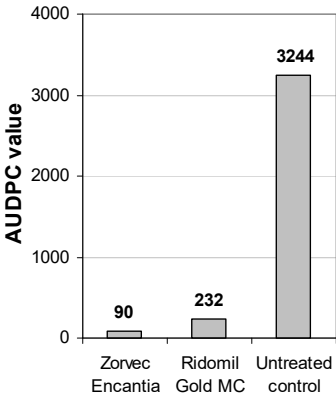
Figure 2. *AUDPC values calculated for the compared variants of treatment (LSD$_{0.95}$ = 67.8)*

The crop capacity corresponded to the late blight development dynamics in the compared variants. In the case of untreated control, it was 8.6 t/ha, whereas the treated control and Zorvec Encantia provided 31.4 and 33.4 t/ha, respectively (Figs. 3, 4). Thus, application of Zorvec Encantia fungicide provided the maximum yield increase (+24.8 t/ha) as compared to the untreated control that exceeded the same value for treated control (+22.8 t/ha). The quality of collected potatoes was evaluated after one-month storage. The level of tuber infection in both Zorvec Encantia and treated control variants was significantly lower (by 25.5 and 25.1%, respectively) than in the untreated control (Figure 5). The marketable fraction of potato in the above-mentioned protected variants was higher than in the untreated control by 50 and 48%, respectively (Figure 4).

Figure 3. *Potato yield and plant appearance (cv. Arizona) in (a) untreated (control) and (b) Zorvec Encantia-based variants of treatment. Each variant included five plants (ARRIP experimental field, Moscow region, September 2017)*
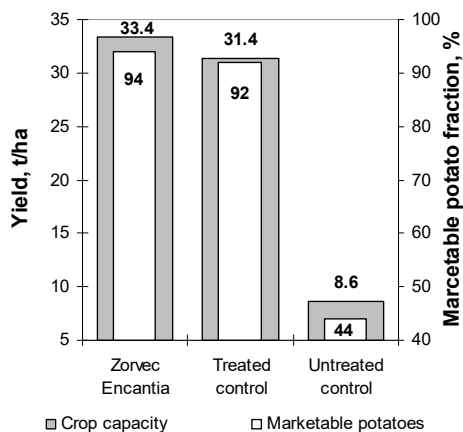
Figure 4. *The total yield (LSD$_{0.95}$ = 5.45) and marketable fraction of potatoes (LSD$_{0.95}$ = 1.5) of the compared variants*
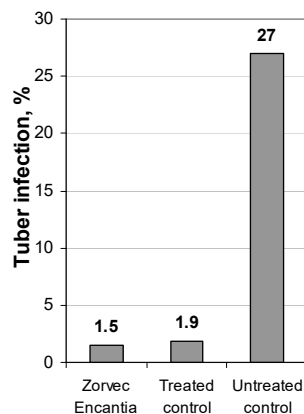
Figure 5. *The level of tuber infection (%) in the compared variants of treatment (LSD$_{0.95}$ = 2.2)*

CONCLUSION

Both studied schemes of treatment showed high efficiency under epiphytotic conditions. Their use made it possible to extend the vegetation period and, therefore, increase potato yield and improve its quality and marketable fraction of tubers. At the same time, scheme, which included Zorvec Encantia, provided the maximum increase in the yield (+24.8 t/ha) and marketable

fraction (+50%) due to its advantage over Ridomil Gold MC (+22.8 t/ha and +48%, respectively) in relation to the suppression of the late blight development.

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Fungicide Resistance in Bavarian *Alternaria solani* and *Alternaria alternata* Field Isolates

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SUMMARY

Alternaria leaf spots caused by *Alternaria solani* and *Alternaria alternata* are a major foliar disease of potatoes. Reduced sensitivity of *A. solani* and *A. alternata* towards Quinone outside inhibitors (QoI) fungicides has been observed in Europe. QoI-insensitive *A. alternata* isolates carry a G143A amino acid exchange caused by a single nucleotide polymorphism (SNP) in the cytochrome *b* gene. *A. solani* evolved a similar F129L mutation. A shift from the predominant *A. solani* genotype I to genotype II, which was exclusively associated with the F129L mutation, was observed in Germany after QoI approval for Alternaria control. Here, we found QoI mutations to be highly abundant in *A. solani* and *A. alternata* field isolates, collected in 2016 in Bavaria, located in southeastern Germany. We observed an increase in the frequency of the F129L mutation but not of *A. solani* genotype II. Instead, we also found the F129L mutation in genotype I, indicating QoI resistance progression through the previously unaffected genotype. In contrast to previous data, QoI mutations were present in all examined areas, indicating a spatial spread. An analysis of SNP diversity pointed to the region of Lower Bavaria as a hotspot for F129L mutation evolution and rather to its dispersal than to multiple independent evolutions. Reduced sensitivity of *Alternaria* spp. towards succinate dehydrogenase inhibitors (SDHI) fungicides is an emerging problem in Europe. We observed the presence of SDHI mutations, but only in combination with QoI mutations, indicating a further adaption to applied fungicides and a selection for dual fungicide resistance.

KEYWORDS

Alternaria alternata, *Alternaria solani*, Fungicide Resistance, Quinone outside Inhibitors (QoI), Succinate Dehydrogenase Inhibitors (SDHI), potatoes

INTRODUCTION

Parasitic species of the Ascomycota genus *Alternaria* cause numerous of plant diseases on diverse crop plants throughout the world (Rotem 1994). *Alternaria solani* Sorauer and *Alternaria alternata* (Fr.) Keissl. are the major *Alternaria* spp. with the potential of causing economic important yield losses of potato (*Solanum tuberosum* L.) (Leiminger and Hausladen 2012). Foliar diseases caused by *A. solani* and *A. alternata* on potato are Early Blight and Brown Spot, respectively. Symptoms of Early Blight are necrotic spots on the leaf surface that typically contain dark concentric rings (bullseye or target spot symptom). The spots may be surrounded by a chlorotic halo. They initially appear a few mm in size before they enlarge to up to 2 cm. They are limited by the leaf veins. First symptoms are observable on older leaves close to the soil surface. Overwintering inoculum in the soil or on plant debris is considered as the source of primary infection. Later in the season, together with the maturation of leaves, Early Blight spots progress upwards. Older spots enlarge, may converge and get brittle. Elongated spots may additionally appear patchy on petioles and stems. Sporulation on the lesions and subsequent wind dispersal of numerous conidia cause secondary infections on the plant, in the field and in neighboring fields. Heavily diseased leaves become premature senescent and necrotic. The reduced photosynthetic capacity of damaged leaves and the premature defoliation of plants can cause yield losses of up to 20% which are rarely exceeded. Brown Spot symptoms resemble Early Blight spots but are smaller in size, darker in color and appear more frequent and occasionally earlier in the season. Large amounts of inoculum on or in the soil can cause tuber lesions in storage when the tubers were wounded at harvesting. (Radtke *et al.* 2000; Stevenson *et al.* 2001) The symptoms of Early Blight and Brown Spot may get confused and both pathogens can co-occur in the same lesions. In Germany, *A. solani* is recognized as the dominant pathogen causing yield losses of potato, whereas *A. alternata* is considered a secondary pathogen with a more opportunistic lifestyle (Rotem 1994; Leiminger *et al.* 2015).

In parts of the world, Early Blight has been a major potato disease for decades and centuries. Reports on problems with Early Blight in the Midwest of the USA date back to 1893 (Pscheidt and Stevenson 1986). In Australia, Early Blight can be of greater importance than Late Blight caused by *Phytophthora infestans* (Dillard *et al.* 1993). In Europe, although having been latent present, Early Blight of potato has not been in the focus of plant protection until reports on an increase in the frequency and severeness of the disease in the field a decade ago (Leiminger 2009). Early Blight was considered as an emerging disease in Europe and has been included as an option in chemical plant protection strategies against Late Blight. Currently especially very susceptible as well as late season varieties might require additional *Alternaria* spp. control.

A range of fungicide active ingredients registered for Late Blight control also contain a good efficacy for *Alternaria* spp. control, e.g. the multi-site inhibitors mancozeb and the related metiram or the Quinone outside inhibitor (QoI) famoxadone (LfL 2018). If those (and others not mentioned) are not used in Late Blight control and if further factors, like heat and drought stress, additionally increase the conduciveness to Early Blight disease, then additional applications of fungicides with a greater efficacy in *Alternaria* spp. control can be of an economic advantage in southern Germany. Current fungicide products registered for *Alternaria* spp. control in potatoes in Germany contain azoxystrobin, pyraclostrobin, boscalid and difenoconazole as antifungal agents and mixtures of them or mixtures with active ingredients against *P. infestans*. Azoxystrobin and pyraclostrobin are QoI (FRAC code 11) with a high risk for fungicide resistance, known resistance in various fungal species and cross resistance between all QoI. Boscalid is a succinate dehydrogenase inhibitor (SDHI) (FRAC code 7) with a medium to high risk for

fungicide resistance and known resistance in several fungal species. Difenoconazole is a triazole of the demethylation inhibitor (DMI) (FRAC code 3) group with a medium risk for fungicide resistance. (FRAC 2018a) Due to observed degradation of sensitivity of some fungal species to sterol biosynthesis inhibitors (SBI) (Price *et al.* 2015), it is recommended not to use one single SBI for the control of one pathogen in multiple applications (FRAC 2018b) as required in the case of *Alternaria* spp. control. For the sake of keeping *Alternaria* spp. populations as long as possible sensitive to antifungal agents, fungicide resistance management practices are of utmost importance.

QoI are single-site respiration inhibitors. Biochemically, they act by binding to the Qo site of cytochrome b of the cytochrome bc_1 complex (complex III) at the inner mitochondrial membrane thereby inhibiting the electron transport and subsequent ATP production (Bartlett *et al.* 2002). Azoxystrobin was registered for Early Blight control in 1999 in the USA and found to be highly effective in the American Midwest, where Early Blight is notoriously difficult to control. A loss of sensitivity of collected *A. solani* field isolates to azoxystrobin was already observed in the year 2000 in lab trials, with a further increase of the EC_{50} values in the year 2001. Notably, this shift in sensitivity was also observed in isolates collected from fields that have not been treated with azoxystrobin. By 2006, 96.5% of U.S. *A. solani* isolates, also collected from potato growing areas outside the Midwest, had a reduced sensitivity to azoxystrobin. The rapid loss of sensitivity towards azoxystrobin was likely due to multiple applications of azoxystrobin as single compound in Early Blight control during the growing seasons (Pasche and Gudmestad 2008).

The loss of sensitivity to QoI fungicides is mainly attributed to single nucleotide polymorphisms (SNPs) in the cytochrome *b* (*cytb*) gene of phytopathogenic fungi leading to amino acid substitutions in the Qo pocket of the cytochrome b protein that abolish QoI 'ligand' binding (Bartlett *et al.* 2002; Gisi *et al.* 2002). The most prominent amino acid substitution is from glycine to alanine at position 143 (G143A). The G143A substitution typically causes strong to complete loss of sensitivity towards QoI fungicides (Gisi *et al.* 2000) and cross-resistance within QoI fungicides (Pasche and Gudmestad 2008). Sequencing of the *cytb* gene of diverse phytopathogenic fungi revealed the presence of an intron directly after the codon coding for glycine at amino acid position 143 in some of the species, which is absent in species showing the G143A substitution. There, a SNP changing the codon to code for alanine would prevent splicing of the intron that would likely be lethal. Therefore, species with such a gene structure are unlikely to evolve the G143A substitution. However, they are able to gain the F129L mutation, where phenylalanine is replaced by leucine at amino acid position 129 in the Qo pocket of cytochrome b (Grasso *et al.* 2006). The F129L substitution was found to cause a more differential loss in sensitivity to QoI. The effect of the F129L substitution reduces the sensitivity towards QoI but does not result in complete resistance and cross-resistance within QoI fungicides (Kim *et al.* 2003; Pasche *et al.* 2005).

A. alternata can gain the G143A substitution, whereas *A. solani* cannot, but can instead gain the F129L substitution (Grasso *et al.* 2006). U.S. *A. solani* F129L mutant isolates were 10-15-fold less sensitive to azoxystrobin in spore germination assays compared to wildtype isolates and showed a robust cross-resistance to pyraclostrobin. However, there was just a subtle cross-resistance with famoxadone, which is a QoI but not a strobilurine. Please note that famoxadone is generally less effective than azoxystrobin in sensitive *A. solani* populations. (Pasche *et al.* 2004; Pasche *et al.* 2005; Pasche and Gudmestad 2008) This loss of sensitivity appears modest when e.g. compared to the 200-fold decrease of sensitivity towards QoI caused by the G143A

substitution in *Blumeria graminis* f.sp*. tritici* (Sierotzky *et al.* 2000). However, the F129L substitution reduced the efficacy of azoxystrobin in Early Blight control in field trials to the level of mancozeb, such that azoxystrobin has lost its superiority in Early Blight control in the USA (Pasche and Gudmestad 2008).

In Germany, azoxystrobin and pyraclostrobin plus boscalid were registered for *Alternaria* spp. control in potatoes in 2007 and 2008, respectively, due to a preceding increase of Alternaria diseases of potato (Leiminger and Hausladen 2012). The leaf blight caused by *Alternaria* spp. is only hardly attributable to *A. solani* or *A. alternata* by eye in the field and both pathogens can co-occur on diseased fields (Hausladen and Leiminger 2007). Species-specific real-time PCR pointed to *A. solani* being the more abundant species, although that was not entirely consistent between years (Leiminger *et al.* 2015). In 2009, first F129L substitutions were detected at a very low frequency (5%) in *A. solani* isolates collected from potato fields in Lower Bavaria in southern Germany. The frequency of *A. solani* F129L mutant isolates rapidly rose to 74% until 2011 in Bavaria although the distribution of the F129L substitution remained patchy in and between potato growing areas. (Leiminger *et al.* 2014) Sequencing revealed two different compositions of introns in the *cytb* gene in isolates collected from German potato growing areas. An additional intron was found 2 codons upstream of the putative F129L mutation site in the *cytb* gene of genotype I isolates that is absent in genotype II isolates. 63% of in total 203 *A. solani* isolates collected between 2005 and 2011 in Germany were of genotype I. Interestingly, the F129L mutation was exclusively found with a frequency of 97% in genotype II isolates and never in the more abundant genotype I (Leiminger *et al.* 2014). It has not become evident why the F129L substitution could only evolve in genotype II in Germany, whereas it could also evolve in genotype I in the USA, where genotype II is more abundant. Regardless, spore germination assays showed that German F129L *A. solani* mutant isolates had a 4-fold reduced sensitivity to azoxystrobin and a 2.5-fold decreased sensitivity to pyraclostrobin, which is less compared to U.S. isolates. This translated to a roughly 50% loss in Early Blight control efficacy by azoxystrobin in a greenhouse trial. (Leiminger *et al.* 2014)

An emerging problem in Early Blight Control is dual resistance to QoI and SDHI fungicides. A loss of sensitivity of *A. solani* field isolates towards the SDHI boscalid was first reported in 2009 from Idaho in the USA (Wharton *et al.* 2012). A rapid increase in the frequency of boscalid-resistant *A. solani* field isolates from 15% in 2009 to 80% in 2011 was subsequently observed (Miles *et al.* 2014). All tested isolates in 2009 and 2010 were insensitive to azoxystrobin and dual resistance to azoxystrobin and boscalid was recognized as emerging. However, insensitivity to pyraclostrobin was not observable in 2009 and with 22% of tested isolates in 2010 at a comparatively low level (Fairchild *et al.* 2013). Cross-resistance within SDHIs was to some extent observable but was diverse, presumably depending on the molecular spatial structure of the respective SDHI that might influence SDHI binding kinetics to their target enzyme complex succinate dehydrogenase (SDH) (Fairchild *et al.* 2013; Miles *et al.* 2014). SDHIs are, like QoIs, single-site respiration inhibitors but act on complex II instead of complex III of the mitochondrial respiratory chain. The SDH complex (complex II) consists of the four subunits SDHA, SDHB, SDHC and SDHD. Oxidation of succinate to fumarate at SDHA provides additional electrons that are loaded onto ubiquinone at the ubiquinone binding site consisting of residues of SDHB, SDHC and SDHD. Reduced ubiquinone (ubiquinol) subsequently travels to the Qo site of complex III. SDHIs physically interfere with electron transfer from complex II to ubiquinone at the ubiquinone binding site of complex II. Several amino acid substitutions in SDHB, SDHC and SDHD have been found to confer to SDHI resistance in phytopathogens (Avenot and Michailides 2010). In

A. alternata these are H277R or H277Y on SDHB, whereas the amino acid substitutions on SDHB are H278R or H278Y in *A. solani*. Further, the H134R substitution on SDHC and the H133R or the D123E substitution on SDHD were found to confer SDHI resistance in both *Alternaria* spp. (Avenot and Michailides 2010; Mallik *et al.* 2014). SDHB and SDHC mutations were also seen to co-occur in *A. solani* (Landschoot *et al.* 2017) and in *A. alternata* isolates (Avenot *et al.* 2014). SDH mutations are generally indicative of a loss of sensitivity towards SDHIs although the associated phenotypic resistance degree varies within isolates showing a given SDH mutation (Mallik *et al.* 2014; Landschoot *et al.* 2017).

A recent study discovered the presence of SDHI resistance mutations in 41% of *A. alternata* and in 70% of *A. solani* field isolates collected from naturally infected potato fields in Belgium in Central Europe (Landschoot *et al.* 2017). All SDH-mutant isolates were generally less sensitive to boscalid, irrespective of which SDH subunit was mutated. Further, 58% of *A. alternata* and 40% of *A. solani* SDH-mutant isolates simultaneously showed SDHI and QoI resistance mutations.

OBJECTIVES

This study aimed to survey the current status of QoI mutations occurrence in *A. alternata* and *A. solani* populations in major potato growing areas in Bavaria, located in Southern Germany in Central Europe. Further aims were to examine the current frequency distribution of genotype I and genotype II of *A. solani* and to explore to which extent genotype I may have evolved the F129L substitution. An analysis of SNPs causing QoI mutations should generate hints on whether Alternaria QoI mutations have evolved independently multiple times. Finally, we aimed to check the presence of SDHI resistance mutations in Bavaria based on the examination of a subset of randomly selected field isolates.

MATERIALS AND METHODS

Collection of isolates

Potato leaves showing Alternaria leaf spots were collected during August and September 2016 from commercial and experimental potato fields in the regions Lower Bavaria, Lower Franconia, Swabia, Upper Bavaria and Upper Palatinate which are major potato growing regions in the state of Bavaria located in Southern Germany. Leaves were kept dry at room temperature for a few days until analysis.

Preparation of single spore isolates

From symptomatic leaves, leaf pieces bearing single lesions were excised and surface-sterilized in 5% NaOCl for 1 minute. After washing the leaf cut in sterile, distilled water, it was placed on synthetic low nutrient agar (SNA, 1 g/l KH_2PO_4, 1 g/l KNO_3, 0.5 g/l $MgSO_4x7H_2O$, 0.5 g/l KCl, 0.2 g/l glucose, 0.2 g/l sucrose, 600 µl 1M NaOH, 22 g/l Agar). To induce sporulation, plates were incubated in a growth cabinet at 20 °C under black light with a 12 h photoperiod for about two weeks. Subsequently, plates were checked for sporulation using a binocular and single spores were transferred to new SNA plates with a fine needle. To obtain single spore colonies, only one conidium of *A. solani*, or a single conidia chain of *A. alternata,* per leaf piece was transferred to fresh SNA plates. Successful isolation of *A. alternata* and *A. solani* cultures was checked microscopically by their characteristic spore morphology and size. The SNA plates were subsequently incubated in a growth chamber at 23 °C under blacklight and 65% relative

Humidity (rH). To obtain pure cultures, the spore isolates were sub-cultured once on SNA plates. Afterwards, small agar plugs of the cultures were transferred to potato-extract dextrose agar (PDA, 4 g/l potato infusion, 20 g/l glucose, 15 g/l Agar) to favor mycelium growth for DNA extraction. The PDA plates were incubated under blacklight for about 30 days at 23 °C and 65% rH until plates were fully colonized with mycelium.

DNA extraction

Genomic DNA was isolated from 80-100 mg mycelium scraped off from fully colonized PDA plates of *A. alternata* and *A. solani* single spore isolates. The fungal mycelium was then transferred into a 2 ml microcentrifuge tube together with 50 µl sterile water and 10-20 glass beads (2 mm diameter, Carl Roth). After the bead-beating step for 3 minutes at 20 Hz (Retsch), DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions (beginning with step 7).

Detection of QoI resistance isolates

The primers AF and AR (Table 1) were used to amplify the QoI mutation prone region of the cytochrome *b* gene in *A. alternata* according to Ma *et al.* (2003). In consequence of the different intron exon composition of the amplified *cytb* gene region in *A. solani* genotype I and genotype II, both the primer pairs As-5f/As-5r for genotype I and As-Gf/As-Gr for genotype II according to Leiminger *et al.* (2014) and Pasche *et al.* (2005), respectively, were used to amplify the appropriate *cytb* gene region for each *A. solani* isolate. PCR reactions were set up in 25 µl volumes containing 1 µl DNA extract, 0.2 µM of each Primer, 0.2 mM of each dNTP (Carl Roth), 1.5 mM MgCl$_2$, 1x Peqlab Taq-Polymerase Buffer Y and 1 U Taq-Polymerase (Peqlab). PCR conditions are described in Table 1. PCR products were separated by agarose-gelelectrophoresis on a 1.5% agarose gel with 1x Tris-Borate-EDTA-(TBE) Buffer and stained with PeqGreen (Peqlab). The appropriate fragments were excised and purified with the QIAquick Gel Extraction kit (Qiagen). Finally, the purified amplicons were sequenced in both directions using the same primers as for the PCR (GATC Biotech).

Detection of SDHI resistance isolates

PCR reactions were carried out in 25 µl-volumes containing 1 µl DNA extract, 0.2 µM of each Primer, 0.2 mM of each dNTP (Carl Roth), 1x Enhancer Solution P (Peqlab), 1x Peqlab Taq-Polymerase Buffer Y and 1 U Taq-Polymerase (Peqlab). Primers and PCR conditions for amplification of the subunits *SdhB, SdhC* and *SdhD* in *A. alternata* as well as *A. solani* are listed in Table 1. Gel extraction and sequencing was done analogous to QoI mutation detection.

Table 1. *Primers and PCR conditions*

Primer	Target	Reference	PCR parameters
AF AR	Cyt b, A. alternata	Ma *et al.* (2003)	95°C for 3 min, 35 cycles (94°C for 40 s, 68°C for 40 s and 72°C for 60 s), 72°C for 10 min
As-Gf As-Gr	Cyt b, A. solani genotype I	Leiminger *et al.* (2014)	95°C for 10 min, 35 cycles (95°C for 60 s, 54°C for 30 s and 72°C for 30 s), 72°C for 10 min
As-5F As-5R	Cyt b, A. solani genotype II	Pasche *et al.* (2005)	95°C for 10 min, 35 cycles (95°C for 60 s, 54°C for 30 s and 72°C for 30 s), 72°C for 10 min
SdhBa-F SdhBa-R	SdhB, A. alternata	Avenot *et al.* (2008)	95°C for 3 min, 40 cycles (94°C for 40 s, 51°C for 50 s and 72°C for 60 s), 72°C for 10 min
SdhCa-F SdhCa-R	SdhC, A. alternata	Avenot *et al.* (2009)	95°C for 3 min, 40 cycles (94°C for 40 s, 51°C for 50 s and 72°C for 60 s), 72°C for 10 min
SdhDa-F SdhCa-R	SdhD, A. alternata	Avenot *et al.* (2009)	95°C for 3 min, 40 cycles (94°C for 40 s, 51°C for 50 s and 72°C for 60 s), 72°C for 10 min
SdhBs-F SdhBs-R	SdhB, A. solani	Mallik *et al.* (2014)	95°C for 2 min, 30 cycles (95°C for 30 s, 60°C for 30 s and 72°C for 60 s), 72°C for 7 min
SdhCs-F SdhCs-R	SdhC, A. solani	Mallik *et al.* (2014)	95°C for 2 min, 30 cycles (95°C for 30 s, 60°C for 30 s and 72°C for 60 s), 72°C for 7 min
SdhDs-F SdhCs-R	SdhD, A. solani	Mallik *et al.* (2014)	94°C for 5 min, 40 cycles (94°C for 30 s, 55°C for 30 s and 72°C for 60 s), 72°C for 7 min

Supplementary Table 1

RESULTS AND DISCUSSION

Frequency of QoI mutant A. alternata and A. solani field isolates
In total 55 isolates of *A. alternata* and 47 isolates of *A. solani* were prepared as single spore cultures from diseased potato leaves. The leaves were collected in 2016 from 26 locations distributed in the major potato growing areas in Bavaria in Southern Germany. Up to three independent isolates per location were analyzed. 74.5% of *A. alternata* isolates carried the G143A mutation (Figure 1) which was exclusively caused by a GGT to GCT SNP in their nucleotide sequences of the *cytb* gene. The G143A mutation typically causes a severe loss in QoI sensitivity (Gisi *et al.* 2000; Pasche and Gudmestad 2008). G143A mutant *A. alternata* isolates were found in all examined regions. *A. alternata* G143A mutant frequencies were highest in Upper Palatinate (100%, n = 7), Lower Franconia (100%, n =2) and Swabia (90%, n = 10), followed by Upper Bavaria (65%, n = 20) and Lower Bavaria (62.5%, n = 16). All multiple *A. alternata* isolates per location were G143A mutated in Upper Palatinate (2 locations) and Lower Franconia (1 location). This was also the case for 3 of 4 locations in Swabia, where the remaining was a mixture of a sensitive and a mutant isolate. This situation was more diverse in the more abundantly sampled regions Upper and Lower Bavaria. Here, locations with all independent isolates being mutated (2 of 7, resp. 3 of 7), locations showing a mixture of sensitive and insensitive isolates (4 of 7, resp. 3 of 7) and locations with all isolates not being mutated (1 of 7 each) were observed.
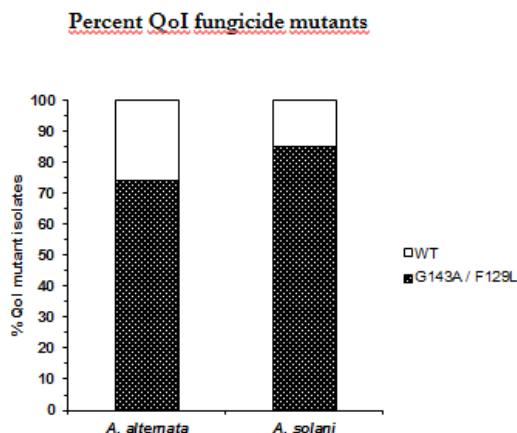
Figure 1. *Percent QoI fungicide mutant field isolates*
74.5% of n = 55 A. alternata isolates showed the G143A mutation and 85.1% of n = 47 A. solani isolates showed the F129L mutation in 2016. WT: wildtype. QoI: Quinone outside inhibitor

The frequency of QoI mutations was comparatively higher in *A. solani* field isolates. 85.1% of analyzed *A. solani* isolates showed the F129L mutation. The F129L mutation causes a loss in sensitivity to QoI fungicides although the degree was seen to vary (Kim *et al.* 2003; Pasche *et al.* 2005). The percentage of *A. solani* F129L mutants was high in all sampled regions. All of the tested isolates carried the F129L mutation in Swabia (n = 10) and Upper Palatinate (n = 8). 88.2% of isolates were F129L mutated in Lower Bavaria (n = 17). The lowest frequency of 63.6% F129L mutant isolates was found in Upper Bavaria (n = 11). In Lower Franconia only one sensitive isolate was obtained from one location. This distribution was somewhat reflected by the composition of sensitive and mutant isolates at locations where at least two independent isolates could get prepared. All multiple isolates per locations were F129L mutated in Swabia (3 locations) and Upper Palatinate (3 locations). In Lower Bavaria a mixture of sensitive and mutated isolates was only found in one of 5 locations whereas at the others all isolates were mutated. Finally, three of 5 locations showed a mixture of sensitive and mutated isolates in Upper Bavaria and the remaining showed only F129L mutated isolates.

Together, QoI fungicide resistance mutations are present at high levels in *A. alternata* and *A. solani* field isolates, also in geographically distinct potato growing areas, in Bavaria in Southern Germany. Leiminger *et al.* (2014) found 74% of German *A. solani* isolates, predominantly sampled in Bavaria, to be F129L mutated in 2011. They further observed a patchy distribution of mutant isolates and sensitive isolates in and between regions. Our results show that F129L mutations are now present in all major Bavarian potato growing regions. Our results further indicate that the frequency of the F129L mutation has increased by roundabout 10% in the period between 2011 and 2016. In the Midwest of the USA, it took only five years from the first observed F129L mutant *A. solani* isolates to 96.5% of sampled isolates being mutated (Pasche and Gudmestad 2008). The increase of the F129L mutation frequency in Bavarian *A. solani* isolates appears to progress slower as it has not reached such a high level since 2009. Nevertheless, in the light of 85.1% of *A. solani* and 74.5% of *A. alternata* isolates

showing mutations for reduced sensitivity against QoIs, it appears at least questionable whether there would be an additional benefit in using single compound QoI fungicides for Alternaria leaf spot control.

QoI mutations in A. solani genotype I and frequencies of A. solani genotypes I and II
As Leiminger *et al.* (2014) exclusively observed F129L mutations in genotype II of German *A. solani*, isolates, we were interested whether the composition of genotypes in the Bavarian *A. solani* population has changed since 2011 and whether genotype I meanwhile gained the F129L mutation. 25.5% of our analyzed *A. solani* isolates (n = 47) belonged to genotype I in 2016 (Figure 2). This resembled the results of Leiminger *et al.* (2014) who found 24.5% (n = 94) of German *A. solani* isolates to belong to genotype I in the year 2011, which were mostly collected in Bavaria. They also showed that the frequency of genotype II rapidly increased from 5.1% in 2009 to 75.5% in 2011. Our data indicate that the shift to genotype II has come to a halt and that the distribution of genotypes may have found a balance.

Frequency of *A. solani* genotypes

A. solani genotypes [%]

25,5

74,5

▩ Genotype I   ▪ Genotype II
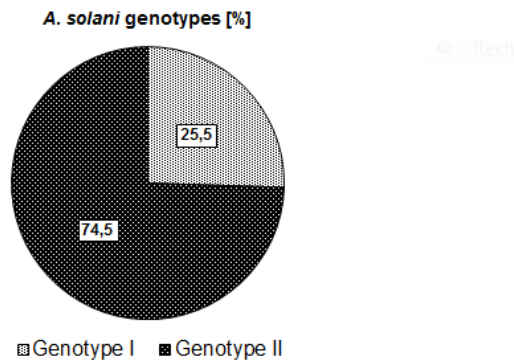
Figure 2.   *Distribution of A. solani genotypes*
25.5% of n = 47 A. solani field isolates were genotype I and 74.5% were genotype II in 2016

Leiminger *et al.* (2014) found F129L mutations with a frequency of 98.6% (n= 71) in genotype II isolates in 2011 and never in genotype I. We likewise found 100% (n = 35) of *A. solani* genotype II isolates to be F129L mutated in 2016. However, we also found F129L mutations with a frequency of 41.7% (n = 12) in genotype I isolates of *A. solani* (Figure 3). Genotype I F129L mutant *A. solani* isolates were identified from Lower Bavaria (n = 2), Swabia (n = 2) and Upper Palatinate (n = 1), but not from Upper Bavaria. This shows that within the five year period between the two sampling time points, genotype I likely has evolved the F129L mutation and that F129L mutant genotype I isolates have undergone positive selection. This further implies that the observed 10% increase of F129L mutant isolates between 2011 and 2016 is very likely due to the evolution of the F129L mutation in *A. solani* genotype I.
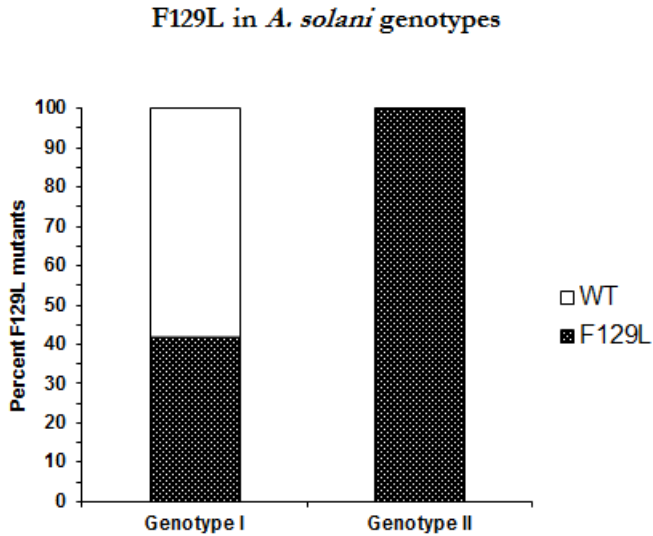
Figure 3. *Percent F129L mutant isolates in A. solani genotype I and genotype II*
41.7% of n = 12 genotype I and 100% of n = 35 genotype II A. solani field isolates showed the F129L
amino acid exchange in 2016. WT: wildtype

SNPs causing F129L in A. solani

In this study, F129L mutant *A. solani* isolates were collected from all examined Bavarian potato
growing regions, which are partly geographically separated. Only seven out of 47 examined
A. solani field isolates did not carry the F129L mutation. These were genotype I isolates from
5 different sites in Lower Bavaria, Upper Bavaria and Lower Franconia. F129L mutant genotype
II isolates were co-isolated from all of these sites except from one site in Lower Franconia where
only one genotype I wildtype isolate was available. Together with the previous finding of a
patchy distribution of F129L mutants within and between regions five years before by Leiminger
et al. (2014), this indicates that the F129L mutation has apparently spatially spread. Therefore
we wondered whether we could get hints on the mechanism of the spread by determining the
distribution of the SNPs CTC, TTA and TTG (wildtype codon: TTC) that can cause the amino acid
exchange from phenylalanine (F) to leucine (L). Diverse SNPs in and between regions would
point to multiple independent evolutions whereas the opposite would rather point to a dispersal
of F129L mutant *A. solani* isolates.

The TTA SNP was found with 88.6% to be the predominant SNP in *A. solani* genotype II
(Figure 4). Further it was the most abundant SNP in each sampled region. In Swabia all F129L
mutations in *A. solani* genotype II isolates (n = 8) were caused by the TTA SNP. The CTC SNP
was found as second SNP to cause the F129L with a frequency of 11.4% of all sampled *A. solani*
genotype II isolates. It was present with a frequency of 15.4% (n=13) in Lower Bavaria and with
14.3% each in Upper Bavaria (n = 7) and Upper Palatinate (n = 7). The TTG SNP was never
found in the examined genotype II isolates. The TTA SNP having been predominant in all regions
might point to a physical spread by e.g. conidia dispersal or by seed potato contamination,
although it cannot be excluded that it preferably evolves. However, the CTC SNP found in the

neighboring potato growing regions Lower Bavaria, Upper Bavaria and Upper Palatinate but not in the more geographically separated Swabia indicates at least one independent evolution of the F129L amino acid substitution in parts of Bavaria.
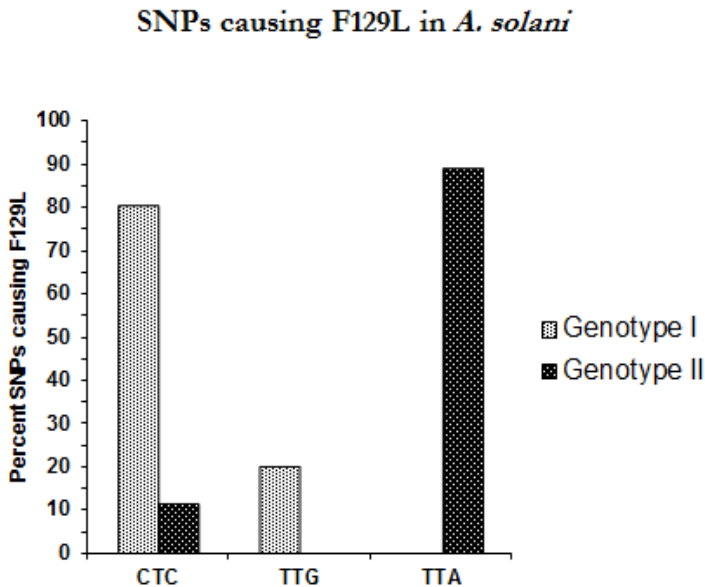
Figure 4 *Frequency of SNPs causing F129L in A. solani genotype I and genotype II*
In genotype I, the F129L amino acid exchange was caused to 80% by the SNP CTC, to 20% by TTG and to 0% by TTA in n = 5 field isolates. In genotype II, the distribution of SNPs was 88.6% TTA, 11.4% CTC and 0% TTG in n = 35 field isolates. SNP: single nucleotide polymorphism. Wildtype codon = TTC. Exchanged nucleotides are underlined

The CTC SNP is in contrast the most abundant SNP causing F129L in *A. solani* genotype I. In total 80% of F129L mutated genotype I isolates (n = 5) showed the CTC SNP, the remaining the TTG SNP, which has not been found in genotype II. The abundantly in genotype II present TTA SNP was not found in genotype I. The CTC SNP was present in F129L mutant *A. solani* isolates in Swabia, Upper Palatinate and Lower Bavaria. The TTG SNP only co-occurred in Lower Bavaria. Like in genotype II, the presence of two different SNPs causing F129L indicates at least one independent evolution of the F129L mutation in *A. solani* genotype I.

The clear dominance of each one distinct SNP in *A. solani* genotype II and genotype I might point to spatial spreading of the F129L mutation as cause for the increase of F129L mutant isolates instead of multiple independent evolutions. However, more research would be required to substantiate that hypothesis. Lower Bavaria could be the hot spot of QoI evolution in Bavaria since it was the sole region where all SNPs causing F129L found in this study were simultaneously present. This first hint would be worth further investigations.

Upcoming SDHI mutations and dual fungicide insensitivity in A. alternata and A. solani

Belgian *A. alternata* and *A. solani* populations showed considerable frequencies in SDH mutations and to some extent also dual mutations against QoI and SDHI fungicides (Landschoot *et al.* 2017). We randomly selected 23 *A. alternata* and *19 A. solani* of our Bavarian isolates and screened them for the presence of SDHI fungicide mutations in the subunits SDHB, SDHC and SDHD. From the examined isolates, a total of 43.5% of *A. alternata* and 42.1% of *A. solani* isolates showed SDH mutations (Figure 5). All SDH mutated *A. alternata* and *A. solani* isolates exhibited a QoI mutation in parallel. The SDHB mutation was with a frequency of 26.1% (60% within SDH mutant isolates) predominant in the screened *A. alternata* isolates. It was caused in 5 out of 6 cases by the H277Y amino acid exchange and in one case by the H277R substitution. Further, the SDHC mutation was detected with a frequency of 17.4% in *A. alternata*. The SDHD mutation was not observed in *A. alternata*. Also in Bavarian *A. solani* isolates only SDHB and SDHC mutations were detected but the ranking in their frequencies was reverse. The SDHC mutation was with a total frequency of 36.8% (87.5% within SDH mutant isolates) clearly more common than the SDHB mutation (H278Y only) with a frequency of 5.3%.

Percent SDHI fungicide mutants
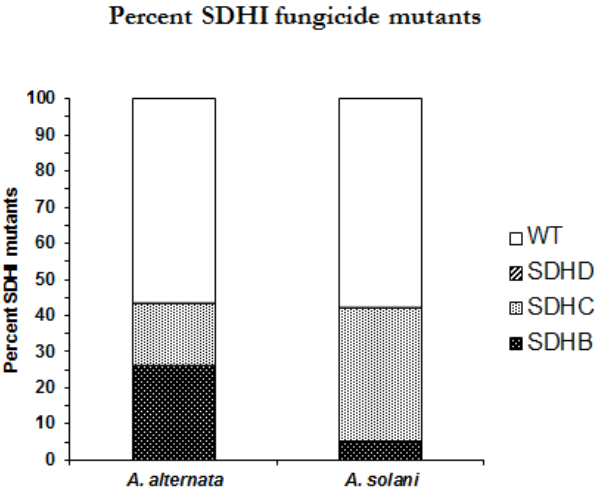
Figure 5. *Frequency of succinate dehydrogenase (SDH) mutations in A. alternata and A. solani*
In A. alternata, 43.5% of n = 23 field isolates showed a SDHI fungicide mutation. The frequencies of mutant isolates were 26.1% SDHB (83.3% H277Y, 16.7% H277R), 17.4% SDHC and 0% SDHD. In A. solani, the total frequency of SDH mutated field isolates was 42.1% of n = 19. 36.8% of isolates showed a SDHC mutation and 5.3% a SDHB (H278Y) mutation. No SDHD mutation was observed. SDHI: Succinate dehydrogenase inhibitor
SDHB, SDHC, SDHD: Subunits of succinate dehydrogenase enzyme complex. WT: wildtype

The total frequency of SDH mutations in Bavarian *A. alternata* isolates resembled the results from Landschoot *et al.* (2017) where 41% of Belgian isolates were SDH mutated. However, SDH mutations were less prevalent in Bavarian *A. solani* isolates compared to Belgian ones, of which

70% were mutated. This might point to differences in selection pressure exerted on *A. solani* populations. The dominancy and the frequency of the SDHB mutation in *A. alternata* were comparable to the results of Landschoot *et al.* (2017), who found 21.21% SDHB mutated isolates in 2015. In contrast to Belgian isolates in which only the H277Y substitution was found, also a H277R exchange was detected in addition to the predominant H277Y in Bavaria. The balance between SDHB and SDHC mutations was more pronouncedly shifted to SDHC mutations in Bavarian *A. solani* isolates collected in 2016 in comparison to Belgian isolates collected in 2015. Landschoot *et al.* (2017) reported 38.10% SDHC mutations and 26.19% SDHB mutations, of which were 2.38% caused by H278R and the overwhelming part by H278Y. No H278R mutation was detected in Bavarian *A. solani* isolates. This might be due to the reduced number of screened isolated in this study. The same might hold true for the absence of SDHD mutations in our *A. alternata* and *A. solani* isolates. SDHD mutations were found in Belgian *A. alternata* isolates with a comparatively low frequency of 9.09% in 2016 and were absent in Belgian *A. solani* isolates (Landschoot *et al.* 2017). In contrast to Landschoot *et al.* (2017) and Avenot *et al.* (2014) who observed dual mutations of the SDHB and SDHC subunits in Belgian *A. solani* and U.S. *A. alternata* isolates, respectively, we could not observe dual SDH mutations in our set of isolates. However, all SDH mutations being accompanied by QoI mutations in Bavarian *A. solani* isolates resembled more the situation in the USA where 99% of screened SDH mutated isolates showed the F129L mutation in parallel (Gudmestad *et al.* 2013) than the situation in Belgium where only 28.76% of isolates showed dual mutations against SDHIs and QoIs and where isolates only being SDH mutated were with 39.76% even predominant (Landschoot *et al.* 2017). This contrast between Bavarian and Belgian isolates was also observed for *A. alternata*, where in Belgium only 44.23% of the in 2015 collected isolates showed dual mutations against QoIs and SDHIs. These differences could possibly be explained by different applied fungicide strategies and active ingredient combinations.

In the overall view, it can be stated that QoI fungicide resistance mutations are fully established in the major potato growing areas in Bavaria. The results of this study further show that dual resistance against QoI and SDHI fungicides are an upcoming problem in chemical Alternaria disease control in potatoes in Bavaria. This is reflected by reports that reach us from agricultural practice.

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Fear of tuber blight: a significant barrier to industry's uptake of IPM

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Introduction

Cultivar resistance should be a crucial component of late blight IPM. However, a major obstacle to the UK potato industry's uptake of cultivar resistance-based IPM is concern that reduced fungicide inputs may increase the risk of tuber blight for many cultivars. The aim of this work was to investigate the suitability of some cultivars for IPM in relation to their 1 to 9 resistance ratings. Field trials were used to determine which combinations of leaf resistance and tuber resistance ratings have the potential to offer acceptable control of tuber blight in a system of reduced fungicide inputs. All cultivars received the same blight fungicide programme which was designed to allow controlled foliar blight development towards the end of the growing season (to simulate the IPM situation that growers are concerned about) coupled with poor direct protection of the tubers through fungicide product choice.

Methods

Test cultivars were selected to give a range of combinations of foliar and tuber blight resistance ratings. The ratings, to genotype 13_A2, were relatively recent (last updated between July 2014 and July 2018 on the AHDB Potato Variety database). It should be noted that cultivar Setanta had two ratings for tuber blight resistance. Not all cultivars were tested in both 2017 and 2018. There were two cultivars that represented susceptible references.

During the tuber protection phase of the trial fungicide programme, fungicides with zero, or very little, efficacy against tuber blight, i.e. mandipropamid or mancozeb, without or with cymoxanil, were applied at full UK label rates at a target 7-day interval. In 2017 the risk of tuber infection was raised by crop desiccation being deliberately delayed until 14 days after the final application of fungicide. All tubers from the centre two rows of the four-row plots were harvested, washed and assessed twice, after weeks and then months of ambient storage. In 2017 240 tubers from each of five replicate plots were assessed per cultivar. In 2018 the corresponding number was 184.

Results

In both 2017 and 2018, conditions were very favourable for tuber infection, as indicated by 4.7% and 12.3% tuber blight respectively in the reference cultivar Shepody (Figures 1 & 2). Very good to good control of tuber blight was achieved in 2017 for Setanta, Harmony, and Gatsby and in 2018 for Carolus, Harmony, Orla, Setanta and Gatsby.
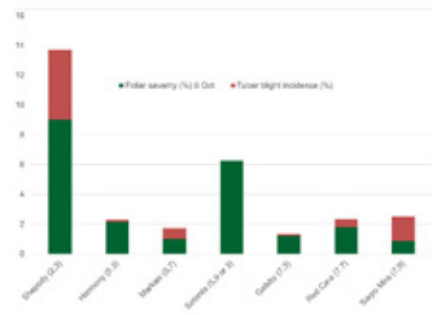
Figure 1: Tuber blight incidence in relation to final foliar blight severity for six test cultivars (2017 trial). Values in parentheses are (foliar blight resistance rating, tuber blight resistance rating)
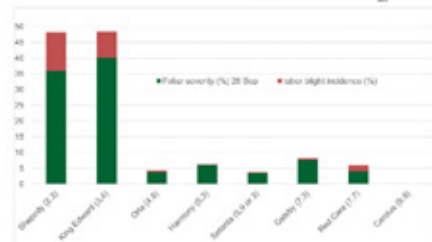
Figure 2: Tuber blight incidence in relation to final foliar blight severity for six test cultivars (2018 trial)

Discussion and Conclusions

These preliminary results suggest that the risk of tuber blight was acceptably low in a simulated IPM system for five of the eight test cultivars. The very good control of tuber disease by cultivars rated 9 for tuber blight (Setanta) or 9 for both phases of the disease (Carolus) was expected but there are few agronomically acceptable cultivars with such high ratings.

The hypothesis being tested in this work was whether cultivars with a combination of moderate ratings for both foliar and tuber blight offered sufficiently effective control of tuber blight through a reduction in inoculum density (because of foliage that is more resistant) to a value below the threshold for infection of tubers with a moderate level of resistance. The hypothesis was correct but the trial results were more complex than anticipated. Suitably effective control of tuber blight was achieved by cultivars with the following combinations of cultivar resistance: 5,3 (Harmony), 7,3 (Gatsby) or 4,8 (Orla) whereas control of tuber blight with the combinations 7,9 (Sarpo Mira), 7,7 (Red Cara) and 5,7 (Markies) was poorer. The discrepancy between official resistance ratings and some of the results obtained may have been due to the ratings being for 13_A2. Although both trials were inoculated with a single isolate of 13_A2 only, by the end of the growing season genotype frequencies had changed substantially. In 2017, from 100% 13_A2 (presumably) to 68% 13_A2, 27% 6_A1 and 5% 8_A1 and in 2018 the corresponding genotype percentages in September were 8% 13_A2, 79% 6_A1 and 13% 37_A2.

Cultivars with a reduced risk of tuber infection could also help to prevent or delay the emergence of resistance to Qil fungicides and fluopicolide by allowing fungicides with different modes of action (generally considered to be less effective) to be used for tuber blight control on these cultivars.

Further experiments in 2019 and 2020 are planned to allow more robust conclusions to be reached.

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Acknowledgements

In quest for new sources of late blight resistance in the VIR collection of wild potato germplasm

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SUMMARY

Seedlings of 40 species wild tuber-bearing *Solanum* spp. maintained in VIR or introduced to VIR from other potato genebanks were assessed for late blight resistance under the field and laboratory conditions. Among 287 *Solanum* accessions, few were resistant (6-16%), half of accessions were susceptible, whereas about one third of accessions were heterogeneous seed populations segregating for resistant, moderately resistant and susceptible individuals. Newly isolated accessions of *S. doddsii* and *S. okadae* are prospective initial sources for breeding late blight resistant potato varieties. When the resistance indices obtained in these studies were compared to the previously obtained data, we observed medium correlation, with the Spearman's coefficient of 0.56 at p<0.05. The specific problems of maintaining and reproducing seed collections of wild potato species are under discussion.

KEYWORDS

Phytophthora infestans, wild tuber-bearing *Solanum* spp., *ex situ* population, potato late blight*, durable resistance

INTRODUCTION

Rapid evolution of the pathogenic oomycete *Phytophthora infestans* Mont. De Bary presents the main obstacle on the road to breeding new potato varieties with high and durable resistance to late blight (LB). The best way to confront this threat is by developing the initial breeding material - potato parental lines that carry several resistance genes, providing in this way for the broad range of specificity towards diverse races of *P. infestans.* Wild tuber-bearing *Solanum* species, which have not yet been involved in commercial breeding, are of particular interest. The VIR collection comprises 2100 accessions of 140 tuber-bearing relatives of *Solanum tuberosum* L. (Kiru, Rogozina, 2017). This gene pool, which has been accumulated for almost a century, has been evaluated annually for numerous agronomic traits including many disease resistances. The objective of this study was to assess the samples of *Solanum* spp. for LB resistance and elucidate whether this resistance is maintained in accessions characterized as resistant in previous studies, a decade or over.

MATERIALS AND METHODS

Plant material and assessment of LB resistance

Nine of 19 tuber-bearing series of Subsection Potato G. Don. represented 40 *Solanum* species were assessed for LB resistance in the field and in the laboratory. Botanical seeds were either the samples (accessions) from VIR or were donated by others potato genebanks: NRSP-6 Potato Genebank (the USA), CGN (Centre for Genetic Resources, the Netherlands) and the Gross Lüesewitz Potato Collection of the IPK Genebank (GLKS, Germany). Species are listed in Table 1 according to J.G. Hawkes (Hawkes, 1990).

Table 1. Plant material tested for LB resistance

Solanum group	Series	*Solanum* spp. (number of accessions)
North American	Bulbocastana	*S. bulbocastanum* (4)
	Pinnatisecta	*S. cardiophillum* (10), subsp. *ehrenbergii* (2), *S. jamesii* (6), *S.× michoacanum* (6), *S. pinnatisectum* (8), *S. trifidum* (4)
	Polyadenia	*S. lesteri* (2), *S. polyadenium* (2)
	Tuberosa (wild)	*S. verrucosum* (6)
	Longipedicellata	*S. fendleri* (18), *S. hjertingii* (6), *S. papita* (4), *S. polytrichon* (15), *S. stoloniferum* (31)
	Demissa	*S. brachycarpum* (4), *S. demissum* (26)
South American	Acaulia	*S. acaule* (2)
	Yungasensa	*S. chacoense* (14)
	Tuberosa (wild)	*S. abancayense* (4), *S. leptophyes* (3), *S. medians* (4), *S. mochiquense* (3), *S. multidissectum* (5), *S. multiinterruptum* (4), *S. sparsipilum* (5), *S. alandiae* (15), *S. avilesii* (3), *S. berthaultii* (7), *S.×doddsii* (7), *S. gandarillasii* (5), *S. gourlayi* (5), *S. hondelmannii* (6), *S. incamayoense* (4), *S. kurtzianum* (2), *S. microdontum* (8), *S. okadae* (8), *S. oplocense* (4), *S. spegazzinii* (4), *S.×sucrense* (4), *S. vernei* (7)

The number of tested accessions of each *Solanum* species ranged between 2 and 31 (Table 1). For each accession, 10 to 20 seedlings were grown in the field, and 3 to 5 seedlings in the greenhouse. LB resistance of wild potato plants was assessed in the field trials at Pushkin (St. Petersburg, Russia) under conditions of natural infestation and scored by 1 to 9-point scale, where 9 corresponds to no visible lesions. The sightings of LB distribution were started when the first lesions appeared on wild potato plants. The final index of LB resistance of each tested accession was defined as the maximum damage score at the end of the growing season. LB resistance of greenhouse plants were assessed in the laboratory test conducted in VIR. Detached leaves test was performed according to the Eucablight protocol (www.euroblight.net) using highly virulent and aggressive isolate of *P. infestans* collected in the Leningrad region. Leaflets collected from 30-55-day-old plants were infected by pathogen isolates containing the race 1,2,3,4,5,6,7,8,9,10,11. Three leaflets per each tested genotype were inoculated with zoospore suspension at the concentration of 30-40 ×1000 per ml according to standard protocol. Scoring by 1 to 9-point scale was carried out 5-7 days after inoculation.

RESULTS AND DISCUSSION

Results of a current study of Solanum spp.

In 2016-2018 a total 287 accessions representing 40 *Solanum* spp. were assessed in the field. During the field trials, the weather conditions in the growing season were not the same; the onset of lesions and the pattern of LB development also varied. The earliest LB manifestation was registered in 2016, when the first symptoms appeared in July, and by the beginning of August all plants of *S. acaule*, *S. fendleri*, *S. hondelmannii*, *S. kurtzianum* and *S. oplocense* were completely affected. Under such conditions of early emergence and rapid LB development, high resistance was observed in accessions of *S. doddsii* k-18240, *S. lesterii* k-24475, and *S. polyadenium* k-24461: here the leaf area affected by LB did not exceed 50% by the end of the growing season. In 2017, the initial LB symptoms were noted in the first decade of August, and disease developed slowly; as a result, by early October, resistant accessions *S. berthaultii* k-19961, *S. famatinae* (*S. spegazzinii* syn.) k-7466, *S. lesterii* k-24475, *S. polyadenium* k-24461, *S. stoloniferum* k-5431, *S. vernei* k-23771, and *S. verrucosum* k-23015, k-24315, k-24427 were scored as 5-7 points. Early in the 2018 growing season, hot weather with heavy if short rainfalls favored the development of *Alernaria* ssp. A significant number of *S. demissum* and *S. fendleri* accessions were completely defeated by the first decade of August, which indicates their extreme susceptibility to this disease. Seedlings of *S. okadae* differed in their response to *Alernaria*. In September, with the onset of LB, we noted disease-resistant accessions of *S. demissum* k-24378, *S. lesterii* k-24232, *S. stoloniferum* k-24189, *S. okadae* k-25394 and *S. tarnii* k-23936. Every year of field testing, responses to LB widely varied among species and among accessions within species. The proportion of highly resistant (7-9 point) and susceptible (1-3 point) accessions notably differed in North and South American groups of *Solanum* species: the percentage of highly resistant accessions was 16 and 6, respectively, whereas for susceptible accessions, the respective indices were 47 and 54%. Both in the North American and South American groups of *Solanum* species under study, we registered the same proportion of accessions (34%) to segregate by resistance in the range of 1 to 7 points. Segregation in LB resistance was observed within accessions of *S. microdontum*, *S. okadae*, *S. vallis-mexici*, and *S. vernei* (Table 2).

Table 2.   *Solanum accessions manifesting high LB resistance*

***Solanum* spp.**	**Catalogue numbers of VIR accessions**	**LB resistance score (field\ laboratory test)**	**The corresponding accessions numbers in other collections***	**Previously reported data ****
S. berthaultii	k-19961	5\n.d	PI 473331	R
S. chacoense	k-7394	9\7	PI 320292	S
	k-2861	8\2.3-2.5	no	
S. demissum	k-2353	9\3.7	no	
"	k-20000	7\n.d.	PI 498230	R
"	k-24378	3-8\n.d.	PI 160221	R
S.× doddsii	k-18240	5-7\n.d.	PI 442690	n.d.
S. famatinae	k-7466	3-7\n.d.	no	
S. lesterii	k-24232	3-7\n.d.	GLKS 2782	n.d.
"	k-24475	5-7\n.d.	no	
S.× michoacanum	k-5763	6\5-7	no	
S. microdontum	k-25385	1-7\n.d.	CGN 20597	VR\M
S. stoloniferum	k-5135	7\n.d.	no	
"	k-5431	5-7\n.d.	no	
"	k-24189	7\n.d.	GLKS 66	n.d.
S. okadae	k-25394	5-8\5.5-7.7	CGN 17998	VR
"	k-25395	1-8\n.d.	CGN 17999	R
"	k-25397	3-9\2.7-8.0	CGN 18279	VR\M
S. tarnii	k-23936	7\n.d.	PI 498043	n.d.
S. trifidum	k-24984	7\n.d.	PI 283064	R
S. vallis-mexici	k-8473	1-7\4-5.7	no	
S. vernei	k-23771	5\n.d.	PI 458373	R
"	k-25413	1-7\n.d.	CGN 18112	R\R
S. verrucosum	k-23015	5-7\n.d.	PI 558482	R
"	k-24315	5\ n.d.	n.d.	
"	k-24427	7\n.d.	PI 275260	R

*PI, accession from NRSP-6 Potato Genebank, the USA; CGN, Centre for Genetic Resources, the Netherlands; GLKS, Gross Lüesewitz Potato Collection of the IPK Genebank, Germany

** R, resistant; VR, very resistant; M, moderately resistant; S, susceptible (see https://www.ars-grin.gov/nr6/ and Pel, 2010); n.d., no data

Detached leaf tests were carried out in 2017. Variation among species and among accessions within species was also observed in this test. Resistance for LB artificial infestation (5-7 score) was found in accessions *S. chacoense, S.× michoacanum, S. mochiquense, S. okadae*. Detached leaf test confirmed the field data for LB resistance in some promising accessions of *S. chacoense* k-7394, *S.× michoacanum* k-5763, *S. okadae* k-25394, whereas *S. okadae* k-25397 and *S. vallis-mexici* k-8473 accessions segregated for resistance (Table 2).

Results in comparison with previous data
Our data do not completely match the evidence obtained with *Solanum* accessions from other collections. While we confirmed high LB resistance in accessions of *S. demissum* k-20000, *S. trifidum* k-24984, *S. verrucosum* k-23015 and k-24427, the accessions of *S. demissum* k-24378 and *S. vernei* k-25413, which were previously reported as resistant, were found to segregate for LB response in our field tests. Meanwhile the accession *S. chacoense* PI 320292 reported as susceptible in the USA tests behaved as resistant in our field and laboratory

experiments (Table 2). We compared our current data with the evidence composed in the previous studies of the VIR collection (Zoteyeva *et al.* 2004). The data for LB resistance from two sources covering 82 accessions of 33 *Solanum* species matched moderately (the Spearman's correlation coefficient of 0.56 at p<0.05). This bulk of these data let us select 20 accessions of nine *Solanum* species with the stable level of LB resistance: *S. demissum* (4), *S. stoloniferum* (5), *S. pinnatisectum* (5), *S. jamesii* (1), *S. cardiophyllum* (1), *S.× michoacanum* (1), *S. avilesii* (1). *S. microdontum* (1) and *S. okadae* (1). Of special interest are the accessions of *S. chacoense* k-2861, *S. demissum* k-2353, *S. famatinae* k-7466, *S.× michoacanum* k-5763, *S. stoloniferum* k-5135, k-5431 and *S. vallis-mexici* k-8473, which were acquired by the VIR collection in 1953-1968 following the introduction of genetic materials from the collection of J. Hawkes and the Gross Lüesewitz Potato Collection. Through past half of a century, many accessions from this pool passed through several multiplication cycles. VIR Pushkin laboratories is the only place engaged in reproduction of wild potato species. Botanical seeds are gathered from plants grown in field and glasshouse. In field, the environmental conditions are most advantageous for LB development. *P. infestans* populations in the Pushkin potato stands changed similarly to those in commercial potato fields (Patrikeeva *et al.*, 2011; Vedenyapina *et al.*, 2002). Such accessions revealed in the VIR collection are extremely valuable for researching into the fundamentals of durable LB resistance of wild potato relatives, which is most important for their further deployment as the initial breeding sources.

Wild relatives of potato are *ex situ* conserved in germplasm banks as samples of seed populations (accessions). A key operation of a genebank that maintains botanical seeds is regeneration (also called "multiplication") in order to expand the amount of stored seeds and/or to increase their viability. This process includes the multiplication of seeds sampled *in situ* from an initial generation (original sample) or from the next generation sample donated by another germplasm holder. Multiplication is an activity that could easily affect the genetic composition of a resulting accession. Genebank Standards for Plant Genetic Resources prepared by FAO (2013) lay down the key principles that make up the core of genebank operations, i.e. the preservation of germplasm identity, maintenance of seed viability and genetic integrity, and the promotion of access. New consumer demands, climatic changes and shifts in the populations of pathogens cohabiting with potatoes as well as enhanced pathogenicity of previously innocuous microorganisms - all these factors call for a systematic study of the diversity in the collections of wild and cultivated potatoes. Today genebanks should take on a new role- not just being a repository providing germplasm resources, but also a research center to advance deeper understanding of genetic diversity (Bethke *et al.*, 2019; Mascher *et al.* 2019). A coupled study of genotypic and phenotypic traits of *Solanum* tuber-bearing species and *P. infestans* strains colonizing potato plants in the VIR collection is an important focus of multi-year research in this country (Chizhik *et al.*, this issue; Fadina *et al.*, 2017). This study produced the characteristics of individual genotypes (clones) representing the wide scope of wild and cultivated *Solanum* species. The next step will be to study the seed populations from the VIR collection in order to better comprehend the diversity of crop plants and their wild relatives and in this way develop new strategies to maintain these collections and successfully deploy them in breeding.

CONCLUSION

Among 287 accessions representing 40 wild tuber-bearing *Solanum* species from the VIR collection, only a small number (6-16%) are homogeneous populations resistant to late blight. About one third of the accessions under study are heterogeneous populations manifesting a wide

range of diversity in plant response to late blight: resistant, moderately resistant and susceptible genotypes. The results of previous studies evaluating late blight resistance of potato accessions from the VIR collection and other potato genebanks are not fully consistent: newly obtained data and the evidence on accessions from the VIR collection assessed earlier did not strongly correlate. Further research will hopefully help to elucidate the causes of these discrepancies.

ACKNOWLEDGEMENTS

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AHDB's blight services
Tools and resources for late blight management

Kathryn Hales

Agriculture and Horticulture Development Board (AHDB)

AHDB's blight services
Tools and resources for late blight management

POTATOES

AHDB

The problem:

➤ Late blight, caused by the pathogen *Phytophthora infestans*, remains the single most important disease for the British potato trade

➤ Spreading quickly in the foliage, a typical blight pressure season can cost the industry approximately £55 million a year

FIGHT AGAINST BLIGHT

- For over 15 years Fight Against Blight (FAB) has been reporting late blight outbreaks

- FAB warns farmers and growers of outbreaks quickly, allowing improvements in management practices

- Blight scouts (growers & advisors who regularly walk potato fields) voluntarily take samples for analysis

potatoes.ahdb.org.uk/online-toolbox/fight-against-blight-tool

- FAB has built a network of over 100 blight scouts who have collected samples from over 2,000 outbreaks since 2003

- Blight incidents are reported on a national level via the FAB webpage, where all users can see where blight has been found across the country

- Nearly 8,000 samples have been genotyped to track the changes in blight strains and help inform management practices

Visit: blight.ahdb.org.uk

Blightwatch

- Blightwatch uses Met Office forecast weather data to predict Hutton Criteria

- Email alerts are sent out for postcode locations to warn of potential Hutton Criteria in the next 24 hours

- Over 15,000 users are signed up to Blightwatch, enabling them to use the alerts to inform spray programmes and management practices

Visit: blightwatch.co.uk

CV34

14 Day

Tmin °C

rH90% Hours

CV8

Key: Clear | Hutton Criteria | Full Hutton Period

Fungicide resistance of Russian potato and tomato pathogens

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Fungicide resistance of Russian potato and tomato pathogens

Lyudmila Kokaeva, Sergey Elansky

Early blight

Potato and tomato leaves with early blight lesions were collected in six regions of the European part of Russia.
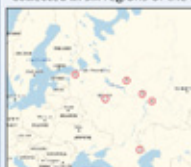
In vitro assessment demonstrated low efficiency of azoxystrobin for Russian isolates. Strains with high level of resistance were registered in all regions.

Alternaria solani

F129L and G137R mutations were not found in the sequences of 28 isolates from three regions of European Russia. However, resistant strains had several other SNPs.

SDHID (sdh gene subunit D)

14 strains had isoleucine instead of valine at the codon 48 (*A. tomatophila?*). One strain had valine → isoleucine substitution at the codon 143. Two SNPs in sdhB and one SNP in sdhC were found in three strains (data not shown).

Alternaria alternata

Allele-specific PCR detection of the G143A mutation. 1-7 – amplification of *A. alternata* DNA with ARF4/ ASR primers (Karaoglanidis et al., 2011); 10-16 – the same DNA with ARF4/ ARR4 primers; 8 – λHindIII marker, 9 – 1kB marker.

No G143A were found in the protein sequences of 38 resistant isolates.

DNA studies did not identify G143A, F129L and G137R mutations in the studied strains. This demonstrates a high level of strobilurin resistance in *Alternaria* species.
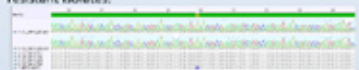
Silver scurf of potato (*Helminthosporium solani*)

We performed a screening for resistance to the fungicides difenoconazole, azoxystrobin, thiabendazole, and colloidal silver. Difenoconazole (EC_{50} < 0.12mg/l) and colloidal silver (EC_{50} < 76 mg/l) were the most effective – no strains resistant to these fungicides were found. In most cases, azoxystrobin was effective against *H. solani* (EC_{50} < 7 mg/l), however, there were several strains with high resistance to this fungicide (EC_{50} > 100 mg/l).

Thiabendazole appears to be effective against the sensitive strains of *H. solani* (EC_{50} < 7.3 mg/l); however, six studied strains from Russia and the Netherlands were found to be extremely resistant to it (EC_{50} > 1000 mg/l).

* – strain RMCh2 with a mutation in the codon 200 resulting in the substitution of Phe (TTC) with Tyr (TAC).
** – strains RMCh24, RKSu10, and H16 with a mutation in the codon 198 resulting in the substitution of Glu (GAG) with Gln (CAG).

The structure of the β-tubulin gene of *H. solani* strains with different levels of resistance to thiabendazole. The codons with mutation are in bold.

Strain	Resistance to thiabendazole, EC50, mg/l	Sequence of codons 197-200 of the β-tubulin gene
RB7	5.4	GACGAGACCTTC
RB11	6.6	GACGAGACCTTC
RMCh2*	> 1000	GACGAGACCTAC
RMCh24**	> 1000	CAGCAGACCTTC
RA/44	0.5	GACGAGACCTTC
RKSu68	5.7	GACGAGACCTTC
RKSu2/2	0.7	GACGAGACCTTC
RKSu10**	> 1000	GACGAGACCTTC
HCh1	0.8	GACGAGACCTTC
RCh8	1.7	GACGAGACCTTC
H16**	> 1000	GACGAGACCTTC
G5	1.0	GACGAGACCTTC
G11	0.5	GACGAGACCTTC
G12	3.2	GACGAGACCTTC
G18	0.5	GACGAGACCTTC
G20	5.8	GACGAGACCTTC
G21	5.5	GACGAGACCTTC

H and G strains isolated from the seed material imported from Germany and the Netherlands.

Location of the sampling sites. Filled circle designates the site where mutant strains were found.

Black dot (*Colletotrichum coccodes*)

Fungicide	EC_{50}, mg/l
Pencycuron	>100
Colloidal silver	1.20-7.00
Azoxystrobin	0.09->100
Fludioxonil	0.05-0.09
Difenoconazole	0.05-0.12
Tiabendazole	0.90-50.30

No strains with high resistance to difenoconazole and colloidal silver were found. Thiabendazole was highly effective in most cases.

Fludioxonil inhibited the growth of *C. coccodes* (EC_{50} was 0.60-0.90 mg/L). However, when cultured for more than twenty days, strains formed stable sectors that grew faster than the original isolate.

C. coccodes growth on fludioxonil

Black scurf (*Rhizoctonia solani*)

Rhizoctonia solani (teleomorph, *Thanatephorus cucumeris*) is a fungal pathogen associated with severe diseases in many crop species. In potato, it causes stem cankers and tuber blemishes.

The sensitivity tests for pencycuron and fludioxonil were performed in vitro using different concentrations of the active ingredients: from 0 to 100 µg per ml of media in three replications. Most of the strains did not show growth on media with fungicides. Several strains were able to grow on media with 1 µg/ml of fludioxonil and even with 1000 µg/ml of pencycuron.

Sensitivity of the *R. solani* isolates to pencycuron

Genotypic and phenotypic variations within 2016-2018 *Phytophthora infestans* French clonal populations

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Genotypic and phenotypic variations within 2016-2018 *Phytophthora infestans* French clonal populations

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Phytophthora infestans is renowned for its fast and dramatic changes in the genotype occurence and for its capacity to adapt, with strong impacts for a durable management of late blight. To examine in real time the ongoing evolution of these populations, an extensive survey of French *P. infestans* populations has been undertaken. In this study, we investigate the genetic distribution within French *P. infestans* populations over the last three years and explore the relationship between genotype and phenotype in dominant clonal lineages. Surveys were conducted during 2016-2018 from potato commercial fields and from some dumps and volunteers.

Genotypic structuration

Samples were randomly collected on FTA cards in the major French potato production areas and analysed for molecular variation at 12 microsatellite (SSR) loci. It revealed :
- Clonal lineages structure and low genetic diversity
- Variations in lineage distribution according to regions and years
- 13_A2: predominant over the various temporal and special scales
- 6_A1 : highly frequent in western France, but almost absent in northern France
- 1_A : old lineage, at low frequency in all areas in 2016, but almost disappeared in northern France in 2018
- 37_A2: emergence in 2016 in North and East, with an extended distribution to western and central France in 2018
- 36_A2: also first detected in 2016 in North and East, but not recovered in 2017 and, on the opposite to 37_A2, rare in 2018.
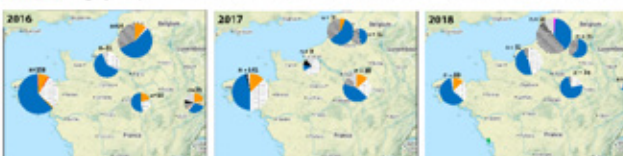
Clonal lineage frequency during the last three years, according to the main potato commercial areas

Phenotypic fitness

Isolations were also performed to obtain alive isolates in 2016 and 2017 for aggressiveness assays on detached leaflets of the susceptible cultivar Bintje. Aggressiveness traits (lesion size and sporangia production) were compared at 5 days post-inoculation.

Aggressiveness characterization under controlled conditions
- 10 leaflecs per isolate
- Incubation : 15°C (8h dark) and 18°C (light)

Number of isolates analysed per year and clonal lineage

Isolates /year and lineage	2016	2017
EU_1_A1	9	6
EU_6_A1	10	16
EU_13_A2	14	20
EU_37_A2	8	9

Aggressiveness traits analysis revealed :
- 13_A2 : the least aggressive isolates
- 6_A1 : the most aggressive ones
- 1_A1 : intermediate, and closed to 13_A2 isolates
- 37_A2 : different aggressiveness level according to the year
  - high in 2016, and closed to 6_A1 isolates
  - lower in 2017, and similar to 13_A2 ones

Discussion and Perspectives

- The three years sampling revealed that French *P. infestans* populations have undergone recent and continuous changes over time and locations.
- In northern France, the 37_A2 clone has displaced the established 1_A1, 6_A1 and 13_A2 lineages.
- Surprisingly, 37_A2 aggressiveness level has changed over the two years and decreased in 2017.
- The widespread occurence of the 13_A2 lineage and its persistence suggest its high potential to adapt to various agro-ecosystems; its many sub-clonal variants may help its adaptation to different environments.

- Climatic conditions could favor some lineages, such as 6_A1 in western France and Great Britain, with an oceanic climate.

These results suggest that :
- aggressiveness tested under controlled conditions does not completely reflect the lineage fitness in the field;
- additional factors, such as fungicide insensitivity, environmental conditions, resistant potato cultivars (*see poster, Mabon et al.*) may also be involved in the selection of *P. infestans* genotypes;
- further investigations are crucial to explain the invasive success of new lineages and their epidemic and fitness potential.

Fitness of different *Alternaria solani* isolates

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Fitness of different *Alternaria solani* isolates

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Background

- Early blight is getting more important in Germany due to climate change

- Quinone outside inhibitors (Qols) like azoxystrobin are very common fungicides

- Reduced fungicide sensitivity has already been reported several times

- **Reason:** F129L mutation in cytochrome b (complex III) of the mitochondria (Pasche et al. 2004)

Alternaria solani Genotype I and Genotype II in Germany

Since 2009 F129L mutations are present in Germany

Two different Genotypes were found (Leiminger et al 2014):

Alternaria solani type I – Europe: Intron at position A126

Alternaria solani type II – USA: No Intron at position A126

Does the genotype and/or mutation has an impact on the fitness?

Structure of F129L mutation site and Azoxystrobin

Azoxystrobin

Leu

Phen

Structural changes due to exchange from am no acid Phenylalanine (Phen) to Leucin (Leu) (model by R. Hückelhoven)

Spore germination
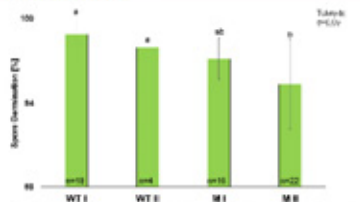
→ Significant difference between Mutant type II and both Wildtype I and II

Mycelium growth
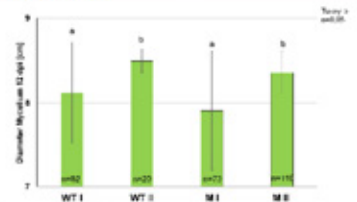
→ Genotype I is significantly different to Genotype II (independent from Wildtype or Mutant)

Material & Methods

Spore germination:

- 2 SNA plates per isolate
- Spore solution on H_2O- Agar
- Incubation for 5h and 26°C
- Counting of 100 spores per plate

Mycelium growth:

- 10 SNA plates per isolate
- Cultivating the plates under near UV light and 22°C
- Diameter measurement after 7 and 12 days
- Counting of 100 spores per plate

Summary & Conclusion

	highest	→		lowest
Mycelium growth	WT II [a]	M II [a]	WT I [b]	M I [b]
Spore germination	WT I [a]	WT II [a]	M I [ab]	M II [b]

→ Overall, F129L-Wildtype isolates show a higher fitness then F129L-Mutants (at least for spore germination and mycelium growth)

→ Hint for a possible shift in population structure towards more wildtype genotypes after suspension of Qols

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Solanum alandiae as a potential source of late blight resistance genes

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SUMMARY

The Bolivian species *S. alandiae* is among the insufficiently researched representatives of *Solanum* series Tuberosa. Accessions of *S. alandiae* and its hybrids with potato varieties were screened with specific SCAR markers of genes for resistance to *Phytophthora infestans* (*Rpi* genes). SCAR amplicons derived from *S. alandiae* genome included the structural homologues of several *Rpi* genes of broad specificity, such as *R2=Rpi-blb3* (94-98% identity), *R8* (98% identity), *R9/Rpi-vnt1* (95% identity) and *Rpi-blb2* (99% identity). *R9/Rpi-vnt1* homologues in *S. alandiae* and other South American potato species merit special interest in the context of evolution in *Solanum* series Tuberosa.

KEYWORDS

Phytophthora infestans, Solanum alandiae, Solanum okadae, late blight, resistance genes, *R2, R8, R9, Rpi-blb2, Rpi-vnt1,* SCAR markers

INTRODUCTION

To contain potato late blight (LB), the introgression breeding depends on continual search, identification, and deployment of new genes for resistance to *Phytophthora infestans* (*Rp*genes). When these genes are of broad specificity to pathogen races and in particular, when they come from the sources that have not been as yet widely exploited by breeders, such genes are not rapidly overcome by the *P. infestans* strains currently dominating the commercial potato plantations and in this way promise durable LB resistance. Many wild relatives and landraces of potato from South America are a prospective lode to mine for these genes (Bethke *et al.*, 2019; Gaiero *et al.*, 2018; Li *et al.*, 2018; Machida-Hirano, 2015; Vossen *et al.*, 2014). Among them, the Bolivian species *S. alandiae* Cárdenas (EBN 2x), a member of *S. brevicaule* Bitter complex (Spooner *et al.*, 2014), is among the insufficiently researched wild species of *Solanum* L. series Tuberosa Rydb. Wild. Some accessions of *S. alandiae* were reported to manifest considerable resistance to *P. infestans* (Bhardwaj *et al.,* 2018; Perez *et al.*, 1999/2000; Zoteyeva *et al.*, 2012); however, the *Rpi* genes of *S. alandiae* have been studied but sporadically (Srivastava

et al., 2018). Here we report on a pilot study of the *Rpi* genes in *S. alandiae* and its hybrids with cultivated potatoes from the VIR collection. Plant resistance to *P. infestans* was assessed in the laboratory and field experiments*,* and the resistant individuals have been maintained as the clonal collections. Plants were screened for structural homologues of the *Rpi* genes using PCR analysis of the plant genome with highly specific sequence characterized amplified region (SCAR) markers, and the identity of amplicons was recognized by comparative phylogenetic analysis of their sequences.

MATERIALS AND METHODS

Plant material and assessment of LB resistance
Several accessions of *S. alandiae* and its hybrids with potato varieties together with wild species and potato varieties that served as positive and negative controls in PCR screening for *Rpi* gene homologues with SCAR markers of these genes come from the clonal collections maintained in VIR and the Institute of Phytopathology (Fadina *et al.*, 2017). The accession of *S. okadae* K-25397 was introduced from the accession CGN 18279 (CGN Potato Collection, the Netherlands) kindly provided by Roel Hoekstra (Wageningen University, the Netherlands). Resistance of this accession to *P. infestans* was reported to depend on the *Rpi-vnt1-2* gene (Foster *et al.*, 2009). Resistance of wild species to *P. infestans* was assessed in the laboratory test with detached leaves according to the Eucablight protocol (www.euroblight.net/) using a highly virulent and aggressive isolate of *P. infestans* N161 (races 1.2.3.4.5.6.7.8.9.10.11, mating type A1) collected in the Moscow region (the collection of the Institute of Phytopathology) and var. Santé as a standard. LB resistance of potato hybrids and varieties was assessed in the field trials at the Institute of Phytopathology (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. The experimental data for LB resistance were transformed to 1-9-point scores.

SCAR markers for Rpi genes
The sequences of primers for SCAR markers recognizing the *Rpi* genes were compiled from several publications (Table 1). Primers for *R8* were further modified in our laboratory. The positions of the markers as related to gene sequences are presented at Figure 1. Primers were verified using potato genotypes reported to comprise the corresponding functional *Rpi* genes.

Table 1. *SCAR markers of Rpi genes*

Gene	Prototype gene	Marker and its size, bp	Position on the prototype genes, bp		Annealing temp., °C	Reference
R1	AF447489	R1-1205	5126-6331	F-cactcgtgacatatcctcacta R-gtagtacctatcttatttctgcaagaat	61	Sokolova *et al.*, 2011
R2=Rpi-blb3	FJ536325	R2-686	4215-5357	F-gctcctgatacgatccatg R-acggcttcttgaatgaa	54	Kim *et al.*, 2012
R3a	AY849382	R3a-1380	1677-3056	F-gtagtacctatcttatttctgcaagaat R-agccacttcagcttcttacagtagg	64	Sokolova *et al.*, 2011
Rpi-blb2	DQ122125	Rpi-blb2-976	3226-4202	F-ggactgggtaacgacaatcc R-atttatggctgcagaggacc	55	Van der Vossen *et al.*, 2005
Rpi-vnt1.3	FJ423046	Rpi-vnt1.3-612	89-701	F-ccttcctcatcctcacatttag R-gcatgccaactattgaaacaac	58	Foster *et al.*, 2009; Pel., 2010
R8	KU53015	R8-1276	73694-74970	F-aacaagagatgaattaagtcggtagc R-gctgtaggtgcaatgttgaagga	62.5	Modified after Vossen *et al.*, 2016
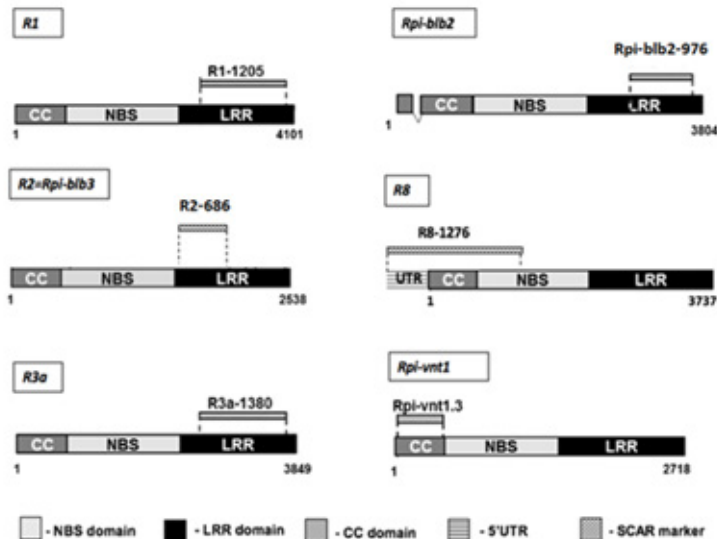
Figure 1. *SCAR markers of the Rpi genes*

RESULTS AND DISCUSSION

We screened 29 potato genotypes: six clones of *S. alandiae* K-21240 (K-21240 D17-329, K-21240-1 - K-21240-5), five clones of *S. alandiae* K-18473 (K-18473-1-K-18473-5), *S. alandiae* K-20408, *S. alandiae* K-19443, three clones of *S. okadae* K-25397 (K-25397-1 - K-25397-3), three parental varieties Atzimba, Elizaveta, Svitanok kievskiy and ten clones of interspecific hybrids (Table 2).

The *R1* and *R3a* genes was absent from *S. alandiae*, and the amplicons of both these genes in the hybrids are apparently derived from a *S. demissum* progenitor. We cloned and sequenced amplicons for the gene markers R2-686, Rpi-blb2-976, R8-1276 and Rpi-vnt1.3-612 derived from *S. alandiae* and *S. okadae* as a positive control (Table 2). Below we discuss only the sequences that are highly identical to the prototype genes and their orthologues with established *Rpi* function (Table 3).

The marker for the *R2* gene was found in *S. okadae* accession K-25397, in *S. alandiae* accession K-18473 (94% identical to the *R2* prototype gene from *S. demissum* and 97% identical to *Rpi-blb3* from *S. bulbocastanum,* DQ122125) and in Atzimba x *S. alandiae* hybrid. The accession of *S. alandiae* K-21240, which was used in the latter cross, was not saved for analysis; nevertheless, we presume that in the hybrid 24-2 (Atzimba x *S. alandiae*), the marker R2-686 arrived from *S. alandiae.* In the hybrid 24-2 x Svitanok kievskyi, both *S. alandiae* and *S. demissum* could be the sources of this marker. The *R2* cluster in the Mexican *Solanum* species on chromosome IV has been described in detail (Destafanis *et al.*, 2015; Lokossou *et al.*, 2009). Our data on *R2* structural homologues in *S. alandiae* and *S. okadae* complement previously reported evidence on *R2=Rpi-blb3* homologues on chromosome IV in another South American species, *S. microdontum* (Lokossou, 2010; Lokossou *et al.*, 2009).

Table 2. SCAR markers of the Rpi genes in S. okadae, S. alandiae and its hybrids with cultivated potatoes (1/0 – presence/absence)

Genotype	VIR accession, clone and pedigree*	R1-1250	R2-686	R3a-1380	Rpi-blb2-976	R8-1259	Rpi-vnt1.3-612	Resistance to *P. infestans*, points
S. alandiae	K-21240 D17-329	0	0	0	1	1	0	5
S. alandiae	K-21240-1	0	0	0	1	1	0	n. d.
S. alandiae	K-21240-2	0	0	0	1	0	0	n. d.
S. alandiae	K-21240-3	0	0	0	1	1	0	n. d.
S. alandiae	K-21240-4	0	0	0	1	1	0	n. d.
S. alandiae	K-18473-1	0	1	0	1	1	1	n. d.
S. alandiae	K-18473-2	0	1	0	1	0	1	n. d.
S. alandiae	K-18473-3	0	1	0	1	1	1	n. d.
S. alandiae	K-18473-4	0	1	0	1	0	1	n. d.
S. alandiae	K-18473-5	0	1	0	1	1	1	n. d.
S. alandiae	K-19443	0	0	0	1	0	0	5
S. alandiae	K-20408	0	0	0	1	1	0	3
S. okadae	K-25397-1	0	1	0	1	1	1	n. d.
S. okadae	K-25397-2	0	1	0	1	1	1	n. d.
S. okadae	K-25397-3	0	1	0	1	1	1	n. d.
Atzimba P1	n. d.	0	0	0	1	1	0	5
Elizaveta P2	acl, adg, dms, phu, sto, vrn	1	0	1	0	0	0	4
Svitanok kievskyi P2	dms (Victoria Augusta, Adretta)	0	1	1	0	1	0	5
24-1		0	0	0	1	1	1	6
24-2		0	1	0	1	0	1	6
117-1	Atzimba x *S. alandiae*	0	0	0	1	0	0	5
117-2		0	0	0	1	1	1	n. d.
25-1-2007	24-1 x Elizaveta	1	0	0	1	0	0	5
25-2-2007		1	0	0	1	0	1	4
134-6-2006		0	0	1	0	1	1	5
134-2-2006	24-2 x Svitanok kievskyi	0	0	0	0	1	1	6
135-1-2006		1	1	1	0	0	1	5
135-2-2006		1	1	1	0	0	0	4

*acl – S. acaule Bitter, adg – S. andigenum Juz. & Bukasov, aln – S. alandiae Cárdenas, dms – S. demissum Lindl., phu – S. phureja Juz. & Bukasov, sto – S. stoloniferum Schltdl., vrn – S. vernei Bitter & Wittm.

The marker of the *R8* gene was found in all accessions of *S. okadae* and in most individuals of *S. alandiae* and Atzimba x *S. alandiae* hybrids. In *S. alandiae*, all cloned markers are 98-99% similar to the *R8* prototype gene from *S. demissum* (KU530153). Four cloned sequences of R8-1259 marker from potato hybrid 24-1 are 94% similar to the prototype gene and R8-1259 marker from *S. alandiae*.

The marker Rpi-vnt1.3-612 was found in *S. okadae* and in *S. alandiae* K-18473; it was absent from Atzimba and three other accessions of *S. alandiae* and present in most Atzimba x *S. alandiae* hybrids. The sequences of the marker for *Rpi-vnt1* gene from hybrids are 84-89% similar to the *Rpi-vnt1-3* gene, whereas the markers from *S. alandiae* and *S. okadae* were 92-93% identical to this gene. Mining for *Rpi-vnt1* alleles across *Solanum* section Petota showed that the three functional alleles were confined within *S. venturii,* whereas only two accessions of the closely related species *S. weberbaueri* Bitter and *S. mochiquense* Ochoa carried *Rpi-vnt1.1* (Pel, 2010). Three orthologues of *Rpi-vnt1* from *S. venturii* and *S. alandiae* are 87-89% identical to the 3307-5925-bp stretch in *R9a* sequence (Armstrong *et al.*, 2019); whereas the region of *Rpi-vnt1.3* in *S. alandiae* spanning 809-1374 bp in the sequence FJ423044 is 99% identical to the corresponding fragment of *R9a*. Comparison of the *Rpi-vnt1* structural homologues from *S. alandiae* with the already reported *Rpi* homologues from other South American species (Pel, 2010; van Weymers *et al.*, 2016) showed 90-94% identity; in particular, the sequences of *Rpi-vnt1* from *S. alandiae* resemble those in *S. okadae* (GU338334) and *S. raphanifolium* (GU338338) with 94% identity. While the *R9* gene is characteristic of the Mexican species *S. demissum*, no *Rpi-vnt1* homologues have been reported to this day in the Central American species.

Table 3. *Structural homologoues of the Rpi genes from S. alandiae as compared to the prototype Rpi genes and their already identified orthologues/homologues, % identity*

Prototype	R2=Rpi-blb3 S. bulbocasta-num DQ122125	R2=Rpi-blb3 S. demissum FJ536325	R2/Rpi-hjt1 S. hjertingii GU563971	R2/Rpi-edn1 S. edinense GU563963	R2/Rpi-blb3 S. okadae*	R2-like S.bulbocasta-num FJ536331	R2/Rpi-snk1 S. schenckii GU563975	R2-like S. demissum FJ536331
R2/Rpi-blb3 from *S. alandiae*	97	94	97	97	98-99	92	97	94
Prototype	*Rpi-blb2 S. bulbocasta-num DQ122125*	*Mi-1.2 S. lycopersi-cum AF091048*						
Rpi-blb2 from *S. alandiae*	91	86						
Prototype	*R9a (Armstrong et al., 2019)*	*Rpi-vnt1.1 S. venturii FJ423044*	*Rpi-vnt1.2 S. venturii FJ423045*	*Rpi-vnt1.3 S. venturii FJ423046*	*Rpi-vnt1-like S. okadae GU338336*	*Rpi-vnt1 S. okadae K-25397*	*Rpi-vnt1-like S. micro-dontum GU338312*	
Rpi-vnt1.3 from *S. alandiae*	86-89	92-93	92-93	92-93	88-89	97-98	90-91	
Prototype	*R8 S. demissum KU530153*	*R8 S. okadae K-25397*	*Sw5-b S. lycopersi-cum AY007366*					
R8 from *S. alandiae*	99	94	85					

*S. okadae - Rpi-vnt1.3-612 cloned from the accession K-25397 in this study

Figure 2. *The structural homologues of the Rpi-vnt1 gene from of S. alandiae and S. okadae as compared to already published sequences of Rpi-vnt1 genes from several South American species (Pel, 2010) and R9a (Armstrong et al., 2019)*

S. alandiae – Rpi-vnt1.3-612 cloned from the accession K-18473 in this study, *S. okadae – Rpi-vnt1.3-612 cloned from the S. okadae Hawkes & Hjert. accession K-25397 in this study, S. bukasovii Juz. GU338315, S. microdontum Bitter GU338312, S. stenotomum Juz. & Bukasov GU338323, S. sucrense Hawkes GU338339, S. raphanifolium Cárdenas & Hawkes GU338338, S. orophilum Correll GU338320, S. medians Bitter GU338344, S. neorossii Hawkes & Hjert. GU338331, S. oplocense Hawkes GU338317, S. okadae GU338334, S. tarijense Hawkes GU338326

CONCLUSION

Genomes of *S. okadae*, *S. alandiae* and interspecific hybrids comprising genetic material from *S. alandiae* contain the structural homologues of genes *R2, Rpi-blb2*, *R8* and *R9* well known to provide the broad specific resistance to *P. infestans*. The homologues of *R2=Rpi-blb3* were 94-99% identical to the prototype genes in *S. bulbocastanum* and *S. demissum* and their orthologues in several other Mexican species, such as *S. edinense, S. schenckii* and *S. hjertjingii*. Among South American potatoes, the closest homologies to these structures is the sequence from *S. microdontum,* which is evolutionarily and functionally different from *S. demissum R2* (Lokossou, 2010; Lokossou *et al.*, 2009; Vossen *et al.*, 2014).

The *Rpi-vnt1* gene is apparently restricted to the South American species of *Solanum* series Tuberosa. The structural homologues of this gene in *S. alandiae* are 92-97% identical to the already known sequences from several species from Argentina, Bolivia and Peru. The evaluation of the breeding potential of the reported structural homologues must wait until we will clone the full-size sequences of the presumed genes and assess their function as the *Rpi* genes.

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Outcome of sexual reproduction in the *Phytophthora infestans* population in the Baltic countries

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Outcome of sexual reproduction in the *Phytophthora infestans* population in the Baltic countries

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INTRODUCTION

Potato crop losses can be substantial in the Baltic countries when the conditions for late blight (*Phytophthora infestans*) development and spread are favorable. These countries have similar potato growing traditions and agricultural background and most people still grow potato for their own food supply. The small conventional farms have potato fields small in size (<1 ha) and located nearby, crop rotations are short and chemical control of late blight is limited. In contrast, large conventional production farms use high-quality certified potato seeds, keep crop rotation at least 3 years, and apply sufficient disease control measures. Nevertheless, initial inoculum from the small fields, which are unprotected against late blight is a serious threat to seed and large production fields especially under late blight favorable conditions.

In this region during the winter the temperature drops below 0°C and the snow covers the ground limiting the asexual survival of the pathogen from one season to another. However, during the sexual phase of its life cycle two isolates with opposite mating types (A1 and A2) mate and produce oospores, which survive unfavorable conditions.

For the production sustainability efficient control of the late blight should be applied aided by the up-to-date knowledge about the pathogen population. Monitoring *P. infestans* population spatio-temporal variation could be useful in making appropriate disease management decisions with respect to cultivar selection and fungicide application.

MATERIAL AND METHODS

- Collecting *P. infestans* isolates from potato late blight outbreaks in 2010-2017 from potato fields in Estonia, Latvia and Lithuania.
- Growing isolates on ryeB agar media at 17°C.
- DNA extraction from the harvested mycelium with DNeasy Plant Mini Kit (QIAGEN).
- Genotyping in The James Hutton Institute with SSR marker 12-plex method following Li et al. (2013) [1].
- Data analysis in MS Office Excel 2013 Add-in GenAlEx version 6.501 and package poppr 2.0.2 within R v.3.1.0

Table 1. Genetic diversity in Estonian (EE), Latvian (LV) and Lithuanian (LT) *Phytophthora infestans* populations in 2010-2012 based on analysis of 11 SSR markers.

	Estonia	Latvia	Lithuania
N	141	125	69
MLG	94	77	44
eMLG	54.8	49.5	44
SE	2.52	2.59	0
A	0.985	0.977	0.903
Es	0.819	0.724	0.378
Hs	0.886	0.845	0.766
Hexp	0.519	0.538	0.490
Ia	0.037	0.011	0.103
P	0.210	0.365	0.096
$\bar{r}_d$	0.004	0.001	0.011
P	0.201	0.394	0.091

Statistics given are the no. of isolates (N), the no. of original multilocus genotypes (MLG), the no. of expected MLG at the smallest sample size based on rarefaction (eMLG), Simpson index (A), evenness (E5), normalized Shannon diversity index (Hs), Nei's unbiased gene diversity (Hexp), the index of association (IA) and the standardized index of association ($\bar{r}_d$) with P-values from 999 permutations calculated based on a clone corrected dataset.

Figure 1. Genotype EU_13_A2 map in 2015-2017 in the Baltic countries.
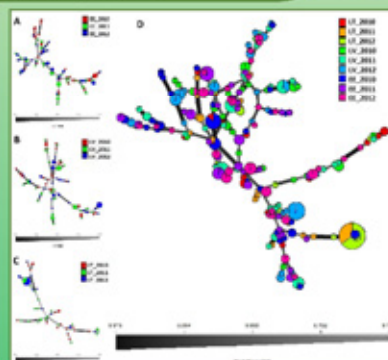
Figure 2. Minimum-spanning network of Estonian (A), Latvian (B), Lithuanian (C) and the Baltic (D) *P. infestans* populations in 2010-2012. Each MLG is represented by one node sized in proportion to its frequency in the populations. Line width and shading represent relatedness; line length is arbitrary.

RESULTS AND CONCLUSION

- Populations adapted in the Baltics are reproducing sexually, which increases pathogen fitness via higher genetic diversity and production of oospores as a source of primary inoculum in the soil. Also short crop rotations between growing potatoes in the small fields facilitate oospore-driven epidemics [2,3].
- Pathogen genotypes do not spread widely within that growing season or re-appear in the following year. Only limited spread and survival of local clones was detected in the region, but also few MLGs were common to EE and LT populations [2,3].
- Clonal lineages common to Western European *P. infestans* populations were not detected in the Baltic countries before 2015 when the invasive genotype EU_13_A2 was found in EE and next years in LT.
- Late blight studies in 2010-2017 revealed that the Baltic *P. infestans* populations are similar to other populations in the Northern and North-Eastern Europe where local clones are adapted to regional climatic conditions, agricultural practices and common host varieties. Most of the fields have been infected with various *P. infestans* genotypes and the spread of the local clones is limited [2,3].

SUGGESTIONS

For effective disease management, current strategies should be adjusted according to the epidemiology of pathogen populations in the region. Potato growers should implement late blight preventive measures such as longer field rotation to prevent oospore infections, especially in Estonia and Latvia, and to grow more disease resistant cultivars for the limiting effect on asexual reproduction of the pathogen as well as on oospore production in case of co-infection of sexually compatible *P. infestans* isolates.

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Structure and dynamics of *Phytophthora infestans* clonal populations as related to potato resistance

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Structure and dynamics of *Phytophthora infestans* clonal populations as related to potato resistance

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Developing more sustainable cropping systems has become a major challenge in the current context of pesticide reduction. A promising way for a complete IPM strategy is to combine different types or levels of host resistance with other control practices. However, pathogens may adapt or overcome host resistance. In the PoTStaR project, funded by "Agence Française pour la Biodiversité" as a part of the national action plan ECOPHYTO II, we investigate the effect of selection by resistant potato genotypes on *Phytophthora infestans* populations. We monitored the composition and pathogenicity of *P. infestans* samples in field trials at the beginning of natural epidemics on the susceptible cultivar Bintje, and then at the end of the season on different resistant genotypes.

1 The PoTStaR project objectives

❖ To evaluate potato late blight resistance of 258 potato genotypes, in naturally infected multilocal field trials: Improved breeding lines from different research programmes maintained in the INRA BrACySoI Biological Resource Center *(not presented in this poster)*

❖ To develop molecular markers linked to late blight resistance: Use SNP data and genetic association mapping. *(not shown in this poster)*

❖ To follow the dynamics of *Phytophthora infestans* clonal populations on resistant potato genotypes.

2 Experimental sites

❖ Two field trials in the North and in the West of France with two different local late blight populations. *(Results shown in poster by M. Guibert)*

Geographic position of the two fields trials
Auchy-les-Mines in northern France and Ploudaniel in western France with different *P. infestans* clonal lineages in each location

3 Sampling characteristics

❖ Sampling strategy in each site in 2018
  • 13 to 19 samples on the susceptible cultivar Bintje at the beginning of epidemics.
  • 15 to 27 samples at the end of season on different genotypes showing partial resistance.

❖ Isolate characterization
  • Genotyping with 12 SSR markers *(European reference method)*
  • Virulence characterization on Black's differential set, in laboratory on detached leaflets.

4 Genotypic characterization of *P. infestans*

Auchy-les-Mines

Population on Bintje n=13

Population on resistant potato genotypes (n= 15)

Population in Auchy-les-Mines, 2018
• the 1/_A2 clonal lineage mainly found in this trial on Bintje at the beginning of the epidemics.
• the 13_A2 clonal lineage mainly recovered in this trial on resistant potato genotypes at the end of the season, except on genotype 03T.9.8

Ploudaniel

Population on Bintje n=19

Population on resistant potato genotypes (n= 27)

Population in West of France, 2018
• the population on Bintje in this trial matches with the population of this potato region, mainly composed of 13_A2, 6_A1 and 1_A1 clones.
• the 13_A2 clonal lineage mostly sampled in this trial on resistant potato genotypes at the end of the epidemics, except on genotype 03T.9.8

❖ Resistant potato genotypes have been in contact with all major clonal lineages present in France.

❖ Isolates sampled on Bintje are different from isolates on resistant potato genotypes.

❖ Two different behaviors of potato genotypes with partial resistance to late blight:
  • Genotype 03T.9.8 is infected by all major clonal lineages.
  • Other potato genotypes are only infected by the 13_A2 lineage.

Both sites together

Population on Bintje n=32

Population on resistant potato genotypes (n= 42)

Population in France in 2018

Legend : The colors indicate clonal lineages: 1_A1, 6_A1, 13_A2, 36_A2, 37_A2, 39_A1, others. Samples for which the genotyping failed.

5 Virulence characterization

Number of R genes overcome by the main clonal lineages (in %)

37_A2 n=8	
13_A2 n=7	
6_A1 n=12	
1_A1 n=6	

Legend: The colors indicate the number of R genes overcome by the isolate: 11 genes, 10 genes, 9 genes, 8 genes, 7 genes, 6 genes, 5 genes.

❖ More than 50% of isolates are virulent on:
  • The 11 R genes : 13_A2 lineage
  • 8 R genes : 37_A2 lineage
  • 6 to 7 R genes : 1_A1 and 6_A1 lineages

Percentage of virulent isolates against 5 R genes, for each clonal lineage

Legend: R2, R5, R6, R8, R9

❖ R genes overcome:
  • R9 by 13_A2 lineage only;
  • R5 by 37_A2 and 13_A2 lineages;
  • R8 by 6_A1 and 13_A2 lineages.

❖ R1, 3, 4, 7, 10, 11 are generally overcome by all the isolates.

6 Conclusion

❖ Resistant potato genotypes influence the structure and the dynamics of *Phytophthora infestans* populations.

❖ In our experiment, the 13_A2 clonal lineage is selected over time by some resistant potato genotypes.

❖ The highly complex virulence patterns of 13_A2 isolates may explain its selection by potato genotypes with race-specific resistance genes.

❖ Two types of behaviors in the resistant potato genotypes studied in this experiment:
  • One type with an high level of selection in *P. infestans* populations = impact of R genes?
  • Another type without selection pressure in the *P. infestans* populations = quantitative resistance?

❖ This study emphasizes the need to incorporate diverse resistance sources, especially non-specific ones, into breeding programs to avoid selection, spreading and dominance of specific *P. infestans* genotypes.

Field disease progress curves of R potato genotypes

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The repertoire of Avr genes in two East European populations of *Phytophthora infestans*

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SUMMARY

Single-strand conformation polymorphism (SSCP) analysis was employed to assess avirulence genes (*Avr* genes): *Avr1*, *Avr2*, *Avr2-like*, *Avr3a*, *Avr3b*, *Avr4*, *Avr8*, *Avr9*, *Avr-blb1*, *Avr-blb2* and *Avr-vnt1* in 20 single-cell lines obtained from the isolates of *P. infestans* collected in the VIR potato genetic collection (Pushkin, Leningrad region), in 16 lines from the isolates collected in the potato stationary field plots of the Institute of Phytopathology (Ramenki, Moscow region), and in seven reference lines from the Western Europe and the USA. SSCP patterns of *Avr* genes were collated with the profiles of simple sequence repeats of these *P. infestans* lines and the complements of the genes for resistance to *P. infestans* in potato genotypes that were colonized by particular pathogen strains.

KEY WORDS

Phytophthora infestans, potato late blight, *Avr* genes, *Rpi* genes, allelic polymorphism, SSCP analysis, SSR genotyping

INTRODUCTION

Elucidating the repertoire of avirulence genes (*Avr* genes) that determine the pathogenicity of *P. infestans* strains would help better understand the mechanisms of rapid pathogen evolution that brings forth the epidemic development of late blight (LB). As an agronomic projection, the express assessment of the repertoire of *Avr* genes in particular agrocenoses would promote early discernment of the changes in pathogen populations crucial for diagnosing LB development and expansion.

Among the *Avr* genes of *P. infestans*, best known are those producing RXLR effectors (Raffaele and Kamoun, 2012). Potato resistance to LB evoked by these virulence genes is usually described by the Flor's "gene-for-gene" paradigm (Flor, 1971) presuming that each RXLR effector is specifically recognized by the corresponding receptor kinase, the product of the race specific gene for resistance to *P. infestans* (*Rpi* gene). The virulence genes of *P. infestans* are

conventionally recognized using the Mastenbroek-Black set of differential plants (Kim *et al.*, 2012) comprising the individual *Rpi* genes. The molecular genetic analysis of *Avr* genes has greatly expanded the scope of identified virulence genes in *P. infestans* and helped evaluate their polymorphisms immediately reflecting their evolution; however, in contrast with the differential set, the molecular studies do not always discriminate between the virulent and avirulent forms of the particular gene.

The SSCP analysis of *Avr* genes of *P. infestans*, when verified by cloning and sequencing individual DNA fragments that comprise SSCP patterns, turned out a robust method of comparing *Avr* profiles in several populations of *P. infestans*. Here we collated SSCP patterns in two East European populations of *P. infestans*, in Leningrad and Moscow regions of Russia, with the SSR profiles of these populations and the spectra of *Rpi* genes in potato plants colonized by these pathogen strains. Some data reported here were previously reported elsewhere (Martynov *et al.*, 2019; Sokolova *et al.*, 2017).

MATERIALS AND METHODS

LB-affected leaves were collected in 2015 in the potato stands of the VIR field genetic collection in Pushkin, St. Petersburg, and in 2018, in the potato stationary field plots of the Institute of Phytopathology (Ramenki, Moscow). *P. infestans* isolates were processed in the Institute of Phytopathology as described elsewhere (Sokolova *et al.*, 2017). Following the SSR genotyping of *P. infestans* isolates collected in 2013 and 2014 from individual potato plants in Pushkin, we presumed that some individual plants in the VIR collection were colonized mutually by at least two different *P. infestans* genotypes. For this reason, single-cell lines were obtained from the 2015 Pushkin and 2018 Ramenki isolates, and further studies of *Avr* genes were carried out only with these lines. In addition, we investigated seven reference lines from the Western Europe and the USA provided as DNA samples by Dr David Cooke (The James Hutton Institute, Dundee, UK) and a highly virulent and aggressive line 161 (races 1.2.3.4.5.6.7.8.9.10.11, mating type A1) collected in the Moscow region (the State Collection of the Institute of Phytopathology). All *P. infestans* lines under study were genotyped at 12 SSR loci (Li *et al.*, 2013).

Two approaches were used to evaluate the repertoire of *Avr* genes and their polymorphisms. Full-length gene sequences were PCR-amplified from genomic DNA with the previously described primers, the amplicons were cloned, and ten randomly selected clones per line were sequenced; the sequences thus obtained were compared with the *Avr* genes identified in the fully sequenced genome of the *P. infestans* isolate T30-4 (prototype genes) and other *Avr* sequences of *P. infestans* deposited in the NCBI Genbank. In the case of SSCP analysis, amplicons obtained with primers known from the literature or developed by the authors were subjected to thermal and chemical denaturation and electrophoretic separation in a non-denaturing polyacrylamide gel. DNA fragments corresponding to the zones of different mobility were excised from gel, and DNA fragments were eluted, cloned and sequenced. The sequences thus obtained were compared with the *Avr* genes identified in the same *P. infestans* lines using the first approach as well as with the sequences deposited in the NCBI Genbank. SSCP patterns are well reproducible, and when the individual electrophoretic zones are validated by sequencing, these patterns can serve as reliable barcoding descriptors for deciphering the *Avr* polymorphisms. Our protocols for DNA isolation, SSR genotyping and SSCP analysis followed previously described standard procedures (Martynov *et al.*, 2019; Sokolova *et al.*, 2017).

RESULTS AND DISCUSSION

*Distribution of allele variants of Avr genes in the geographically distant samples of P. infestans*
We explored the polymorphisms of eleven *Avr* genes of *P. infestans*: *Avr1, Avr2, Avr2-like, Avr3a, Avr3b, Avr4, Avr8=Avr-Smira2, Avr9=Avr-Smira1, Avr-blb1=ipiO, Avr-blb2*, and *Avr-vnt1* in 36 single-cell lines from Pushkin (Leningrad region) and Ramenki (Moscow region) and the reference lines with genotypes 4_A1, 8_A1, 5_A1, US8, EC-1, 13_A2 and 6_A1 (C1, C2, C4, C6, C7, 3928a and 4100a, respectively) from the Western Europe and the USA plus the line 161 from the collection of the Institute of Phytopathology. The *Avr2-like* gene is considerably different from *Avr2* (Gilroy *et al.*, 2011), and therefore we treat it separately.

The allelic profiles of the genes *Avr3b, Avr4* and *Avr8* as revealed by SSCP analysis were monomorphic, and all other profiles were very polymorphic (Figure 1). Three geographically diverse samples of *P. infestans* widely differed in their SSCP patterns and in the frequencies of these patterns. In the case of the Pushkin and Ramenki pathogen populations, such differences apparently stem from the fact that different potato varieties with dissimilar *Rpi* genes are grown in these two regions of the European Russia, under diverse agroclimatic conditions. The reference lines seem to reflect even more remote situations as regards climate and potato varieties colonized by *P. infestans* lines.

The *Avr1* gene was present in all Pushkin line of *P. infestans* and 72% of the reference lines, whereas it was considerably less frequent in the Ramenki lines (29%). All *Avr1* sequences reported in this study correspond to virulent alleles and differ in several single nucleotide substitutions, two of them apparently representing evolutionary hotspots. The absence of the avirulent *Avr1* allele can probably reflect high frequency of the *R1* gene in potato varieties grown in this area. Pattern 2 of the *Avr2* gene dominated the Pushkin and Ramenki lines (80 and 88%, respectively) as compared to 57% in the reference lines. This allele corresponds to already described $Avr2^K$ gene recognized by the *R2* gene (Saunders *et al.*, 2012). With Avr2 effector recognized by R2 kinase, one would expect that plants comprising the *R2* gene would manifest resistance to most *Avr2* lines in this study. However, as reported recently, the presence of the intact *Avr2* gene does not always imply that it is expressed (Stefańczyk *et al.*, 2017). The virulent homologue *Avr2-like* corresponded to alleles $Avr2-like^{TV}$ and $Avr2-like^{MI}$; this homologue dominated all pathogen populations under study and was most polymorphic.

All Pushkin and Ramenki lines of *P. infestans* contained only virulent variants of the *Avr3a* gene, whereas the reference lines also comprised the heterozygotes combining the virulent and avirulent alleles. Here we observed only random and synonymic substitutions. In contrast, the *Avr3b* gene was found only in some Pushkin and reference lines, whereas in the Ramenki lines its frequency was as high as 43%. *Avr3b* sequences described here differ by several SNPs from those previously reported. Characteristically, the Pushkin and Ramenki lines were similar in this aspect and differed from the reference lines. The *Avr9* gene was found in all Pushkin and most Ramenki and reference lines of *P. infestans* and was represented by its virulent allele except in the Pushkin line 87/2-2 and the Ramenki lines 194, 3 and 5. The presence of the virulent *Avr9* gene in *P. infestans* populations would decrease LB resistance in potato varieties comprising the *R9=Rpi-Smira1* gene.
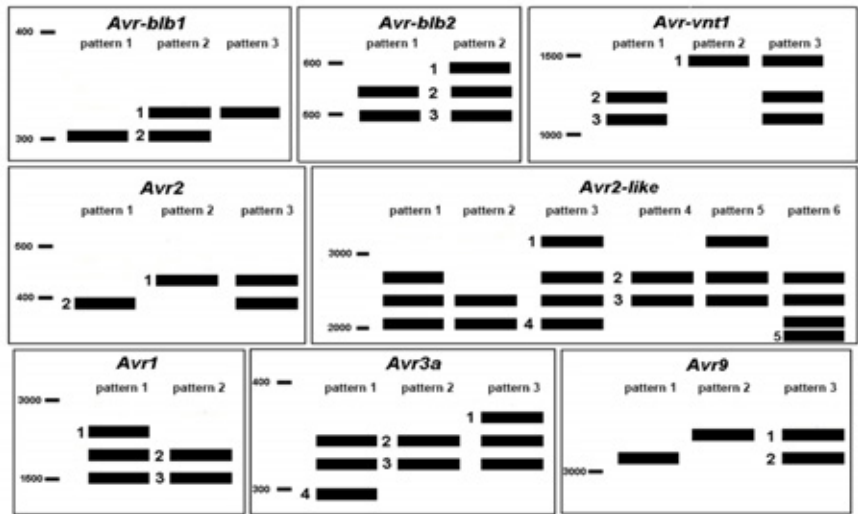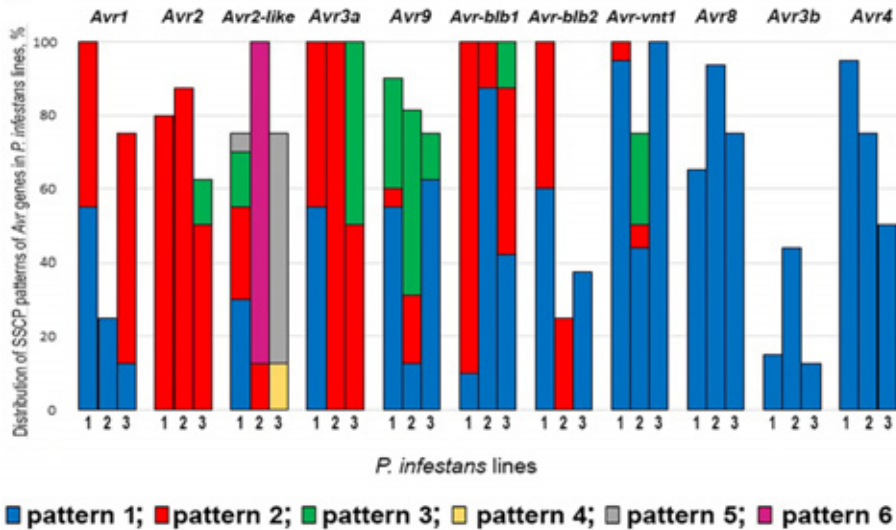
Figure 1. A. SSCP patterns of Avr genes in P. infestans lines. B. Frequencies of particular Avr patterns in P. infestans samples under study. 1 - the Pushkin lines; 2 – the Ramenki lines; 3 – the reference lines

While the *R9* and *Rpi-vnt1* genes are structural homologues (Aguilera-Galvez *et al.*, 2018), we failed to recognize any extended homology between the *Avr9* and *Avr-vnt1* sequences, except in the conserved domains RXLR and s/dEER. The case of *Avr2* is directly opposite: the corresponding effector is recognized by two genes of diverse structures, *R2* and *Rpi-mcq1* on chromosomes IV and IX (Aguilera-Galvez *et al.*, 2018). Apparently, in these cases the structural evolution of gene predecessors was ahead of their functionalisation.

We found the *Avr-vnt1* gene in all Pushkin and reference lines and in many Ramenki lines. Earlier the gene was reported to exist in three allelic variants V1, V2 and V3 (Pel, 2010). Our samples were dominated by the V1/V3 combination and the homozygous form V2 was found in the lines 87/2-2 and 163. All three alleles combined only in the Ramenki lines.

The family of *Avr-blb1* genes is very polymorphic, and only distinct classes in this family differ in their functions. We therefore designed class-specific primers recognizing the conserved stretches of these genes. All lines contained one variant of the *Avr-blb1* gene, predominantly of class I, whereas line 5_A1 comprised only class II gene. Class I alleles of the *Avr-blb1* gene are recognized by the *Rpi-blb1* gene and therefore are avirulent, while class II genes are virulent only in the absence of the class I genes (Champouret *et al.*, 2009). We therefore conclude that all *P. infestans* lines under study will turn avirulent towards potato hybrids comprising the *RB/Rpi-blb1* gene.

Avirulent and virulent alleles of the *Avr-blb2* gene (Oh *et al.*, 2009) produce a contrasting pattern in the lines under study. The highly aggressive lines 13A_2 and 6A_1 comprise various avirulent alleles of this gene. It follows that the hybrids comprising the *Rpi-blb2* gene are prospective sources for potato breeding.

Most lines under study contained the *Avr8* gene with its sequence completely identical to the prototype gene in the isolate T30-4. High conservation of this gene was previously reported by Jo (Jo, 2013).

The phylogenetic analysis of Avr genes based on sscp patterns
The diversity of SSCP patterns that characterize the *Avr* genes in three samples of *P. infestans* lines fall into five clusters (Figure 2A).
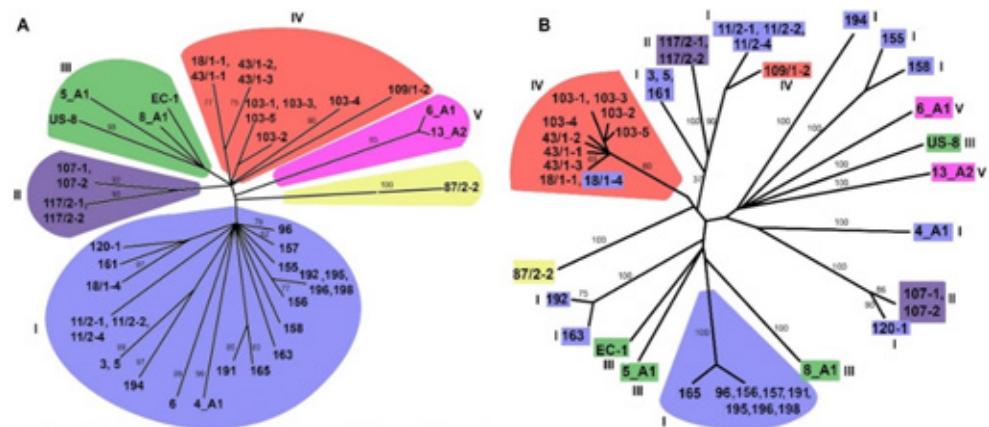
Figure 2. *The phylogenetic analysis of SSCP patterns of P. infestans lines based on (A) seven polymorphic Avr genes and (B) 41 polymorphic SSR loci. The dendrogram is built with SplitsTree 4.10 using uncorrected P with gaps included in the analysis and a UPGMA network. Bootstrap support for main clades (1000 replicates) is indicated by the smaller numbers on specific branches. The Roman numbers and colour of clusters in Figure 2B follow Figure 2A*

Half of Pushkin lines share cluster I with all Ramenki lines plus line 161, also collected in the Moscow region; here we also find the reference line 4_A1. Besides, many Pushkin lines are found in cluster IV and some in cluster II. Single-cell lines produced from one and the same isolate are usually clustered together. The strikingly aggressive West European lines 6_A1 and 13_A2 (Cooke *et al.*, 2012) form the isolated cluster V, whereas most other reference lines are in cluster III. The isolated position of 6_A1 and 13_A2 lines is apparently related to the fact that these lines are devoid of several *Avr* genes, such as *Avr1*, *Avr4*, *Avr8* and *Avr9*; in addition, 13_A2 also lacks the *Avr2* gene.

SSR genotyping produced less compact clusters (Figure 2B); nevertheless, the general arrangement of pathogen lines in clusters I, IV and V is compatible in two cases. The line 87/2-2 characterized by low aggressiveness and predominantly avirulent alleles holds a specific position in both SSCP and SSR distributions.

The profiles of the Avr genes in P. infestans lines as compared to the complements of late blight resistance genes in colonised potato plants

The composition of *Rpi* genes in potato genotypes colonized by *P. infestans* lines under study has been established previously (Fadina *et al.*, 2017). The profiles of *Avr* and *Rpi* genes and virulence factors registered with the differential set are presented in Table 1. Within the gene-for-gene paradigm, the profiles of *Avr* and *Rpi* genes match pretty well, except in the case of the *Avr3b* gene. This gene was found in the Pushkin line 11/2 and the Ramenki lines 155, 156, 157 and 196, which colonized potato hybrids comprising the *R3b* gene. However, the *Avr3b* sequences described in this study contain several non-synonymic substitutions, which would prevent its recognition by the corresponding kinase and render the gene non-functional. In hybrids 14/8-09 and 120 (118/6-2011) in var. Robijn, we registered marker R2-1137 and did not find marker R2-686, another marker of the same gene (Fadina *et al.*, 2017) indicating some changes in the gene structure. In this case, we treated this gene as absent. In the pathogen lines derived from potato hybrids comprising the *Rpi-blb2* gene, we found the virulent alleles of *Avr-blb2* (Oh *et al.*, 2009).

In four cases from the Pushkin population, 11/2-2 and 11/2-4, 43/1-1 and 43/1-2, 18/1-1, and 103B, the lines derived from the same isolates were dissimilar by one *Avr* gene presuming the possibility that an individual plant was simultaneous colonized by two independent although related pathogen lines.

Table 1. Repertoire of Avr genes as compared to the complement of virulence factors assessed with the Mastenbroeck-Black differentials and the profile of the Rpi genes

Potato clones; *Rpi* genes	Lines	*Avr* genes	Virulence factors assessed with differential plants
Pushkin lines			
139 (4/1-2012); *R1, Rpi-blb1/Rpi-sto1, Rpi-blb2*	120-1	*avr1, Avr2_K, avr3a_EM, avr4, Avr8, Avr9* (I)*, Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	12347891011
106 (171-3); *R3b*	11/2-1	*avr1, Avr2-like_TV/MI, avr3a_EM, Avr3b, Avr8, Avr9* (I, II)*, Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	134781011
113(50-1KWA); *R1, Rpi-blb2*	103-1, 103-2, 103-3, 103-5	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8, Avr9* (I)*, Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	123467891011
27; *R1, Rpi-blb1, Rpi-blb2*	107-1	*avr1, Avr2_K, avr3a_EM, avr4, Avr9* (I)*, Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	123478910
Robijn	87/2-2	*avr1, Avr2_K, Avr2-like_TV/MI, avr3a_EM, avr4, Avr8, Avr9* (II)*, Avr-blb1* (I)*, Avr-blb2, Avr-vnt1* (V2)	12378
160-17; *Rpi-blb2*	43/1-1	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8, Avr9* (I)*, Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	12347891011
18/40-2000; *R1, Rpi-vnt1.3*	18/1-1	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8, Avr9* (I)*, Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	1234567891011
18/40-2000; *R1, Rpi-vnt1.3*	18/1-4	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8*, *Avr-blb1* (I, II)*, Avr-blb2, Avr-vnt1* (V1, V3)	1234567891011
Ramenki lines			
2372-60; *R1, R2, R3a, R3b*	96	*Avr2_K, Avr2-like_MI, avr3a_EM, Avr8, Avr-blb1* (I)*, Avr-vnt1* (V1/V3)	nd
13/11-09; *R3b, Rpi-blb1, Rpi-vnt1.3*	155	*Avr2_K, Avr2-like_MI, avr3a_EM, Avr3b, Avr-blb1* (I)*, Avr-vnt1* (V1/V3)	nd
14/8-09; *R3a, R3b, Rpi-vnt1.3*	156	*Avr2_K, Avr2-like_MI, avr3a_EM, Avr3b, Avr8, Avr9* (I, II)*, Avr-blb1* (I)	nd
16/27-09; *R1, Rpi-blb1, Rpi-blb2*	158	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, Avr3b, avr4, Avr8, Avr9* (I, II)*, Avr-blb1* (I)*, Avr-blb2, Avr-vnt1* (V1/V3)	nd
18/40-2000; *R1, Rpi-vnt1.3*	163	*Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8, Avr9* (I)*, Avr-blb1* (I)*, Avr-blb2, Avr-vnt1* (V2)	nd
134-6-2006; *R3b, Rpi-vnt1.3*	194	*avr1, Avr2_K, avr3a_EM, avr4, Avr8, Avr9* (II)*, Avr-blb1* (I)	nd
135-1-2006; *R2, R3a, R3b, Rpi-vnt1.3*	196	*Avr2_K, Avr2-like_MI, avr3a_EM, Avr3b, Avr8, Avr9* (I, II)*, Avr-blb1* (I)*, Avr-vnt1* (V1/V3)	nd
Easterling	3	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8, Avr9* (II)*, Avr-blb1* (I, II)*, Avr-vnt1* (V1/V2/V3)	nd
Robijn	5	*avr1, Avr2_K, Avr2-like_MI, avr3a_EM, avr4, Avr8, Avr9* (II)*, Avr-blb1* (I, II)*, Avr-vnt1* (V1/V2/V3)	nd
Gloria; *R1, R3a, R3b*	6	*Avr2-like_MI, avr3a_EM, Avr8, Avr9* (I, II)*, Avr-blb1* (I)	nd

Avr9 (I) and Avr9 (II), virulent and avirulent alleles, respectively; nd, no data

In the history of introgression breeding, some *Rpi* genes, such as *R1* and *R2*, were early arrivals to potato varieties, as compared to *R9, Rpi-blb1, Rpi-blb2,* and *Rpi-vnt1*, which are met in a small number of new varieties and hybrids. Meanwhile many *P. infestans* lines described in this communication comprise the *Avr-blb2* and *Avr-vnt1* genes corresponding to the *Rpi* genes as yet uncommon in potato varieties. Similarly, the lines 6_A1 and especially 13-A2, which are devoid of the *Avr* genes corresponding to the typical profiles of *Rpi* genes in widespread potato varieties, rapidly expand, displace the already present pathotypes and inflame most dramatic LB epidemics (Cooke *et al.*, 2012; Knaus *et al.*, 2019). Of special interest is the line 87/2-2, which differs from all other lines in both the SSCP and SSR patterns (Figure 2). This line colonised the susceptible var. Robijn, with the single *R2* gene; this line comprises mostly avirulent alleles of the *Avr* genes.

CONCLUSIONS

Screening, by SSCP analysis, *P. infestans* lines from the Leningrad and Moscow regions of the European Russia as compared to the reference lines from the Western Europe and the USA revealed the wealth of *Avr* allelic variants, with their frequencies different as regards to the agroclimatic characteristics of the territories.

The evidence from the SSCP screening doe not completely match the results of the SSR genotyping apparently because the genome loci assessed by two methods evolve at contrasting speeds (Raffaele and Kamoun, 2012). It is also essential that, in contrast to SSR loci, the *Avr* genes assayed by the SSCP analysis are immediately related to pathogenicity of *P. infestans* lines under study.

The repertoire of the *Avr* genes revealed by the SSCP analysis does not completely match the profile of virulence genes (factors) examined with the Mastenbroek-Black differential set. The latter provides indirect evidence bases on plant response to infection; in addition the currently employed differential set is devoid of many important *Rpi* genes recognizing such *Avr* genes as *Avr-blb1, Avr-blb2* and *Avr-vnt1*.

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Rapid discrimination of *Phytophthora infestans* (a)virulence genes by SSCP analysis

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SUMMARY

Single-strand conformation polymorphism (SSCP) analysis is a workable and high-performance method to discern DNA polymorphisms due to SNP-dependent conformation changes revealed by thermal and chemical denaturation of DNA fragments. We employed SSCP analysis to examine eleven *Avr* genes in single-cell *P. infestans* lines and validated the SSCP electrophoretic patterns by cloning and sequencing individual bands and comparing the sequences to the *Avr* prototype genes deposited in the NCBI Genbank. Such annotation allows to further employ SSCP patterns as barcoding descriptors for genotyping pathogen strains and rapid assessing pathotype profiles in *P. infestans* populations. With the reference pathogen strains maintained in genetic collections, SSCP analysis provides a reliable tool for early predicting the dramatic changes in *P. infestans* pathogenicity.

KEY WORDS

Phytophthora infestans, virulence genes, SSCP analysis, genotyping, diagnostics

INTRODUCTION

The fight against late blight disease is greatly complicated by rapid changes in the composition of the populations of its causative agent *Phytophthora infestans* (Mont.) de Bary. New pathotypes appear as a result of pathogen evolution and migration and affect potato varieties that were previously considered resistant to late blight, sometimes completely destroying the potato crop (Cooke *et al.*, 2012). Therefore, it is hard to overestimate the importance of early discerning these changes in pathogen populations.

Molecular markers make it possible to discriminate *P. infestans* pathotypes, which is important for characterizing the pathogen populations and assessing their potential harmfulness. Most of these methods are based on direct or indirect analysis of polymorphisms in functional loci (peptidase and glucose 6-phosphate isomerase spectra, genes that determine the mating type or resistance to metalaxyl) or anonymous genome fragments (RAPD, AFLP, RFLP, SSR analysis)

and identification of mitochondrial DNA haplotypes (Cooke and Lees, 2004; Martin *et al.*, 2019). The recently established international system for monitoring and predicting the expansion of the most dangerous *P. infestans* pathotypes is based on the SSR analysis of isolates at 12 loci (Li *et al.*, 2013). However, all these methods of molecular genotyping have a common disadvantage: they employ descriptors associated with genome loci comprising housekeeping genes rather than those directly involved in pathogen virulence.

In contrast, virulence genes (*Avr* genes), which determine the pathogenicity of *P. infestans*, are located in the relatively mobile part of the genome, free of housekeeping genes and rich in transposable elements (Fry, 2016). Many of these genes encode effector proteins that allow pathogens to overcome defense barriers of host plants and disrupt plant physiological processes. Determining the composition of *Avr* genes would help evaluate the aggressiveness and harmfulness of *P. infestans* strains. Using the methods of molecular genetic analysis of *Avr* genes for early detecting new *P. infestans* strains and assessing the pathogenicity of these strains would make more efficient fungicide application and help predict crop losses in varieties that carry already known resistance genes.

Single-strand conformation polymorphism (SSCP) is among the ready available and high-throughput methods for studying DNA polymorphisms (Sheffield *et al.*, 1993). Following thermal and chemical denaturation of single DNA strands, single nucleotide substitutions produce unique changes in conformation of DNA molecules, which affect their mobility during electrophoresis in non-denaturing polyacrylamide gel (PAAG). Validation of SSCP patterns by sequencing individual electrophoretic bands allows further use of these patterns as descriptors for recognition of pathogen strains and early prediction of harmfulness based on results of SSCP analysis.

MATERIALS AND METHODS

Material
P. infestans isolates and derived single-cell lines were obtained in the Institute of Phytopathology as described elsewhere (Sokolova *et al.*, 2017). In addition, we investigated seven reference lines from the Western European and USA collections of *P. infestans* kindly provided as DNA samples by Dr David Cooke, the James Hutton Institute, Dundee, UK.

Table 1.   *Primer sequences and PCR conditions*

Avr gene	Sequence 5' –3'		Fragment length bp	T°C anneal.	Reference
Avr1	Avr1F	CCGGATTCGACCACGACAAGG	557	69	Du *et al.*, 2015
	Avr1R	AAATGGTACCACAACATGTCCACCAAGC			
Avr2	AVR2F4	ATGCGTCTCGCCTACATTTT	340	61	Gilroy *et al.*, 2011
	AVR2R4	TGTCACCCTTAATTTTCAAATGC			
Avr2-like	avr2F6	AGGCTCTCGATCTTGTGCY	392	61	Gilroy *et al.*, 2011
	avr2R6	TAGCTCCTTTTGTTACCCTTACTTTGT			
Avr3a	Avr3aSF	GTTTAATGTGGCTGCGTTG	239	53	*
	Avr3aR	CTGAAAACTAATATCCAGTGA			
Avr3b	Avr3bF	TACTCGACTTCAAAGGGGGA	378	60	Wang *et al.*, 2017
	Avr3bR	TTAGAAATTGTTCTTTGCGGTCA			
Avr4	Avr4SF	GGCACGCCACTGGAAAAAGTC	220	61	*
	Avr4SR	GCGCAACCCACCTAAGGAGC			
Avr8	Avr8F2	ACAAGTATCCCTCTCGTTCCTTC	661	66	*
	Avr8Sm2R	TTACGATGTTTTCGCTTCTTTAAAAAGC			
Avr9	AvrSm1F1	ATGCGTCTAAGCTCCACATTTCT	717	65	*
	AvrSm1R	TTATCCGGAGGGGTTTAGC			
Avr-blb1 (*ipiO*)	IPIOF	CATCCAAGATTCGCTTTCTGTCG	266	63	*
	IPIO1R	GCTTATCGGCGTCTCTCCGG			
	IPIO4R	GGATGCTTGTTCTTGTAGCTAGC	283		
Avr-blb2	Avrblb2F	AATCCCCGACGWGTCTCG	307	65	*
	Avrblb2R	GTCTACCCCTTTCTCGAAGTC			
Avr-vnt1	Avr-vnt1-SP-F	GTAACGACCCCGACCAAGTT	393	66	Pel, 2010
	Avr-vnt1-R	TCAAGCTCTAATAGGATCAAGC			

* Primers designed by the authors

DNA isolation
Genomic DNA was isolated using AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, USA). DNA concentration was measured with an UV/Vis NanoPhotometer P300 (IMPLEN, Germany).

PCR amplification
Primer sequences and PCR amplification conditions for *Avr1*, *Avr2*, *Avr3b* and *Avr-vnt1* genes were as described previously (Du *et al.*, 2015; Gilroy *et al.*, 2011; Wang *et al.*, 2017). Primers for amplification of *Avr3a*, *Avr4*, *Avr-smira2* (*Avr8*) and *Avr-blb1* genes were designed by the authors. Primers for amplification of *Avr-smira1* (*Avr9a*) and *Avr-blb2* were modified after Rietman *et al.* (2012) and Oh *et al.* (2009), respectively (Table 1). Positions and sizes of amplified fragments of *Avr* genes are shown in Figure 1.

SSCP analysis
One to four microlitre of PCR product was mixed with seven volumes of denaturing buffer comprising 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol, incubated for 10 min at 95°C, immediately put on ice and then loaded on a 8% PAAG. Electrophoresis in 0.5× TBE buffer was run for 4.5 hours at 4°C and a constant voltage of 200 V.

Gels were stained in 1X SYBR Green I solution for 30 min and visualized under UV at 312 nm wavelength.
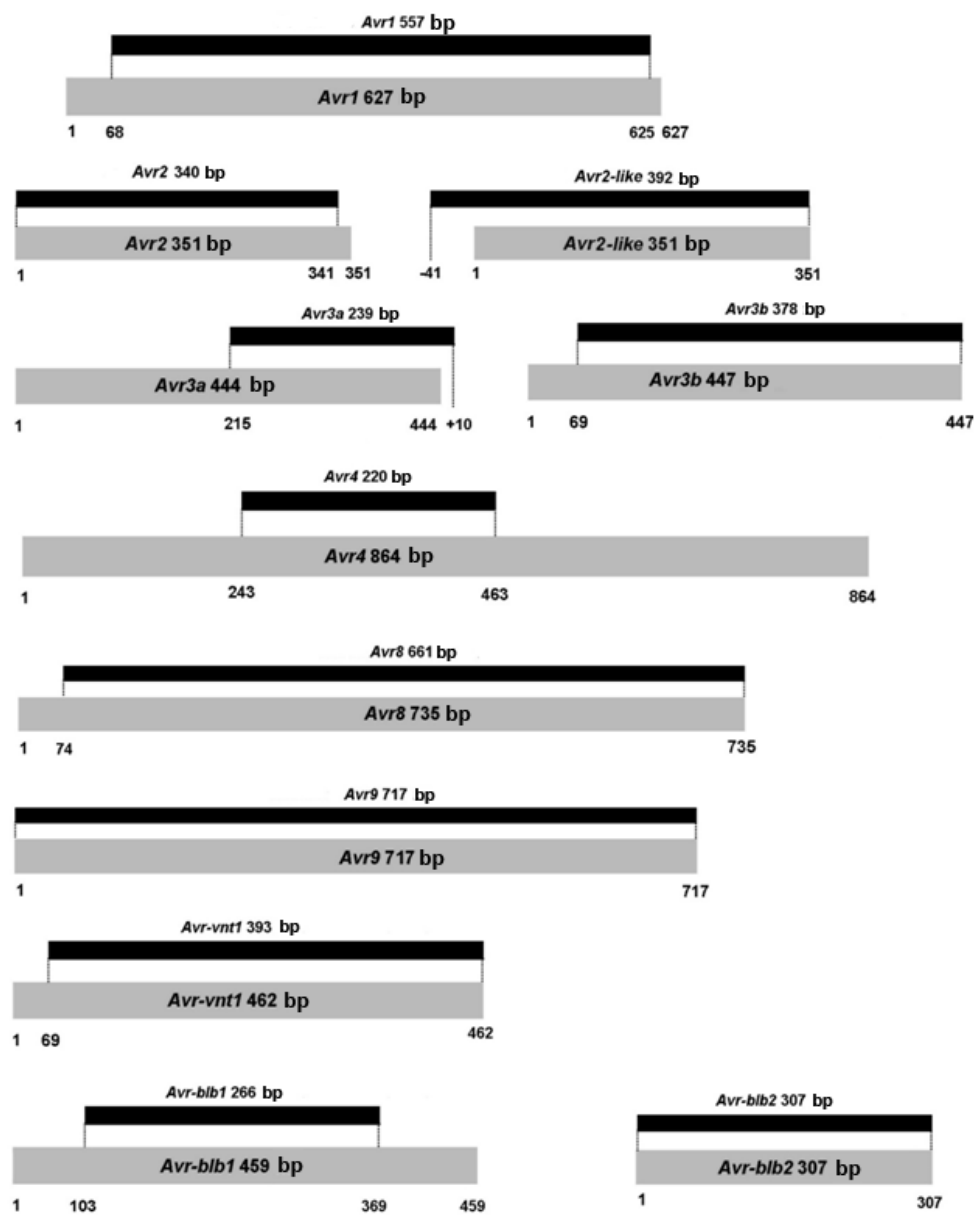
Figure 1. *Schematic representation of the Avr genes and the regions amplified for SSCP analysis*

Cloning and sequencing

SSCP bands were excised from the gel under UV. The gel slices were crushed into fine slurry, which was soaked in TE buffer for 36 hours at room temperature, the slurry was centrifuged, and the supernatant recovered with a pipette. Cloning was performed in pGEM/T Vector (Promega, USA). Isolated plasmids were sequenced using a NANOFOR 05 analyzer (Institute for Analytical Instrumentation RAS, Russia). Nucleotide sequences of the *Avr* genes were deposited at the NCBI GenBank (https://www.ncbi.nlm.nih.gov/) and compared with the already published *Avr* sequences using the LaserGene software (https://www.dnastar.com/software/).

RESULTS AND DISCUSSION

We validated the SSCP patterns of eleven *Avr* genes encoding the RXLR effectors Avr1, Avr2, Avr2-like, Avr3a, Avr3b, Avr4, Avr8, Avr9a, Avr-blb1, Avr-blb2 and Avr-vnt1. All studied lines produced unique SSCP profiles of *Avr* genes. The patterns of *Avr3b*, *Avr4*, and *Avr8* genes are monomorphic. All other genes reveal two to five well reproducible SSCP patterns suitable for discerning and identifying *P. infestans* strains (Figure 2). Table 2 lists the NCBI Genbank accession numbers for DNA sequences of the individual (allelic) zones from these patterns. These sequences were compared with the reference sequences of *Avr* genes in a fully sequenced genome of *P. infestans* isolate T30-4. The isolates corresponding to these nucleotide sequences are maintained in the State Collection of Phytopathogenic Microorganisms in the Institute of Phytopathology (www.vniif.ru) and can be used to verify this method in other laboratories.
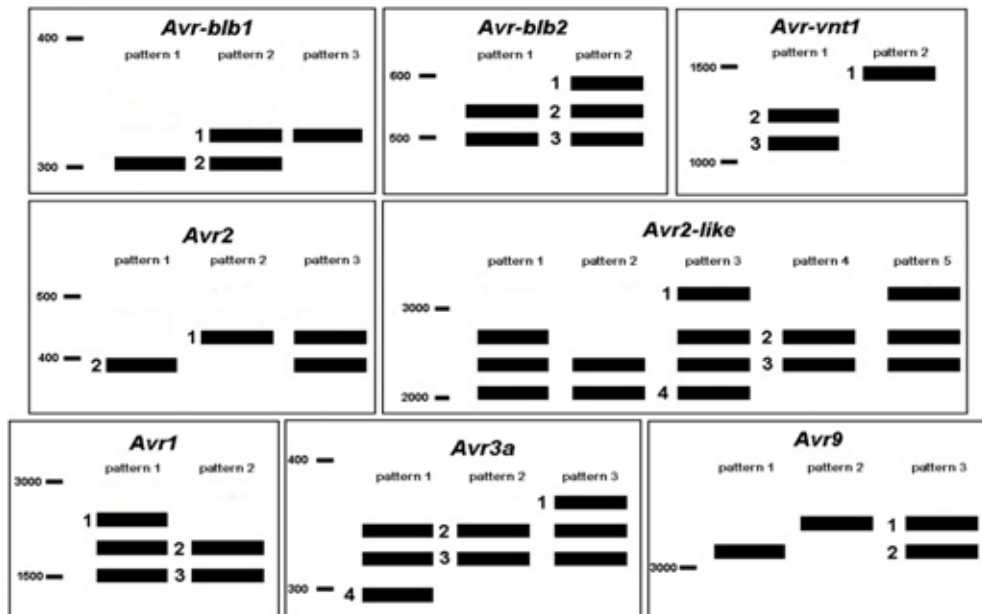
Figure 2. *Polymorphic SSCP patterns of Avr genes; 1 – 4 – electrophoretic zones; at the left, fragments of the DNA ladder, bp*

We also compared the SSCP patterns of studied lines with the composition of virulence genes (races) identified with the Mastenbroek-Black differential set and with the results of SSR genotyping of these lines. Comparison of the genotyping results obtained by SSCP analysis with the race profiles identified by differentials did not always give matching results, probably because of the limitations inherent to both methods. The traditional classification of *P. infestans* races is based on the method of indirect analysis using differential plants that carry individual resistance genes. The widespread Mastenbroek-Black differential set initially comprising only resistance genes of *Solanum demissum* recognizes the limited number of virulence genes as compared to the SSCP method. On the other hand, SSCP analysis does not always distinguish between virulent and avirulent forms of the same gene.

Table 2. *Sets of nucleotide sequences characteristic of the SSCP patterns produced by the Avr genes under study and the P. infestans lines in which these patterns were found. Pattern numbers indicated in the table cells correspond to those shown in Figure 2*

Gene	Pattern number	NCBI accession Nos. of pattern-composing sequences	Reference isolates and their numbers in the database of the Institute of Phytopathology
Avr1	1	MH450289-MH450291	2015.L.O./K.G. 43/1-1
	2	MH450290-MH450291	2015.L.O./K.G. 43/1-2
Avr2	1	KY354765	2015.L.O./K.G. 43/1-1
	2	KY354764	5M*
	3	KY354764-KY354765	6_A1 (4100)*
Avr2-like	1	MF956407-MF956409	2015.L.O./K.G. 18/1-1
	2	MF956408-MF956409	2015.L.O./K.G. 103-1
	3	MF956401 MF956407-MF956409	2015.L.O./K.G. 11/2-1
	4	MF956407-MF956408	2015.L.O./K.G. 161
	5	MF956401 MF956407-MF956408	2015.L.O./K.G. 87/2-1
Avr3a	1	MH450287-MH450288	2015.L.O./K.G. 18/1-1
	2	MH450287-MH450288	2015.L.O./K.G. 107-1
	3	KF154428 MH450287-MH450288	6_A1 (4100)*
Avr3b	1	MK287366	2015.L.O./K.G. 11/2-4
Avr9	1	MH423620	2015.L.O./K.G. 103-5
	2	MH423619	2015.L.O./K.G. 87/2-2
	3	MH423619 MH423620	2015.L.O./K.G. 109/1-2
Avr-blb1	1	MH220873	2015.L.O./K.G. 87/2-2
	2	MH220872-MH220873	2015.L.O./K.G. 109/1-2
	3	MH220872	5_A1 (C4)*
Avr-blb2	1	MK776760 MK776761	2015.L.O./K.G. 103-5
	2	MK776760-MK776762	2015.L.O./K.G. 87/2-2
Avr-vnt1	1	MH423615-MH423616	2015.L.O./K.G. 103-5
	2	MH423614	2015.L.O./K.G. 87/2-2

* These patterns were found in isolates not included to the collection of the Institute of Phytopathology

The results of discerning the single-cell lines of *P. infestans* using SSCP analysis of *Avr* genes also do not completely correlate with the outcome of SSR genotyping, primarily because these two methods analyze evolutionarily diverse parts of the *P. infestans* genome. SSR loci correspond to expressed housekeeping genes, while *Avr* genes that determine the pathogenicity are located in another, relatively mobile part of the genome, free of housekeeping genes and rich in mobile elements. Thus, in contrast to the widely used method of SSR genotyping of *P. infestans* strains, SSCP analysis reveals polymorphisms directly related to pathogen virulence.

CONCLUSIONS

We were first to employ the SSCP analysis in order to reveal the allelic polymorphisms of *Avr* genes of *P. infestans*. SSCP patterns are robust barcoding descriptors of polymorphism that can be used to distinguish between pathogen strains. When these descriptors are validated by sequencing in the reference strains maintained in the genetic collections, SSCP technology becomes reliable, fast, cost-effective and sensitive enough for population and gene geographic studies and for early discriminating rare *Avr* variants, which is crucial when new pathotypes of *P. infestans* appear in potato fields.

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Virulence and fungicide sensitivity of the most prevalent genotypes collected in Belgium in 2017-2018

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Summary

A total of 119 isolates of *Phytophthora infestans* were collected in the southern part of Belgium (Wallonia) in several potato fields, volunteers, dumps and field trials during the years 2017 and 2018. The isolates were analyzed using standardized 12 plex Euroblight SSR genotyping (Euroblight monitoring). Most of isolates were tested for several phenotypic characteristics, such as virulence and fungicide resistance.

Materials and Methods

Phytophthora infestans isolates : 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.

Genotyping: The isolates were analyzed using standardized 12 plex Euroblight SSR genotyping. Genotypes were determined by comparing fragment sizes with isolates previously genotyped (Euroblight monitoring).

Virulence: virulence was determined using Black's differential set of potato clones, each having one of the R1-R11 resistance genes. Virulence was also determined by detached leaf assay on several commercial varieties which have a good rating about resistance in field (rated 1 (very sensitive) to 9 (resistant). Each leaflet was inoculated with 10µl droplet of sporangial suspension and incubated in humid chambers under controlled conditions (18°C). After seven days of incubation, the sporulation was evaluated (High sporulation, low sporulation and no sporulation).

Fungicide resistance: Metalaxyl resistance was assessed using a floating leaf disk method (leaf of potato cv. Bintje). Leaf disk were floated abaxial side up in Petri dishes each containing water or metalaxyl at concentrations of 0 to 100 µg/ml. Each disk was inoculated with 10µl droplet of sporangial suspension. After seven days of incubation, isolates sporulating on the disks floating on water containing 100 µg/ml metalaxyl were rated resistant. Fluazinam resistance was evaluated on potato leaf discs (Bintje) by mixing fungicide at different concentrations (0,1 - 1 - 10 - 30 and 100 µg/ml) with the sporangial suspensions. After seven days of incubation, the sporulation was evaluated and isolates sporulating with 100 µg/ml fluazinam were rated resistant and isolates sporulating with 30 µg/ml were rated intermediate.
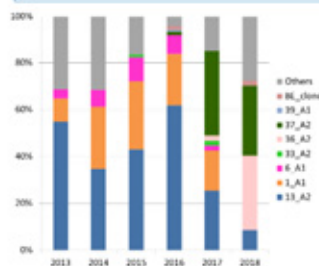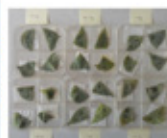
Results

Until 2016, 3 clonal lineages dominated in Belgium: the most prevalent was EU_ 13_A2 clone which made up 50% of the population. The two others were EU_1_A1 and EU_6_A1 and found at low frequency. In 2017 and 2018, monitoring highlighted the emergence of two new clonal lineage, named EU_36_A2 and EU_37_A2. They have displaced other lineages genotypes and are now dominant in Belgium. The genetically diverse "Others" samples comprised 30% of the sampled population in 2018.

All known virulence genes were found in Wallonia isolates. EU_ 13_A2 had a more complex virulence profile (1-2-3-4-(5)-6-7-(8)-(9)-10-11) than others genotypes. EU_36_A2 and EU_37_A2 had the same virulence profile (1-3-4-(5)-7-10-11).

Significant differences were observed between genotypes regarding their virulence on resistant varieties. EU_13_A2 was the more virulent genotype. EU_36_A2 and EU_37_A2 were virulent on the same varieties.

EU_13_A2 was resistant to metalaxyl whereas others genotypes were sensitive. EU_37_A2 and some EU_36_A2 were resistant to fluazinam whereas others genotypes were sensitive. Some EU_36_A2 were intermediate.