VI Monitoring population dynamics and assessing fungicide sensitivity of *Phytophthora infestans* in Denmark

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Introduction

Phytophthora infestans causes late blight, which is the most devastating disease in potato production in Denmark. Fungicides are key to the management of late blight (Abuley et al., 2023). Potato is the one most sprayed crop in Denmark. *Phytophthora infestans* has a high evolutionary capacity, enabling the pathogen to evolve and overcome control measures such as fungicides. As a part of the EuroBlight network we have monitored the population dynamics as well as evaluated the sensitivity of the Danish *P. infestans* to key fungicides used in late blight management. In this paper, we present the population dynamics of *P. infestans* from 2017 to 2023 and fungicide resistance testing in 2022 and 2023.

The *Phytophthora infestans* population in Denmark

The *P. infestans* population in Denmark was recorded during the years 2017 to 2023 (Figure 1), indicating that the population of *P. infestans* consists of genetically diverse genotypes ("Other") and mainly two clonal lineages, namely EU_41_A2 and EU_43_A1, with varying frequencies over the years. The genotypes of *P. infestans* were determined via simple sequence repeat (SSR) markers at the James Hutton Institute. The *P. infestans* population in Denmark has until 2022 been characterised by genetically diverse *P. infestans* genotypes (i.e. other genotypes), accounting for >50% of the *P. infestans* population in Denmark. The majority of these "other" genotypes are considered to be the result of sexual recombination. The EU_13_A2 *P. infestans* genotype with reduced sensitivity to metalaxyl was found at low frequencies (<1%) in 2017, 2020 and 2023 (Figure 1), whereas the EU_36_A2 was found in one and two fields in 2017 (~2%) and 2019 (~4%), respectively, in the Danish *P. infestans* population. The EU_41_A2 genotype was the dominant clone in Denmark from 2017 to 2020, after which the EU_43_A1 genotype took over as the dominant clone in Denmark from 2021 to 2023 (Figure 1). The EU_43_A1 genotype emerged for the first time in Denmark in 2018, and it has increased gradually to become the dominant *P. infestans* clone in Denmark since 2021. In 2022, the EU_43_A1 became the most dominant *P. infestans* genotype surpassing all genotypes including the "Other" genotypes. This drastic increase in the frequency of the EU_43_A1 was linked to its resistance to one of the active ingredients used in Denmark: mandipropamid (Abuley et al., 2023). In 2023, however, the frequency of EU_43_A1 declined, mainly because of the decreased usage of mandipropamid.

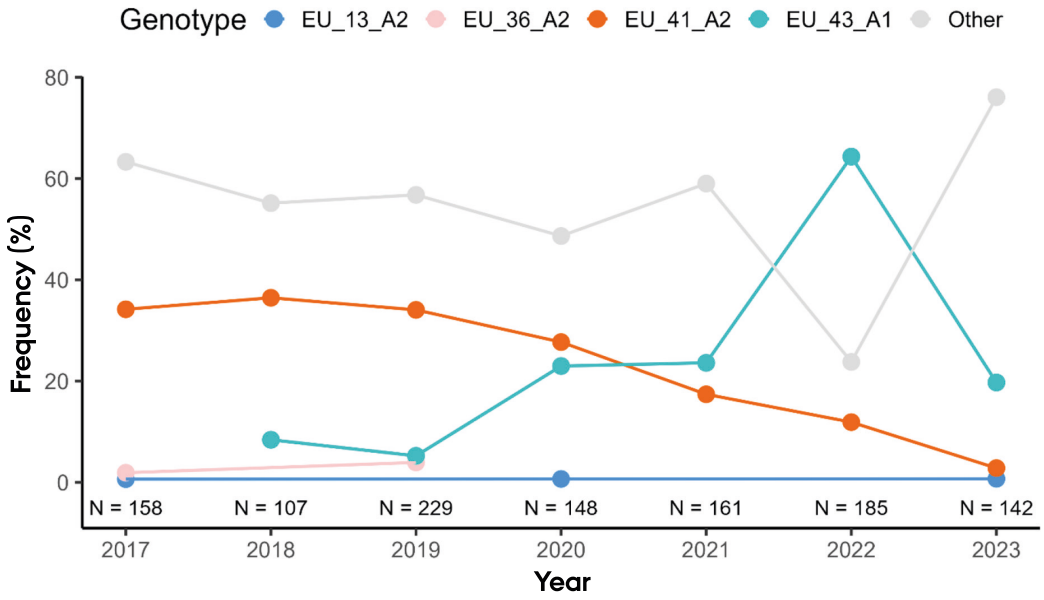

Figure 1. Frequency of *Phytophthora infestans* genotypes across different years in Denmark.

Fungicide resistance monitoring in 2022

In 2022, in response to the widespread report of reduced efficacy of mandipropamid in potato fields in Denmark, we sampled >100 potato leaves infected with late blight from several fields in Denmark. Single spore isolations were done to isolate the associated *P. infestans* from the lesion. Genotype (SSR) analysis was carried out on 72 of the samples to determine their genotype. Of the 72 samples, 63 were EU_43_A1, 5 were EU_41_A2 and 4 were "Other" genotypes. Fungicide sensitivity analysis was done using a floating disc assay, using 25 EU_43_A1 and 5 EU_41_A2 isolates. The results are shown in Figure 2. The EU_43_A1 genotype infected the leaf discs at all tested concentrations, whereas the EU_41_A2 showed a clear dose-response with an EC_{50} value of about 0.5 µg/ml (Figure 2). A further test was done to investigate the sensitivity of the EU_43_A1 genotype to higher concentrations (up to 100 µg /ml) of mandipropamid, and the result is shown in Figure 3. Compared with the other genotypes tested, the EU_43_A1 was able to infect the leaf disc at all tested concentrations, with average disease incidence exceeding 70%. In conclusion, we determined that the EU_43_A1 genotype has a strong resistance to mandipropamid, with an EC_{50} value probably exceeding the highest dose we have tested (100 µg/ml).

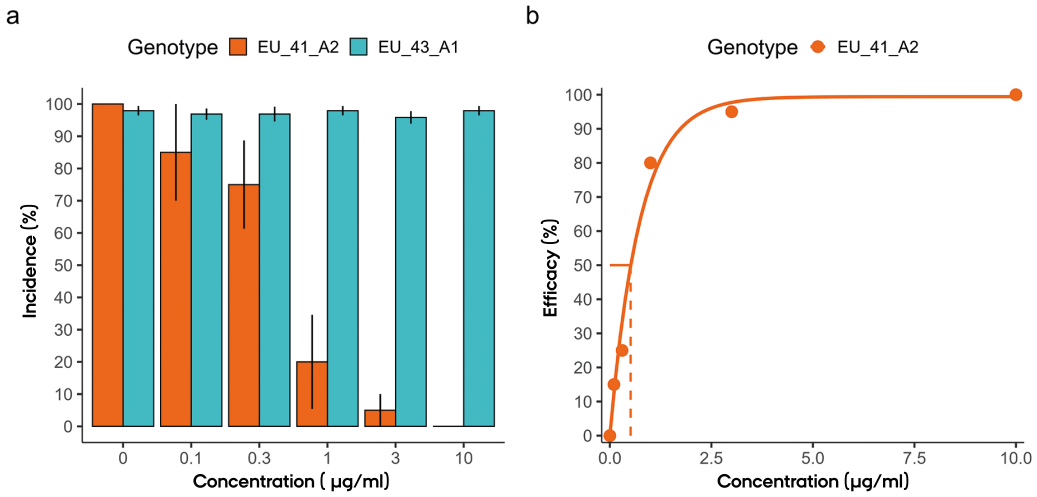

Figure 2. Incidence of late blight (a) and fitted dose–response curves (b) showing the sensitivity of the EU_41_A2 (n = 5) and EU_43_A1(n = 25) genotypes of *Phytophthora infestans* to mandipropamid from the in vitro leaf disc test. The vertical black line associated with each bar in (a) is the bootstrapped confidence interval (95%). The dashed vertical line in (b) indicates the dosage (µg/ml) at which 50% disease control is obtained (EC_{50} value).

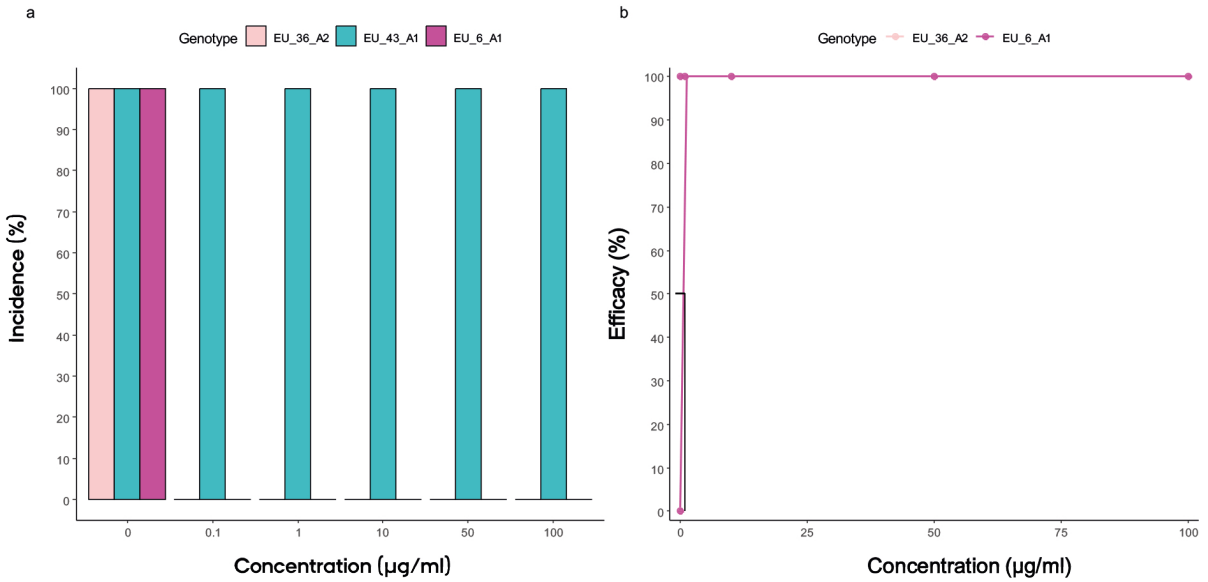

Figure 3. Incidence of late blight (a) and fitted dose–response curves (b) showing the sensitivity of the EU_36_A2 (n = 1), EU_43_A1 (n = 5) and EU_6_A1 (n = 1) genotypes of *Phytophthora infestans* to mandipropamid from the in vitro leaf disc test. The vertical black line in (b) indicates the dosage (µg/ml) at which 50% disease control is obtained (EC_{50} value).

Sensitivity of *Phytophthora infestans* isolates to fluazinam

In early 2023, sensitivity tests were conducted on 5 EU_43_A1, 1 EU_41_A1 and 1 other *P. infestans* genotypes against fluazinam. The aim was to assess the sensitivity of Danish isolates to fluazinam, the primary protectant fungicide for the 2023 growing season, due to reported resistance to mandipropamid. Tests were performed on pea agar with varying fluazinam concentrations (0, 250, 500, 750 and 1000 µg/ml). Results indicated high sensitivity among all Danish isolates to fluazinam, with an EC_{50} value of less than 0.2 µg/ml (Figure 4).

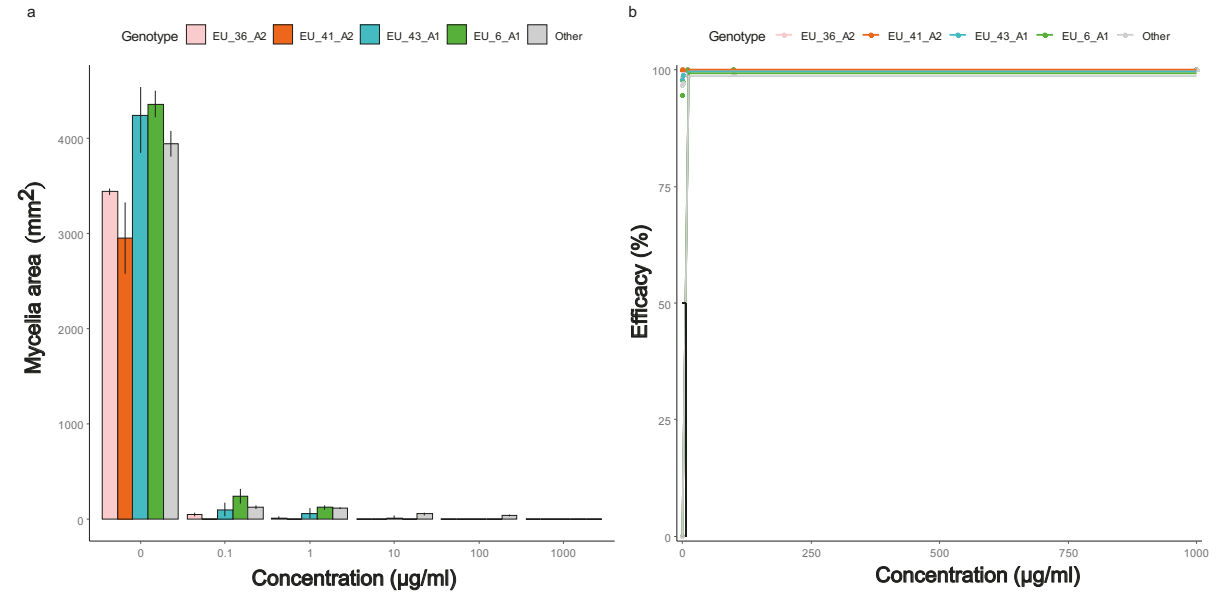

Figure 4. (a) Mycelia growth of various *P. infestans* genotypes at different fluazinam concentrations. (b) Dose-response curve for these genotypes to fluazinam. The vertical black line associated with each bar in (a) is the bootstrapped confidence interval (95%). The vertical black line in (b) indicates the dosage (µg/ml) at which 50% disease control is obtained (EC_{50} value).

Fungicide sensitivity monitoring in 2023

In 2023, over 100 *P. infestans* isolates were sampled in different potato fields in Denmark. Thirty-one of the *P. infestans* isolates collected during the 2023 growing season were tested against the commonly used active ingredients for late blight control: mandipropamid (Revus), fluazinam (Shirlan Ultra), propamocarb (Sporax), cymoxanil (Cymbal WG) and oxathiapiprolin (Zorvec Enicade). The tested isolates were genotyped and found to be EU_43_A1 (n = 4) and "Other" (n = 27) genotypes. The results, which are shown in Figure 5 and Table 1, indicate that most Danish isolates were sensitive to the tested fungicides, with EC_{50} values well below their field rates. However, EU_43_A1 showed resistance against mandipropamid (Figure 5; Table 1).

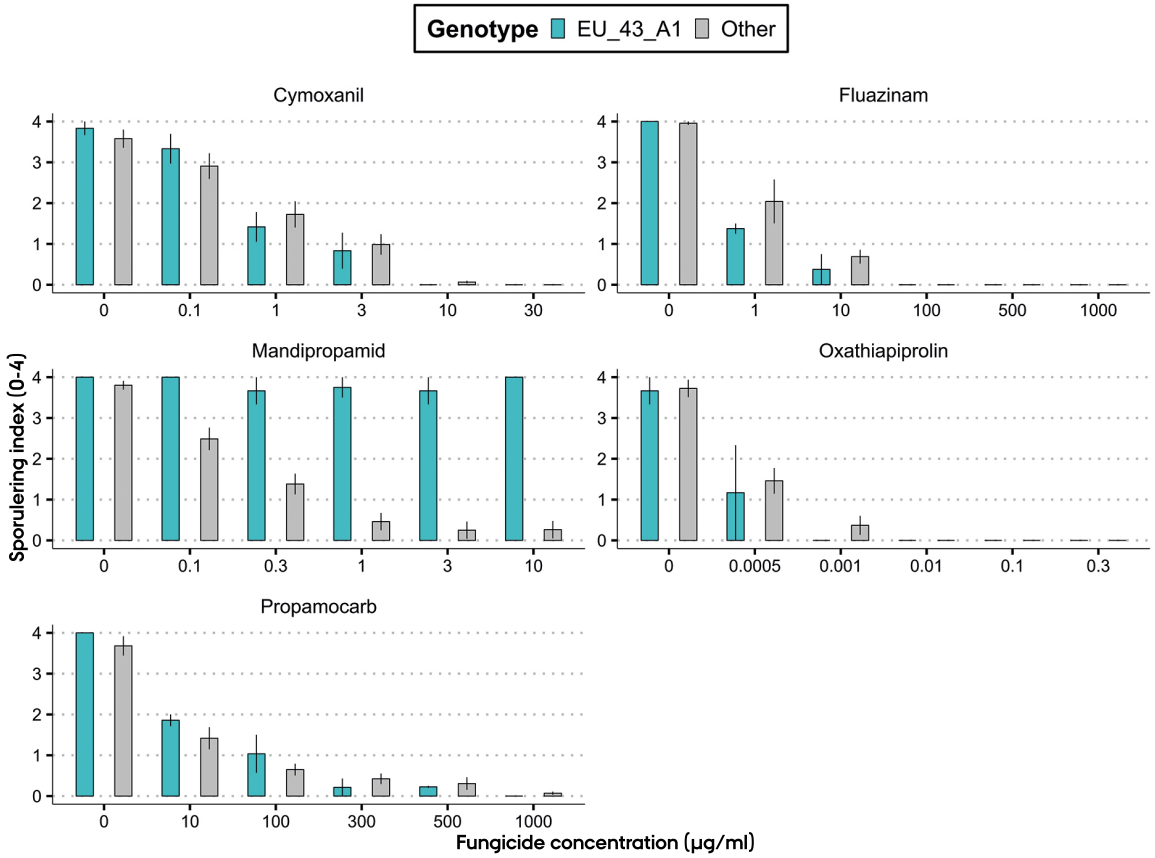

Figure 5. The sensitivity of EU_43_A1 (n = 3) and "Other" *Phytophthora infestans* genotypes to different fungicide active ingredients. Sensitivity was measured by assessing the sporulation index per leaf disc (from 0 = "no sporulation" to 4 = "heavy sporulation"). The vertical black line associated with each bar is the bootstrapped confidence interval (95%).

Table 1. The effective concentration at which 50% late blight control is achieved for different fungicide active ingredients for EU_43_A1 and "Other" *Phytophthora infestans* genotypes.

Active ingredient	Genotype*	Mean EC_{50} of tested isolates (µg/ml)**
Cymoxanil	EU_43_A1	0.59 (0.00013)
	Other	0.91 (0.0007)
Propamocarb	EU_43_A1	9.1 (0.00041)
	Other	6.1 (0.00047)
Fluazinam	EU_43_A1	0.1 (0.0001)
	Other	1 (0.00021)
Mandipropamid	EU_43_A1	>10
	Other	0.165 (0.000071)
Oxathiapiprolin	EU_43_A1	0.000256 (0.00000009)
	Other	0.000287 (0.00000009)

*The number of isolates tested per genotype are 4 (EU_43_A1) and 27 (Other).
**Values in brackets are the standard error of the mean EC_{50} values.

Acknowledgements

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References

Abuley, I. K., J. S. Lynott, J. G. Hansen, D. E. L. Cooke and A. K. Lees (2023). The EU43 genotype of *Phytophthora infestans* displays resistance to mandipropamid. Plant Pathology 72: 1305-1313.