WUR – SPECIAL REPORT NO 19 – 2019, 127-136

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?

ROSELYNE CORBIERE[1], BRITT PUIDET[2], ROMAIN MABON[1], MICHELE GUIBERT[1], CATHERINE CHATOT[3], GUILLAUME SAUBEAU[3], FREDERIQUE AUROUSSEAU[4], PAULINE DEWAEGENEIRE[5], DIDIER ANDRIVON[1]

[1] INRA, Institut National de la Recherche Agronomique, UMR IGEPP, 35653 Le Rheu, France
[2] EULS, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Estonia
[3] Germicopa Breeding and Florimond-Deprez Veuve & Fils, 29520 Châteauneuf du Faou, France
[4] Comité Nord / SIPRE, 76110 Bretteville Du Grand Caux, France
[5] Sipre and FN3PT - RD3PT, 62217 Achicourt, France

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).

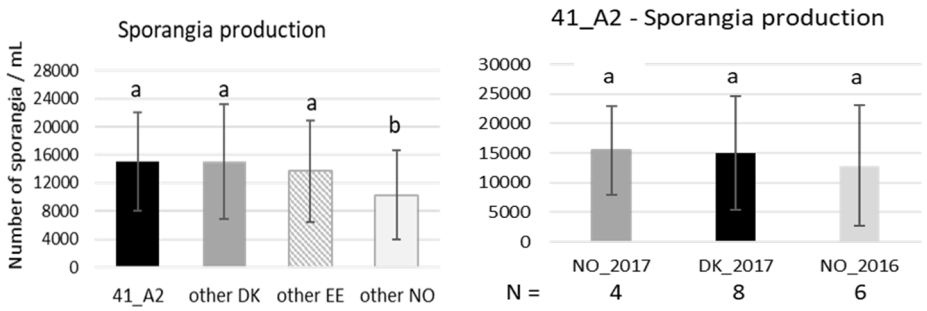

Figure 1. *Spore production (number of sporangia per lesion) of 2017 P. infestans isolates on potato cv. Bintje detached-leaflets (mean of 10 replicates). Measurements made after five incubation days, at 15°C night / 18°C light (16 h light period)*

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.

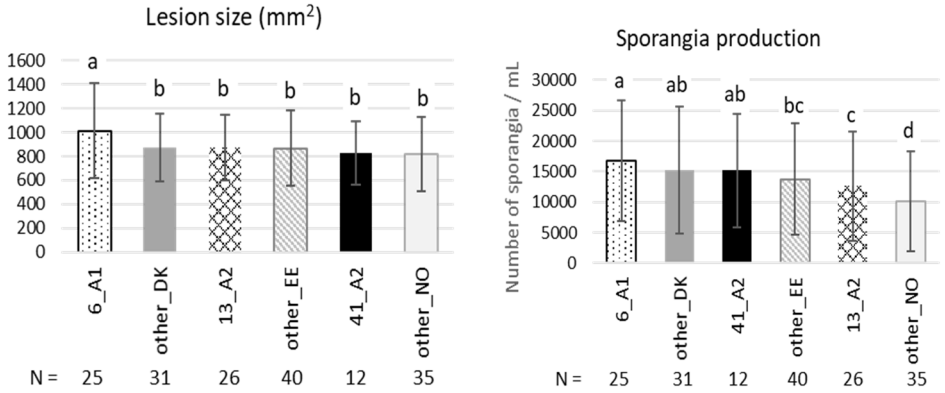

Figure 2. *Aggressiveness traits of Nordic and Baltic P. infestans isolates (EU_41_A2, "other" from Denmark, Estonia and Norway) compared to EU_6_A1 and EU_13_A2 clonal lineage isolates from France and Great Britain. Bioassay performed on cv. Bintje detached leaflets*
Left: mean lesion size and right: 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.

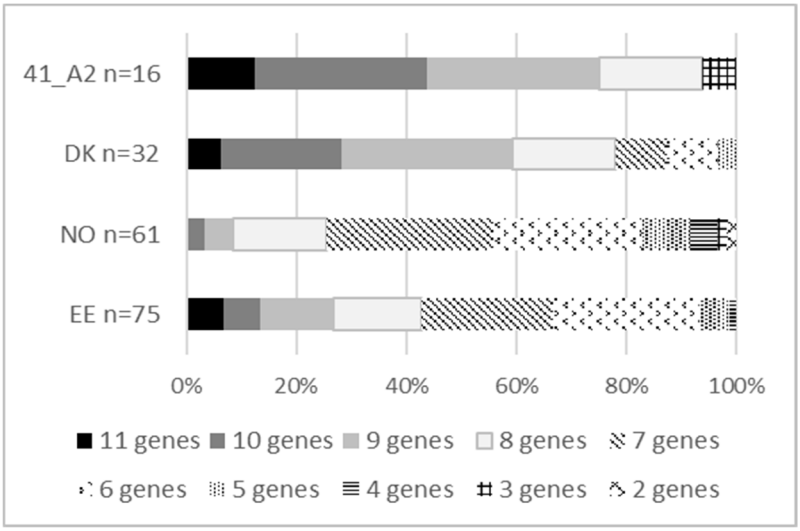

Figure 3. *Mean number of overcome R genes (R1 to R11 from the Black's differential set) by the isolates sampled in 2016-2017 (in percent). First line: EU_41_A2 isolates, 2nd line: Danish "other" isolates, 3rd line: Norwegian "other" isolates; 4th line: Estonian "other" isolates. Each color presented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

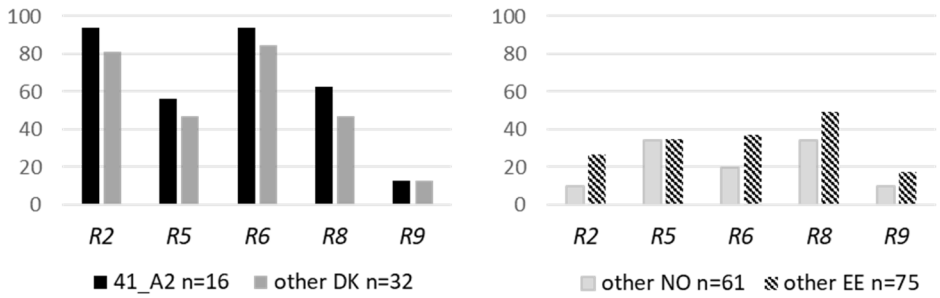

Figure 4. *Percentage of virulent 2016-2017 P. infestans isolates against five R specific genes (R2, R5, R6, R8, R9) from the Black's differential set, based on a detached leaflet bioassay*
Right: virulence of EU_41_A2 isolates (in black) and Danish "other" isolates (in dark grey). Left: virulence of "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.

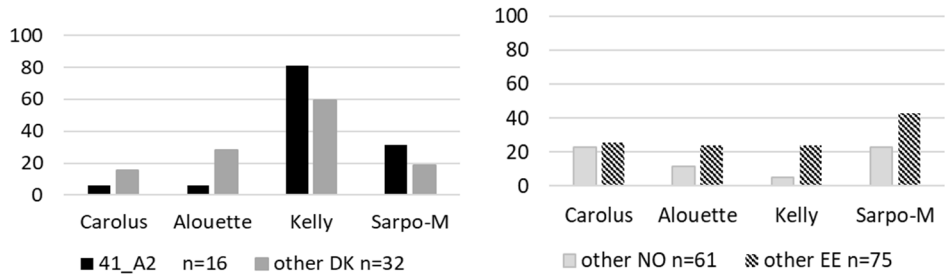

Figure 5. *Frequency of virulent 2016-2017 P. infestans isolates against four potato cultivars, Carolus, Alouette, Kelly and Sarpo-Mira. Bioassay performed on detached leaflets*
Right: virulence of EU_41_A2 isolates (in black) and Danish "other" isolates (in dark grey). Left: virulence of "other" isolates 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.

ACKNOWLEDGEMENTS

We thank our partners in the IPMBlight2.0 project: Håvard Heikemo and Vinh Hong Le (Norsk institutt for bioøkonomi (NIBIO), Norway), Bent Jørgen Nielsen (Aarhus University, Denmark), Riinu Kiiker (Estonian University of Life Sciences (EULS), Estonia) and all the collectors for providing isolates. We are grateful to David Cooke (the James Hutton Institute, UK), Jens Hansen and Poul Lassen (Aarhus University, Denmark) for EuroBlight pathogen data base. We thank INRA Genetic Resources Center BraCySol of Ploudaniel for providing us potato seed tubers used in phenotyping bioassays. This work was supported by IPMBlight2.0 project from C-IPM ERA-Net (Coordinated Integrated Pest Management in Europe) and funded in France by the French Agency for Biodiversity (AFB) and SMaCH metaprogram from INRA.

REFERENCES

Abuley, I. and Hansen, J.G. (2019). Trap Nursery data in the IPM context; Results from the trap nurseries. 17th EuroBlight Workshop, York, UK, 12-15 May 2019. http://agro.au.dk/fileadmin/euroblight/Workshops/York/Presentations_and_posters/Tuesday/S6/16_Trap_nursery_Euroblight.pdf

Andrivon, D., Avendaño-Corcoles, J., Cameron, A., Carnegie, S., Cooke, L.R., Corbière, R., Detourné, D., Forisekova, K., Griffin, D., Hannukkala, A., Lebecka, R., Lees, A.K., Niepold, F., Polgar, Z., Shaw, D.S., Thompson, J., Trognitz, B., van Raaij, H. and Zimnoch-Guzowska, E. (2011). Stability and variability of virulence of *Phytophthora infestans* assessed in a ring test across European laboratories. Plant Pathology 60, 556-565.

Andrivon, D., Corbière, R., Hansen, J.G., Eikemo, H., Puidet, B., Gaucher, D., Chatot, C., Aurousseau, F., Barbary, A., Glorvigen, B., Lees, A.K and Cooke, D.E. (2017). IPMBlight2.0: using pathogen population information to improve late blight control. In: Proceedings of the 16th EuroBlight Workshop, PAGV-Special Report no. 18, Schepers HTAM (ed.), Aarhus, Denmark, 14-17 May 2017, p. 33-38.

Cooke, D., Kessel, G., Hansen, J.G., Lassen, P., Andrivon, D., Corbière, R., *et al.* (2019). The European population of *P. infestans* in a global context. 17th EuroBlight Workshop, York, UK, 12-15 May 2019. http://agro.au.dk/fileadmin/euroblight/Workshops/York/Presentations_and_posters/Monday/S1/Cooke_Euroblight_2019final_forweb.pdf

EuroBlight plateforme: https://agro.au.dk/forskning/internationale-platforme/euroblight/pathogen-characteristics-and-host-resistance/about-pathogen-monitoring/

Kiiker, R., Skrabule, I., Ronis, A., Cooke, D.E.L., Hansen, J.G., Williams, I.H., Mänd, M. and Runno-Paurson, E. (2019). Diversity of populations of *Phytophthora infestans* in relation to patterns of potato crop management in Latvia and Lithuania. Plant Pathology 68, 1207-1214.

Lehtinen, A., Hannukkala, A., Andersson, B., Hermansen, A., Le, V.H., Nærstad, R., Brurberg, M.B., Nielsen, B.J., Hansen, J.G. and Yuen, J. (2008). Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. Plant Pathology 57, 277–234.

Lehtinen, A., Andersson, B., Le, V.H., Nærstad, R., Rastas, M., Ketoja, E., Hannukkala, A.O., Hermansen, A., Nielsen, B.J., Hansen, J.G. and Yuen, J. (2009). Aggressiveness of *Phytophthora infestans* on detached potato leaflets in four Nordic countries. Plant Pathology 58, 690-702.

Mariette, N., Androdias, A., Mabon, R., Marquer, B., Corbière, R., Montarry, J. and Andrivon, D. (2016). Local adaptation to temperature in populations and clonal lineages of the Irish potato famine pathogen *Phytophthora infestans.* Ecology and Evolution 6, 6320-6331. DOI: 10.1002/ece3.2282.

Runno-Paurson, E., Kiiker, R., Joutsjoki, T. and Hannukkala, A. (2016). High genotypic diversity found among population of *Phytophthora infestans* collected in Estonia. Fungal Biology 120, 385-392.

Sjöholm, L., Andersson, B., Högberg, N., Widmark, A.K. and Yuen, J. (2013). Genotypic diversity and migration patterns of *Phytophthora infestans* in the Nordic countries. Fungal Biology 177, 722-730.