# Fungicide resistance in *Phytophthora infestans*

## Resistance to mandipropamid and oxathiapiprolin in Swedish field isolates

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## Abstract

*Phytophthora infestans* is the most serious pathogen on potato worldwide. The pathogen has several problematic characteristics such as a mixed reproduction system, polycyclism and a high genetic plasticity rendering it prone to adapt to new situations, e. g. by developing resistance to fungicides. Resistance has previously been reported for fungicides such as metalaxyl, propamocarb, mandipropamid (MPD) and oxathiapiprolin (OTP). This study surveys *P. infestans* isolates collected in Swedish fields during the season 2023 for resistance to MPD and OTP, resistances which have not been found in Sweden before. A floating leaf disc assay with concentration series for both fungicides was deployed for phenotyping the isolates. To connect any resistance to SNPs found to cause resistance in previous research, target genes PiCesA3 for MPD and ORP1 for OTP were Sanger sequenced. In addition, some isolates were sent to the James Hutton institute in Scotland for microsatellite genotyping in order to investigate if resistance can be connected to a specific SSR genotype of *P. infestans*.

One *P. infestans* isolate collected in a conventional field plot were able to infect and sporulate on leaf discs in all fungicide concentrations of the floating leaf disc assay while all other isolates were completely inhibited at higher concentrations. Unfortunately, Sanger sequencing failed and for this particular isolate and it was not among the isolates sent for microsatellite genotyping. Hence, resistance could not be connected to any specific SNP or SSR genotype. The 10 isolates displaying normal sensitivity could not be attributed any specific SSR genotype but were classified as ‘other’, as is the normal situation for Swedish *P. infestans* isolates. The resistance found in this study is of interest for future fungicide use. However, since it was only found in a single isolate, more comprehensive studies are needed to assess the situation and produce a more representative picture of the in-field situation in Sweden.



*Keywords: Phytophthora infestans*, mandipropamid, oxathiapiprolin, fungicide, resistance

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# Abbreviations

| | |
|---|---|
| SLU | Swedish University of Agricultural Sciences |
| MPD | Mandipropamid |
| OTP | Oxathiapiprolin |
| FRAC | Fungicide Resistance Action Committee |
| RF | Resistance factor |
| IPM | Integrated Pest Management |
| MoA | Mode of action |
| SSR | Single sequence repeat |
| SNP | Single Nucleotide Polymorphism |

# 1. Introduction

The pathogen *Phytophthora infestans* is causing foliar late blight and tuber blight of potato, resulting in yield losses representing a monetary value in the magnitude of one billion € annually throughout the European Union (Haverkort et al. 2008). The damage of potato late blight and tuber blight is destructive, in contrast to other diseases on potato such as scabs and scrubs which are mostly cosmetical. Data from long term field trials in Scandinavia reveal that late blight epidemics have gradually become more common, more severe and with an earlier onset during the last century, especially since the mid-1990s (Hannukkala et al. 2007; Wiik 2014). A crop rotation with subsequent cropping seasons of potato led to an earlier onset of epidemics after the year 1998 as compared to earlier years, suggesting an introduction of soil borne inoculum around this time (Hannukkala et al. 2007). This coincides with the first reports of mating type A2 and detections of *P. infestans* oospores in Sweden and other parts of northern Europe (Andersson et al. 1998; Hohl & Iselin 1984), leading to the complex and diverse population with mixed reproduction found in North-Western Europe today (Brurberg et al. 2011; Sjöholm et al. 2013; Widmark et al. 2007; Widmark et al. 2011).

A study by Wiik (2014) analysing data from 30 years of Swedish field trials found fungicide programmes to increase blight free tuber yield by 15,5 – 21,5 tonnes per ha and year during the last 20 years, an increase of up to 58%. These data in combination with reports of earlier onset and increased severity of *P. infestans* epidemics highlights the importance of, and elevated reliance on, fungicides in potato late blight management (Hannukala et al. 2007; Wiik 2014). Consequently, emergence of strains carrying resistance to fungicides would be very problematic for potato production. *Phytophthora infestans* is a highly adaptable organism and has previously developed resistance to fungicides such as the widely used fungicide metalaxyl and propamocarb-HCl (Haas et al. 2009; Lehtinen et al. 2008).

In 2022, *P. infestans* isolates of SSR genotype EU43 collected in Danish fields were found to be unresponsive to doses of 10 µg $ml^{-1}$ of the fungicide mandipropamid (MPD), in contrast to $EC_{50}$ values of 0,35-0,75 µg $ml^{-1}$ for sensitive isolates and earlier reports of $EC_{50}$ values as low as 0,01 µg $ml^{-1}$ (Abuley et al. 2023; Blum et al. 2012). MPD is a highly specific fungicide targeting the gene PiCesA3 involved in cell wall biosynthesis of oomycetes. Resistance have

previously been connected to single point mutations in the CesA3 gene causing substitution of glycine in amino acid location 1105 (Blum et al. 2010a; Sierotzki et al. 2011). Another fungicide with previous recordings of in-field resistance is oxathiapiprolin (OTP), targeting the oxysterol binding protein 1 in oomycetes (Miao et al. 2020). Already at the stage of OTP field trials in 2013, isolates with resistance factors over 1000 were found in *P. infestans* populations in the Netherlands (Mboup et al. 2022). In this case, single point mutations in the ORP1 gene causing substitution of asparagine (N) with leucine (L) in position 837 was found in the resistant isolates. With this in mind, the present study aimed to survey occurrence of similar resistances in Swedish *P. infestans* isolates.

# 2. Aims and limitations

The aim of this study was to investigate the occurrence of resistance to mandipropamid and oxathiapiprolin in *P. infestans* isolates collected in Swedish fields. A combination of phenotyping and genotyping of obtained isolates aimed at answering the following research questions:

i) Do any of the collected isolates display resistance to either one or both of the fungicides mandipropamid and oxathiapiprolin?

ii) Can resistance be connected to certain SSR genotypes of *P. infestans* or to any of the two main mutations previously connected to resistances, i. e. SNPs corresponding to amino acid position 1105 in gene PiCesA3 for mandipropamid and amino acid position 837 in the ORP1 gene for oxathiapiprolin?

The study was limited to a small number of isolates collected at three different locations and obtained results cannot be considered representative for all of Sweden. Fungicide doses used for in-lab phenotyping cannot be interpreted as field doses as they were set to range from very low concentrations meant to exert very little effect on the pathogen to concentrations far above recommended field doses, in which only highly resistant isolates would survive.

# 3. Background

## 3.1 *Phytophthora infestans* biology and epidemiology

*P. infestans* is a heterothallic oomycete plant pathogen infecting a range of domestic and wild solanaceous species, some of which are considered weeds with prospects of acting as alternative hosts (Abreha et al. 2018; Andersson et al. 2003; Garry et al. 2005; Grönberg et al. 2012; Seidl Johnson & Gevens 2014). Symptoms on potato plants include dark green, waterish, usually amorphous leaf and stem lesions evolving to brown-black necrotic blotches unrestricted by plant veins. The lesions are usually surrounded by light green or yellow chlorotic edges and especially in wet weather, sporangia can cause a soft white mildew-looking area around the lesions (figure 1). Infections normally result in plant death. Infections of tubers start at tuber eyes and result in brown sunken lesions extending into the tuber tissue.


*Figure 1. a) Leaf lesions and b) stem lesion caused by* Phytophthora infestans. *Picture: Björn Andersson*

The pathogen has a mixed reproduction system with two mating types. When mating types A1 and A2 infect a host plant simultaneously they can recombine and form new genotypes through production of sexual spores called oospores (figure

2a). Oospore production is favoured by slow epidemics which can occur as a result of fungicide applications, use of partly resistant varieties or environmental conditions with maximum oospore production attained at 10°C and high humidity. At 25°C and 5°C, oospore production is almost completely inhibited (Drenth et al. 1995; Romero-Montes et al. 2008). Oospore conducive conditions are prevalent in Scandinavia and could be an explanatory factor to the diverse *P. infestans* population found here. Oospores can remain infectious for years and after exposure to various environmental conditions, contrasting the low resilience of asexual structures such as mycelia, sporangia and zoospores which do not survive in field between seasons (Andersson et al. 1998; Fay & Fry 1997; Kirk 2003; Medina & Platt 1999). Prior to the introduction of the A2 mating type during the 1980s, the European populations of *P. infestans* relied on the asexual reproduction cycle for propagation and survival as mycelia in infected tubers, with volunteer plants, infected seed and refuse piles being primary inoculum sources (Hohl & Iselin 1984; Zwankhuizen et al. 1998).

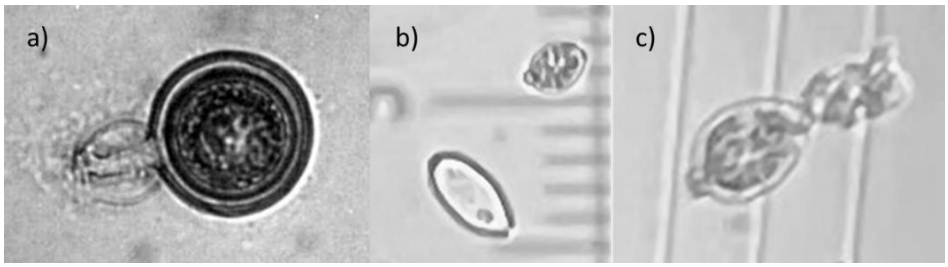


*Figure 2. a) Oospore of* Phytophthora infestans*. Licensed under CC BY-SA 2.5. b) and c) showing sporangium before, during and after zoospore release. Pictures b) and c): Lisa Ericsson*

From the epidemic starting point of oospores in the soil or infected seed tubers, mycelia grow upwards through the shoot and sporangia then emerge through stomata (Johnson 2010). Mature sporangia are dispersed by water splashes or wind. Higher sporangial viability rates have been recorded in temperatures <19°C in combination with cloudy weather, as survival is negatively affected by UV radiation (Sunseri et al. 2002). However, even at short survival spans wind can carry sporangia for distances in the magnitude of kilometres (Aylor et al. 2001, Aylor et al 2011; Sunseri et al. 2002). In infected leaf lesions, hundreds of sporangia are produced per $mm^2$ of leaf tissue with larger numbers recorded in humid conditions (Flier et al. 2007; Johnson 2010). Once dispersed, sporangia can germinate directly and cause late blight lesions when landing on host tissue or indirectly by releasing motile flagellated zoospores when landing on soil. Both direct and indirect germination require a relative humidity above 90% (Glendinning et al. 1965). Soil splashes containing spores or direct contact between host plant tissue and soil can cause late blight (Johnson 2010). Infection of potato tubers causing tuber blight is

dependent on zoospore release (figure 2b-c), favoured in temperatures <16°C, and zoospore motility which attains maximum longevity around 10°C (Sato 1979). Prior to infection, zoospores encyst and produce germ tubes and sometimes appressoria (Grenville-Briggs et al. 2005). While the sexual reproduction cycle is monocyclic, the asexual life cycle is polycyclic and is completed in less than a week under favourable conditions (Lapwood 1966; Yang et al. 2021). Generally, optimum temperatures for late blight epidemics are 16-23°C with little to no disease <7° and >28°C. (Hyre 1954; Maziero et al. 2009; Rotem et al. 1971). A schematic overview of the *P. infestans* life cycle is presented in Figure 3.

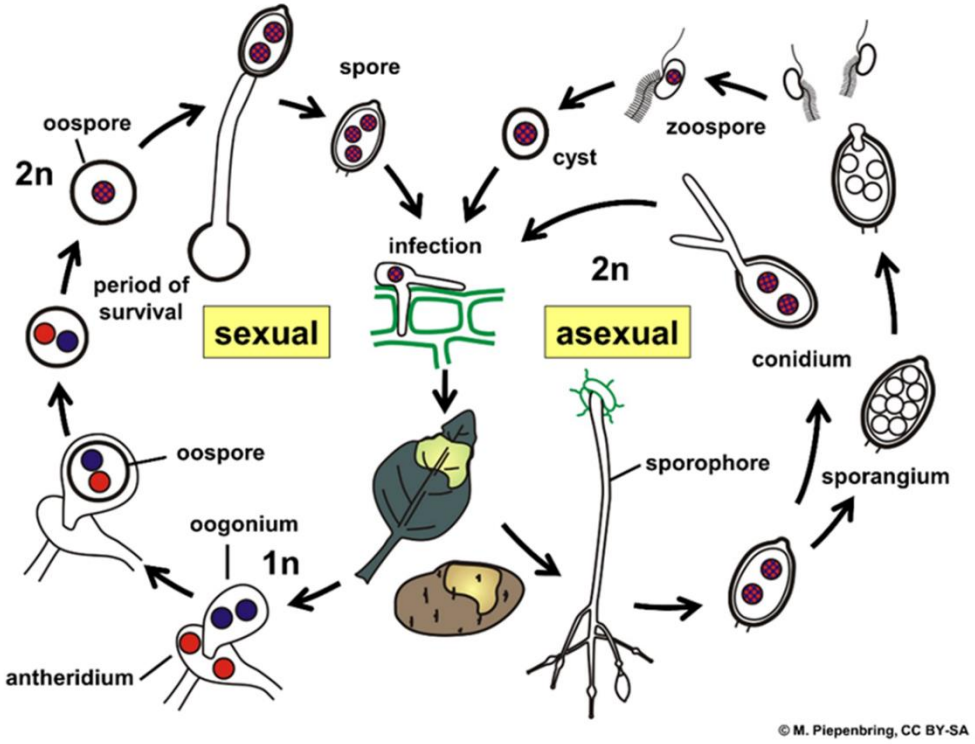


*Figure 3. "Phytophthora infestans on potato, Peronosporales, Oomycota " by M. Piepenbring. Licensed under CC BY-SA 3.0. Showing the life cycle of* P. infestans.

## 3.2 Integrated Pest Management

Based on data from the Swedish Central Bureau of Statistics (SCB), potato was grown on less than 1% of the Swedish arable land in 2022 and yet almost 10% of the total hectare-doses of fungicides sold in Sweden in 2022 targeted *P. infestans* on potato (https://www.scb.se/contentassets/12e94ca362884cbf835924425a3b4a04/mi0501

_2022a01_br_mi31br2301.pdf). This indicates a high reliance on fungicides for potato production, posing challenge to strategies such as the Integrated Pest Management (IPM), implemented in the EU according to the European Parliament and Council Directive (2009/128/EC) aimed at limiting fungicide use by shifting focus to prevention of disease through agricultural management and alternative control measures. While nothing has been able to replace fungicides, a lot of effort has been put into minimizing fungicide use in late blight control. Perhaps the most prominent research in this area has been aimed at finding and introgressing R-genes into potato cultivars, resulting in discoveries of a large number of R-genes in wild potato relatives (Paluchowska et al. 2022). In a decade long project called Durable Resistance in Potato against Phytophthora (DuRPh), scientists found that introduction of R-genes from crossable relatives (cisgenes) through genetic transformation of potato resulted in protection equivalent to 100% fungicide dose at a 25% dose rate for potato with a single introduced R-gene, and at a 0% dose rate for plants with several stacked R-genes. Even though successful control was achieved, in-field monitoring during these trials found pathogenicity towards all single R-genes employed in the trials in different *P. infestans* isolates, pointing to the high adaptability of the pathogen (Haverkort et al. 2016). Whereas effective R-genes could eradicate or at least reduce fungicide use, many of the effectors that are the target of R-genes are located in the 74% of the *P. infestans* genome classified as highly dynamic, rendering the pathogen very adaptable and prone to overcome R-genes (Haas et al. 2009). Introduced R-genes have been defeated in several instances, even when various R-genes were stacked in the same cultivar (e. g. Black et al. 1953; Malcolmson 1969). Monitoring the *P. infestans* populations and replacing or withdrawing R-genes before they are defeated can restart the adaptation process of the pathogen, thus increasing longevity of R-genes and saving the money- and time-consuming process of identifying and introducing novel R-genes (Haverkort et al. 2016).

Another promising research area is the identification and silencing of *P. infestans* genes important for the infection process. Gene silencing induced by either production of small RNA (sRNA) in genetically transformed potato plants (HIGS) or by spraying double stranded RNA (dsRNA) onto potato plants (SIGS) have been found to impair important features in *P. infestans* such as production of sporangia in vitro (Jahan et al. 2015; Kalyandurg et al. 2021; Resjö et al. 2017).

Fungicide use may also be reduced by implementing bioprotection, both in the form of living agents (biological control) and biologically derived compounds. For instance, single strains or combinations of strains of bacteria in genera *Pseudomonas, Bacillus*, *Streptomyces* and *Trichoderma* have been found to exert a biologically controlling effect on various life stages of *P. infestans,* such as mycelial growth and sporulation along with decreased overall disease severity in field (De Vrieze et al. 2019; Islam et al. 2022; Jin et al. 2023). However, the effects are often

specific to the strain of the biocontrol agent, can differ with potato cultivars and may not be consistent when transferred from lab to field conditions (Bengtsson et al. 2015; De Vrieze et al. 2019; Jin et al. 2023). Several biologically derived compounds with effects *in vitro* could possibly be combined with fungicides to reduce fungicide doses. Examples of such compounds are BABA, an amino acid derivative inducing plant resistance by callose build-up and ROS response (Harrison 1992; Liljeroth et al. 2010), bergamot and orange oils with reducing effects on sporulation abundance and lesion sizes (Messgo-Moumene et al. 2015) and sugar beet root extract, inducing responses in detached leaves leading to reduced lesion sizes and sporulation while not toxic to *P. infestans* itself (Moushib et al. 2013).

Crop management can also be adjusted to minimize inoculum buildup from sources such as infected seed tubers, volunteer plants and oospores. Hence, use of certified seed potatoes and efficient weed control are important tools for inoculum reduction. Infections originating from oospores can be avoided with crop rotations. A markedly greater effect on early infections have been reported when three cropping seasons or more surpass between each potato crop (Bødker et al; 1998; Hannukkala et al. 2007). Removal of nightshade of the species *Solanum physalifolium*, previously identified as an alternative *P. infestans* host, from potato growing areas could also lead to inoculum reduction (Andersson et al. 2003). As a high humidity is conducive for late blight epidemics, irrigation can be adapted to minimize the amount of time with high humidity in the field. Tuber blight might be reduced by selecting cultivars with longer stolons, distributing tubers away from the stem where a channel guiding zoospore-containing water downwards can appear as a result of stem wind movements (Lacey 1966; Lacey 1967). Maximizing the soil barrier by hilling and selecting cultivars with deep tubers can also act to minimize tuber blight along with killing infected haulm prior to tuber harvest (Lacey 1966; Nærstad et al 2007).

While fungicides remain the most prevalent control method for late blight outbreaks, decision support systems (DSS) are an important tool to help farmers decide when spraying is required. When comparing to routine spray treatments, using DSSs reduced the input of fungicide by 8-62% in a European assessment of DSSs for control of potato late blight in 2001 (Hansen et al. 2002).

## 3.3 Fungicides

### 3.3.1 Resistance development

As fungicide use is the most important tool for managing potato late blight, the longevity of their protectant abilities is an important issue as the process of inventing and getting new active ingredients approved can be both lengthy and

costly. Thus, avoiding development of resistance in the target organism should be a key factor for fungicide deployment. Cross resistance between fungicides with similar Modes of actions (MoAs) is a recurring phenomenon (e. g. Ziogas et al. 2006) and therefore availability of fungicides of alternative modes of action is advantageous for fungicide perseverance. In 2023, fungicides of 9 different modes of action were available for oomycete pathogens in Sweden according to the Swedish Chemicals Agency (KI) (www.kemi.se).

Fungicide resistance can exist in a pathogen population as part of the natural variation or occur as a result of inheritable mutations (Toffolatti et al. 2018). Mechanisms for fungicide resistance include ejection of the active ingredient through efflux pumps, overexpression of the target site, detoxification of the fungicide (e. g. through degradation or other modifications) or mutations at the target site inferring changes to the binding site of the fungicide (Lucas et al. 2015). Depending on which mechanism is causing the resistance, the pathogen responds differently to increased doses of fungicide. For instance, in the case of target site mutations, the fungicide no longer matches with its binding site and thus increased doses will have little effect on the pathogen. Van den Bosch et al. (2011) defines three phases in development of resistance, namely i): the emergence phase during which mutation(s) give rise to a resistant strain, ii): the selection phase when the resistant strain is allowed to amplify due to selection pressure from the active ingredient to which it is resistant and iii): the adjustment phase in which the control strategy is adapted to the new, resistant pathogen population. The risk for emergence of resistance is higher for single site compared to broad spectrum or multi-site fungicides because a single mutation is in some cases all that is needed to acquire resistance to single-site fungicides (Blum et al. 2010a; Brent & Holloman 2007). This poses increased threats to fungicide perseverance as there has been a shift in fungicide use from multi-site to single-site fungicides since the start of their use. Pathogen related risk factors for emergence of resistance are a short generation time, high spore production and dispersal potential (Brent & Holloman 2007).

*In vitro* mutagenesis studies aimed at identifying and estimating risks for mutations causing fungicide resistance and analysing stability of said resistance have in some cases found associations between resistance and fitness penalties, diminishing the effect of selection pressure from the fungicide in field (Cohen et al. 2007; Ziogas et al. 2006). Even if resistance is not associated with fitness penalties, the selection phase of resistance development can be slowed down by reducing selection pressure from the active ingredient. Applying alternating fungicides with different modes of action or mixtures of fungicides reduces selection pressure on the pathogen, as long as the same level of protection is maintained (Staub & Sozzi 1984). Application of metalaxyl (a phenylamide fungicide) to a *P. infestans* population with resistant isolates already existing in the population shifted resistance to almost a 100% in 4 generations while a mixture with metalaxyl +

mancozeb shifted resistance to 50% over the same period (Staub & Sozzi 1984). A reduced selection pressure is also achieved by reduced fungicide doses, as was reported in 9 out of 11 studies reviewed by Van den Bosch et al (2011). However, when it comes to *P. infestans*, the controlling effect of reduced doses may vary with seasons and potato cultivars. A 50% dose rate can provide the same protection as a 100% dose rate in years unfavourable for *P. infestans* but not in years with weather favouring the pathogen (Wiik et al. 2019).

### 3.3.2 Anti-resistance strategies

Working groups in the Fungicide Resistance Action Committee (FRAC) develop and provide recommendations for fungicide anti-resistance management according to MoAs. The fungicides included in the scope for this paper fall under FRAC working group 40, the carboxyl acid amides (CAA; mandipropamid), and FRAC working group 49, the oxysterol binding protein inhibitors (OSBPIs; oxathiapiprolin). Resistance risks for CAA fungicides are classified as low to medium whereas OSBPIs are at medium to high risk. Resistance management is necessary for both fungicide groups. In practice, FRAC working groups resistance management recommendations have implications on number, order and timing of applications along with application mixtures and alternations. No more than 50% of the total number of fungicide applications over a cropping season can be constituted by CAAs, while the corresponding number is 33% for OSBPIs. The number of consecutive applications cannot surmount 2 for CAAs and 3 for OSBPIs. Preventative application and alternation with fungicides of other MoAs are recommended for both groups. CAAs are preferably used in fungicide mixtures while OSBPIs can only be used in mixture with a partner where cross resistance is not recorded (a so-called anti-resistance partner). In the FRAC anti-resistance sheet for CAAs, it is mentioned that good agricultural practices should be implemented to minimise inoculum, i. e. an integrated pest management (IPM, see section 3.2) strategy should be considered. In high-risk areas, e. g. where potato crops are grown for several subsequent seasons, or where resistance is already present, there are further restrictions imposed on the above-mentioned recommendations, such as fewer consecutive applications and increased requirements on mixture application and fungicide alternations (FRAC 2023a; FRAC 2023b).

The FRAC anti-resistance strategy was assessed in a study by Toffolatti et al (2018), examining field populations of *Plasmopara viticola*, another oomycete plant pathogen, where resistance to CAAs was already present in one population and resistance alleles but no phenotypic resistance was present in a second population. Implementation of anti-resistance strategies excluding CAAs resulted in decreasing portions of resistance over the years of the study in the first population whereas implementing an anti-resistance strategy where CAA applications were included according to FRAC recommendations still resulted in the emergence of

resistance in the second population. The study conclude that anti-resistance strategies recommended by FRAC have effect but should be complemented with resistance monitoring to achieve its objectives over time.

### 3.3.3 Mandipropamid

Mandipropamid is an active substance belonging to the carboxyl acid amides (CAA) which has previously been classified as a low-risk substance regarding resistance development by several authors. One study inducing mutations with UV radiation and chemical mutagens produced mandipropamid resistance in *P. infestans* generation 0 but failed to create a stable resistance that could be transferred through several generations (Rubin et al. 2008). Another study applying selection pressure in the form of different MPD doses on various *P. infestans* isolates failed to produce resistance, but found some differences in MPD sensitivity distributed on a continuous scale leading to the proposal that MPD resistance is either a multi-locus trait, the inheritance of resistance genes is recessive or resistance is connected to severe fitness penalties (Cohen et al. 2007).

*Phytophthora infestans* is generally sensitive to low doses of MPD if applied preventatively, while the curative effect is limited and requires higher doses (Cohen & Gisi 2007). Using radioactive labels on *in vitro* applied MPD showed that the fungicide does not enter the pathogen cell but exerts its effect from outside of the cell and therefore it is primarily a contact fungicide (Blum et al. 2010a). The inhibitory effect is also reversible by washing with water (Cohen & Gisi 2007). By applying CAAs to different stages in the asexual life cycle of *P. infestans*, Cohen & Gisi (2007) discovered that the applied fungicides primarily inhibited germination of encysted zoospores. After observation of MPD amended germinating cysts, Blum et al. (2010a) observed growth inhibition and swellings at germ tube tips, leading to assumptions that the MPD mode of action is linked to cell wall production or structure. A study by Grenville-Briggs et al. (2008) found cellulose synthesis to be essential for host infection and identified cellulose synthase gene orthologs CesA1, CesA2, CesA3 and CesA4 to be upregulated during cyst germination and formation of appressoria. Several other studies devoted to identifying the mode of action and possible sources of resistance for various CAAs identified point mutations in the gene CesA3 of *P. infestans* and other related oomycetes conferring resistance to MPD (table 1). In *P. viticola*, only individuals homozygous for the SNP conferring resistance to MPD had the resistant phenotype, supporting the theory that resistance is a recessive trait. Among representatives from several clades of oomycetes, Phytophthora species were found to be the most sensitive to MPD (Blum et al. 2012). However, there are examples of oomycete species related to Phytophthora where MPD resistance already exist in the population, e. g. *P. viticola* (Toffolatti et al. 2018). Cross resistance between MPD

and other CAAs, e. g. benthiavalicarb, have been recorded for *P. viticola* as early as 2004 (Gisi et al. 2007).

*Table 1. Locations in oomycete genomes where mutations have been found to cause resistance to mandipropamid.*

| Pathogen species | Gene | Location(s) | Ref |
|---|---|---|---|
| P. viticola | PvCesA3 | 1105 | Delmas et al. 2017 |
| P. viticola | PvCesA3 | 1105 | Gisi et al. 2007 |
| P. viticola | PvCesA3 | 1105 | Blum et al. 2010b |
| P. infestans | PiCesA3 | 1105 | Blum et al. 2010a |
| Perenosporales clade | CesA3 | 1109, 1111 | Blum et al. 2012 |
| Phytophthora capsici | PcCesA3 | 1073, 1105, 1109 | Cai et al. 2021 |

### 3.3.4 Oxathiapiprolin

Oxathiapiprolin (OTP) is the first fungicide in the oxysterol binding protein inhibitor group (FRAC code 49) targeting the oxysterol binding protein 1 in oomycetes. The exact function of the target protein is unknown (Pasteris et al. 2016). Various studies have found a range of oomycetes, including *P. infestans*, to be strongly inhibited by OTP at almost all life stages (Cohen 2015; Gray et al. 2018; Miao et al. 2016; Qu et al. 2016; Wang et al. 2023). When compared to fungicides ethaboxam, fluopicolide, mandipropamid and mefenoxam, OTP had the lowest EC50 values for four different species of *Phytophthora* infecting citrus (Gray et al. 2018). OTP can translocate from a plant to drain water and between plants of tomato and potato via root exudates, protecting untreated neighbouring plants from *P. infestans* infection (Cohen & Weitman 2023). Allocation of OTP within the plant is bidirectional (true systemic), and in an Israelian study, a single application of OTP and benthiavalicarb to the soil early in the season provided durable protection against *P. infestans* infections in potato plants in a dose dependent manner for the remnant of the cropping season (Cohen & Rubin 2020; Cohen & Weitman 2023). According to FRAC, resistance to OTP was found in a *P. infestans* population (RF>1000) in the Netherlands already during field trials (FRAC 2023b; Mboup et al. 2022). In the study by Mboup et al. (2022), a single point mutation in amino acid position 837 of the ORP1 gene was detected in the resistant isolate. Mutations in position 837 was also found in an OTP resistant population of *Plasmopara viticola* (RF>30 000) and studies on genetically modified *Phytophthora capsici* and *Phytophthora sojae* confirmed that mutations in this position confer resistance to OTP in these oomycete species as well (Massi et al. 2023; Miao et al. 2018; Miao et al. 2020). Several additional mutations in the ORP1 gene of various oomycetes have also been connected to OTP resistance (Bittner et al. 2017; Miao et al. 2016b;

Wang et al. 2022). When evaluating the resistance risk of the novel fungicide fluoxapiprolin, also in the oxysterol binding protein inhibitor group, Li et al. (2022) found cross resistance between fluoxapiprolin and OTP in *P. infestans* and low to no fitness penalties in resistant isolates.

# 4. Materials and methods

## 4.1 Overview of methods

To answer the research questions posed in section 2, the study was divided into three main analyses. Presence of resistance to MPD and OTP was surveyed by phenotyping *P. infestans* isolates in a potato leaf disc assay (section 4.3). To investigate connections between resistance and known SSR genotypes defined in the EUROBLIGHT project, DNA from *P. infestans* isolates was genotyped with microsatellites (section 4.4). For detection of any SNPs previously connected to resistances to MPD and OTP, DNA from target genes CesA3 and ORP1 was extracted from *P. infestans* isolates and Sanger sequenced (section 4.1).

## 4.2 Sampling and isolation

*Phytophthora infestans* isolates included in the analyses described above were collected in Swedish fields during 2023 or provided as reference samples by Aarhus university, Denmark. Isolates were collected from potato leaves displaying late blight symptoms at the sampling occasion. Potato leaves with late blight lesions were sampled on August 9 (batch 1, samples 1-120) or provided at a later date by Hushållningssällskapet (batch 2, samples 1.2-20.2) and SLU Alnarp (batch 3) (table 2). In addition, reference samples of SSR genotype EU43 with MPD resistances as reported by Abuley et al. (2023) were provided by Aarhus university, Denmark. Samples of batch 1 were collected in untreated fields whereas samples of batch 2 were collected in a field where MPD and OTP were included in the spraying regime in fungicides Revus Top and Zorvec, respectively. At the sampling occasion for samples of batch 1 & 2, *P. infestans* lesions were divided in two with one half put in individual plastic zip-lock bags for transportation and the other half pressed onto FTA papers to capture and preserve *P. infestans* DNA. The half of the lesions transported in plastic bags were transferred to slices of potato tubers later on the same day and incubated in room temperature until mycelia was visible.

Rye-pea agar was prepared by soaking 30 g of organic rye in deionized water overnight and boiling the mixture for 15 min before adding 60 g of organic frozen

peas and boiling for an additional 45 min followed by sieving through a strainer and dilution with deionized water to 1 l. 15 g of agar was added before the medium was autoclaved and poured onto petri dishes at a volume of ~20 ml per plate. For the streptomycin amended plates, streptomycin was pipetted, spread out and allowed to diffuse into the agar plates after they had cooled off to room temperature.

Mycelia from viable isolates was transferred to a rye-pea agar medium on petri dishes (Ø=9 cm), incubated in room temperature and checked for contaminations. Contaminated isolates were discarded or in viable cases transferred to fresh agar plates. Some isolates containing bacteria were transferred to rye-pea agar amended with the antibiotic streptomycin at a concentration of 10 µg $mL^{-1}$ medium. When axenic isolates were achieved, they were stored in darkness at 12°C and maintained by transfer to new agar plates when the old plate was covered by mycelia.

*Table 2. Information on the isolates sampled for this study. Mandipropamid is the active ingredient in Revus Top and oxathiapiprolin is the active ingredient in Zorvec, used for treatment in the field where samples of batch 2 were collected.*

| Samples | | Cultivar | Location | Treatment (date) |
|---|---|---|---|---|
| Batch 1 | *1-40* | Folva | Lilla Böslid, Halland | Untreated |
| | *41-80* | Folva | Mosslunda, Kristianstad | Untreated |
| | *81-120* | Tinca | Mosslunda, Kristianstad | Untreated |
| Batch 2 | *1.2-20.2* | Dartiest | Sölvesborg | Ranman Top (2023-06-19)<br>Infinito (2023-07-05)<br>Revus (Top 2023-07-20)<br>Leymay + Zorvec (2023-08-02)<br>Revus Top (2023-08-16)<br>Revus Top (2023-08-30) |
| Batch 3 | - | N/A | Mosslunda, Kristianstad | Untreated |
| References | *DKXXX* | N/A | Denmark | N/A |

## 4.3 DNA extraction, amplification and Sanger sequencing

Prior to the DNA extraction process, agar plugs of all viable isolates were transferred to liquid rye-pea medium. The liquid medium was prepared in the same way as the solid medium described in paragraph 4.2 with the exception of agar exclusion and addition of 5 g sugar per litre medium. Isolates were cultivated in liquid medium on petri dishes (10 ml medium per plate) in room temperature for 6 days. Mycelia was harvested from the liquid medium, transferred to plastic tubes with glass beads and freeze dried at -83 °C for 24 hrs prior to vortexing of the tubes to break the cell walls. DNA was extracted with the magnet bead based NucleoMag® Macherey-Nagel Plant kit and Mealstrom 4800 extraction robot.

Concentrations of the extracted DNA in resulting samples corresponding to each of the isolates were measured with a Nanodrop and diluted to concentrations of 1 ng $\mu l^{-1}$.

Sequences for primer pairs corresponding to PiCesA3 (forward (CTACGACTCGGTGCTGTATCC)/reverse (CTCGGGGTCTTCTTCATGGC)) and ORP1 (forward (GACTTGATGCTGTACGCA) /reverse (CTCCAGTACGTCTTGTTG)) were provided by Syngenta and published by Mboup et al (2022), respectively, and ordered from LabLife Nordic. Standard PCR mixtures for both primer pairs were prepared and mixed with DNA from each of the isolates before a PCR programme was run (table 3). Gel electrophoreses were performed at 300A and 180V for 20 minutes to confirm a successful PCR. PCR products were purified using Sera-Mag magnetic carboxylate, a PCR Clean-up reagent based on magnetic bead technology and sent to Macrogen Europe for Sanger sequencing. Obtained sequences were assembled and compared using SeqMan Pro®.

*Table 3. PCR program used for amplification of PiCesA3 and ORP1.*

| | Stage 1 | Stage 2 | | | | Stage 3 |
|---|---|---|---|---|---|---|
| Temperature | 95°C | 95°C | 58°C | 72°C | 72°C | 4°C |
| Time | 0:03:00 | 0:00:30 | 0:00:30 | 0:01:00 | 0:05:00 | ∞ |
| No of cycles | 1x | 35x | | | | 1x |

## 4.4 Phenotyping

To determine sensitivity of the *P. infestans* isolates to fungicides mandipropamid and oxathiapiprolin, a floating leaf disc assay was performed. For this purpose, 24 well plates (well size Ø=15 mm) were utilized. FRAC recommendations for testing of fungicide sensitivities (Edel & Sierotzki 2007; Jaworska et al. 2017) were modified to fit the 24 well plates and according to this, six concentrations of mandipropamid (0, 0.1, 0.3, 1, 3 and 10 µg $ml^{-1}$) and oxathiapiprolin (0, 0.000064, 0.00032, 0.0016, 0.040 and 1 µg $ml^{-1}$), were prepared using fungicides Revus (active ingredient MPD) and Zorvec Enicade (active ingredient OTP). According to the setup schematically presented in table 4, 1,5 ml of each concentration was put in a well for each of the isolates. This was repeated four times for every isolate. 12 mm leaf discs were punched out from leaves of approximately 4 weeks old potato plants (cv. Solist) and placed abaxial side up in the wells.

Inoculum was prepared by transferral of agar plugs with mycelia onto potato slices which were subsequently incubated in room temperature for four days to induce sporulation. Sporangia were harvested and the sporangial concentration adjusted to $10^4$ sporangia/ml suspension. Prior to inoculation, the suspensions were

incubated in 4 °C for two hours to induce the release of zoospores. 20 µl of suspension was then pipetted onto each leaf disc. All plates were incubated in a climate-controlled chamber in 17 °C, 85% relative humidity and a 12 hrs light/darkness regime for six days before *P. infestans* infection of the leaf discs were scored as follows: 0 = no sporulation, 1 = very few sporangia, 2 = thinly spread sporangia, 3 = full sporangia coverage. Incidence of disease was calculated using the median values of scores ($Y_{median}$) divided by the score representing full coverage (3) using Equation 1. Efficacy of the disease control exerted by the fungicides was calculated using Equation 2 where $Y_{ci}$ represents the incidence of disease for the cth concentration in the ith isolate and $U_{ci}$ represents incidence of disease on the negative control discs for the cth concentration in the ith isolate. Values obtained for efficacy of disease control were used for fitting of a logistic curve and extraction of $EC_{50}$ values for individual isolates.

Eq 1. $$I = \frac{Ymedian}{3} \times 100$$

Eq 2. $$E = 1 - \frac{Yci}{Uci} \times 100$$

To analyse phenotypic differences between isolates, a Kruskal-Wallis test followed by a Dunns test with a Bonferroni-correction was carried out in Matlab (2023) for each concentration of MPD and OTP, using code built with the help of ChatGTP (2024).

*Table 4. Schematic view of the setup of the 24 well plates used for the floating leaf disc assay with A, B, C and D representing repetitions of the same isolates with increasing fungicide concentrations from left to right.*

| Concentration / Repetition | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | | | | | | |
| B | | | | | | |
| C | | | | | | |
| D | | | | | | |

## 4.5 Genotyping

At the sampling occasion, the abaxial side of each leaf was pressed onto an FTA card to capture DNA of *P. infestans*. The FTA papers were air-dried and stored in room temperature. After isolation of *P. infestans* samples, the FTA papers corresponding to successfully isolated samples were sent to the James Hutton institute in Scotland for genotyping according to standardised protocols available at www.euroblight.net (Cooke 2020; Cooke et al. 2020). As described by Li et al.

(2013), microsatellite primers targeting 12 different single sequence repeats (SSRs) loci were used in a multiplex PCR amplifying all 12 loci of one isolate simultaneously in a single assay. Fluorescent labels were attached to all primers in order to identify the resulting DNA segments. Results from this genotyping reveal what SSR genotype the *P. infestans* isolates belong to. For analysis of the microsatellite genotyping, a Neighbour joining tree was constructed using Bruvo distance in R version 4.2.2 with the POPPR package version 2.9.3 (Kamvar et al. 2014; R Core Team 2023).

# 5. Results

## 5.1 Sampling and isolation

Due to a very late onset of the 2023 *P. infestans* epidemic, potato plants had already lost vigour at the time of sampling for reasons other than being infected with late blight. As a result, many samples were contaminated with various other organisms and therefore the numbers of viable *P. infestans* isolates decreased by approximately 90% during the isolation process. Additionally, samples 1.2-20.2 (batch 2, collected in fungicide treated plots) were very weak on the rye-pea medium. Samples in batch 3 were heavily contaminated with bacteria and could not be included in any analyses. A summary of remaining viable isolates included in microsatellite genotyping, Sanger sequencing and phenotyping is presented in table 5.

*Table 5. Summary of isolates viable for phenotyping and Sanger sequencing after the isolation process along with the samples sent for microsatellite genotyping from FTA cards.*

| Samples | | Phenotyping | Sanger sequencing (CesA3 and ORP1) | Genotyping |
|---|---|---|---|---|
| Batch 1 | *1 – 40* | 2, 9, 33, 36 | 2, 9, 33, 36 | 2, 9, 33, 36 |
| | *41 – 80* | 63, 66 | 63, 66, 76 | 63, 66, 76 |
| | *81 – 120* | 107, 112, 120 | 107, 112, 120 | 107, 112, 120 |
| Batch 2 | *1.2 – 20.2* | 10.2 | 7.2, 10.2 | 1.2, 2.2, 3.2, 4.2, 5.2 |
| Refs | *DKXXX* | - | DK015, DK079, DK085, DK105 | - |
| Sum | | 10 | 16 | 16 |

## 5.2 DNA extraction, PCR and Sanger sequencing

DNA extraction was successful for all samples included in the Sanger sequencing process (table 5). Reference sample DK72 was initially included but was lost in preparation for the PCR due to equipment malfunction. The PCR had to be repeated

several times with adjustments to the PCR program before products were obtained for both primer pairs.

Sanger sequencing of ten samples from Lilla Böslid and Mosslunda (batch 1, samples 1-120 in table 5) showed no SNPs in the location connected to MPD resistance and therefore no substitution of the amino acid in position 1105 in CesA3. Unfortunately, the Sanger sequencing of the CesA3 gene failed for samples 7.2, 10.2 and the Danish reference isolates (DKXXX in table 5). Sequencing of ORP1 failed for all isolates.

## 5.3 Phenotyping

In phenotyping, no significant differences were found between any isolates from batch 1 (samples 1-120). However, isolate 10.2 (batch 2) was significantly different from all other isolates at MPD concentrations of 3 and 10 µg $ml^{-1}$ and at an OTP concentration of 1 µg $ml^{-1}$. At an MPD concentration of 1 µg $ml^{-1}$, isolate 10.2 was significantly different from isolates 2, 9, 21, 33, and 107. At an OTP concentration of 0,04 µg $ml^{-1}$, isolate 10.2 was significantly different from isolates 9, 21, 63 and

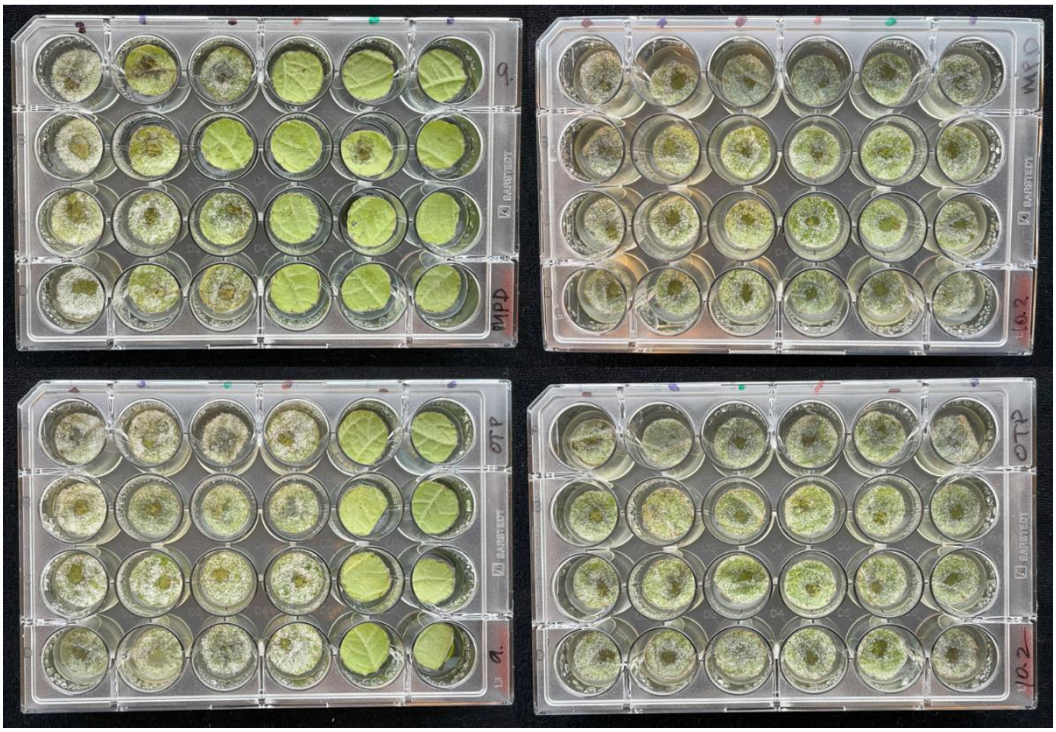


*Figure 4. Responses of* phytophthora infestans *to mandipropamid (top row) and oxathiapiprolin (bottom row) with isolate 9 (sensitive) to the left and isolate 10.2 (resistant) to the right. Fungicide concentrations from left to right on each plate are 0, 0.1, 0.3, 1, 3 and 10 µg ml-1 and 0, 0.000064, 0.00032, 0.0016, 0.040 and 1 µg $ml^{-1}$ for MPD and OTP, respectively. In infected wells, sporangia are visible as a white mildew-looking coating. Green leaf discs are not infected. Picture: Lisa Ericsson*

112. Resistance to both fungicides was observed in isolate 10.2 as there was no change in disease incidence for this isolate from the lowest to the highest concentration of either MPD or OTP (figure 4-5). All other isolates were sensitive and displayed a dose response with decreasing incidence of disease at increasing fungicide concentrations and EC50 values ranging from 0,1-0,3 µg $ml^{-1}$ for MPD and 0,0081-0,0252 µg $ml^{-1}$ for OTP (figure 5, Appendix 1).

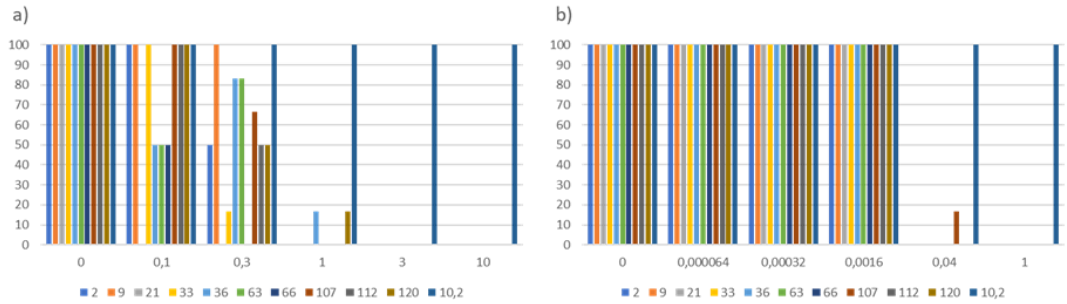


*Figure 5 Incidence of disease (%) for a) mandipropamid and b) oxathiapiprolin with fungicide concentrations µg $ml^{-1}$ on the x-axis and isolates represented by coloured bars.*

## 5.4 Microsatellite genotyping

Results from the Microsatellite genotyping conducted at the James Hutton institute in Scotland revealed that all isolates sampled on August 9 (samples 1-120) were genetically different from all previously defined SSR genotypes of *P. infestans* and therefore classified as "other". Some isolates were so closely related they could be considered clones of the same individual (figure 6). Groups of clones were sampled in the same locations; isolates 9 and 36 in Lilla Böslid and isolates 63, 107, 112 and 120 in Mosslunda. All five of the isolates originating in the fungicide treated plot in Sölvesborg (samples 1.2-20.2) sent for genotyping were classified as SSR genotype EU43 (data not shown). Isolate 10.2 was not among the isolates sent for SSR genotyping.

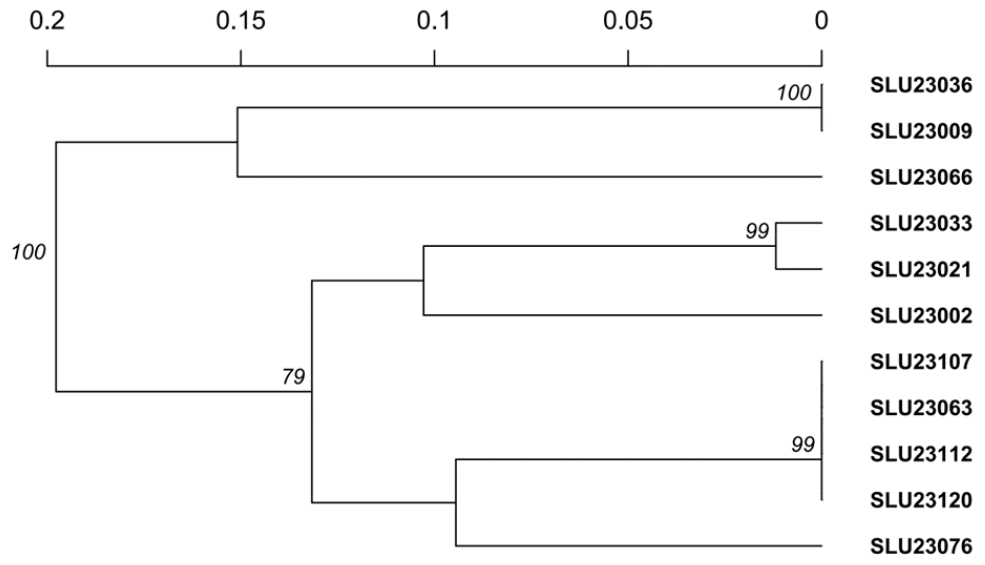


*Figure 6. Neighbour-Joining tree for* Phytophthora infestans *isolates sampled in two field trials (Samples 1-120) in Sweden 2023. The tree was built using Bruvo's distance and clustering. The robustness of the node was assessed using bootstrap resampling (n = 1000 boots). Cut-off value was set to 75.*

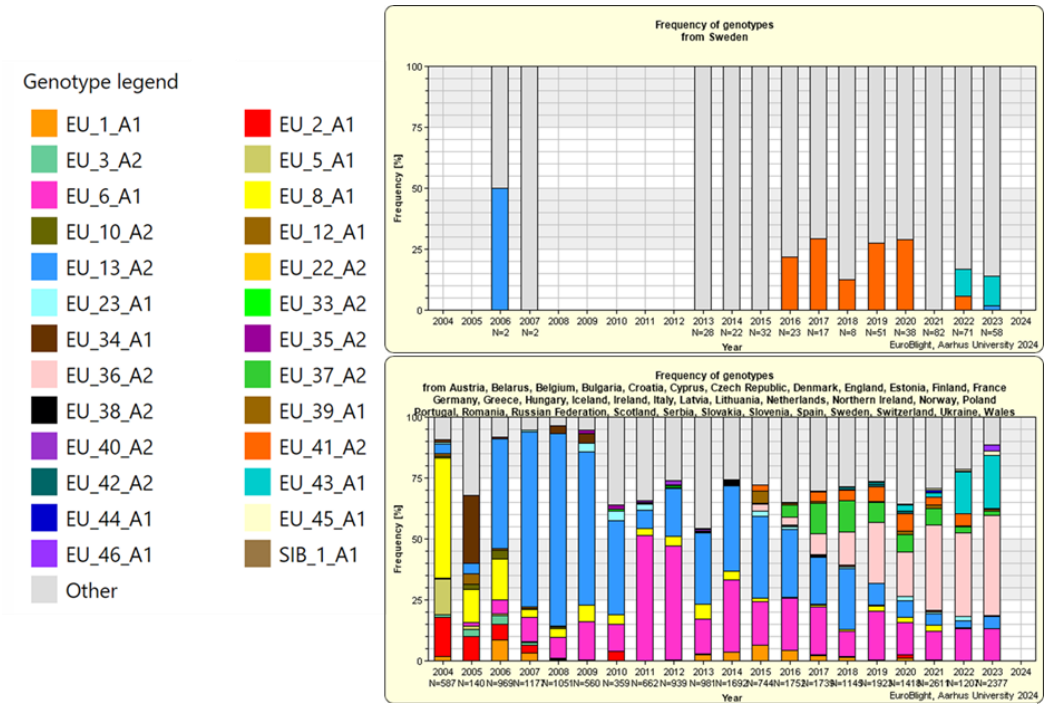


*Figure 7.* P. infestans *SSR genotypes in Sweden (top right) and the rest of Europe (bottom right). Coloured bars represent specific SSR genotypes while the grey represent a wide variety of undefined genotypes. Genotype frequency charts extracted from Euroblight.net (2023).*

# 6. Discussion

This study reports the first incidence of resistance to both MPD and OTP in a single *P. infestans* isolate sampled in Swedish fields. The resistance in isolate 10.2 was obvious in phenotyping (figure 4-5) but unfortunately, it could not be connected to SNPs previously reported to confer resistance since Sanger sequencing was unsuccessful for both CesA3 and ORP1 in this particular isolate. No $EC_{50}$ values could be calculated for isolate 10.2 as incidence of disease was 100% throughout all fungicide concentrations with maximum concentrations of 10 µg $ml^{-1}$ for MPD and 1 µg $ml^{-1}$ for OTP, contrasting previous records of $EC_{50}$ values of 0,35-0,75 µg $ml^{-1}$ and 0,001-0,03 µg $ml^{-1}$ for MPD and OTP, respectively (Abuley et al. 2023; Mboup et al. 2018). For the sensitive isolates phenotyped in this study (batch 1), $EC_{50}$ values were comparatively low for mandipropamid (0,1-0,3 µg $ml^{-1}$) and within the previously reported range for oxathiapiprolin (0,0081-0,0252 µg $ml^{-1}$).

Along with obvious issues connected to loss of fungicide efficiency in affected regions, resistance to OTP also means the loss of a unique MoA as OTP is the only fungicide in FRAC group 49 approved in Sweden for oomycete control. Such a loss may affect long term resistance management considering that one important tool in anti-resistance programmes is minimising selection pressure by alternating MoAs or using them simultaneously in mixtures (see section 3.3.2). The immediate consequence for potato production in affected regions would be an increase in regulations on fungicide use – MPD would have to be applied in mixtures with a maximum of two consecutive applications or in strict alternation and OTP would have further restrictions on consecutive applications than previously suggested (FRAC 2023a, FRAC 2023b).

In Sweden, OTP for *P. infestans* control is sold in one of two products, Zorvec Endavia or Zorvec Enicade. While OTP is the only active ingredient in Zorvec Enicade, it is combined with anti-resistance partner benthiavalicarb in Zorvec Endavia. Benthiavalicarb is a fungicidal ingredient belonging to the CAA fungicides, hence sharing its MoA with MPD. In a field treated with Zorvec Endavia, *P. infestans* isolates carrying resistance to only one of the active ingredients would still be affected by the treatment. However, a previous study reported cross resistance between MPD and benthiavalicarb, rendering the possibility that isolates like 10.2 could carry resistance to both anti-resistance partners OTP and benthiavalicarb in Zorvec Endavia (Gisi et al. 2007). In a case

like this, there would be implications on the future deployment of Zorvec Endavia as FRAC recommends OSBPI be used only in mixtures with partners where cross resistance have not been recorded (FRAC 2023b). However, a more comprehensive study including benthiavalicarb in the phenotyping part would be needed to confirm presence of resistance to both OTP and benthiavalicarb. Considering that the discovery of resistance in *P. infestans* was restricted to a single field in this study, performing similar studies on a material with larger distribution throughout Sweden would be useful for resistance monitoring in the future.

While resistance to OSBPIs have previously been characterised to have low to no fitness penalties, studies on resistance towards the CAA fungicide MPDin *P. viticola* populations have found that resistant isolates are less prevalent in fields where selection pressure by fungicide application is not applied, suggesting fitness penalties to the resistance (Li et al. 2022; Toffolatti et al. 2018). Field observations during 2022-2023 points to fitness penalties of *P. infestans* as well since the resistant genotype EU43 have repeatedly been found in MPD treated fields but never in untreated fields. Going forward, this hypothesis should be tested for *P. infestans* in the Swedish context as this could be of consequence for future use of MPD and OTP. In the case of *P. viticola*, a decline in the frequence of resistance in affected fields was recorded after three years or more of MPD free spraying regimes (Toffolatti et al. 2018). In the present study, isolates connected to resistance were very weak on rye-pea medium, suggesting either a fitness penalty or selection between sensitive and resistant isolates by the medium itself. To avoid this type of differentiation, it would be favourable to be able to extract DNA for Sanger sequencing directly from FTA papers and to use an alternative method for isolation to increase the recovery rate of samples. A possible issue with the method of floating leaf discs in pre-prepared fungicide solutions used in this study is the abilities of the fungicides to translocate in the plant. OTP is a true systemic fungicide as opposed to MPD which is contact-based, possibly causing some differences in the disease response (Blum et al. 2010a; Cohen & Weitman 2023).

Traditionally, the Swedish *P. infestans* population is diverse and the genotype distribution does not correspond to the European situation where a few strains dominate the populations (figure 7). The most common genotype in Sweden is “other”, meaning that isolates are genetically not close enough to each other or to other known genotypes to be classified as a specific SSR genotype. This situation is also true for isolates of batch 1, collected in untreated plots during this study, which could not be classified as any specific SSR genotype (isolate 1-120). In Denmark, MPD resistance has so far only been found in SSR genotype EU43 (Abuley et al. 2023). Unfortunately, isolate 10.2 displaying resistance in this study were not sent for microsatellite genotyping. However, other isolates from batch 2, collected in the same fungicide treated field as 10.2 were classified as EU43, leading to the conclusion that this particular isolate should be sent for genotyping

in the future as the probability of it being a EU43 is high. The connection between the EU43 genotype and resistance should be investigated closer for Swedish populations as it would be time and cost effective to use microsatellite genotyping as a differentiator for further resistance studies.

In conclusion, resistance to both MPD and OTP was found in a single *P. infestans* isolate but no connections could be made between resistance and any specific SNPs or SSR genotypes as sequencing and genotyping were not successfully performed for this isolate. The natural next step would be to successfully complete these processes for the resistant isolate and to carry out more comprehensive studies to extend the search for fungicide resistance outside the field in southern Sweden where it was found.

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# Popular science summary

Potato late blight is a disease caused by a fungal-like pathogen called *Phytophthora infestans*. Potato plants infected by *P. infestans* are heavily affected and usually die within days or weeks after infection. A result of this is large losses of potato yield, equivalent to billions of Euros worldwide, every year.

*P. infestans* can reproduce and amplify quickly, produce survival structures with the ability to overwinter in harsh conditions and spread by infected plant parts or soil, by wind and water splashes. It thrives in humid and relatively cool conditions such as those prevalent during summers of the temperate regions. Some alternative management methods can slow the pathogen down but none matches the efficiency of chemical plant protectants called fungicides that are available on the market today. The effect of this is a heavy reliance on fungicides for potato crop protection which means that if *P. infestans* develop resistance to fungicides, production is negatively affected. In this study, *P. infestans* sampled in Swedish fields are investigated for resistance to two fungicides: mandipropamid (MPD) and oxathiapiprolin (OTP). *P. infestans* resistance to MPD has previously been found in Denmark in 2022 and in the Netherlands in 2013. By placing *P. infestans* infected potato leaf discs in a series of concentrations of MPD and OTP ranging from low to high, we found that one of the *P. infestans* individuals were able to grow even in the highest concentrations and was thereby deemed resistant two both fungicides. The target of these fungicides are specific genes in the *P. infestans* DNA, a gene called CesA3 for MPD and a gene called ORP1 for OTP. In this study, these two genes were sequenced to search for specific mutations which have previously been found to be the cause for resistance to the two fungicides. Unfortunately, sequencing mostly failed and no mutations could be found in the resistant individual. This is the first discovery of *P. infestans* resistance to MPD and OTP in Sweden and might result in consequences for the way these fungicides are used in the future. If we spray a field where resistance to a fungicide is present, only the resistant individuals will survive and be able to reproduce, e. g. going from a fraction of resistance to up to almost 100% resistance in that field. To avoid this, restrictions can be imposed on the allowed number of applications, which fungicide mixtures to apply and how they should be altered. However, as only one resistant isolate was found in this study, more comprehensive investigations are needed for more conclusive results regarding the Swedish *P. infestans* population.

# Acknowledgements

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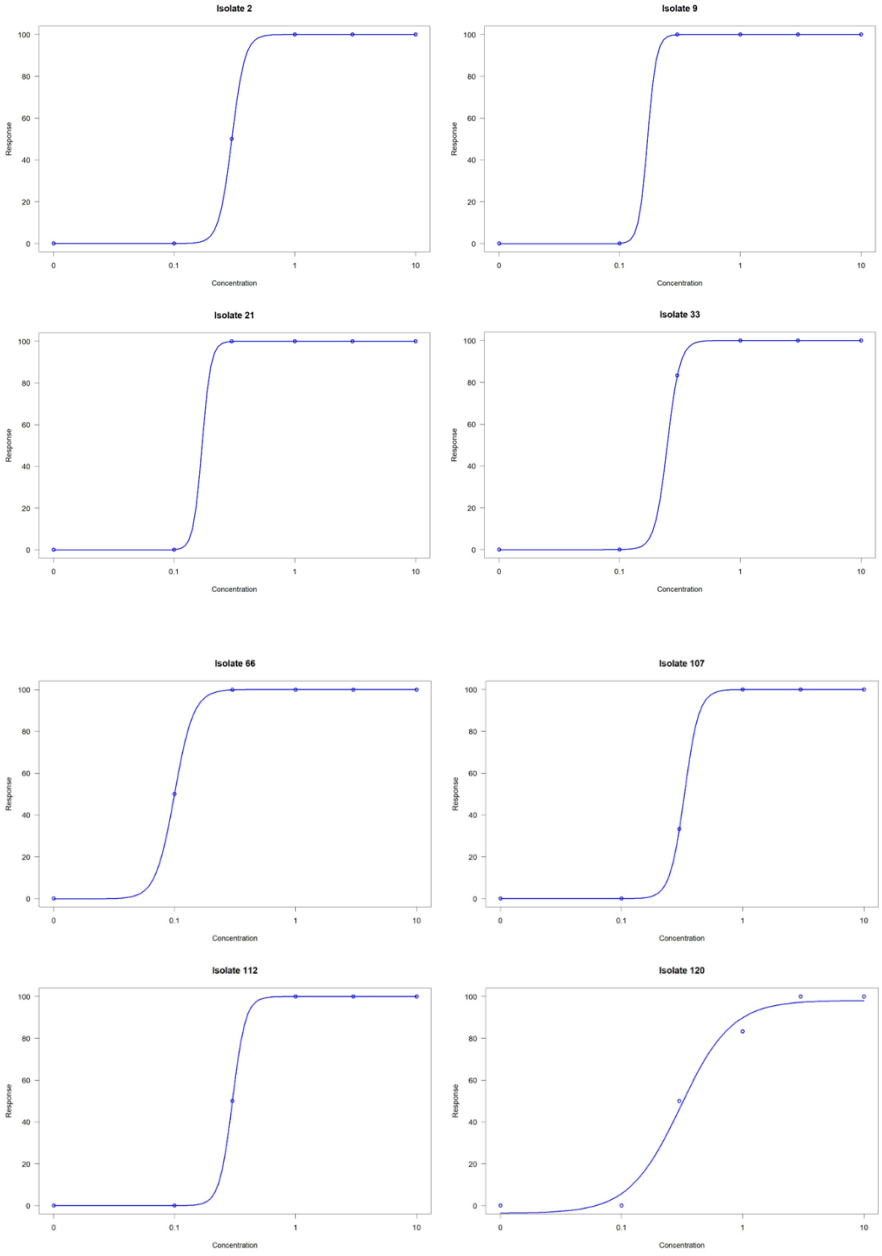
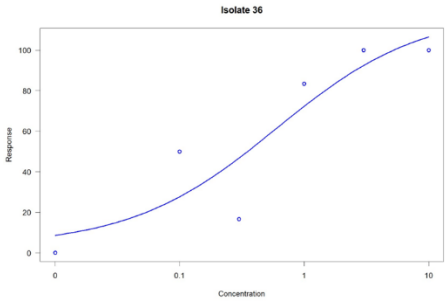
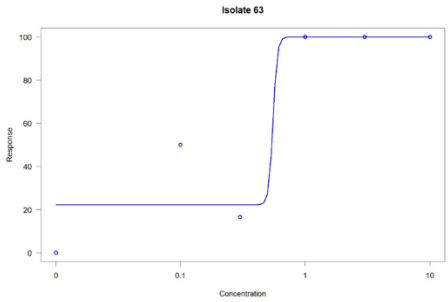
# Appendix 1

$EC_{50}$ curves for mandipropamid; one diagram for each sensitive isolate (10 curves), and for oxathiapiprolin, with identical responses for all sensitive isolates except 107 (2 curves). Table of $EC_{50}$ values with standard errors below.



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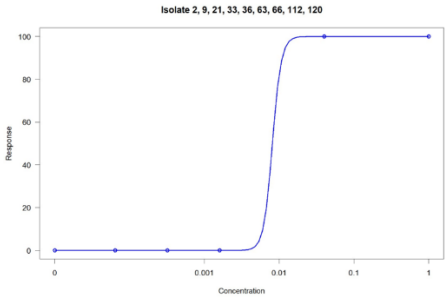


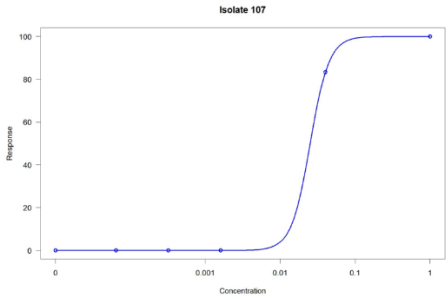


| *Isolate* | *EC50 CAA* | *Std error* | *EC50 OSBP* | *Std error* |
|---|---|---|---|---|
| *2* | 0,3 | 0 | 0,0081 | 0,00001 |
| *9* | 0,1702 | 0,0075 | 0,0081 | 0,00001 |
| *21* | 0,1702 | 0,0075 | 0,0081 | 0,00001 |
| *33* | 0,2477 | 0,0026 | 0,0081 | 0,00001 |
| *36* | 0,5669 | 1,1621 | 0,0081 | 0,00001 |
| *63* | 0,5522 | 182,6 | 0,0081 | 0,00001 |
| *66* | 0,1 | 0,0001 | 0,0081 | 0,00001 |
| *107* | 0,3301 | 0,0017 | 0,0252 | 0,00001 |
| *112* | 0,3 | 0 | 0,0081 | 0,00001 |
| *120* | 0,3057 | 0,0573 | 0,0081 | 0,00001 |

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