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Population structure of the current population of Phytophthora infestans in Brazil and sensitivity to oomycides

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Thesis submitted to the Plant Pathology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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ABSTRACT

SILVA, Mariana Guimarães, D.Sc., Universidade Federal de Viçosa, August, 2024. **Population structure of the current population of Phytophthora infestans in Brazil and sensitivity to oomycides**. Adviser: Eduardo Seiti Gomide Mizubuti.

Pathogen populations change over time due to the inexorable action of evolutionary processes. The analysis of the current pattern of genetic variation in the population of plant pathogens may lead to: a better understanding of the evolutionary history; forecast the future profile of variants; and infer their epidemiological implications. Populations of *Phytophthora infestans*, the causal agent of potato and tomato late blight, have been studied for a long time, but the epidemics still cause yield losses of both crops worldwide. In this study, isolates of *P*. *infestans* obtained from 2020 and 2024 in Brazil were characterized using phenotypic and genotypic markers: mating type, mitochondrial DNA haplotypes, microsatellite genotypes, and sensitivity to mefenoxam. Additionally, the sensitivity of the population to mefenoxam, mandipropamid, zoxamide, and oxathiapiprolin was assessed by different methods: sporangia germination, mycelial growth, and sporulation on leaf disc. The present study also reports the efficacy of zoxamide and oxathiapiprolin on potato and tomato late blight control in field conditions and the stability of the sensitivity to these oomycides during the season. The results show that the population of *P*. *infestans* from Brazil is composed by mating types A1 and A2, self-fertile isolates were identified from samples of Santa Catarina state, the Ia and Ib mitochondrial DNA haplotypes are occuring associated with potato and only the Ib occurs in tomato samples. Three clonal lineages are present here in Brazil, the EU_2_A1, EU_37_A2, and SA_1_A2, the new one. The European lineages occur associated with potato while the new one, SA_1_A2, with tomato. The sensitivity to mefenoxam is varied among the isolates tested. Most isolates were intermediately resistant and five isolates were resistant to mefenoxam. The majority of isolates were sensitive to mandipropamid and no one grew or sporulated at 10 µg/mL of the compound. Likewise, there was no evidence of reduced sensitivity to zoxamide or oxathiapiprolin. The best treatments to control potato and tomato late blight were oxathiapiprolin + dimethomorph and zoxamide + chlorothalonil interleaved (7 days) with cymoxanil + mancozeb. No decrease in sensitivity to oxathiapiprolin was observed at the end of a pressurization experiment. This study updates the status of the population of *P*. *infestans* in Brazil.

Keywords: population biology; late blight; potato ; tomato ; plant disease control

RESUMO

SILVA, Mariana Guimarães, D.Sc., Universidade Federal de Viçosa, agosto de 2024. **Estrutura populacional da população atual de Phytophthora infestans no Brasil e sensibilidade a diferentes oomicidas**. Orientador: Eduardo Seiti Gomide Mizubuti.

As populações de patógenos mudam ao longo do tempo devido à ação inexorável dos processos evolutivos. A análise do padrão atual de variação genética na população de patógenos de plantas pode levar a: uma melhor compreensão da história evolutiva; prever o perfil futuro das variantes; e inferir suas implicações epidemiológicas. As populações de *Phytophthora infestans*, o agente causal da requeima da batateira e do tomateiro, são estudadas há muito tempo, mas as epidemias ainda causam perdas de rendimento de ambas as culturas em todo o mundo. Neste estudo, isolados de *P. infestans* obtidos de 2020 a 2024 no Brasil foram caracterizados usando marcadores fenotípicos e genotípicos: tipo de acasalamento, haplótipos de DNA mitocondrial, genótipos de microssatélites e sensibilidade ao mefenoxam. Além disso, a sensibilidade da população ao mefenoxam, mandipropamida, zoxamida e oxatiapiprolina foi avaliada por diferentes métodos: germinação de esporângios, crescimento micelial e esporulação em disco foliar. O presente estudo também relata a eficácia da zoxamida e da oxatiapiprolina no controle da requeima da batateira e do tomateiro em condições de campo e a estabilidade da sensibilidade a esses oomicidas durante a estação. Os resultados mostram que a população de *P. infestans* do Brasil é composta pelos tipos de acasalamento A1 e A2, isolados autoférteis foram identificados em amostras do estado de Santa Catarina, os haplótipos de DNA mitocondrial Ia e Ib estão ocorrendo associados à batata e apenas o Ib ocorre em amostras de tomate. Três linhagens clonais estão presentes aqui no Brasil, a EU_2_A1, EU_37_A2 e SA_1_A2, a nova. As linhagens europeias ocorrem associadas à batata, enquanto a nova, SA_1_A2, ao tomate. A sensibilidade ao mefenoxam é variada entre os isolados testados. A maioria dos isolados foi intermediáriamente resistente e cinco isolados foram resistentes ao mefenoxam. A maioria dos isolados foi sensível à mandipropamida e nenhum cresceu ou esporulou a 10 µg/mL do composto. Da mesma forma, não houve evidência de sensibilidade reduzida à zoxamida ou oxatiapiprolina. Os melhores tratamentos para controlar a requeima da batateira e do tomateiro foram oxatiapiprolina + dimetomorfe e zoxamida + clorotalonil intercalados (7 dias) com cimoxanil + mancozebe. Nenhuma diminuição na sensibilidade à oxatiapiprolina foi observada no final de um experimento de

pressurização. Este estudo atualiza o status da população de *P*. *infestans* no Brasil.

Palavras-chave: biologia de população ; requeima ; batata ; tomate; controle de doenças

Summary

General Introduction

The oomycete *Phytophthora infestans* (Mont.) de Bary is the (in)famous pathogen that causes late blight, a recurrent disease highly destructive to potato and tomato crops (Haverkort et al. 2008; Judelson and Blanco 2005). The pathogen affects the whole plant, causes direct damage to leaves, tubers, and fruits (Fry and Mizubuti 1998), reduces yield, and increases production costs (Haverkort et al. 2008). *P. infestans* has been considered a highly destructive pathogen since The Great Famine in Ireland, a historic event that had catastrophic consequences to the Irish people (Jordan 1997; Ristaino 2002). Furthermore, the elucidation of this oomycete as the causal agent of late blight is considered the beginning of plant pathology as a science (Amorin et al. 2019; Ristaino 2002).

Phytophthora infestans has cellulose as the main constituent of cell walls, is diploid with one haploid phase in its life cycle, and does not have pigments (Judelson and Blanco 2005). This oomycete is a hemibiotrophic pathogen with a biphasic infection strategy, i.e. behaves as a biotrophic and a necrotrophic pathogen in the first and second phases of infection, respectively (Zuluaga et al. 2016). *P. infestans* is classified as a heterothallic pathogen and two mating types (A1 and A2) are required for sexual reproduction to take place. Each mating type can reproduce asexually by means of zoospores that are produced in sporangia. The sporangium can germinate directly or by releasing biflagellate spores that move in water, the zoospores. Temperature drives how sporangium germinates: high temperatures favor direct germination while low temperatures (below 10°C) favor zoospore formation (Judelson and Blanco 2005). The sexual reproduction only occurs between two individuals of opposite mating types and the sexual spore, the oospore, is produced. However, isolates able to produce oospores by itself, the self-fertile isolates (Casa-Coila et al. 2017; Fyfe and Shaw 1992; Orona et al. 2013; Zhu et al. 2016) and with the both mating types have already been described (Casa-Coila et al. 2017; Lehtinen et al. 2008; Santana et al. 2013; Zanotta 2019; Zhu et al. 2016). The oospore is the survival structure of *P. infestans* and the pathogen does not produce chlamydospores (Judelson and Blanco 2005). Oospores contribute as a source of variability due to recombination, whereas the sporangia are essential to population growth due to the two strategies of germination and easy dispersal by wind (Fry 2020; Fry and Mizubuti 1998; Judelson and Blanco 2005).

The population of *P. infestans* worldwide is predominantly clonal (Fry 2020) and resistance to oomycide, i. e. chemical compounds active specifically against oomycetes, is

reported in association with the clonal lineages. Even though asexual reproduction is more frequent, population composition changes very fast due to the migration of clonal lineages (Fry 2020). The displacement of lineages is a common fact in *P. infestans* and was well documented in studies of global population biology of the pathogen (Fry 2016). Also, the change in the population composition might affect the sensitivity to oomycides and the virulence of the pathogen on potato and tomato cultivars. This can explain the constant occurrence of late blight epidemics and continued challenges in disease control (Fry 2016, 2020). Therefore, the knowledge of local population conformation is valuable information to delineate suitable management strategies.

Among the markers available to monitor populations of *P. infestans*, mating type, mitochondrial DNA haplotypes (mtDNA), sensitivity to mefenoxam, isoenzyme profile, restriction fragment length polymorphisms (RFLPs), and simple sequence repeats (microsatellite or SSRs) have greatly contributed to the understanding of the population of *P. infestans*. The analysis of mating type dynamics allowed important epidemiological conclusions regarding migration events, including the inference about the center of origin of *P. infestans* based on the distribution of mating types. Polymorphisms at mtDNA marker allowed to trace the uniparental evolutionary history of the pathogen (Martin et al. 2019). The sensitivity to mefenoxam was very useful when there was tight association with RFLP genotypes. The isoenzymes and the RFLPs were important to study the population genetics and diversity of *P. infestans* but were replaced by new markers, such as SSRs (Cooke and Lees 2004). The SSRs can detect a high number of polymorphisms, enable the identification of pathogen migration and the distinction between clonal and recombinant populations, and provide more information about pathogen history, dispersal, and evolution (Cooke and Lees 2004; Fry 2016; Martin et al. 2019). The use of microsatellites for *P. infestans* emerged as an attempt to describe the structure of the pathogen population in some countries in Europe and to compare the pattern with other populations (Knapova and Gisi 2002). Several SSR markers have been developed and used for *P. infestans* (Cooke and Lees 2004; Knapova and Gisi 2002; Lees et al. 2006; Li et al. 2010). Currently, a set of 12 SSRs, the 12-plex SSR, is widely used as a fast and reliable tool for lineage genotyping (Li et al. 2013; Lindqvist‐Kreuze et al. 2020).

Few studies have been published about the Brazilian population of *P. infestans*. For many years, a host-structured, clonal population of *P. infestans* was reported in Brazil. Individuals of the A1 mating-type, from the US-1 lineage, occurred on tomato, while A2 individuals, from the BR-1 lineage, occurred on potato (Brommonschenkel 1988; Goodwin et

al. 1994; Reis et al. 2003, 2006; Suassuna et al. 2004). Other variants of lineages were present in the country, but in low frequencies (Reis et al. 2003).

From 2006 to 2013, two studies reported the co-occurrence of individuals of the A1 and A2 mating types in potato fields, but evidence of sexual recombination was reported only between 2003 and 2005 (Oliveira 2010; Santana et al. 2013). Recent studies found self-fertile individuals (homothallic isolates) and reported sexual recombination in potato fields (Casa-Coila et al. 2017; Zanotta 2019). Furthermore, the predominant lineage in potato fields has changed from BR-1 to EU_2_A1 (Zanotta 2019).

When regarding oomycide sensitivity of the Brazilian population, the past populations were assessed for their sensitivity to mefenoxam, chlorothalonil, mancozeb, cymoxanil, propamocarb, fenamidone, dimethomorph, mandipropamid, and cyazofamid (Oliveira 2010; Reis et al. 2005; Santana et al. 2013; Zanotta 2019). There was evidence for resistance to mefenoxam only, with consistent detection of insensitive isolates over the years (Oliveira 2010; Reis et al. 2005; Santana et al. 2013). These molecules comprise different chemical groups, such as phenylamides, chloronitriles, dithiocarbamates, cyanoacetamideoxime, carbamates, quinone outside inhibitors (QoI), carboxylic acid amides, and quinone inside inhibitors (QiI).

The phenylamides, introduced in the 1970s, act on nucleic acid metabolism, preventing mycelial growth and sporangia formation by inhibiting RNA polymerase I (De Miccolis Angelini et al. 2015). This group comprises the active ingredients of metalaxyl, mefenoxam (metalaxyl-M); oxadixyl; and benalaxyl (Gisi and Sierotzki 2015). Field resistance of *P. infestans* to metalaxyl was reported in the early 1980s (Davidse et al. 1981, 1983; Delp and Dekker 1985; Hartill et al. 1983), including in Brazil (Reis et al. 2005, 2006).

The cloronitriles and dithiocarbamates are chemicals with multi-site activity. In the chloronitrile class the protectant fungicide chlorothalonil is widely used for late blight management, while in the dithiocarbamate class, mancozeb is commonly used for late blight management. The dithiocarbamates also include the maneb, metiram, propineb, thiram, and ziram (Hermann and Stenzel 2019).

Cymoxanil is a cyanoacetamideoxime compound. The exact mode of action of cymoxanil has not been unraveled yet (Hillebrand et al. 2019). This compound is frequent used in combination with another oomycide to improve residual effect (Hillebrand et al. 2019).

The carbamates target site is cell membrane permeability, lipid synthesis, and membrane integrity (Hermann and Stenzel 2019). The representative active ingredient of the

carbamate group used to control oomycetes is propamocarb, but this class also includes the iodocarb, and prothiocarb.

The QoIs are a group of fungicides commonly referred to as strobilurins and include nine chemical classes. Some compounds have good activity against oomycetes such as azoxystrobin, famoxadone, and fenamidone (Sierotzki 2015). The QoIs inhibit mitochondrial respiration by preventing electron transport in cytochrome bc1 (complex III - ubiquinol oxidase) (De Miccolis Angelini et al. 2015; Gisi and Sierotzki 2015).

The carboxylic acid amides act on membrane permeability and the mode of action is the inhibition of the cellulose synthase (De Miccolis Angelini et al. 2015; Gisi and Sierotzki 2015; Hermann and Stenzel 2019). Examples of these oomycides used to manage late blight include dimethomorph, fluomorph, benthiavalicarb, iprovalicarb, and mandipropamid (Brent and Hollomon 2007). Field resistance to dimethomorph has not been reported (Stein et al. 2004) and laboratory mutation studies have found resistance to flumorph (Yuan et al. 2006). Resistance to mandipropamid has been reported in commercial potato fields in Sweden (Ericsson 2024).

The QiIs are a class of fungicides that acts on the Complex III of respiration: cytochrome bc1 (ubiquinone reductase) at the quinone inside site (Hermann and Stenzel 2019). Cyazofamid and amisulbrom are compounds of QiIs class that can be applied to control foliar diseases caused by oomycetes.

Besides the chemical groups described above, the benzamide and piperidinyl-thiazole-isoxazoline deserve attention due to potent and novel mode of action against *P. infestans*. The sensitivity of *P. infestans* population from Brazil to these compounds have not been assessed or reported.

The benzamide class comprises derivatives of benzoic acid that interfere in cell division. Two active ingredients are available: zoxamide and fluopicolide (Zhang et al. 2020). Zoxamide is a site-specific fungicide, and its target site is β-tubulin. The molecule prevents the nuclear division of zoospore cysts during germination by cellular microtubule disruption in the cytoskeleton (Young and Slawecki 2001; Zhang et al. 2020). The microtubule disruption occurs because the molecule presents a high specific covalent bind to the β-subunit of tubulin (Young and Slawecki 2001). The interruption of cellular division inhibits pathogen germ-tube elongation and makes pathogen penetration difficult in host tissue (Bi et al. 2011; Young and Slawecki 2001).

The piperidinyl-thiazole-isoxazolines chemical group comprises the oxathiapiprolin and fluoxapiprolin. The oxathiapiprolin target site is an oxysterol-binding protein homolog

(OSBP) (Pasteris et al. 2016, 2019) . Studies suggest that OSBP or OSBP-like proteins are responsible for binding lipids, transporting them to plasmatic membranes, and regulating cellular signals (Saheki and De Camilli 2017; Weber-Boyvat et al. 2013; Yan and Olkkonen 2008) (Weber-Boyvat et al. 2013; Saheki and De Camilli 2017; Yan and Olkkonen 2008). As reported for mandipropamid, resistance to oxathiapiprolin was reported in *P. infestan*s from Swedish commercial fields (Ericsson 2024).

The analysis of the Brazilian population over time, the perceptions of change in the late blight epidemic in Brazil, and the phenotype differences between isolates obtained from potato and tomato suggest that there have been recent changes in the Brazilian population, regarding genetic composition and sensitivity to oomycides. Thus, this study aimed at **i.** characterizing the current population of *P. infestans* in Brazil based on mating type, mitochondrial DNA haplotypes, microsatellite genotype, and mefenoxam sensitivity; and **ii.** to assess the sensitivity of *P. infestans* to mefenoxam, mandipropamid, zoxamide, and oxathiapiprolin.

Chapter 1. Phenotypic and Genotypic Characterization of the Population of *Phytophthora infestans* in Brazil

Abstract

Recently, phenotypic variations and differences in virulence have been observed among Brazilian isolates of *P. infestans*. Analysis of phenotypic variability can be useful to understand the pathogen population and how variants may affect late blight epidemics. Thus, this study aimed to characterize the population of *P. infestans* in Brazil using mating type, mitochondrial DNA haplotype, microsatellite genotype, and mefenoxam sensitivity. The A2 mating type is reported in isolates obtained from tomato for the first time and both mating types are present in potato fields. The Ia and Ib mitochondrial DNA haplotypes were detected in Brazil. The Ia is associated with isolates from potato and the Ib with isolates obtained from potato and tomato hosts. A new genotype, the SA_1_A2, is associated with isolates from tomato, and the EU_37_A2 and EU_2_A1 genotypes were detected among isolates sampled from potato fields. The sensitivity to mefenoxam varied: sensitive, intermediately insensitive, and insensitive isolates are present in potato and tomato crops. There is evidence of population structure by genotype and host origin in Brazil. This study provides valuable information about the Brazilian population of *P. infestans*.

Introduction

Populations of *Phytophthora infestans* (Mont.) de Bary have been studied and characterized for many decades and in several parts of the globe. The agricultural importance of this pathogen explains the large number of groups worldwide dedicated to studying its genetics, ecology, and population evolution, with the final goal of improving knowledge about late blight epidemiology, management strategies and, ultimately, reducing yield losses.

During an epidemic, *P. infestans* can reproduce by asexual and sexual means. However, sexual reproduction only happens when both mating types (A1 and A2) co-occur in the same area (Brylińska et al. 2018; Gallegly and Galindo 1958; Smoot et al. 1958). Although the two reproductive modes possibly occur in the population, in most regions, the asexual cycle always takes place while the sexual cycle is less commonly observed (Fry 2020).

Migration of clonal lineages or genotypes around the world is one of the causes of changes in the genetic structure of populations over time (Fry 2016, 2020). A clonal lineage can be defined as a group of asexual descendants of one individual (Anderson and Kohn 1995). The *P. infestans* individuals of a lineage share genotypic and phenotypic characteristics. These characteristics are assessed using markers, and the *P. infestans* individuals are identified as belonging to specific lineages according to the pattern they share. Therefore, the monitoring of genotypic and phenotypic characteristics and the knowledge about the genetic structure of the *P. infestans* population can be useful to anticipate severe epidemics and avoid yield losses (Fry et al. 1992).

In Brazil, the first study of the population of *P. infestans* was performed in 1988. Back then, isolates of A1 mating type were reported to be associated with tomato, while A2 isolates were associated with potato (Brommonschenkel 1988). In 1994, it was confirmed that two clonal lineages of *P. infestans* were present in Brazil: US-1 (A1 mating type) and BR-1 (A2), associated with tomato and potato and plants, respectively (Goodwin et al. 1994). Despite the presence of individuals of the two mating types for at least a decade in the fields, sexual recombination was not detected in the Southern, Southeast, and Central-West of Brazil (Reis et al. 2003, 2006). Later studies demonstrated that the population of *P. infestans* remained structured by host (Reis et al. 2003, 2006) and a host specialization was reported (Reis et al. 2003). Although isolates from one host can infect the other host, the host-genotype association resulted in differences in pathogen virulence: the US-1 isolates

were more virulent to tomato while BR-1 isolates were more virulent to potato (Suassuna et al. 2004).

By further studies, several facts suggested changes in the pathogen population in Brazil. Individuals of the US-1 genotype and A1 mating type were found to cause late blight on potato in the Southern region (Santana et al. 2013). In Rio Grande do Sul state, individuals of both mating types were found co-occurring in potato fields, prompting for the possibility of sexual recombination (Santana et al. 2013). Additionally, self-fertile isolates of *P. infestans* were reported for the first time in Brazil, i.e. isolates able to form oospores by themselves and when paired with isolates of either mating type (Santana et al. 2013). Even with the presence of individuals of A1 and A2 mating types now occurring simultaneously on potato, evidence of sexual reproduction happening in Brazil was only found in the Southern region between 2003 and 2005 (Oliveira 2010; Santana et al. 2013).

In the latest study conducted in Brazil, self-fertile isolates were reported in fields sampled in Paraná state in 2011 and 2012 (Casa-Coila et al. 2017). Also, oospores were found in naturally infected potato leaves in four fields sampled in São Paulo, Minas Gerais, and Paraná states (Zanotta 2019). Additionally, EU_2_A1 was reported as the most frequently found genotype in potato fields in these states in the 2015 season (Zanotta 2019). This suggests that EU_2_A1 could have displaced BR-1 as the predominant lineage associated with potato in Brazil (Zanotta 2019).

The dynamics of the *P. infestans* population over the past decades in Brazil indicates important changes from evolutionary and epidemiological perspectives. Migration of new lineages or even rare events of sexual recombination may have altered the structure of the population and interfered in the efficiency of management strategies. Therefore, populations of *P. infestans* should be monitored constantly to detect alterations that may result in severe epidemics.

The most recent assessment of the genetic structure of the population of *P. infestans* from Brazil was conducted in 2017, using the 12-plex SSR and mating type (Zanotta 2019). However, phenotypic variations and differences in virulence among isolates of *P. infestans* have been observed in Brazil, leading us to hypothesize that there was a shift in the population of *P. infestans* in Brazil. Thus, this study aimed to (i) characterize the current Brazilian population of *P. infestans* in mating type, mitochondrial DNA haplotype, 12-plex SSR genotype, and mefenoxam sensitivity, and (ii) to conduct a phylogenetic analysis of the isolates sampled from tomato and potato fields to check for any evidence of host association.

Material and Methods

Collection of *P. infestans* isolates. Leaves, fruits (tomato) and stems of potato and tomato plants with typical late blight lesions were collected from fields located in Bahia (BA), Minas Gerais (MG), Espírito Santo (ES), São Paulo (SP), Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS) states, from 2020 to 2024. The infected plant material was kept in paper bags until they arrived at the laboratory. The samples were incubated in a humid chamber at 18°C in the dark to induce sporangia formation. The tomato samples were separated into two groups: 1) leaves with late blight lesions only, and 2) leaves with late blight lesions and necrotic spots caused by other pathogens. The samples of the first group were used for direct isolation and leaves from the second group were used for indirect isolation procedures. The pathogen isolation by direct method was attempted by removing sporangia by means of insulin needle from sporangiophores formed on the interface between the necrotic region of a single lesion and the green tissues of plant material. Sporangia were transferred to pea-agar medium (PAM) (150 g pea, 15 g agar, 0.05 g beta-sitosterol per liter) or V8 10 % unclarified amended with rifampicin (0.02 g/L), ampicillin (0.10 g/L), and tebuconazole fungicide (Folicur®) (10 mg a.i./L) or thiabendazole fungicide (Tecto®) (10 mg a.i./L). The Petri dishes containing sporangia were kept at 18 °C in darkness for 5 to 10 days and were visually inspected at every two days under a stereomicroscope.

Colonies with coenocytic hyphae, forming limoniform and papillated sporangia on branched sporangiophores with the distinctive morphological aspects of *P. infestans* were selected and mycelial discs of colonies were transferred to PAM or to PARP medium (500 mL of Pea Agar, 0.125 g ampicillin, 0.05 g PCNB, and 0.005 g rifampicin) and kept at 18 °C in darkness for 14 days. To obtain monozoosporic cultures, pure cultures were washed with 8 ml of cold sterile deionized water (4 °C) and the resulting sporangia suspensions were kept at 4 °C for 2 to 3 h to stimulate zoospores release. An aliquot was collected with a platinum loop, spread on the surface of PAM in a zigzag pattern, previously marked on the bottom of the Petri dish, or an aliquot of 100 µL of the zoospore suspension was spread on the surface of the water-agar medium (20 g agar per liter) by Drigalski handle. The Petri dishes were kept at 10 °C in darkness for 12 h to allow for germination of the zoospores. A germinated zoospore was selected with the help of a microscope (Olympus CX31 model CX31RBSFA) at 400X, transferred to PAM, and kept at 18 °C in darkness for 14 days.

For the indirect isolation method on tomato samples, small pieces of leaves (~ 5.0 x 5.0 mm) were taken from the intersection area between the necrotic region of a single lesion and the green tissues using a sterilized scalpel. The tissue fragments were washed in 1 % sodium hypochlorite for 30 s, followed by two washes in autoclaved distilled water for 30 s each and immediately placed on filter paper to dry the excess water and then transferred to Petri dishes containing PARP medium with the abaxial part facing up. The plates were kept in an incubator at 18 °C. Colonies were visually inspected daily with the aid of a stereomicroscope (Olympus SZ61 model SZ2-ILST) at 10X.

In addition to isolation to culture medium, *P. infestans* isolates from tomato plants were kept as living colonies in asymptomatic tomato leaves. One leaflet of tomato samples, from each sampled area, with a single sporulating lesion of *P. infestans* was selected and placed in non-chlorinated water at 4 °C for 2 h to induce zoospore release. Subsequently, asymptomatic tomato leaves from greenhouse-grown plants were inoculated with 15 µL-drops of the zoospore suspension obtained. Four to five drops were deposited per leaflet. The inoculated leaves were kept in a humid chamber in an incubator at 18 °C in the dark. Lesions formed in inoculated leaflets were subsequently and periodically transferred to asymptomatic leaves at every 10 days.

DNA extraction. Mycelial discs of the isolates were transferred to pea broth (120 g pea/L) and kept at 18 °C in darkness, for 8 to 15 days (Lindqvist‐Kreuze et al. 2020). After colony development, mycelia were washed twice with sterile distilled water, completely dried at a laminar flow cabinet, and kept at -20 °C until DNA extraction.

The DNA was extracted with the Wizard® Genomic DNA Purification (Promega Corporation). The quality and quantity of DNA were accessed using Nanodrop, visualized in 1 % agarose electrophoresis gel, and standardized at a concentration of 20 ng/µL for genotypic characterization and 100 ng/µL for characterization of mating type, mitochondrial DNA, and genes sequencing.

Mating type characterization. The mating type of each isolate was characterized using a PCR-based method and the results were confronted with pairings on culture medium. The mating type classification as A1 or A2 was done by PCR using standard DNA sent by Dr. David Cooke (James Hutton Institute - JHI). Subsequently, random isolates were paired on culture medium (PAM) with known testers (A1 = Pi94; and A2 = Pi88).

For the PCR-based identification of mating type primers W16-1 (AACACGCACAAGGCATATAAATGTA) and W16-2 (GCGTAATGTAGCGTAA-CAGCTCTC) were used (Judelson et al. 1995). The PCR was

conducted in a 15 µL reaction mixture with 2.5 µL 5X Green GoTaq® Reaction Buffer (Promega Corporation), 0.25 µL $MgCl_2$, 0.25 µL, dNTPs, 0.1 µL GoTaq® Polymerase, 0.2 µL each primer, 9.5 µL of sterile Milli-Q water, and 2.0 µL DNA. The PCR conditions were as described by Brylińska et al. (2018). After PCR amplification, amplicons were subjected to enzymatic digestion with restriction endonuclease from *Bacillus sphaericus* (BshFI) (Jena Bioscience) that recognizes and cuts the 5'-GGCC-3′ sequence (Vlatakis et al. 1989) on mixture contained 10 µL of PCR product, 1 µL of the enzyme, 5 µL of universal buffer, and 34 µL of sterile Milli-Q water. The digestion reaction conditions recommended by the manufacturer were incubation at 37 °C for 10 min and inactivation at 80 °C for 20 min. The PCR and enzymatic digestion products were separated and visualized in 1 % and 2 % agarose electrophoresis gel, respectively. The presence of fragments of approximately 100, 500, and 600 bp identify the A1 mating type and the presence of amplicons of near to 100 and 500 bp identify the A2 mating type.

In the pairing test, isolates were randomly selected and paired with A1 and A2 tester isolates previously identified by the PCR-based method. A strip of the culture medium and mycelia of unknown isolate (~ 3 cm length x 0.5 cm width) from 7 to 15-day-old colonies was placed in the middle of a Petri dish containing PAM. On the right and left sides of the unknown mating type isolate strip (~ 3 cm distance). Mycelial strip of the A1 tester isolate was placed to the right of the centered-placed unknown isolate and another strip of the A2 tester, respectively. Petri dishes were kept at 18 °C in darkness for 3 to 4 weeks and the presence of oospores was visualized in a microscope (Olympus CX31) at 400X. If the isolate formed oospores with the A1 tester it was identified as A2 mating type, if the isolate formed oospores with the A2 tester it was identified as A1 mating type, if the isolate produced oospores with both testers it was deemed as A1/A2, and if the isolate produced oospores by himself it was identified as self-fertile (SF). The PCR-based identification of mating type and pairings on culture medium were conducted twice for all isolates.

Mitochondrial DNA haplotypes identification. The mitochondrial DNA haplotypes were identified using a set of primers that anchor in the P2 and P4 gene regions (Griffith and Shaw 1998). The PCR was conducted in a 15 µL reaction mixture with 1.25 µL 10X Reaction Buffer Complete (Cellco Biotech), 1.0 µL $MgCl_2$, 0.25 µL, dNTPs, 0.1 µL Taq DNA Polymerase, 0.5 µL each primer Fwd/Rev, 9.0 µL of sterile Milli-Q water, and 2.0 µL DNA. The PCR reactions were performed under the following conditions: one cycle of 94 °C for 90 s, followed by 40 cycles of 94 °C for 40 s, 55 °C for 60 s, and 72 °C for 90 s, plus a final extension period of 72 °C for 5 min.

The expected sizes of the PCR products were 1.2 kb for P2 and 0.96 kb for P4. The amplification products of the P2 region were digested with *MspI* restriction enzyme (New England Biolabs) that recognizes and cuts the 5'-CCGG-3' site and the amplification products of P4 region were digested with *EcoRI* restriction enzyme (Cellco Biotech) that recognizes and cuts the 5'–GAATTC–3' sequence, according to manufacturer protocols. Fifteen μL of the digested product was stained with GelRed™ (Biotium, Inc.), separated by electrophoresis on 2 % agarose gels, visualized using standard techniques, and the haplotypes were determined as previously described by Griffith & Shaw (1998).

Mefenoxam sensitivity. Technical grade metalaxyl-M or mefenoxam (1000.0 μg/mL) dispersible oil suspension was provided by Syngenta. An aliquot of the technical grade was used as the stock solution and stored at 4 °C in darkness. Serial dilutions were prepared from the stock solution (1000.0 μg/mL) to reach the final concentrations of 100.0, 50.0, 10.0, and 5.0 μg/mL of mefenoxam

The mefenoxam sensitivity was assessed for isolates obtained from potato using a method of agar previously reported, with slight modifications (Reis et al. 2005). The isolates were grown in PAM for 15 days and transferred to four plates (four replicates) with pea glucose agar (150 g pea, 5 g glucose, and 15 g agar per liter) amended with mefenoxam (5.0 or 100.0 μg/mL) or DMSO 0.1 % (v/v) (control). After 9 days, the mycelial growth was assessed by measuring colony diameter in two perpendicular directions using a digital caliper. The test was done twice for each isolate. The isolates obtained from tomato were assessed regarding their sensitivity to mefenoxam by sporulation on leaf disc method (Fungicide Resistance Action Committee (FRAC) 2017). The abaxial side of the leaves was sprayed with 3 mL of mefenoxam suspension (100.0 and 5.0 μg/mL of i.a.) using a Devilbiss sprayer connected to an air compressor at 15 PSI (Air Plus Schulz MS2.3). After the mefenoxam application, leaves were dried in a laminar flow for 20 min, cut with a cork borer, and immediately placed in wells of 24-well plastic plates containing 1.5 mL of 0.4 % water-agar. After 24 h of mefenoxam spray, a 30 μL-drop sporangia suspension (2 x 10^4 sporangia/mL), kept for 2 h at 4°C to induce zoospore release, was placed in a center of leaf discs. The suspension was prepared by washing sporangia of *P. infestans* isolates cultivated on detached leaves of tomato cv. Santa Clara with sterilized non-chlorinated water. The 24-well plastic plates were kept at 18 °C under 18 h of light alternated with 6 h of darkness for 10 to 15 days. Two days after inoculation, the remaining drop of sporangia suspension was dried with tissue paper. At the end of the incubation period, sporulation was visually observed using a stereomicroscope (Olympus SZ61 model SZ2-ILST) at 10X, and grades were assigned

according to the scale described by Sozzi (1991). Each treatment was assessed using four leaf discs. The experiment was conducted twice for each isolate.

SSR genotyping. The isolates were genotyped by one-step SSR multiplex (Li et al. 2013) using the 12 loci. DNA of eight standard genotypes was kindly provided by Dr. David Cooke (JHI) in Whatman FTA cards. The FTA cards were prepared to be used in the PCR multiplex by (a) cutting the cards with mini punching (2.0 mm); (b) adding 150 µL of QIAcard FTA Wash Buffer (Qiagen), shaking slowly in the vortex for 3 min, and removing the reagent with a pipette, (c) repeating step "b"; (d) adding 150 µL of TE-1 Buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 8.0), shaking slowly in the vortex for 3 min, and removing the reagent with a pipette; (e) repeating step "d"; and (f) air drying disc for 2 h at room temperature.

The PCR was conducted in a 13.5 µL total volume and the protocol was adapted from Euroblight (Cooke et al. 2010). The protocol consisted of 4.81 µL of water, 6.25 µL of 2X Type-IT Multiplex PCR Mix (Qiagen), 1.0 µL of Primer Mix 1 (Cooke et al. 2010), 0.3 µL Pi4B forward and reverse (Fwd/Rev) primer (10 µM), 0.4 µL SSR8 Fwd/Rev primer (10 µM), 0.2 µL D13 Fwd/Rev primer (10 µM), 0.08 µL Pi04 Fwd/Rev primer (4.7 µM), and 2 µL of DNA. The PCR conditions were the same as described by Li et al. (2013).

The fragment analysis of the PCR products was done at Macrogen Inc. The allele patterns were manually sized with the GeneMaker v3.0.1 (SoftGenetics). In the first run, the standard genotypes were analyzed, and the sizes of alleles were adjusted and calibrated according to Li et al. (2013) (Table S1).

Temporal analysis. The current Brazilian population was compared to the isolates collected in Brazil from 1998 to 2010 (“old population”) (Oliveira 2010). The data of allele patterns of the isolates from 1998 to 2010 were obtained (Oliveira 2010) and the analysis was done using the data of six SSR loci common to all isolates: Pi02, Pi04, Pi63, D13, Pi70, and G11 (Knapova and Gisi 2002; Lees et al. 2006).

Phylogenetic study. The phylogenetic relation between *P. infestans* isolated from tomato and potato was investigated by sequencing of Beta-tubulin (*Btub*), TigA gene fusion protein (*TigA*), Internal transcribed spacer (*ITS*), and Mitochondrial cytochrome C oxidase subunit I (*CoxI*) loci. These loci were chosen so that it would be possible to inspect any difference among *Phytophthora* species and identify new ones (Coomber et al. 2023).

The primer set used to amplify the *Btub* locus was Btub_F1 and Btub_R1 (Blair et al. 2008; Kroon et al. 2004) and the PCR conditions were one cycle of 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 64.9 °C for 30 s, and 72 °C for 1 min, plus a final extension

period of 72 °C for 15 min. The *TigA* locus was amplified by two primer sets: Tig_for and Tig_rev, and G3PDH_for and G3PDH_rev (Blair et al. 2008). To amplify the primer set Tig_for/Tig_rev the PCR conditions were: one cycle of 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 63.2 °C for 30 s, and 72 °C for 1 min, plus a final extension period of 72 °C for 15 min; and the amplification of G3PDH_for/G3PDH_rev set was under one cycle of 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 64 °C for 30 s, and 72 °C for 2 min, plus a final extension period of 72 °C for 5 min. The ITS4 and ITS6 (Cooke et al. 2000) were used to amplify the *ITS* locus under PCR conditions of one cycle of 95 °C for 1.25 min, followed by 34 cycles of 95 °C for 35 s, 62 °C for 35 s, and 72 °C for 55s, and a final extension period of 72 °C for 10 min. The COXF4N and COXR4N were used (Kroon et al. 2004) to amplify the *CoxI* locus according to the conditions described by Coomber et al. (2023). The PCR products were separated and visualized in 1 % agarose electrophoresis gel, purified using ExoSAP-IT™ (Affymetrix™), and sent to Macrogen (South Korea) to be sequenced.

Data analysis. To classify the isolate according to their sensitivity to mefenoxam, the mycelial growth and sporulation on leaf disc of each isolate grown in the control was considered as 100 %, and the mycelial growth and sporulation on leaf disc grades on 5.0 and 100.0 µg/mL were transformed in percentage in relation the control. The isolates were classified as sensitive (growth or sporulation less than 40 % of the control at 5.0 and 100.0 µg/mL), intermediate (growth or sporulation greater than 40 % of the control at 5.0 µg/mL and less than 40 % of the control at 100.0 µg/mL), or resistant (growth or sporulation greater than 40 % of the control at both 5.0 and 100.0 µg/mL) (Therrien et al. 1993).

To proceed with the genotyping, a new dataset with 173 reference isolates of genotypes described in North and South America and Europe was created with an updated version of the dataset of Saville and Ristaino (2019) study using methods in line with the Euroblight scoring system (Euroblight UPD), the dataset of Coomber et al. (2023) study and available at *P. infestans* classifier (T-BAS) (Coomber et al. 2023), and the data of standard genotypes provided by Dr. David Cooke (JHI). The Bruvo's genetic distance was calculated for the new dataset with the bruvo.dist() function and a cut-off value was estimated with function cutoff_predictor() of R (R Core Team 2024) in package *poppr* version 2.9.6 (Kamvar et al. 2014). The new dataset was updated with the data of the current population. The Bruvo's genetic distance was calculated for the new dataset updated as described before, and the cut-off value was used to identify the genotypes of each isolate of the current

population. When the Bruvo's genetic distance was lower than the estimated cut-off value, a genotype match was considered.

To complete the identification of the genotypes, a Neighbor-Joining tree (NJ) with 1000 bootstraps and a minimum spanning network (MSN) were generated from Bruvo's genetic distance, respectively, by functions bruvo.boot() and bruvo.msn() of package *poppr*.

General population genetics statistics were estimated for the Brazilian population using the package *poppr*. As the initial steps, a genotype accumulation curve was plotted by the function genotype_curve(), the number of observed alleles per locus and group, the percentage of missing data, and the observed and expected heterozygosity were estimated by function summary() and info_table(). The Simpson index, Nei's 1978 gene diversity, and evenness for each locus were calculated by the locus_table() function. The genotypic diversity was obtained using the poppr() function, the statistics estimated were: number of individuals observed (N), number of multilocus genotypes (MLG) observed, number of expected MLG at the smallest sample size ≥ 10 based on rarefaction (eMLG), Standard error based on eMLG (SE), Shannon-Wiener Index of MLG diversity (Shannon 1948) (H), Evenness E5 (Grünwald et al. 2003; Ludwig and Reynolds 1988; Pielou 1975) (E.5), Nei's unbiased gene diversity (Nei 1978) (Hexp), index of association IA (Brown et al. 1980; Smith et al. 1993) (Ia), and standardized index of association $\bar{r}_d$ (rbarD). An MLG histogram was generated to examine the diversity of MLGs by host, state of origin, mating type, mitochondrial DNA haplotype, and genotype by mlg.table() function. The clone correction was done individually by host, state of origin, mating type, mitochondrial haplotype, and genotype using the function clonecorrect(). The datasets clone corrected, i.e. each dataset formed by one representative of each haplotype, were used to evaluate the population structure.

Deviations from Hardy-Weinberg equilibrium (HWE) were checked by hw.test() function of *pegas* package (Paradis 2010). The population was checked for linkage disequilibrium (LD) using the function ia() with 999 permutations from package *poppr*.

The population structure was assessed using model-based Bayesian clustering in STRUCTURE v.2.3.4 (Pritchard et al. 2000). The parameters used were burn-in of 20,000 repeats, 1,000,000 Markov chain Monte Carlo (MCMC) repeats, and the admixture model was chosen. Independent runs of the model used K values from 1 to 10 with 20 iterations (runs) of each K value. The best K was estimated by the Evanno method (Evanno et al. 2005) at structureHarvester.py v0.7 (Earl and VonHoldt 2012). The graphical output was constructed using the Clumpak (Kopelman et al. 2015). Analysis of molecular variance

(AMOVA) was conducted by amova.poppr() function of package *poppr* to estimate the percent variation between and within the host, state of origin, mating type, mitochondrial haplotype, and genotype. The significance of estimated variance components was assessed based on 10,000 random permutations by function randtest() of *ade4* package (Dray and Dufour 2007). To validate the population clustering by the best K and the significant variation estimated, an analysis of principal components (PCA) and discriminant analysis of principal components (DAPC) was done by functions prcomp() and dapc() of *stats* (R Core Team 2024) and *adegenet* (Jombart 2008) packages, respectively. An MSN was generated individually to host, state of origin, mating type, mitochondrial haplotype, and genotype from Bruvo's genetic distance, as described before.

The sequences of the four loci were manually inspected and the contig sequence was generated using SeqAssem (Hepperle 2004). The contigs were submitted to database query (BLAST) to confirm the similarity of gene and pathogen. The sequences were aligned in MEGA 11 (Tamura et al. 2021) with reference sequences available (Coomber et al. 2023). The phylogenetic trees were inferred for each locus individually, for all loci concatenated, for loci *Btub*, *TigA*, and *ITS* concatenated, and for loci *Btub* and *TigA* concatenated by the Bayesian method.

The inference by the Bayesian method was under the best likelihood model for each locus, MCMC with 5,000,000 repetitions at MrBayes on XSEDE (Ronquist et al. 2012). The statistical selection of the best-fit evolutionary model of nucleotide substitution for each locus was estimated using the Bayesian Information Criterion (BIC) at jModelTest2 on ACCESS (Darriba and Posada 2014). The phylogenetic trees were plotted on FigTree v1.4.4 (Rambaut et al. 2014) and exported to the graphic edition in CorelDRAW® 2021.

Results

Mating type characterization. One hundred thirty-five isolates were characterized by mating type using PCR-based method and 52 were paired on culture medium. Both mating types, A1 (67 isolates) and A2 (68 isolates), were identified in the Brazilian population of *P. infestans* by molecular markers. The pairing on culture medium identified the mating types A1 (42 isolates) and A2 (13 isolates), two A1/A2 isolates, and zero SF (Table 1).

After enzymatic digestion of the PCR product, 67 isolates presented amplification fragments of the A1 type (~ 100, 500, and 600 bp) and 68 isolates of the A2 type (~ 100 and

500 bp). The proportion of mating type (A1:A2) according to the state of origin was: 0:5 in BA, 30:31 in MG, 0:10 in ES, 7:0 in SP, 15:6 in PR, 12:0 in SC, and 3:14 in RS state (Figure 1A). Both mating types were identified co-occurring in the same fields in MG and PR states.

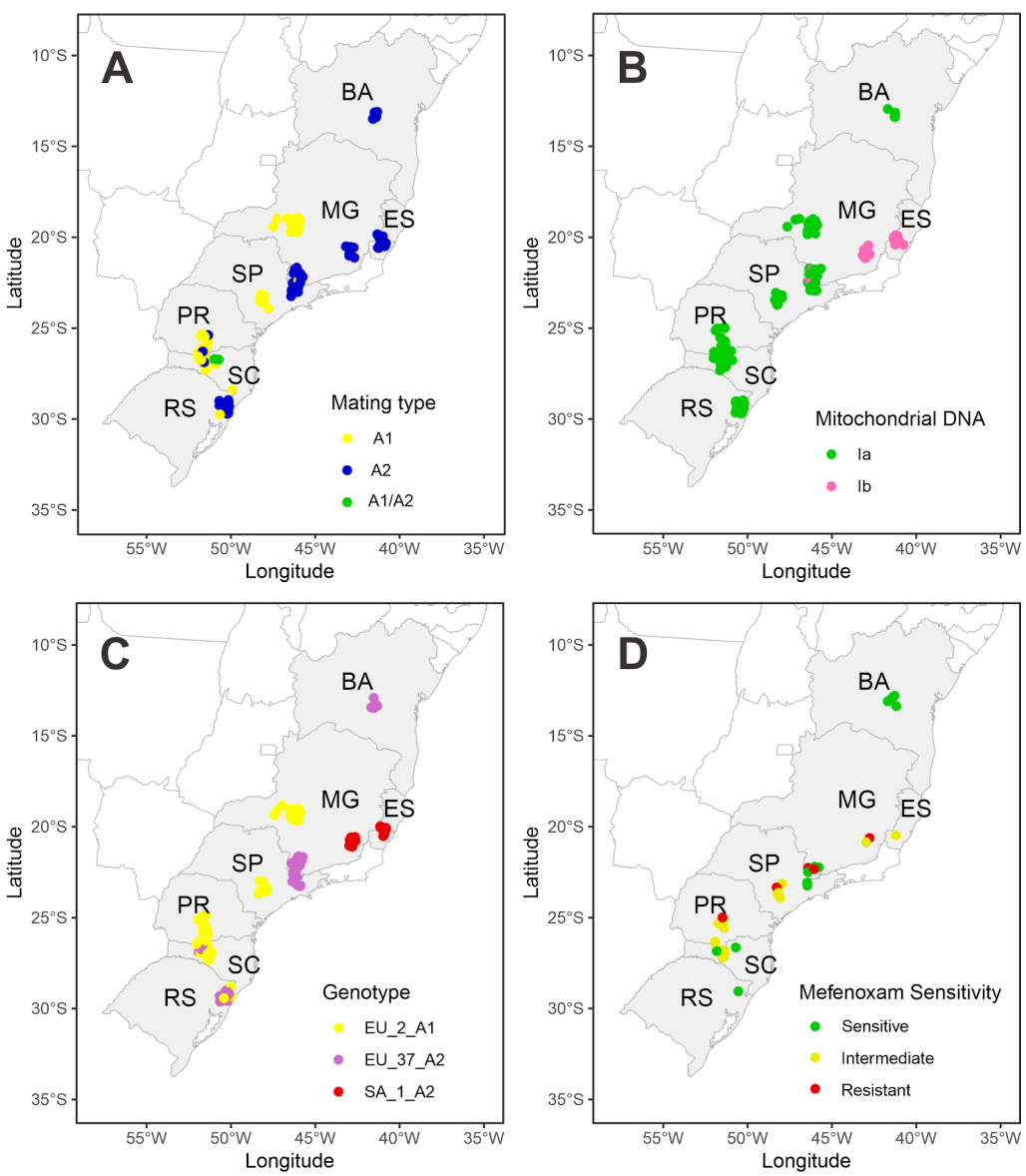


Figure 1. Isolate sites (dots) according to the state of origin: BA = Bahia, MG = Minas Gerais, SP = São Paulo, PR = Paraná, SC = Santa Catarina, and RS = Rio Grande do Sul. The color dots are according to phenotypic or genotypic characteristics as indicated on legends. A: mating type; B: Mitochondrial DNA haplotype; C: Genotype; and D. Mefenoxam sensitivity.

Forty-two isolates formed oospores in the pairing assays on culture medium with the A2 tester, thus these isolates were characterized as A1 mating type; 13 were identified as A2 mating type because formed oospores with the A1 tester, and two formed oospores with both A1 and A2 testers and were characterized as A1/A2. Most isolates identified by pairing as A1

and A1/A2 were identified as A1 by the PRC-based method. Five isolates from tomato were identified as A1 by pairing and as A2 by the PCR-based method, and all isolates identified as A2 by pairing culture were also identified as A2 by the PCR-based method. The proportion of mating type identified by the pairings in culture medium according to the state of origin was the same as described by the PCR-based method except for the ES and SC states. The isolates identified as SF were sampled from the SC state. The proportion of mating type (A1:A2:A1/A2) was 1:14:2 to SC state. Regarding the host of origin, the isolates from potato were identified as A1, A2, and A1/A2, and isolates from tomato were identified as A1 or A2.

Mitochondrial haplotypes identification. The mitochondrial haplotypes of 134 isolates were characterized. The haplotypes Ia (n = 110) and Ib (n = 24) were present in the Brazilian population of *P. infestans* (Table 1). The distribution of mitochondrial haplotypes (Ia:Ib) according to the state of origin was: 5:0 in BA, 50:11 in MG, 0:10 in ES, 0:7 in SP, 20:1 in PR, 12:0 in SC, and 17:0 in RS (Figure 1B). The isolates from the potato host were identified as Ia and Ib, and the ones from the tomato were identified as Ib haplotype.

Mefenoxam sensitivity. Thirty-two isolates, 27 from potato and five from tomato, were characterized by mefenoxam sensitivity. Fourteen isolates (12 from potato and two from tomato) were sensitive to mefenoxam, 14 from potato were intermediate to mefenoxam, and five isolates (two from potato and three from tomato) were resistant (Table 1).

The sensitive isolates were obtained from BA (n = 2), MG (n = 5), ES (n = 2), PR (n = 1), SC (n = 1), and RS (n = 1) states (Figure 1D). The intermediate isolates are from MG (n = 1), SP (n = 3), PR (n = 5), and SC (n = 5) (Figure 1D). The resistant isolates are from MG (n = 1), ES (n = 3), and SP (n = 1) (Figure 1D).

SSR genotyping. One hundred thirty-five isolates obtained from potato and tomato-producing areas sampled in the Brazilian states between the 2020 and 2024 seasons were genotyped by 12-plex SSR. A cut-off value estimated was 0.128.

From those 135 isolates, 67 were identified as EU_2_A1 genotype, 49 as EU_37_A2, and 19 did not match any genotype described before and were named as SA_1_A2 genotype (Table 1). The MSN shows isolates from Brazil grouping with isolates previously identified as EU_2_A1 and EU_37_A2 genotype and some isolates not grouping with any known genotype (Figure 2). The EU_2_A1 genotype were identified in MG, SP, PR, SC, and RS states, the EU_37_A2 were from BA, MG, PR, and RS states, and the SA_1_A2 were present in MG and ES states (Figure 1C).

Population variability. In the entire population, 63 alleles were observed. The average number of alleles was 5.25. The D13 locus had the highest number of alleles (Allele

num = 12), and the SSR11 and SSR2 loci had the lowest allele number (Allele num = 2) (Table 2). The SSR4 locus has the highest diversity according to the Simpson index (1-D = 0.82), and the SSR11 locus had the highest evenness (Evenness = 1.00) (Table 2). The percentage of missing data was 0.19 %. The observed heterozygosity was 1 for all loci, and the expected heterozygosity varied between 0.50 (SSR2) to 0.85 (SSR4) with 0.69 of the mean (Table 2).

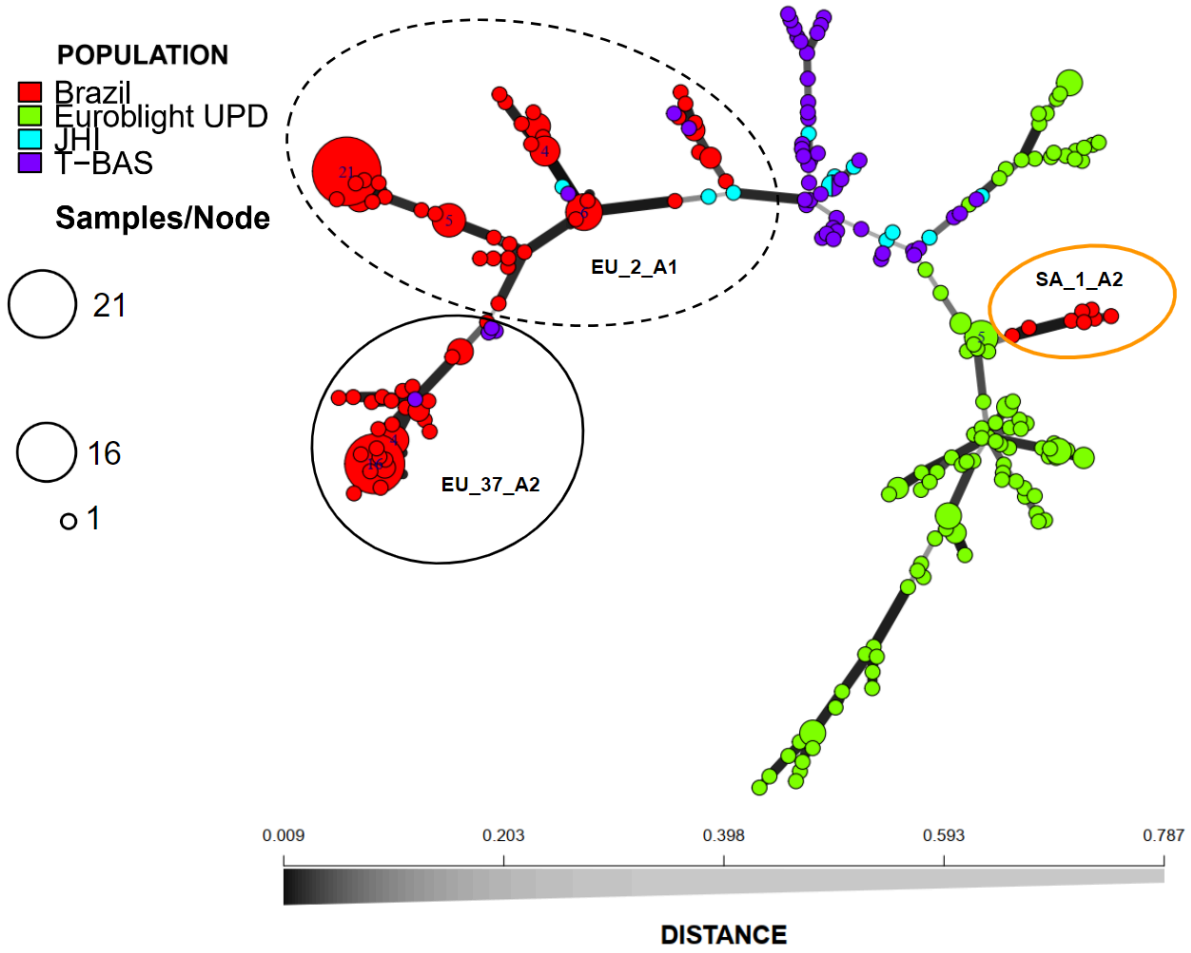


Figure 1. Minimum spanning network of genotypes of *Phytophthora infestans* based on 12-plex simple-sequence repeats (SSRs) using Bruvo's distance. Nodes are colored by the data set: red, green, blue, and purple indicate the isolates from current population from Brazil, isolates from dataset of Saville and Ristaino (2019) study using methods in line with the Euroblight scoring system (Euroblight UPD), control isolates sent by Dr. David Cooke (JHI), and isolates from dataset of Coomber et al. (2023) study lineages in *P. infestans* classifier (T-BAS). The nodes represent the multilocus genotypes (MLGs), and their size increases according to the number of isolates sharing the MLGs. The thickness and darkness of branches represent Bruvo's genetic distance between adjacent nodes. The circle with a black dotted line grouped the isolates from the EU_2_A1 genotype, the circle with a black continuous line grouped isolates from the EU_37_A2 genotype, and the circle with an orange continuous line grouped the isolates from the SA_1_A2 genotype.

Table 1. Characterization of the isolates from the Brazilian population of *Phytophthora infestans* by geographic origin (Municipality), year of sample (Year), host of origin (Host), mating type by molecular markers and by pairing on culture medium, mitochondrial DNA haplotype, mefenoxam sensitivity, and SSR genotype.

Isolate	Municipality	Year	Host	Mating type		Mitochondrial DNA	Mefenoxam Sensitivity *	SSR Genotype
				by molecular markers	by pairing			
Pi1	Viçosa - MG	2020	Potato	A2	-	Ib	-	SA_1_A2
Pi2	Patrocínio - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi3	Perdizes - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi4	Perdizes - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi5	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi6	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi7	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi8	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi9	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi10	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi11	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi12	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi13	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi14	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi15	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi16	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi17	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi18	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi19	São Gotardo - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi20	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1

Pi21	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi22	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi23	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi24	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi25	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi26	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi27	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi28	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi29	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi30	Viçosa - MG	2020	Tomato	A2	-	Ib	-	SA_1_A2
Pi31	Rio Paranaíba - MG	2020	Potato	A1	A1	Ia	-	EU_2_A1
Pi32	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi33	Ipuiúna - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi34	Espírito Santo do Dourado - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi35	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi36	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi37	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi38	Camanducaia - MG	2020	Potato	A2	A2	Ia	S	EU_37_A2
Pi39	Ipuiúna - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi40	Ipuiúna - MG	2020	Potato	A2	A2	Ia	R	EU_37_A2
Pi41	Espírito Santo do Dourado - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi42	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi43	Ipuiúna - MG	2020	Potato	A2	-	Ia	-	EU_37_A2

Pi44	Espírito Santo do Dourado - MG	2020	Potato	A2	-	Ib	-	EU_37_A2
Pi45	Espírito Santo do Dourado - MG	2020	Potato	A2	A2	Ib	S	EU_37_A2
Pi46	Ipuiúna - MG	2020	Potato	A2	-	Ib	-	EU_37_A2
Pi47	Espírito Santo do Dourado - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi48	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi49	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi50	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi51	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi52	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi53	Palmas - PR	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi54	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi55	Guarapuava - PR	2021	Potato	A1	A1	Ia	I	EU_2_A1
Pi56	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi57	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi58	São Francisco de Paula - RS	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi59	Ipuiúna - MG	2020	Potato	A1	-	Ia	-	EU_37_A2
Pi60	Água Doce - SC	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi61	Água Doce - SC	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi62	Guarapuava - PR	2021	Potato	A1	A1	Ia	-	EU_2_A1
Pi63	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi64	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi65	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2

Pi66	Guarapuava - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi67	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	S	EU_37_A2
Pi68	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi69	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi70	Guarapuava - PR	2021	Potato	A2	A2	Ia	I	EU_2_A1
Pi71	Palmas - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi72	Palmas - PR	2021	Potato	A1	-	Ib	-	EU_2_A1
Pi73	Água Doce - SC	2021	Potato	A1	-	Ia	I	EU_2_A1
Pi74	Palmas - PR	2021	Potato	A1	-	Ia	I	EU_2_A1
Pi75	Calmon - SC	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi76	Guarapuava - PR	2021	Potato	A1	-	Ia	I	EU_2_A1
Pi77	Guarapuava - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi78	Ipuiúna - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi79	Bom Repouso - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi80	São José dos Ausentes - RS	2021	Potato	A1	-	-	-	EU_2_A1
Pi81	Guarapuava - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi82	Camanducaia - MG	2020	Potato	A2	A2	Ib	S	EU_37_A2
Pi83	Camanducaia - MG	2020	Potato	A2	A2	Ia	S	EU_37_A2
Pi84	Camanducaia - MG	2020	Potato	A2	-	Ia	-	EU_37_A2
Pi85	Palmas - PR	2021	Potato	A2	-	-	-	EU_37_A2
Pi86	Guarapuava - PR	2021	Potato	A1	-	Ia	R	EU_2_A1
Pi87	Guarapuava - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi88	Água Doce - SC	2021	Potato	A1	A1	Ia	I	EU_2_A1
Pi89	Água Doce - SC	2021	Potato	A1	-	Ia	-	EU_2_A1

Pi90	Tatuí - SP	2021	Potato	A1	-	Ia	I	EU_2_A1
Pi91	Tatuí - SP	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi92	Tatuí - SP	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi93	Guarapuava - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi94	Mucugê - BA	2021	Potato	A2	A2	Ia	S	EU_37_A2
Pi95	Itapetininga - SP	2021	Potato	A1	A1	Ia	I	EU_2_A1
Pi96	Mucugê - BA	2021	Potato	A2	-	Ia	S	EU_37_A2
Pi97	Tatuí - SP	2021	Potato	A1	A1	Ia	I	EU_2_A1
Pi98	Tatuí - SP	2021	Potato	A1	A1	Ia	R	EU_2_A1
Pi99	Palmas - PR	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi100	Mucugê - BA	2021	Potato	A2	A2	Ia	-	EU_37_A2
Pi101	Tatuí - SP	2021	Potato	A1	A1	Ia	I	EU_2_A1
Pi102	Calmon - SC	2021	Potato	A1	A1/A2	Ia	S	EU_2_A1
Pi103	Mucugê - BA	2021	Potato	A2	-	Ia	S	EU_37_A2
Pi104	Água Doce - SC	2021	Potato	A1	-	Ia	I	EU_2_A1
Pi105	Água Doce - SC	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi106	Água Doce - SC	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi107	Mucugê - BA	2021	Potato	A2	A2	Ia	S	EU_37_A2
Pi108	Santa Maria de Jetibá - ES	2021	Tomato	A2	-	Ib	I	SA_1_A2
Pi109	Afonso Cláudio - ES	2021	Tomato	A2	-	Ib	-	SA_1_A2
Pi110	Afonso Cláudio - ES	2021	Tomato	A2	-	Ib	-	SA_1_A2
Pi111	Afonso Cláudio - ES	2021	Tomato	A2	-	Ib	-	SA_1_A2
Pi112	Coimbra - MG	2021	Tomato	A2	-	Ib	-	SA_1_A2
Pi113	Palmas - PR	2021	Potato	A2	-	Ia	-	EU_37_A2

Pi114	Palmas - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi115	São Francisco de Paula - RS	2021	Potato	A2	-	Ia	-	EU_37_A2
Pi116	Água Doce - SC	2021	Potato	A1	-	Ia	I	EU_2_A1
Pi117	Palmas - PR	2021	Potato	A1	-	Ia	-	EU_2_A1
Pi118	Palmas - PR	2021	Potato	A2	A2	Ia	S	EU_37_A2
Pi119	Viçosa - MG	2022	Tomato	A2	-	Ib	R	SA_1_A2
Pi120	Viçosa - MG	2022	Tomato	A2	-	Ib	-	SA_1_A2
Pi121	Palmas - PR	2021	Potato	A1	A1	Ia	I	EU_2_A1
Pi122	São Francisco de Paula - RS	2022	Potato	A1	-	Ia	-	EU_2_A1
Pi123	Viçosa - MG	2023	Tomato	A2	A2	Ib	-	SA_1_A2
Pi124	Espírito Santo do Dourado - MG	2022	Potato	A2	A2	Ia	S	EU_37_A2
Pi125	Espírito Santo do Dourado - MG	2022	Potato	A2	A2	Ia	R	EU_37_A2
Pi126	Calmon - SC	2022	Potato	A1	A1/A2	Ia	-	EU_2_A1
Pi128	Viçosa - MG	2023	Tomato	A2	A1	Ib	I	SA_1_A2
Pi132	Domingos Martins - ES	2024	Tomato	-	A1	-	-	-
Pi133	Castelo - ES	2024	Tomato	A2	-	Ib	-	SA_1_A2
Pi134	Santa Maria de Jetibá - ES	2024	Tomato	A2	-	Ib	-	SA_1_A2
Pi135	Santa Maria de Jetibá - ES	2024	Tomato	A2	A1	Ib	-	SA_1_A2
Pi136	Santa Maria de Jetibá - ES	2024	Tomato	A2	A1	Ib	-	SA_1_A2
Pi137	Santa Maria de Jetibá - ES	2024	Tomato	A2	A1	Ib	-	SA_1_A2
Pi138	Castelo - ES	2024	Tomato	A2	-	Ib	-	SA_1_A2

*S = sensitive; I = intermediate; and R = resistant to mefenoxam.

Table 2. Population statistics by locus observed in the Brazilian population of *Phytophthora infestans*.

Locus	Allele num	1-D	Hexp	Evenness
D13	12.00	0.62	0.62	0.56
SSR8	3.00	0.63	0.63	0.93
SSR4	11.00	0.82	0.83	0.87
Pi04	5.00	0.67	0.68	0.95
Pi70	3.00	0.42	0.42	0.67
SSR6	3.00	0.50	0.50	0.96
Pi63	3.00	0.61	0.61	0.88
G11	10.00	0.78	0.78	0.73
Pi02	6.00	0.57	0.57	0.66
SSR11	2.00	0.50	0.50	1.00
SSR2	2.00	0.12	0.12	0.50
Pi4B	3.00	0.40	0.40	0.75
mean	5.25	0.55	0.55	0.79

Allele num = number of alleles observed; 1-D = Simpson index; Hexp = Nei's unbiased gene diversity

A total of 74 original multilocus genotypes (MLGs) were observed across 135 triploid individuals based on 12 codominant loci. Thirteen MLGs occurred in more than one sampled individual. The genotype EU_2_A1 presented a higher number of MLGs than the other genotypes presented in the population and was the most diverse according to the Shannon-Wiener index (H = 2.79) (Table 3). However, the genotype SA_1_A2 had the highest evenness statistic (E.5 = 0.89) (Table 3). The potato host presented high MLG diversity (H = 3.47), but low evenness (E.5 = 0.47) (Table 2). The subpopulation of RS state was the most diverse (H = 2.64), and those of BA and ES had the highest evenness (H = 1 and 0.96, respectively) (Table 3). According to the index of association, asexual reproduction is predominant in the Brazilian population in all subpopulation assignments (host, state, etc.) (Ia and $\bar{r}_d \neq 0$).

The result of the HWE test indicated that all loci are not in HWE, across all 135 individuals. When the original population and the clone corrected dataset were checked for linkage disequilibrium, the results indicated that the population is on linkage disequilibrium (LD) ($P < 0.001$ and $\bar{r}_d=0.418$), a characteristic observed in clonal populations.

Table 3. Population summary statistics for *Phytophthora infestans* from Brazil. The population is sorted by host, state of origin, and genotype.

	N	MLG	eMLG (SE)	H	E.5	Hexp	Ia*	$\bar{r}_d$*
All	135	74	8.57 (1.12)	3.75	0.46	0.56	3.08	0.32
By Host								
Potato	117	59	13.30 (1.74)	3.47	0.47	0.52	2.99	0.35
Tomato	18	15	15.00 (0.00)	2.63	0.89	0.49	0.21	0.03
By State of origin								
MG	61	24	6.32 (1.35)	2.43	0.49	0.54	3.69	0.40
RS	17	15	9.18 (0.71)	2.64	0.89	0.42	3.77	0.48
PR	21	14	7.93 (1.02)	2.47	0.82	0.50	3.61	0.48
SC	12	7	6.15 (0.66)	1.70	0.72	0.44	-0.45	-0.15
SP	7	5	5.00 (0.00)	1.48	0.82	0.49	0.37	0.07
BA	4	4	4.00 (0.00)	1.39	1.00	0.44	-0.28	-0.16
ES	13	12	9.42 (0.49)	2.46	0.96	0.49	0.28	0.04
By genotype								
SA_1_A2	19	16	16.00 (0.00)	2.70	0.89	0.49	0.20	0.02
EU_2_A1	67	31	11.80 (1.71)	2.79	0.46	0.49	0.32	0.05
EU_37_A2	49	27	12.40 (1.62)	2.75	0.46	0.33	-0.34	-0.14

N = number of individuals; MLG = multilocus genotype; eMLG = multilocus genotype expected; SE = standard error; H = Shannon-Wiener index of MLG diversity; E.5 = Evenness, E_5 ; Hexp = Nei's unbiased gene diversity; Ia = index of association; $\bar{r}_d$ = standardized index of association; * clone corrected value.

Population structure. The cluster analysis shows that the Brazilian population of *P. infestans* is structured into three groups associated with the SSR genotypes (Figure 2). The MSN also grouped the isolates into three groups, by genotype, and the Bruvo's distance between them is higher (Figure 3A). The population also was grouped into three groups defined by the host of origin. For the red group all isolates but one came from tomato plants. The other two groups comprised isolates sampled from potato (Figure 3B). The NJ constructed with Bruvo's distance grouped the isolates into three well supported clades (bootstrap > 70%) (Figure 4).

The AMOVA results indicate that there was significant variation between genotype (75.7 %, $p < 0.001$), and host (51.2 %, $p < 0.001$). The PCA and DAPC support the population clustering into 3 groups, by genotype (Figure 5).

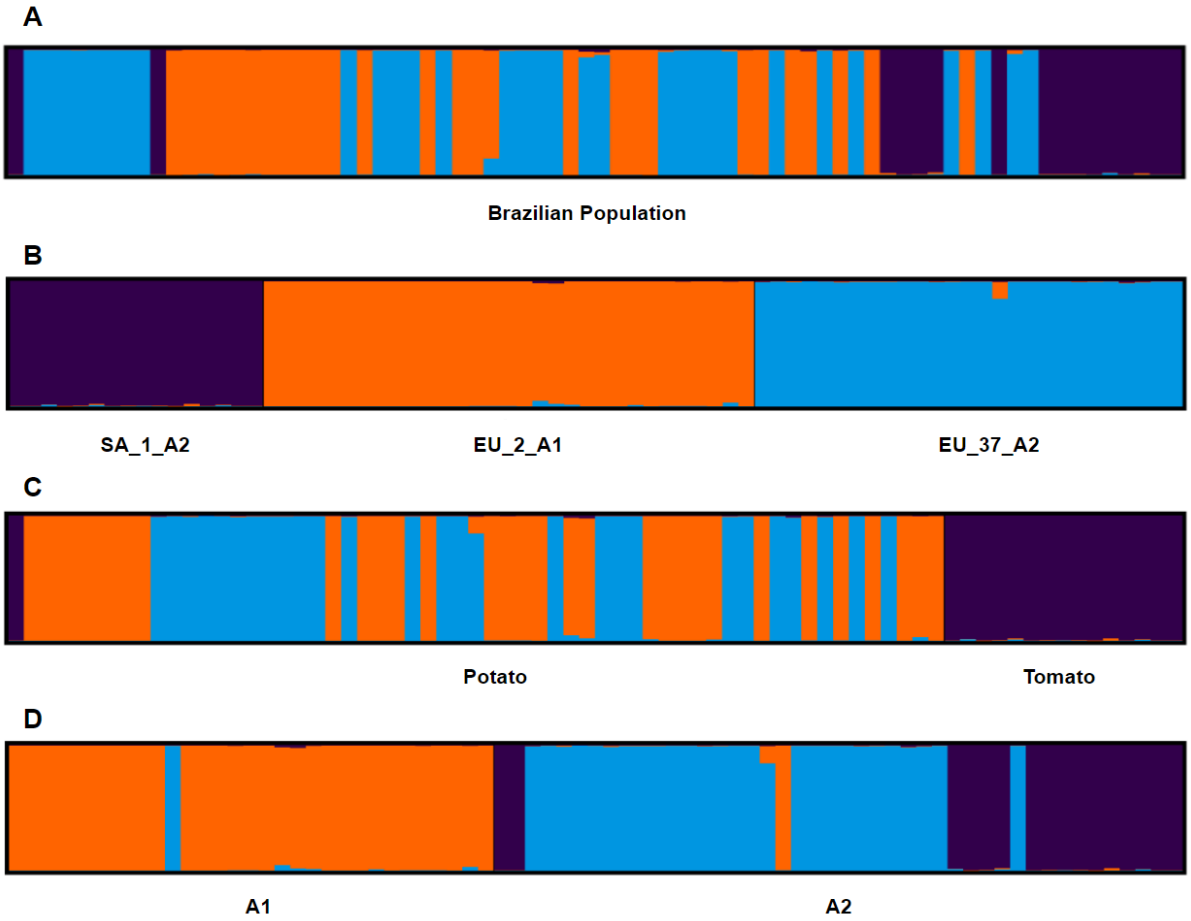


Figure 2. Cluster analysis of the current Brazilian population of *Phytophthora infestans*. The analysis was done considering the entire population without phenotype information (A); the genotypes identified (B); the host of origin (C); and the mating type characterized by molecular markers. The population was analyzed assuming three genetic groups (K = 3). Each bar in the figures represents a multilocus genotype (MLG) colored by the proportion of each MLG variation that belongs to one of the three K groups.

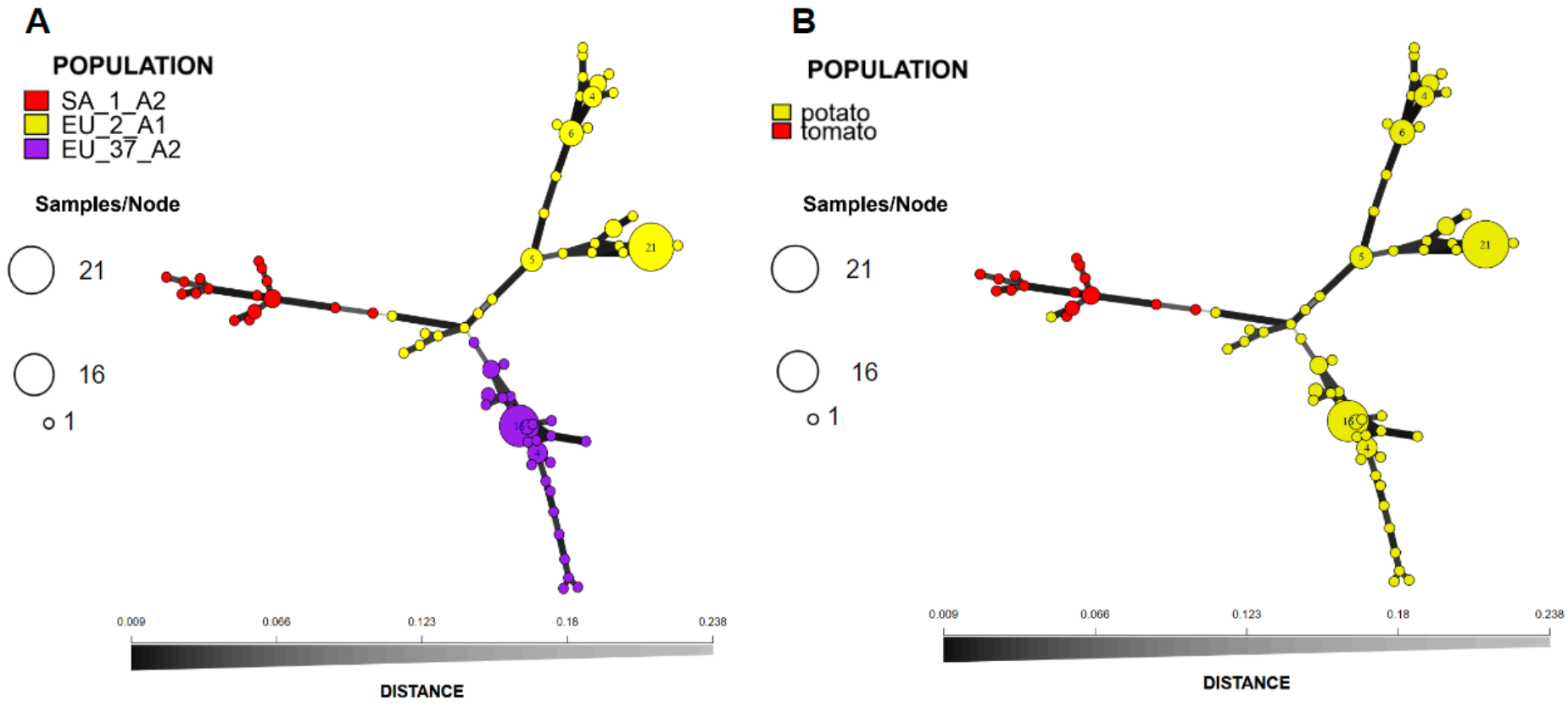
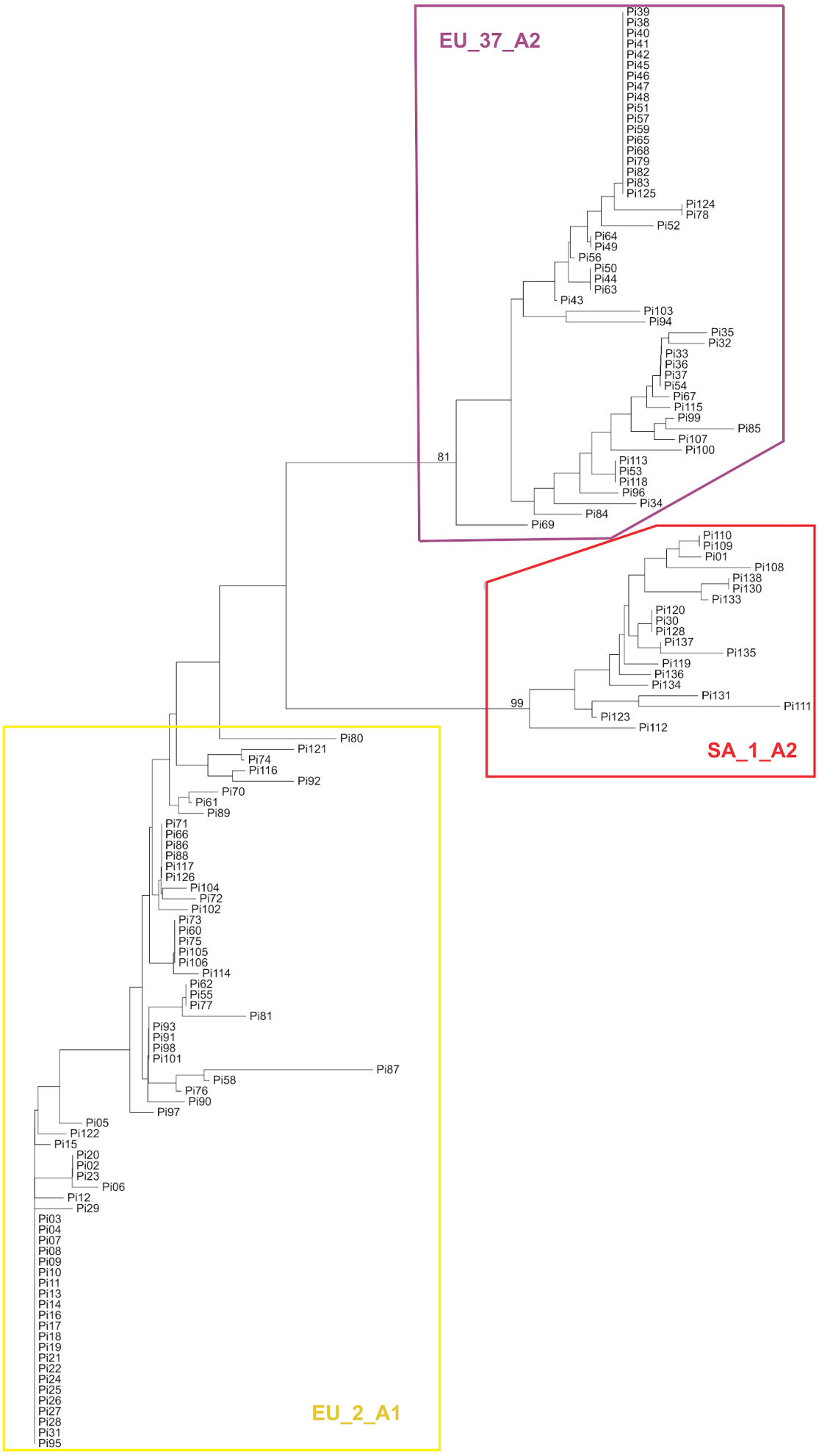


Figure 3. Minimum spanning network of Brazilian population of *Phytophthora infestans* based on 12-plex simple-sequence repeats (SSRs) using Bruvo's distance. Nodes are coloured by genotypes (A) or host (B), represent the multilocus genotypes (MLGs), and their sizes increase according to the number of isolates sharing the MLGs. The thickness and darkness of branches represent the Bruvo's genetic distance between adjacent nodes.


EU_37_A2
Pi39
Pi38
Pi40
Pi41
Pi42
Pi45
Pi46
Pi47
Pi48
Pi51
Pi57
Pi59
Pi65
Pi68
Pi79
Pi82
Pi83
Pi125
Pi124
Pi78
Pi52
Pi64
Pi49
Pi56
Pi50
Pi44
Pi63
Pi43
Pi103
Pi94
Pi35
Pi32
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Pi36
Pi37
Pi54
Pi67
Pi115
Pi99
Pi85
Pi107
Pi100
81
Pi113
Pi53
Pi118
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Pi01
Pi108
Pi138
Pi130
Pi133
Pi120
Pi30
Pi128
Pi137
Pi135
Pi119
Pi136
Pi134
Pi131
Pi111
99
Pi123
Pi112
SA_1_A2
Pi80
Pi121
Pi74
Pi116
Pi92
Pi70
Pi61
Pi89
Pi71
Pi66
Pi86
Pi88
Pi117
Pi126
Pi104
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Pi18
Pi19
Pi21
Pi22
Pi24
Pi25
Pi26
Pi27
Pi28
Pi31
Pi95
EU_2_A1

Figure 4. Neighbor-joining tree of Brazilian population of *Phytophthora infestans* sampled between 2020 and 2024, based on 12-plex simple-sequence repeats (SSRs) using Bruvo's genetic distance. The isolates are color labeled according to the genotype. Purple: EU_37_A2 genotype; Red: SA_1_A2 genotype; and yellow: EU_2_A1 genotype.

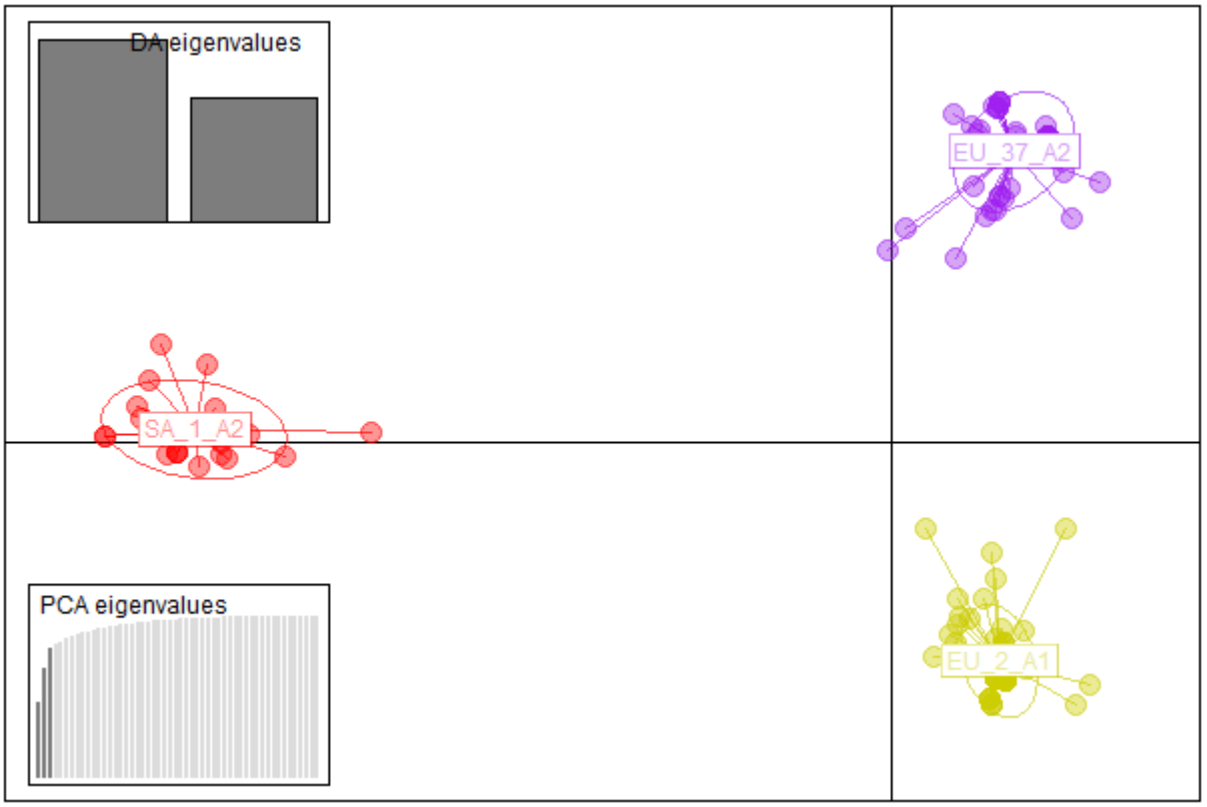


Figure 5. Discriminant analysis of principal components of the Brazilian population of *Phytophthora infestans* sampled between 2020 and 2024. The isolates were grouped by their respective genotypes.

Temporal analysis. A total of 170 MLGs were observed across 301 triploid individuals from 1998 to 2024 by six codominant loci. The 1998-2010 subpopulation had the highest diversity and evenness according to the Shannon-Wiener index and E.5 index (H = 4.58; E.5 = 0.70) (Table 4). There is evidence of the probable occurrence of sexual reproduction in the 1998-2010 population (Ia = 0.09; $\bar{r}d$ = 0.02) (Table 4).

Table 4. Population summary statistics for *Phytophthora infestans* from Brazil. Subpopulation defined by year.

	N	MLG	eMLG (SE)	H	E.5	Hexp	Ia*	$\bar{r}d$*
All	301	170	89.70 (4.02)	4.64	0.45	0.65	0.77	0.155
2020-2024	135	50	50.00 (0.00)	3.17	0.48	0.65	1.83	0.37

1998-201 0	166	120	101.20 (2.36)	4.58	0.70	0.50	0.09	0.02

N = number of individuals; MLG = multilocus genotype; eMLG = multilocus genotype expected; SE = standard error; H = Shannon-Wiener index of MLG diversity; E.5 = Evenness, E_5 ; Hexp = Nei's unbiased gene diversity; Ia = index of association; $\bar{r}_d$ = standardized index of association; * clone corrected value.

The mean number of observed alleles per locus for the 1998 to 2024 subpopulation was 11.5 (Table 5). The total number of alleles observed was 58 and 37 for the 2020-2024 and 1998-2010 population, respectively. The diversity of locus G11 was the highest (1-D = 0.83) and the Pi04 locus had highest evenness (Evenness = 0.64) (Table 5). The percentage of missing data was 0.17 %. The observed heterozygosity was 1 in both populations (2020-2024 and 1998-2010) and the heterozygosity expected ranged between 0.66 (Pi70) to 0.83 (G11) to 2020-2024 population and from 0.53 (Pi02) to 0.79 (G11) to 1998-2010 population (Table 5).

Table 5. Population statistics of 2020-2024 and 1998-2010 populations, by locus observed in the Brazilian population of *Phytophthora infestans*.

Locus	Allele num	1-D	Hexp	Evenness
Pi02	10	0.67	0.67	0.64
G11	17	0.83	0.83	0.62
Pi04	10	0.66	0.66	0.74
Pi63	6	0.61	0.61	0.73
Pi70	6	0.39	0.39	0.58
D13	20	0.74	0.74	0.56
mean	11.5	0.65	0.65	0.64

Allele num = number of alleles observed; 1-D = Simpson index; Hexp = Nei's unbiased gene diversity

The clustering of the Brazilian population between 1998 and 2024 based on the best K value of STRUCTURE estimated by the Evanno method and Bruvo's distance shows a division into 3 and 5 groups. The 2020-2024 subpopulation was better divided into three groups (K = 3) while for the 1998-2010 subpopulation the best number of groups was K = 5 (Figure 7). The MSN with Bruvo's distance shows the separation of the populations defined by sampling year, with low values between them (Figure 8). The AMOVA results indicate significant variation within samples (65.60 %, $p < 0.001$).

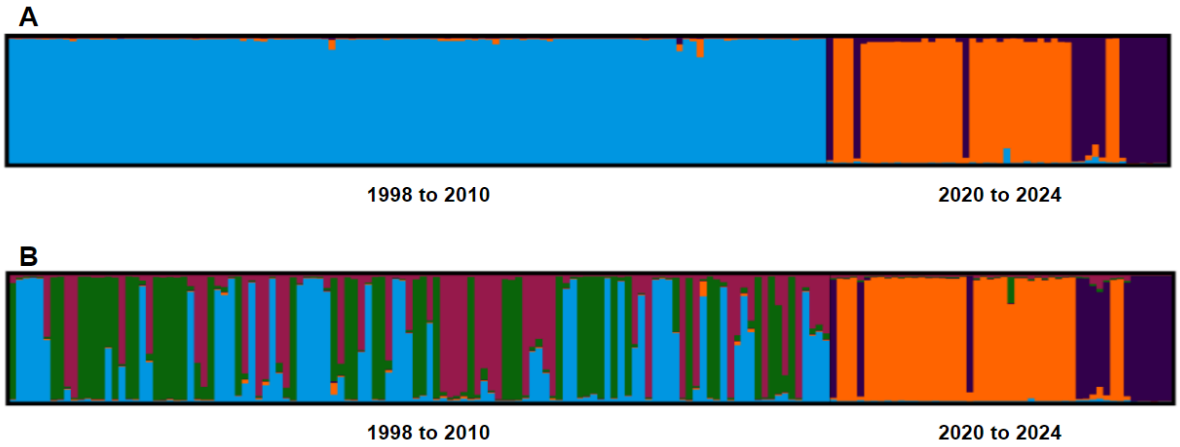


Figure 7. Cluster analysis of the past (1998 to 2010) and current (2020 to 2024) Brazilian population of *Phytophthora infestans*. The population was analyzed assuming three (A) or five (B) genetic groups (K = 3 or K = 5). Each bar in the figures represents a multilocus genotype (MLG) defined based on SSR markers colored by the proportion of each MLG variation that belongs to one of the three or five K groups.

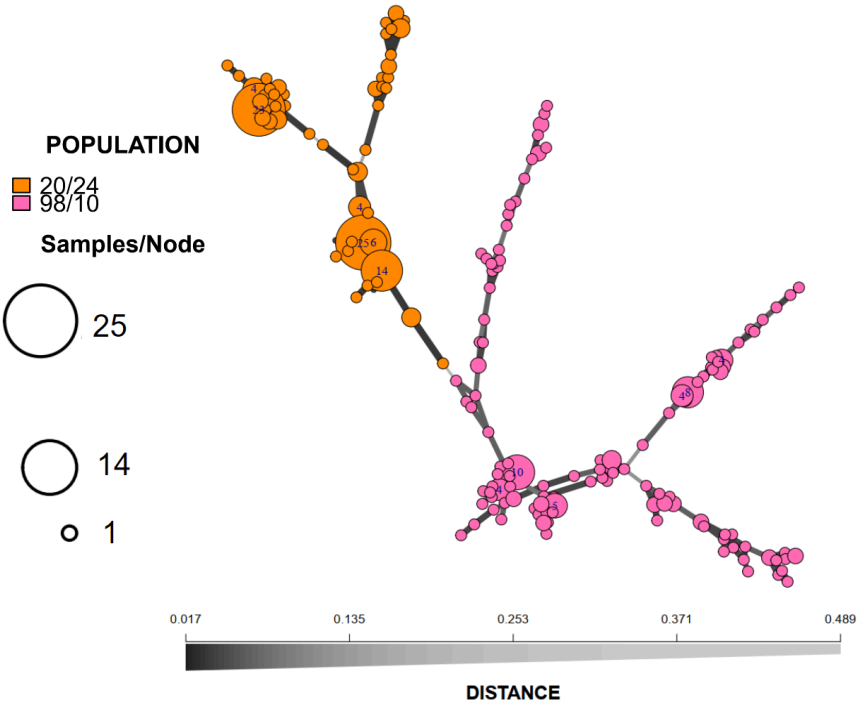


Figure 8. Minimum spanning network of Brazilian population of *Phytophthora infestans* sampled from 1998 to 2010 (pink) and from 2020 to 2024 (orange) based on 6 simple-sequence repeats (SSRs) using Bruvo's distance. Nodes are colored by genotypes and represent the multilocus genotypes (MLGs) defined according to SSR markers, and their size increases according to the number of isolates sharing the MLGs. The thickness and darkness of branches represent Bruvo's genetic distance between adjacent nodes.

Phylogenetic study. Twenty-seven isolates from potato (n = 11) and tomato (n = 16) had the *Btub*, *TigA*, *ITS*, and *CoxI* loci sequenced. The best-fit evolutionary model chosen was GTR+G (nts = 6; rates = gamma) for *Btub*, GTR+I+G (nts = 6; rates = invgamma) for

G3PDH (*TigA* loci), GTR+I (nts = 6; rates = propinv) for Tig (*TigA* loci) and *CoxI*, and HKY+I (nts = 2 rates = propinv) for *ITS* loci.

The aligned sequences from *Btub* (987 aligned characters), G3PDH (527 aligned characters), TIG (576 aligned characters), *ITS* (726 aligned characters), and *CoxI* (648 aligned characters) resulted in 94, 73, 86, 71, and 96 single nucleotide polymorphisms, respectively.

Initially, the phylogenetic analysis was done with all species of *Phytophthora* Clade 1. As the Brazilian isolates grouped in Clade 1c in all analyses, we proceed with the analysis only with species of this clade. The phylogenetic analysis of *ITS* and *CoxI* were able to separate the *Phytophthora* species among their clades but were not able to differentiate the species inside clade 1c (Figure S2, and S3). To compare potato and tomato isolates, the *ITS* and *CoxI* sequences were not used in the multigene analysis.

The multigene analysis was done with *Btub* and *TigA* loci. The alignment for the three concatenated genes (*Btub*, G3PDH, and TIG) contained 2,635 characters, including alignment gaps. The Bayesian phylogeny of the three genes distinguished the species: *P. phaseoli*, *P. mirabilis*, *P. andina*, *P. ipomoeae*, and *P. infestans*. The *P. infestans* isolates from Brazil of SA_1_A2 genotype, EU_2_A1 and EU_37_A2 genotypes grouped separately (Figure 6).

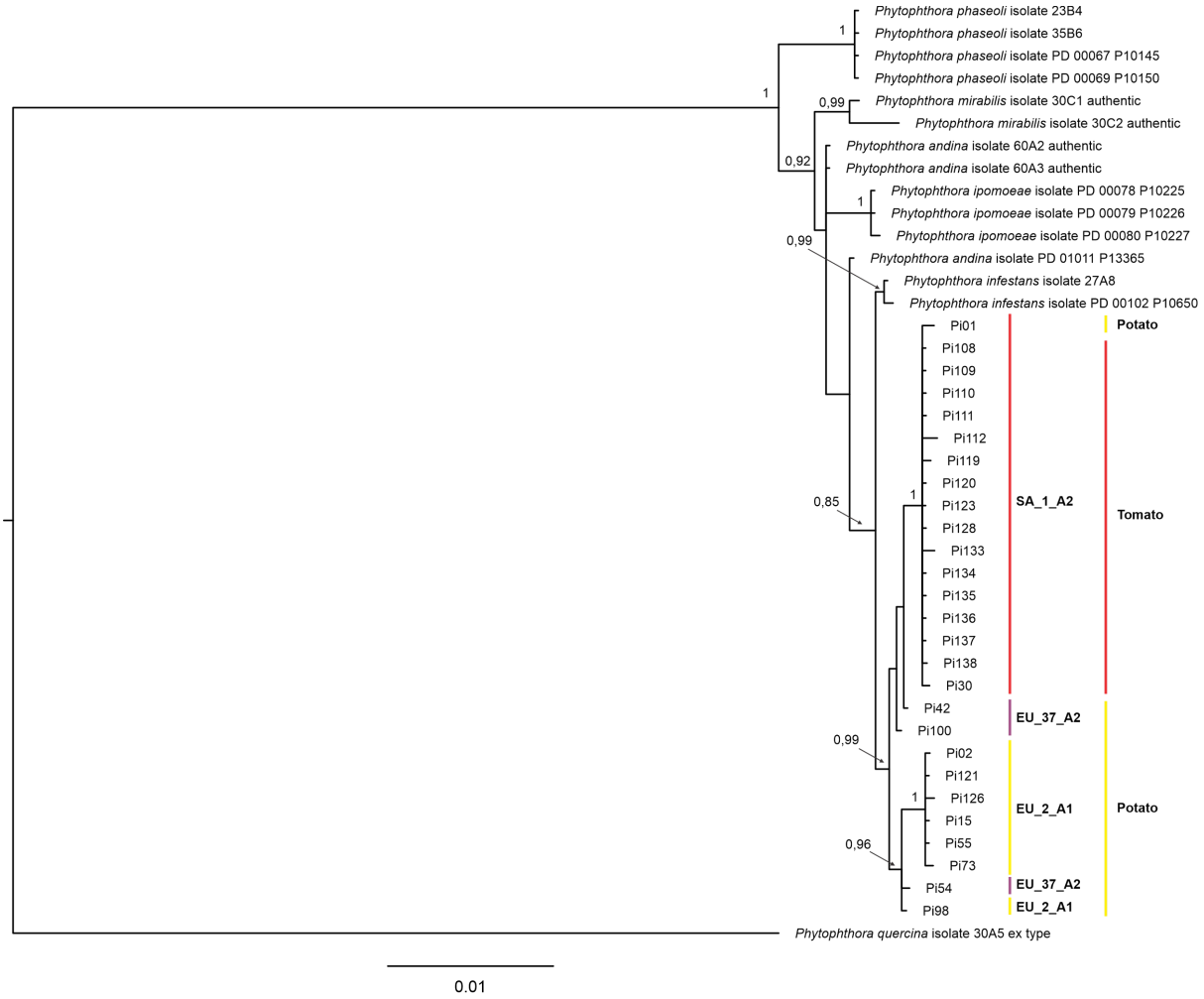


Figure 6. Bayesian 50 % majority rule consensus tree based on the *Btub*, G3PDH, and TIG genes of 27 Brazilian isolates of *Phytophthora infestans* obtained from potato (n = 11) and tomato (n = 16) and reference sequences retrieved from GenBank (isolate description shown in the tree). Bayesian posterior probabilities were also calculated and values are shown at each node > 0.80. The tree was rooted in *P. quercina* (Clade 3). The scale bar represents the expected number of substitutions per site. The Brazilian isolates used in the analyses are indicated by their code in bold. The genotypes and host of origin of isolates from Brazil are indicated by a color bar and the respective genotype or host. Red, purple, and yellow bars adjacent to the node labels followed by SA_1_A2, EU_37_A2, or EU_2_A1 indicate these genotypes in that order. Yellow and red bars on the right side followed by Potato or Tomato indicate the hosts of origin.

Discussion

The genetic profile of the current Brazilian population of *P. infestans* is different from the previous ones. The presence of A1, A2, and A1/A2 isolates when mating types were assessed already indicates major changes in the population of the pathogen. Consequently, shifts in other markers are also noteworthy: Ia and Ib mitochondrial DNA haplotypes, and three clonal lineages EU_2_A1, EU_37_A2, and SA_1_A2, the new lineage, co-occurring in different potato and tomato producing regions. The sensitivity to mefenoxam, formerly

established as marker in characterization studies, did not indicate any changes in the population; i.e., populations of *P. infestans* still harbor sensitive, intermediate, and resistant individuals. In addition, supporting the phenotype differences observed in isolates from both hosts, Bayesian phylogeny distinguished two host-defined populations: one associated with potato and another with tomato. This study provides an update on the *P. infestans* population in Brazil, reported the A2 mating type in tomato fields for the first time, and described a new genotype associated with tomato.

The current Brazilian population of *P. infestans* has a new configuration. Until now, individuals of A1 mating type were almost exclusively sampled from tomato fields (Brommonschenkel 1988; Goodwin et al. 1994; Oliveira 2010; Reis et al. 2003, 2006; Santana et al. 2013; Zanotta 2019). In this study, all isolates obtained from tomato samples were characterized as A2 mating type by PCR-based. Thus, this is the first report of A2 mating type individuals occurring in tomato fields in Brazil. Furthermore, this signals a major shift in the population of *P. infestans* that causes late blight on tomato. Despite the genetic implications of this finding, its epidemiological impacts need to be better assessed because tomato isolates were obtained in only two states in Brazil.

For a long time, the mating type most commonly reported in isolates obtained from potato fields was the A2 (Brommonschenkel 1988; Goodwin et al. 1994; Oliveira 2010; Reis et al. 2003, 2006; Santana et al. 2013) but recently, the mating type A1 was reported as dominant in potato fields (Zanotta 2019). The present study shows both mating types occurring at similar ratios on this host. These results can be explained by the current two genotypes occurring in potato fields in Brazil, EU_2_A1 and EU_37_A2 in very similar ratios.

The mating type characterization done by the PCR-based method was supported by the traditional pairing method in culture medium. The set of primers W16-1/W16-2 agreed 90.38 % with the results of pairing. The disagreement between methods occurred for five isolates obtained from tomato, and in isolates from potato identified as A1/A2 due to the formation of oospores with both testers. The first disagreement can be explained by the lack of consistency observed when pairing isolates from tomato among replicates (inconsistencies), probably due to large variation in time for isolates to grow, i.e. different mycelial growth rates. The second disagreement must be caused because the set of primers used was not able to identify A1/A2 mating type.

The oligonucleotides more commonly used are W16-1/W16-2 (Judelson et al. 1995), S1-A/S1-B (Judelson 1996), and PHYB-1/PHYB-2 (Kim and Lee 2002). These

oligonucleotides allow the identification of both A1 and A2 mating types, respectively. Considering that efficient molecular markers for mating-types identification should present minimal error rates compared with pairing tests, primer validation is necessary due to the genetic variation of each population (Brylińska et al. 2018). The use and validation of molecular markers to identify the mating type is a practice that has often being used (Beketova et al. 2014; Brylińska et al. 2018; Chowdappa et al. 2015; Dangi et al. 2021; Mazáková et al. 2010; Wharton et al. 2023; Zhang et al. 2006).

The Brazilian population was assessed for mating type using the three primer pairs (Zanotta 2019). In the present study, only the set of primers W16-1/W16-2 (Judelson et al. 1995) were useful, i.e. were reliable and agreed with the pairing results. The other primer pairs were not reliable to the tested isolates due to incongruences in different PCR runs and were not used for mating type determination. The results observed in this study provide additional evidence that the primer pair W16-1/W16-2 (Judelson et al. 1995) is useful and reliable for mating type characterization of the population of *P. infestans* in Brazil.

The PCR-based method allows fast mating type determination and does not require pathogen isolation, a very laborious procedure, mainly for tomato isolates. On the other hand, the markers available cannot identify A1/A2 or SF isolates. The A1/A2 and SF isolates are identified as the A1 mating type in this study and also in the study conducted by Zanotta (2019). A new molecular marker able to identify A1/A2 and SF isolates is necessary to replace the pairing completely and assess mating type changes in the population faster.

Isolates able to produce oospore with both mating types or by themselves in Brazil have been reported since 2013 (Casa-Coila et al. 2017; Santana et al. 2013; Zanotta 2019). The occurrence of isolates producing oospore with both mating types was reported in the states of RS (Casa-Coila et al. 2017; Santana et al. 2013) and in SC, in this study. The SF isolates were reported in PR, SP, and MG states (Casa-Coila et al. 2017; Zanotta 2019), The occurrence of A1/A2 and SF individuals increases the chance of recombination by sexual reproduction, as well the co-occurrence of both mating types in fields of MG and PR states reported in the present study and in studies conducted previously (the states of RS, MG, SP, and PR) (Gomes et al. 2007; Oliveira 2010; Zanotta 2019).

The occurrence of sexual reproduction can affect the epidemiology of late blight (Fry and Mizubuti 1998; Mayton et al. 2000; Shattock et al. 1986) because it increases genetic diversity and allows the pathogen to survive for a long time without a host. Thus, given the reports of possible events of sexual reproduction periodical monitoring of the population

should be adopted to support the choice of more appropriate late blight management strategies for disease control.

Another sign of alteration in the population is the presence of two genotypes identified in association with potato and a new lineage occurring on tomato. The lineages already reported in Brazil were US-1 on tomato and potato, BR-1 (Brommonschenkel 1988; Goodwin et al. 1994; Reis et al. 2003, 2006; Santana et al. 2013) and EU_2_A1 on potato (Zanotta 2019). Of the genotypes identified in this study, only one has been previously described in Brazil, the EU_2_A1. This was the most commonly found lineage in Europe before 2006, when it was replaced by EU_13_A1, which spread to eastern Africa by imported seeds (Beninal et al. 2022; Puidet et al. 2023). The genotype EU_37_A2, also identified in isolates from potato, was described for the first time in 2013 in the Netherlands and quickly spread in Europe from 2015 (Puidet et al. 2023; Schepers et al. 2018). This genotype increased its frequency and competed well with other lineages (e.g. EU_13_A1) due to characteristics such as resistance to fluazinam, shorter latent period, and higher lesion growth rate (Puidet et al. 2023). The SA_1_A2 genotype, identified in all isolates from tomato and one from potato, has a DNA fingerprint not described before. Although one isolate from potato (Pi01) has been identified as SA_1_A2, the municipality of origin of Pi01 was Viçosa - MG, near an important tomato-producing region and the migration from the surrounding tomato fields to potato plants has been postulated before (Oliveira 2010; Reis et al. 2003). For these reasons, the new genotype seems to be almost exclusively associated with tomato host.

The mtDNA haplotypes Ia and Ib were reported before associated with isolates obtained from potato and tomato (Oliveira 2010). The Ib mtDNA haplotype in isolates of A2 mating type were associated to be resulted from recombination by sexual reproduction or migration of new genotypes of *P. infestans* (Oliveira 2010). Isolates of A2 mating type and Ib haplotype were identified in tomato fields associated with the new genotype.

The presence of new genotypes of *P. infestans* in Brazil can be explained by the import of infected potato seeds or rare events of recombination. The potato producers in Brazil import seeds tubers mainly from The Netherlands, France, Germany, Canada, and United States (Oliveira 2010). In Europe, the recombination by sexual reproduction was reported in The Netherlands and Nortic countries (Brurberg et al. 2011; Drenth et al. 1994). Also, in Canada the occurrence of sexual recombination was reported recently (Babarinde 2024). Considering the evidences of clonality in the population of *P. infestans* in Brazil, the origin of the new SSR genotypes is likely to be due to migrations from European or North

American countries, where recombination can take place generating new genotypes and USA and Canada are also sources potato seed tubers to Brazil.

The sensitivity to MFX observed in the present study were similar to the previous populations reported before in Brazil (Reis et al. 2003, 2006; Santana et al. 2013). The population of *P. infestans* in Brazil remains composed of sensitive, intermediate, and resistant individuals and no association between sensitivity to MFX and genotype lineage was detected.

Historically, the Brazilian population of *P. infestans* is structured by host and previous studies provided evidence for host specialization (Reis et al. 2003, 2006; Santana et al. 2013; Suassuna et al. 2004). In addition, it has been reported that one subpopulation that used to be associated with tomato responds differently from the subpopulation associated with potato regarding the effects of temperature on growth, sporulation, and virulence (Maziero et al. 2009; Suassuna et al. 2004). More recently, phenotypic differences in isolates from tomato were observed: recalcitrance in isolation, low mycelial growth rate on culture medium, long latent period, and long time to zoospore release (data not shown). Considering phenotypic variation can help to forecast pathogen evolution and, consequently, epidemic changes (Ayala-Usma et al. 2020), we hypothesized a shift in the population of *P. infestans* in Brazil is occurring. Our hypothesis can be supported by the results presented in this study: (i) the occurrence of A2 mating type in tomato fields for the first time; (ii) a new genotype, the SA_1_A2, associated with isolates from tomato; (iii) the presence of EU_37_A2 genotype in potato fields for the first time, (iv) the occurrence of A1 and A2 mating types in similar ratios in potato fields; and (v) the support of phylogenetic analysis in distinguish the Brazilian isolates from tomato and potato between them.

In the multigene phylogenetic analysis, the distinction between isolates of the SA_1_A2 genotype (tomato host) and the other isolates (potato host) is well supported (Figure 6). This result can add to the body of evidence of host specialization of *P. infestans* in Brazil. In the phylogenetic analysis of the single genes, the tree of G3PDH provides the most convincing evidence for isolate divergence according to the host of origin. This gene has an important function in the initial stages of plant-pathogen interactions (Laxalt et al. 1996; Shan et al. 2004). The phylogenetic difference between isolates from both hosts can explain the phenotypic variation observed in isolates obtained from tomato such as longest latent period, and longest time to zoospore release compared to isolates from potato.

The evidence of change in genetic profile of the population of *P. infestans* in Brazil can be well visualized by the temporal analysis of the population from 1998 to 2024. The

analysis of the 1998-2010 and 2020-2024 subpopulations show the contrast of genetic composition of the Brazilian population over the years. The higher diversity of the 1998-2010 subpopulation can be explained by migration of new genotypes and by the sexual recombination events evidenced by the index of association and detected occurred between 2003 and 2005 (Oliveira 2010).

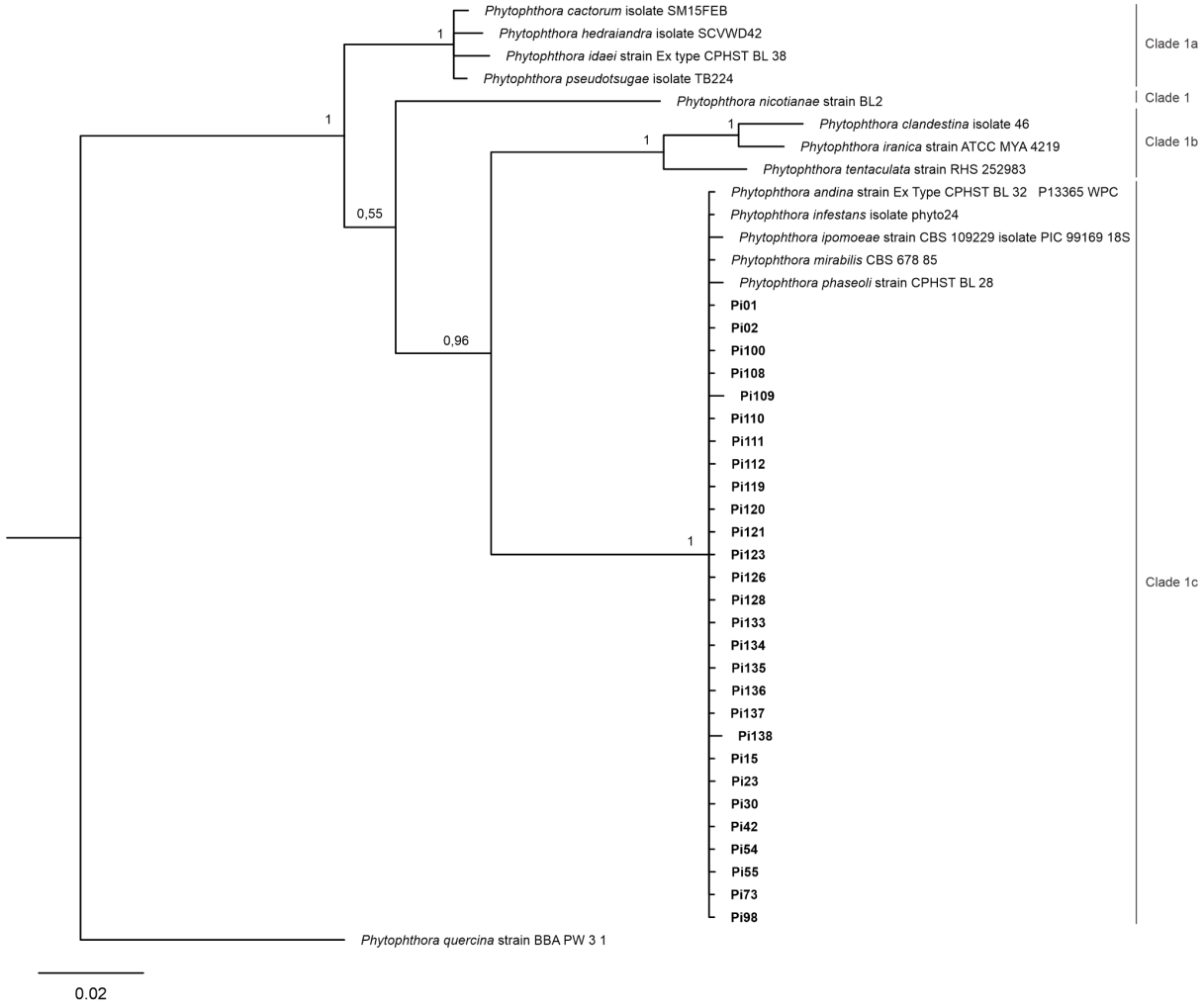
The population of *P. infestans* from Brazil, despite predominant asexual characteristics, has undergone changes over the years. This study characterized the current Brazilian population of *P. infestans* mating type, mitochondrial DNA haplotypes, 12-plex SSR genotype, and mefenoxam sensitivity. For the first time, the A2 mating type is associated with isolates from tomato in Brazil. Also, three genotypes are co-occurring in the population and a new lineage seems to occur exclusively in tomato fields. Phylogenetic evidence of two host-defined populations supported the phenotype differences observed in isolates from tomato and potato. The update of the Brazilian population of *P. infestans* and the separation of isolates from potato and tomato are very important information about this pathogen in Brazil that should be considered to take adequate late blight management actions to each host.

Supplementary material

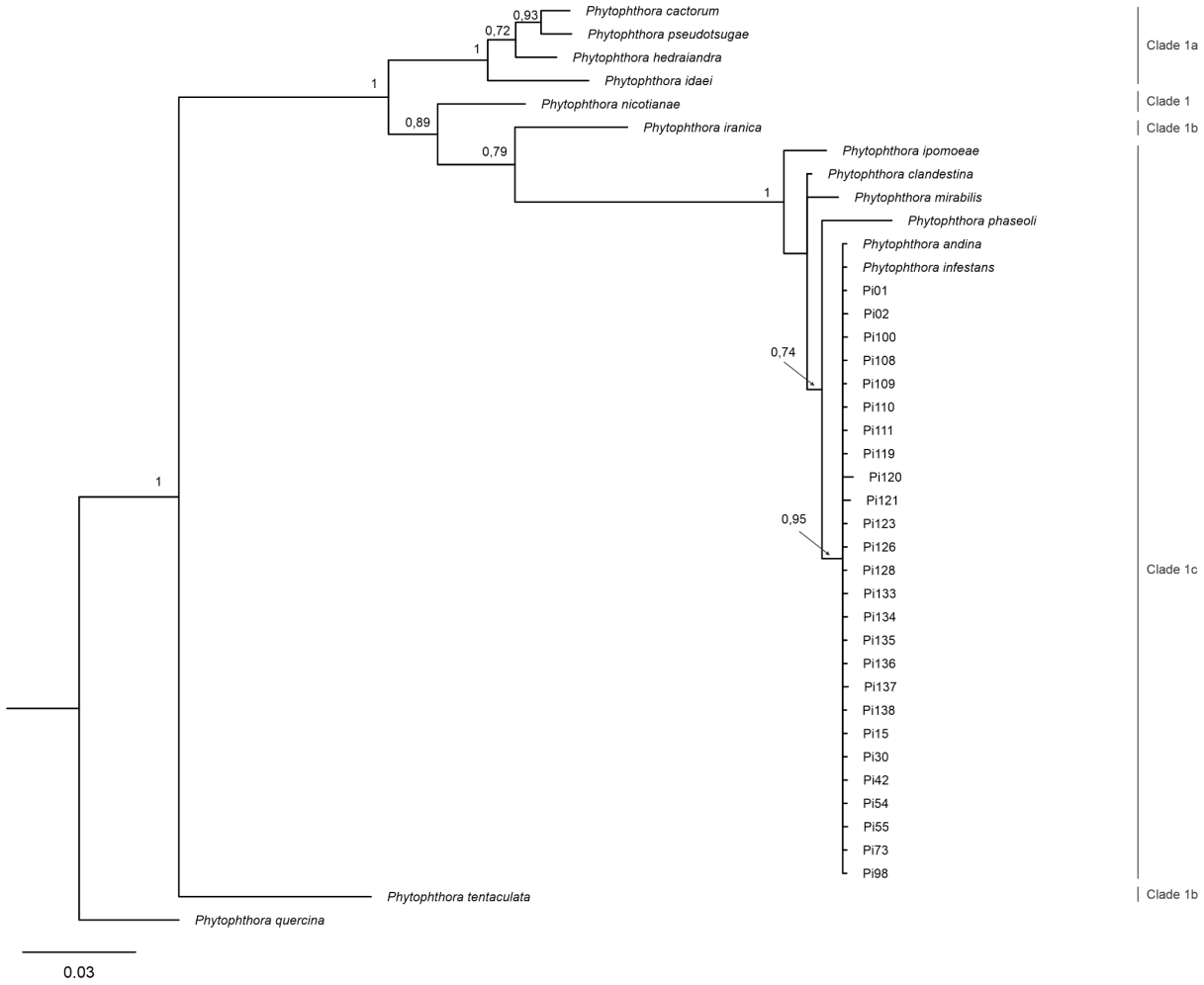
Table S1. SSR allele names and sizes adapted from Li et al. (2013) to Brazilian isolates of *Phytophthora infestans* according control isolates sent by Dr. David Cooke (JHI).

G11	**Allele name**	**130**	**132**	**138**	**140**	**142**	**146**	**148**	**150**	**152**	**154**	**156**	**158**	**160**	**162**	**164**	**166**	**168**	**170**
	Allele size	130.3	132.3	138.5	140	142	147.1	149.4	151.4	153.1	155.2	157	159.4	161.1	163.1	165.1	167	169.2	171.4
	Allele name	**172**	**176**	**198**	**200**	**202**	**204**	**206**	**208**	**210**	**214**	**218**	**220**	**222**					
	Allele size	173.2	176.7	198.3	200.2	202	204.1	206	207.7	210	213.6	217.5	219.5	221.4					
Pi04	**Allele name**	**160**	**166**	**168**	**170**	**172**													
	Allele size	167	171.2	173.5	175.4	179													
Pi4B	**Allele name**	**203**	**205**	**209**	**211**	**213**	**215**	**217**	**221**	**225**									
	Allele size	206	207	210.5	212.5	215	218	220	224	229									
Pi63	**Allele name**	**270**	**273**	**279**															
	Allele size	272	275	280															
Pi70	**Allele name**	**189**	**192**	**195**	**198**														
	Allele size	187	191	194	196														
D13	**Allele name**	**108**	**110**	**112**	**114**	**116**	**118**	**120**	**122**	**124**	**126**	**128**	**130**	**132**	**134**	**136**	**138**	**140**	**142**
	Allele size	106.2	108.2	110	112.1	114.2	116.3	119	120.9	123.1	125.1	127.1	128.8	130.6	132.6	135	137	138.9	141.1
	Allele name	**144**	**146**	**148**	**150**	**152**	**154**	**156**	**158**	**160**	**162**	**164**	**166**	**168**	**170**	**172**	**174**	**176**	**178**
	Allele size	143.3	145.6	147.8	150.2	152.3	154.3	156.4	158.6	160.6	162.4	164.4	166.6	168.4	170.4	172.5	174.4	176.6	178
	Allele name	**184**	**188**	**190**	**208**	**210**	**212**	**214**	**216**										
	Allele size	185.1	188.9	190.8	208.7	210.8	212.7	214.7	217										
SSR2	**Allele name**	**163**	**167**	**173**	**175**	**177**													

	Allele size	163.5	167.3	173.3	175.4	177.4													
Pi02/SSR3	Original allele name	152	158	160	162	164	166	168											
	Allele name	**258**	**264**	**266**	**268**	**270**	**272**	**274**	**262**										
	Allele size	260	266	268	270	272	274	276	264.7										
SSR4	**Allele name**	**283**	**285**	**287**	**289**	**291**	**293**	**295**	**297**	**299**	**301**	**303**	**305**	**307**	**309**	**311**	**313**	**315**	**317**
	Allele size	281.4	285	288	289	290	292	294	296	298	299.7	301.9	304	305.7	307.9	309.9	312.1	314	316.3
SSR6	**Allele name**	**232**	**236**	**240**	**242**	**244**	**246**	**254**	**256**	**258**	**260**	**262**							
	Allele size	234	236.1	245	245	247	249	251	253	255	259.8	262.7							
SSR8	**Allele name**	**260**	**264**	**266**															
	Allele size	262	266	268															
SSR11	**Allele name**	**331**	**341**	**356**															
	Allele size	331	341	356															



Supplementary 2. Bayesian 50 % majority rule tree based on the *ITS* loci of 27 Brazilian isolates of *Phytophthora infestans* obtained from potato (n = 11) and tomato (n = 16) and reference sequences retrieved from GenBank (isolate description shown in the tree). Bayesian posterior probabilities (%) were also calculated and values are shown at each node > 0.50. The tree was rooted in *P. quercina* (Clade 3). The scale bar represents the expected number of substitutions per site. The Brazilian isolates used in the analyses are indicated by their code in bold. The *Phytophthora* clade of isolates are indicated by bar and the respective clade name.



Supplementary 3. Bayesian 50 % majority rule tree based on the *CoxI* loci of 27 Brazilian isolates of *Phytophthora infestans* obtained from potato (n = 11) and tomato (n = 16) and reference sequences retrieved from GenBank. Bayesian posterior probabilities (%) were also calculated and values are shown at each node > 0.70. The tree was rooted in *P. quercina* (Clade 3). The scale bar represents the expected number of substitutions per site. The Brazilian isolates used in the analyses are indicated by their code in bold. The *Phytophthora* clade of isolates are indicated by bar and the respective clade name.

Chapter 2. Sensitivity of *Phytophthora infestans* isolates from Brazil and field efficacy of oomycides in the control of potato and tomato late blight

Abstract

Late blight, caused by *Phytophthora infestans*, is a disease that affects potato and tomato crops. This disease is characterized by a fast and destructive epidemic that can damage the entire field in a few days. The main strategy of late blight management is the use of chemical compounds. The successive and intensive use of oomycides to avoid huge yield loss is associated with selection pressure, resistance emergence, and establishment of pathogen resistant population. The knowledge of the sensitivity of the pathogen population to oomycides frequently used for its control is essential to choose management actions. In this study, the sensitivity of the current Brazilian population of *P. infestans* to mefenoxam (MFX), mandipropamid (MAD), zoxamide (ZOXA), and oxathiapiprolin (OXTP) was assessed. Also, the present study provides information about the efficacy of zoxamide and oxathiapiprolin on potato and tomato blight control in field conditions and with the stability of sensitivity of the isolates to these oomycides. The population of *P. infestans* sampled between 2020 and 2024 is composed of isolates sensitive to MFX, MAD, ZOXA, and OXTP; intermediate sensitive to MFX and MAD; and insensitive to MFX. The combination of ZOXA + chlorothalonil + cymoxanil + mancozeb and OXTP + dimethomorph were the best treatments evaluated with these compounds to manage potato and tomato blight under field conditions. In a pressurization experiment, the sensitivity to ZOXA and OXTP remained stable after three applications. The information presented by this study is essential to late blight management in Brazil.

Introduction

Successful plant disease management includes integrated strategies such as avoidance, exclusion, eradication, protection, resistance, and therapy. The association of integrated strategies with the knowledge of the biology of the plant pathogen and the dynamics of the plant disease epidemic is key for determining the most effective actions to manage a plant disease. Although the theory of plant disease management is well established, the real situation observed in commercial fields is the use of a few or a single (chemical) strategy to manage disease on plants. Thus, resistant pathogen populations have become a relevant concern and frequent issue in commercial fields.

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is the most severe disease that affects potato and tomato worldwide. As commonly deployed in many crops, chemical control is the common method adopted to manage this disease due to the lack of commercially available resistant cultivars (Cooke and Lees 2004). The pathogen *P. infestans* is a hemibiotrophic, heterothallic oomycete, with a short life cycle, and it has a particular set of resources for virulence that results in fast and severe epidemics associated with high yield loss in potato and tomato crops worldwide (Haverkort et al. 2008; Zuluaga et al. 2016). Thus, a high number of chemical sprays are required to achieve satisfactory control levels due to the fast change of the pathogen population (Olave et al. 2022). Frequent oomycide sprayings often result in risks to the environment, higher selection pressure on the pathogen, and selection of resistant individuals (Cooke and Lees 2004).

Based on the biology of *P. infestans*, the speed and destructiveness of the late blight epidemics, and the history of resistance reports, the Fungicide Resistance Action Committee (FRAC) includes the pathogen in the group of medium risk to develop resistance (Fungicide Resistance Action Committee 2019). In cases where the resistance risk is medium to high, the FRAC recommends continuous resistance monitoring. In this context, the efficient monitoring of pathogen sensitivity to oomycides (or fungicides) used for its control is essential to delay pathogen resistance and to extend the shelf-life of commercial products. In the best scenario, the monitoring of sensitivity must be done before the pathogen is exposed to the molecule in the field; i.e. to establish baseline sensitivity; and sensitivity tests over the seasons to keep up with eventual changes in the pathogen population that can compromise the performance of the chemical compounds (Gomes et al. 2014; Gray et al. 2018; Russell 2004).

Currently, there are 212 products registered to manage late blight in Brazil (Agrofit 2024). The most commonly used are phenylamides, benzamides, quinone outside inhibitors, quinone inside respiration inhibitors, carbamates, oxysterol binding protein homologs inhibitors, carboxylic acid amides, cyanohydroxyiminoacetamides, dithiocarbamates, and chloronitriles. Considering the importance of chemical molecules in late blight management and periodic monitoring sensitivity of the pathogen, this study aimed to assess (i) the sensitivity of *P. infestans* to mefenoxam, mandipropamid, zoxamide, and oxathiapiprolin; (ii) evaluate the field efficacy of oxathiapiprolin and zoxamide in potato and tomato late blight control; and (iii) the stability of oxathiapiprolin and zoxamide in potato and tomato late blight control.

Materials and methods

***Phytophthora infestans* isolates.** The isolates used in the sensitivity assays were sampled in many producing areas of potato and tomato in Brazil. The states of Bahia (BA), Minas Gerais (MG), São Paulo (SP), Espírito Santo (ES), Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS) were sampled.

Leaves, stems, and fruits with typical late blight lesions were collected, kept in paper bags, and taken to the Plant Pathogens Population Biology Laboratory of the Department of Plant Pathology at the Universidade Federal de Viçosa, Minas Gerais, Brazil. The samples were kept in a humid chamber made of plastic box (11cm x 11 cm x 3cm high - germbox) lined with sterilized paper towels soaked in water. Germboxes were kept at 18 °C in darkness to induce pathogen sporulation.

Pathogen isolation was attempted by direct and indirect isolation procedures. Tomato samples were submitted to indirect isolation only. Potato samples were processed either by direct or indirect isolation. The direct isolation was done by removing sporangia from lesions and placing them in a Petri dish with Pea Agar medium amended with rifampicin (0.02 g/L), ampicillin (0.10 g/L), and tebuconazole fungicide (Folicur®) (10 mg a.i./L) or thiabendazole fungicide (Tecto®) (10 mg a.i./L) or PARP medium (500 mL of Pea Agar + 0.125 g ampicillin + 0.05 g PCNB + 0.005 g rifampicin). The indirect isolation was done by cutting leaf fragments of 5 mm^2 from symptomatic lesions, followed by disinfestation in sodium hypochlorite 1% for 30 s, and sterilized water for 30 s twice. After double washing, each leaf fragment was immediately placed on a filter paper to dry. Once completely dry, the fragments

were inserted into Petri dishes containing PARP medium with the abaxial part facing up. The isolation plates were kept at 18 °C in the dark for 15 days, and every two days the presence of pathogen structures was checked under a stereomicroscope. The isolates were morphologically identified as *P. infestans* by the presence of sporangia, hyaline, and non-chambered hypha. The tomato isolates were multiplied in tomato leaves by inoculation in new tomato leaves every 10 days.

Sensitivity assays. The sensitivity of *P. infestans* isolates was assessed to mefenoxam (MFX), mandipropamid (MAD), zoxamide (ZOXA), and oxathiapiprolin (OXTP). The methodologies used were germination of sporangia (OXTP), mycelial growth (OXTP, ZOXA, MAD, and MFX), and sporulation on leaf disc (OXTP, ZOXA, MAD, and MFX). Doses and methods used for each compound are described in Table 1.

Table 1. Description of oomycides doses and assay method used to assess the sensitivity of Brazilian isolates of *Phytophthora infestans*.

Oomycide	**Dose**	**Methodology**
MFX	0, 5, 100 μg/mL	mycelial growth sporulation on leaf disc
MAD	0, 0.1, 10 μg/mL	mycelial growth sporulation on leaf disc
ZOXA	0, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 0.5, and 1 μg/mL	mycelial growth
ZOXA	0, 1, 5, 10, 20, and 40 μg/mL	sporulation on leaf disc
OXTP	0, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 μg/mL	germination of sporangia mycelial growth sporulation on leaf disc

Technical grade metalaxyl-M dispersible oil suspension and mandipropamid concentrated suspension (Revus 250SC) were provided by Syngenta. Technical grade zoxamide (240 g a.i./L a.i.) dispersible oil suspension was supplied by Gowan Produtos Agrícolas LTDA and technical grade oxathiapiprolin (100 g a.i./L a.i.) dispersible oil suspension was provided by Corteva Agriscience. A stock solution of each oomycide was prepared (1000.0 μg/mL), diluted in dimethyl sulfoxide (DMSO), and stored at 4 °C in darkness. Serial dilutions were prepared from the stock solution to reach the desired concentrations. The final concentration of DMSO in the treatment was standardized at 0.1% (v/v).

Sporangia germination

The sporangia germination was assessed by a microtiter test on 96-well flat-bottom plastic plates based on methods recommended by FRAC (2005). Aliquots of stock solutions of OXTP were dissolved in sterilized water to reach final concentrations of 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1, and 1.0 µg/mL. A suspension of sporangia was prepared by washing the surface of the 15 days-old colony grown on PAM supplemented with saturated beta-sitosterol (Ayala-Usma et al. 2020) at 18 °C in the dark with 8 mL of a nutrient solution (300 g peas and 10 g glucose per liter). The suspension was filtered through gauze and adjusted to 2 x 10^4 sporangia/mL. A 50 µL-drop of fungicide suspension and 50 µL-drop of sporangia suspension was dispensed in each well The plates were kept at 18 °C in the dark for 5 days. The optical density was assessed using a photometer Multiskan GO 1.01.12 at 405 nm after the incubation. The treatments were assessed using three absorbance readings in different wells, i.e. three pseudoreplicates. The experiment was conducted at least twice for each isolate.

Mycelial growth

The *P. infestans* isolates were grown on PAM at 18 °C, in the dark for 15 days. Then, the sensitivity of the pathogen to the oomycetes was assessed by mycelial growth on pea-glucose-agar medium (PGA) (150 g pea, 5 g glucose, and 15 g agar per liter) amended with the respective compound. Mycelial discs (5 or 9 mm) were cut with a cork borer and transferred to PGA amended with doses tested of each treatment (Table 1). Measurements of diameter of colonies in two perpendicular directions, discounting the initial diameter of the plug, were taken using a digital caliper nine days after incubation at 18 °C in darkness. Each treatment was assessed using four replicates. The test was conducted at least twice for each isolate.

Sporulation on leaf disc

The sporulation on leaf disc test was done according to procedures recommended by FRAC (2006; 2017). Potato and tomato plants were grown under greenhouse conditions on three-liter plastic pots containing a mix of soil, substrate, and cattle manure in the same proportion. Plants were fertilized with N-P-K formula 4-14-8, ammonium nitrate (tomato) and pests were controlled by using Abamectin (Abamectina®), Propargite (Omite®), and Thiamethoxam (Actara®). Leaves were taken from one-month-old potato plants (cv. Agata) and 45-day-old tomato plants (cv. Santa Clara) and the abaxial side was sprayed with 3 mL of oomycide suspension (Table 1) using a Devilbiss sprayer connected to an air compressor at 15 PSI (Air Plus Schulz MS2.3).

After the oomycide spray, leaves were dried in laminar flow for 20 min. Leaf discs were cut with a cork borer and immediately placed, with the abaxial side up, in wells of plastic with 24-well plates containing 1.5 mL of 0.4% water-agar. The plates were kept at 18 °C in white light for 18 h and in darkness for 6 h until the next day.

The inoculation of leaf discs was conducted 24 h after the oomycides application. The sporangia of *P. infestans* isolates, from potato, grown on PAM at 18 °C, in the dark for 15 to 21 days, were washed with 8 mL of sterilized water to obtain a suspension adjusted to 2 x 10^4 sporangia/mL. The sporangia of *P. infestans* isolates, from tomato, grown on tomato leaves were washed with non-chlorinated water to obtain a suspension adjusted to 2 x 10^4 sporangia/mL. The sporangia suspension was kept for 2 h at 4 °C to induce zoospore release. A 30 µL-drop of sporangia suspension was placed in the center of each leaf disc. The plates were kept at 18 °C under 18 h of light alternated with 6 h of darkness for 6 to 8 days for potato isolates or 10 to 15 days for tomato isolates. After 2 days, the remaining drop of sporangia suspension was dried in laminar flow for three hours.

The sporulation was visually observed after incubation period, using a stereomicroscope (Olympus SZ61 model SZ2-ILST) at 10X. Sporulation grades were assigned according to the following scale: 0 - no symptoms visible; 1 - necrosis; 2 - 5 %; 3 - 5 to 20 %; 4 - 20 to 50 %; 5 - greater than 50 % of the leaf disc with sporulation (Sozzi 1991). The grades were transformed into a percentage of sporulation based on the midpoint of the scale: 0 - 0%; 1 - 0%; 2 - 5%; 3 - 12.5%; 4 - 35%; and 5 - 75%. Each treatment was assessed using four-leaf discs and the experiment was conducted twice for each isolate.

Oomycides efficacy under field conditions. Field trials were conducted at the experimental area of the Experiment station of Teaching, Research, and Extension Unit of Universidade Federal de Viçosa. Two experiments with potato and one with tomato to test the oomycides was conducted. The potato trials were conducted from May to August 2022, and the tomato field experiment was conducted from March to May 2024. Soil analysis of each experiment area was done before the experiment was installed. The soil correction and fertilization were done according to the crop recommendation.

The weather variables such as relative humidity, temperature, and precipitation/irrigation were recorded throughout the experiments. The disease progress curve (DPC), the area under the disease progress curve (AUDPC), the climate graph, and graphs of yield were plotted for all experiments.

Inoculum preparation

The potato experiment to test zoxamide field efficacy, the first field experiment set up, was artificially inoculated. The other field trials on potato and tomato were naturally inoculated. Untreated rows spaced 1 m apart the plots were inoculated to serve as an inoculum source. The artificial inoculation was done with 520 mL of zoospore suspension (10^3 zoospore/mL), 25 days after plant emergence (DAE). The zoospore suspension was prepared with five isolates, from potato, randomly selected from five distinct states of origin (MG, SC, PR, SP, and RS). The isolates were grown on PAM for 14 days at 18 °C darkness, the sporangia were washed with sterile water to obtain a sporangial suspension, the suspensions were kept for 3 h at 4 °C, inoculated on potato leaves, the inoculated leaves were kept in a room at 22 °C for approximately four days, to pathogen infect and sporulate. The leaves with pathogen sporulation were put in sterile water and kept for 3 h at 4 °C, the zoospore suspensions were inoculated onto new potato leaves. This process was repeated two times. The sporangia suspension used to inoculate the plants was prepared using fresh sporulating lesions. Equal volume (200 mL) of zoospore suspension of each isolate was prepared and adjusted to a concentration of 10^3 zoospores/mL. The zoospore suspensions of the five isolates were mixed, resulting in the final zoospore suspension was spray-inoculated in plants of the spreader rows.

Potato trials

The potato field experiments were carried out with the susceptible Ágata potato cultivar. The experiment was set in a complete randomized experimental design on a checkerboard pattern with three treatments and three replications per treatment. The experimental plots measured 3.0 x 4.0 m (12 m^2), containing five rows of plants. Sprinkler irrigation was done every two days or according to necessity, and the volume was assessed by a pluviometer. Late blight severity on plants from each plot was estimated every three days, starting at 34 DAE, using a modified diagrammatic scale (Fry 1977). Potato yield was assessed by weighing the tubers of all the plants from each plot after maturation. Yield data was converted into kg/ha. Two potato trials were conducted, one to test the zoxamide and one to test the oxathiapiprolin field efficacy.

Zoxamide field efficacy

In this trial, the plots also consisted of 3.0 x 4.0 m rows spaced surrounded by untreated rows and artificially inoculated. The oomycide applications were initiated 20 days after plant emergence (DAE). The treatments were applied three times with 15 days of intervals (20; 35; and 50 DAE) using a backpack sprayer pressurized by CO^2 with a spray volume of 1000 liter/ha. The treatments were: 1) control, 2) zoxamide, 3) zoxamide +

chlorothalonil interleaved (7 days) with Cymoxanil + Mancozeb (Curzate® at the commercial dose of 2 kg/ha) (Table 2).

Table 2. Details of treatments sprayed at potato and tomato field trials

Trials	Treatments	Doses
Potato		
Zoxamide field efficacy	T1) Control	untreated
	T2) Zoxamide	320 mL/ha
	T3) Zoxamide + Chlorothalonil / Cymoxanil + Mancozeb	320 mL/ha + 1.8 kg/ha / 80 g/kg + 640 g/kg
Oxathiapiprolin field efficacy	T1) Control	untreated
	T2) Oxathiapiprolin (sublethal dose)	7.5 g.ia/ha
	T3) Oxathiapiprolin + Dimethomorph	15 g/L + 300 g/L
Tomato	T1) Control	untreated
	T2) Oxathiapiprolin (sublethal dose)	7.5 g.ia/ha
	T3) Oxathiapiprolin + Dimethomorph	15 g/L + 300 g/L
	T4) Zoxamide	320 mL/ha
	T5) Zoxamide + Chlorothalonil / Cymoxanil + Mancozeb	320 mL/ha + 1.8 kg/ha / 80 g/kg + 640 g/kg

Oxathiapiprolin field efficacy

In the potato trial to test oxathiapiprolin field efficacy, the inoculation occurred naturally. Upwind from this trial there was an older trial that was artificially inoculated and late blight epidemics was advanced (approximately 10 days after inoculation) and inoculum was naturally dispersed to the OXTP field efficacy trial. The applications were initiated 50 days after plant emergence (DAE). The treatments were applied three times at 50; 69; and 90 DAE using a backpack sprayer pressurized by CO^2 with a spray volume of 1000 liter/ha. The treatments were: 1) control, 2) oxathiapiprolin at a sublethal dose, 3) oxathiapiprolin + dimethomorph (Zorvec Entido® at a commercial dose for potato of 1.3 L/ha) (Table 2).

Tomato trial

Tomato cv. Santa Clara was used in this trial. The experiment was carried out in a completely randomized design with five treatments and four replications per treatment. Each

plot was 3.0 m long x 5.0 m wide, being 4 rows containing ten plants spaced 0.5 m apart and 1.0 m between rows, totaling forty plants per plot.

No artificial inoculation was necessary and the epidemic started naturally at 25 days after transplanting (DAT). The applications of treatments started at 30 DAT and were applied at 30; 45; and 60 DAE using a backpack sprayer pressurized by CO_2 with a spray volume of 1000 liter/ha. The treatments were 1) control, 2) oxathiapiprolin (sublethal dose), 3) oxathiapiprolin + dimethomorph (Zorvec Entido® at a commercial dose used for tomato of 1.6 L/ha), 4) zoxamide, 5) zoxamide + chlorothalonil interleaved (7 days) with Cymoxanil + Mancozeb (Curzate® at commercial dose of 2 kg/ha) (Table 2).

Late blight severity was assessed every three days, starting at 28 DAT, in 10 plants previously selected, at two internal rows at each plot using a diagrammatic scale (Corrêa et al. 2009). The severity mean of the 10 plants was estimated and used as plot severity. The late blight effect on tomato production was assessed at the last severity evaluation by the total number of fruits with and without blight symptoms. Tomato yield was assessed by weighing the fruits of all plants from each plot and converted into kg/ha.

Samplings

In the pressurization assays five potato or four tomato leaves were taken at the beginning, middle, and final stages of the epidemics from sprayed and unsprayed plots. Leaves were collected seven days after oomycide application. Sporangia from lesions were used for sensitivity assessment. Sensitivity to oxathiapiprolin and zoxamide was measured with a discriminatory dose by leaf disc assay method, as described before, or using a detached leaf. The leaves sampled from the field trials were placed in germbox with wet paper at the bottom and incubated at 18 - 22 °C for 24 h period overnight. After incubation, the leaves were dipped in sterile water at 4 °C to prepare sporangia suspension. The suspension was kept for 4 h to release zoospores.

Leaves of potato plants cv. Ágata grown under greenhouse conditions for approximately 40 days or of 30-day-old tomato cv. Santa Clara were collected, dipped into water amended with DMSO (control) or oomycides at a discriminatory dose (OXTP = 1 μg/mL and ZOXA = 20 μg/mL) for 10s, naturally dried for 30 min, and placed in germbox with filter paper moistened with distilled water at the bottom. For each treatment, five potato or four tomato leaves were dipped.

On the day after the dipping or spraying of oomycides, leaves were inoculated with 20 drops of 15 μL of zoospore suspension. Germbox with inoculated leaves were kept at 18 - 22 °C under light for 12 h and 12 h in the dark. The presence or absence of the lesions and

sporulation were assessed at 7 and 10 days post inoculation using a stereomicroscope. Insensitive isolates were considered as those with sporulation greater than 75 % in the discriminatory dose compared to the control.

Data analysis. All analyses were done using the R software (R Core Team 2024). The relative mycelial growth and sporulation on leaf disc were calculated, to MFX and MAD. The isolates were classified as sensitive (growth or sporulation less than 40 % of control at 5 and 100 µg/mL), intermediate (growth or sporulation greater than 40 % of control at 5 µg/mL and less than 40 % of control at 100 µg/mL), or resistant (growth or sporulation greater than 40 % of control at 5 and 100 µg/mL) (Therrien et al. 1993).

The effective concentrations of OXTP to inhibit sporangia germination, mycelial growth, and sporulation on leaf discs, and of ZOXA to inhibit mycelial growth and sporulation on leaf discs by 50 % values (EC_{50}) were estimated by regression analyses. The dependent variables, colony diameter, percentage of sporulating area, and absorbance were regressed on correspondent concentration dose.

The package drc (Ritz et al. 2015) was used to choose the best-fitting nonlinear dose-response curve model by function ‘mselect()’. Models checked were four-parameter log-logistic models (LL.4 and LL2.4), three-parameter log-logistic models (LL.3 and LL2.3), Brain-Cousens hormesis models (BC.4 and BC.5), Cedergreen-Ritz-Streibig models (CRS.4a, CRS.4b, and CRS4c), and three-parameter Weibull models (W1.3, W1.4, W2.3, and W2.4). The best-fitting model was chosen based on the lowest Akaike’s Information Criterion (AIC) value for most isolates assessed and according to model characteristics fit data (Noel et al. 2018; Wang et al. 2017). The package ec50estimator (Alves 2022) was used for generating dose-response curves and estimating EC_{50} values by function ‘estimate_EC50()’.

For the field trial analyses, the area under the disease progress curve (AUDPC) was estimated by function ‘AUDPC()’ of the package epifitter (Alves and Del Ponte 2021). The covariance analysis, residuals normality, and comparisons of AUDPC means were done by function ‘dbc()’ of package ExpDes.pt (Ferreira et al. 2021).

The model of the disease progress curve (DPC) of each treatment was fitted using the function ‘fit_multi()’ of the package epifitter (Alves and Del Ponte 2021). The Logistic and Gompertz models were selected based on the nature of epidemic data and curve patterns to compare Lin's coefficient (CCC) and root square error, to choose the better model fitted. The same model was chosen for all treatment epidemics to compare the rate (slope) and initial inoculum (intercept) of the epidemics by t-statistic.

Results

***P. infestans* isolates sensitivity to mefenoxam.** Twenty-seven *P. infestans* isolates collected from potato plants were assessed for their sensitivity to mefenoxam by mycelial growth (Figure 1). The sensitivity assessment for five isolates obtained from tomato plants was conducted by means of leaf disc sporulation assay (Figure 2).

Fourteen isolates (12 collected from potato and 2 from tomato) were sensitive to mefenoxam, 14 from potato were intermediately insensitive to mefenoxam, and 5 isolates (2 from potato and 3 from tomato) were insensitive.

***P. infestans* isolates sensitivity to mandipropamid.** The sensitivity of 30 potato isolates to mandipropamid of were assessed by mycelial growth (Figure 3), and for the five isolates collected from tomato were assessed by sporulation on leaf disc (Figure 4). Thirty-one isolates were sensitive to mandipropamid (28 from potato and 3 from tomato), and four isolates were intermediately insensitive (2 from each host). All isolates were sensitive to 10 μg/mL.

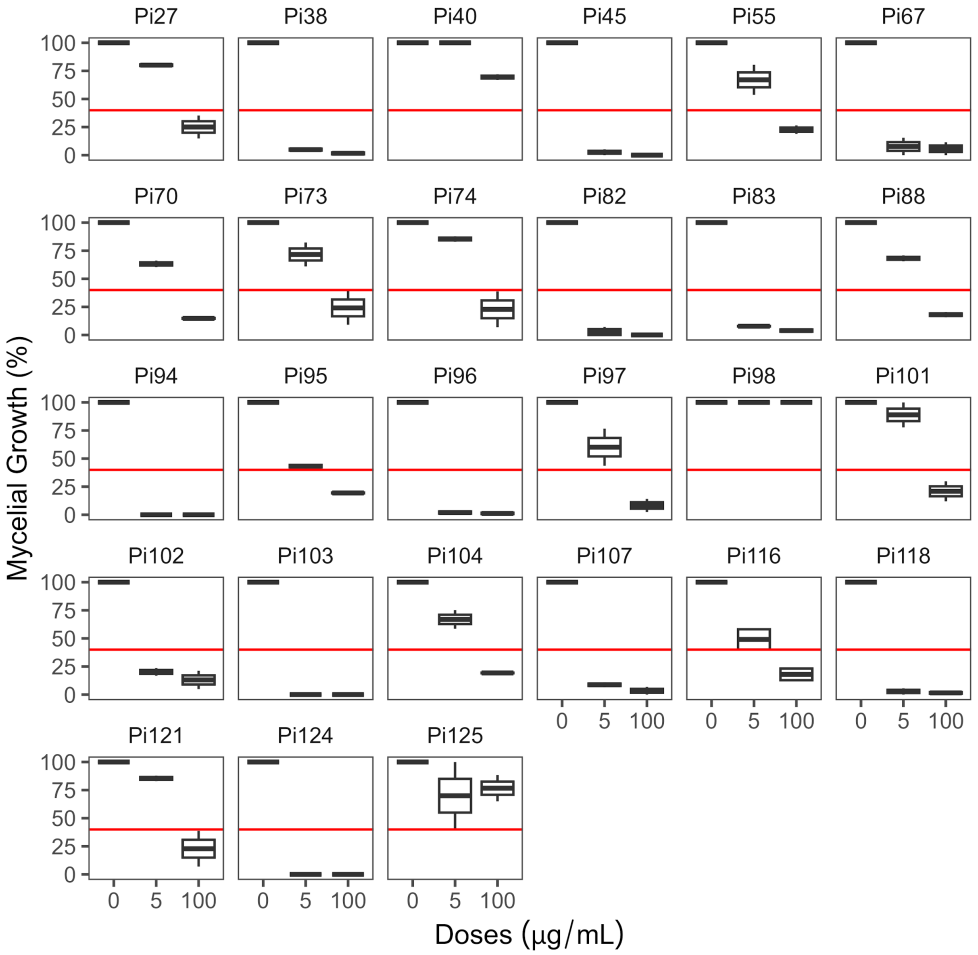


Figure 1. Boxplot of data distribution of mycelial growth of isolates of *Phytophthora infestans* from potato in response to the different doses of mefenoxam relative to the control. The red line delimits the 40 % to separate the sensitive class. Sensitive class: isolates growth less than 40 % (below the red line) at 5 and 100 µg/mL; Intermediate class: isolates growth of more than 40 % (above the red line) at 5 µg/mL and less than 40 % (below the red line) at 100 µg/mL; Resistant class: isolates growth of more than 40 % (above the red line) at 5 and 100 µg/mL.

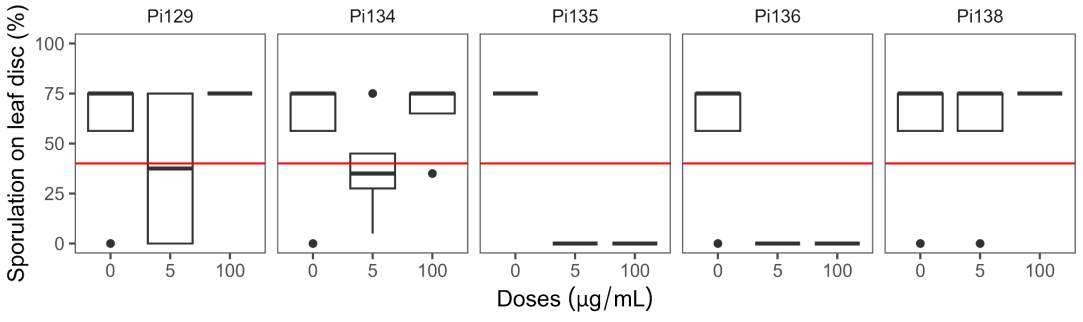


Figure 2. Boxplot of data distribution of sporulation on leaf disc of isolates of *Phytophthora infestans* from tomato in response to the different doses of mefenoxam relative to the control. The red line delimits the 40 % to separate the sensitive class. Sensitive class: isolates sporulated of less than 40 % (below the red line) at 5 and 100 µg/mL; Intermediate class:

isolates sporulated of more than 40 % (above the red line) at 5 µg/mL and less than 40 % (below the red line) at 100 µg/mL; Resistant class: isolates sporulated of more than 40 % (above the red line) at 5 and 100 µg/mL.

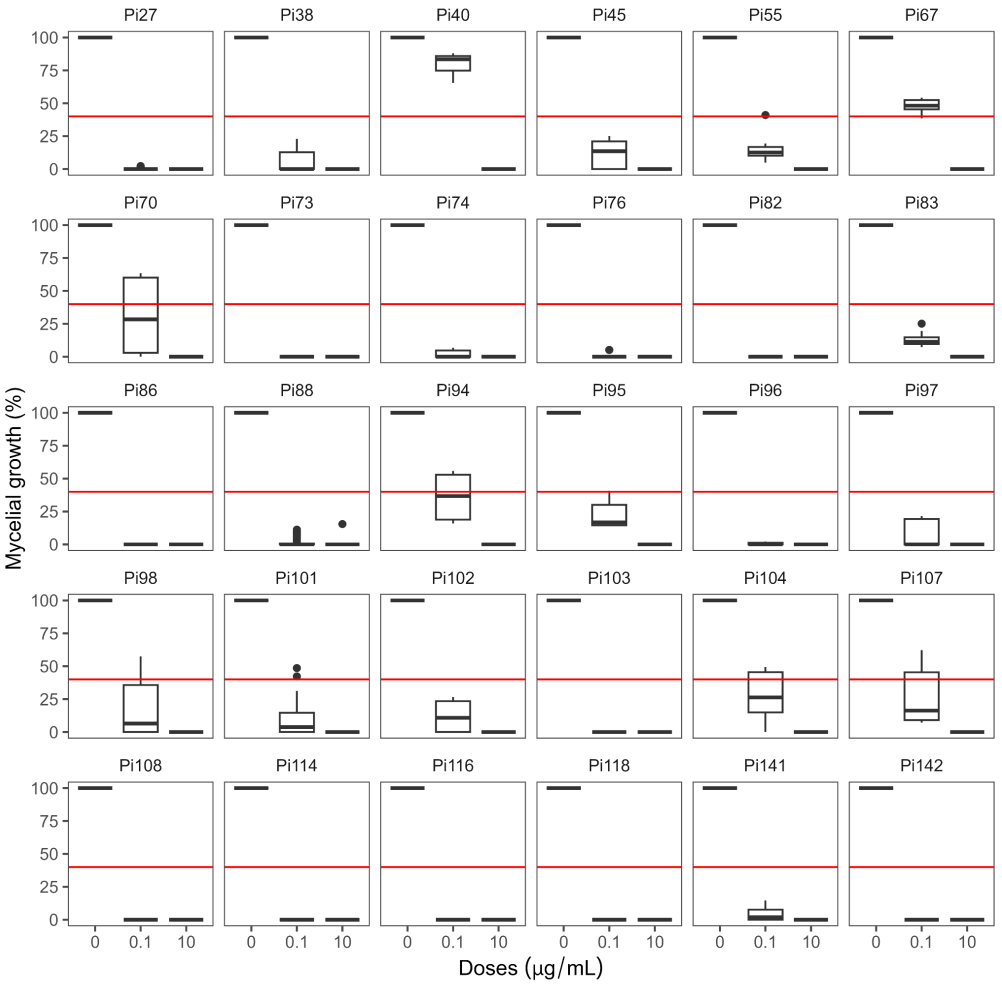


Figure 3. Boxplot of data distribution of mycelial growth of isolates of *Phytophthora infestans* from potato in response to the different doses of mandipropamid relative to the control. The red line delimits the 40 % to separate the sensitive class. Sensitive class: isolates growth of less than 40% (below the red line) at 0.1 and 10 µg/mL; Intermediate class: isolates growth of more than 40 % (above the red line) at 0.1 µg/mL and less than 40 % (below the red line) at 10 µg/mL; Resistant class: isolates growth of more than 40 % (above the red line) at 0.1 and 10 µg/mL.

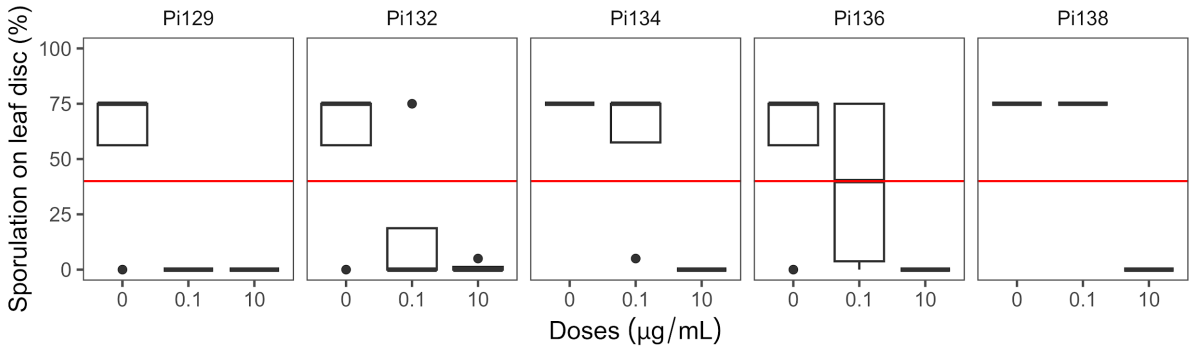


Figure 4. Boxplot of data distribution of sporulation on leaf disc of isolates of *Phytophthora infestans* from tomato in response to the different doses of mandipropamid relative to the control. The red line delimits the 40 % to separate the sensitive class. Sensitive class: isolates sporulated of less than 40 % (below the red line) at 0.1 and 10 µg/mL; Intermediate class: isolates sporulated of more than 40 % (above the red line) at 0.1 µg/mL and less than 40 % (below the red line) at 10 µg/mL; Resistant class: isolates sporulated of more than 40 % (above the red line) at 0.1 and 10 µg/mL.

Sensitivity to zoxamide. The sensitivity of the isolates to zoxamide was assessed by mycelial growth (n = 39) and sporulation on leaf disc (n = 14). All isolates tested were collected from potato.

The models used to estimate the EC_{50} were W2.3 and W1.3 for mycelial growth and sporulation on leaf disc, respectively. The EC_{50} estimated ranged between $2.28x10^{-3}$ to $1.13x10^{-1}$ µg./mL^{-1}, the median was $1.47x10^{-2}$ µg/mL to mycelial growth, and ranging from 1.16 to 19.10 µg/mL and median of 6.25 µg/mL to sporulation on leaf disc (Figure 5).

The Kruskal-Wallis rank sum test was not significant to the EC_{50} values of the isolates obtained from different states of origin for the mycelial growth (p-value = 0.563), and sporulation on leaf disc (p-value = 0.047) (Figure 6).

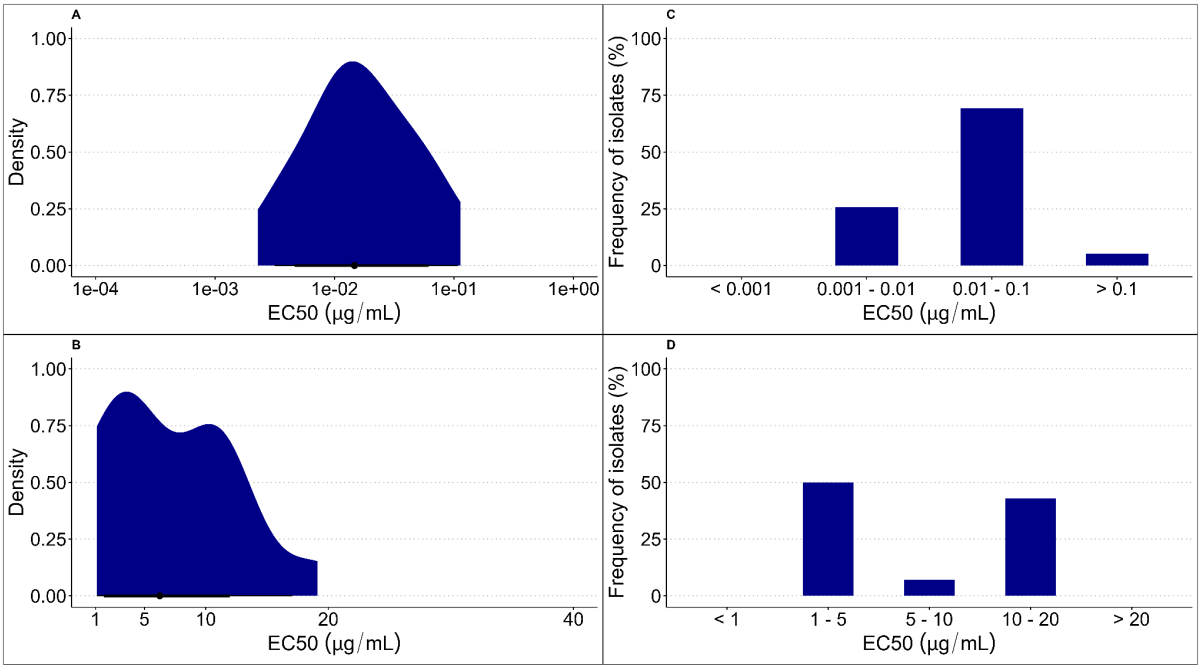


Figure 5. Density (A and B) and frequency (C and D) distribution of EC_{50} values of zoxamide. A. Density of EC_{50} values estimated by mycelial growth of *Phytophthora infestans*; B. Density of EC_{50} values estimated by sporulation on leaf disc; C. Frequency of EC_{50} value intervals assessed by mycelial growth; and D. Frequency of EC_{50} value intervals assessed by sporulation on leaf disc.

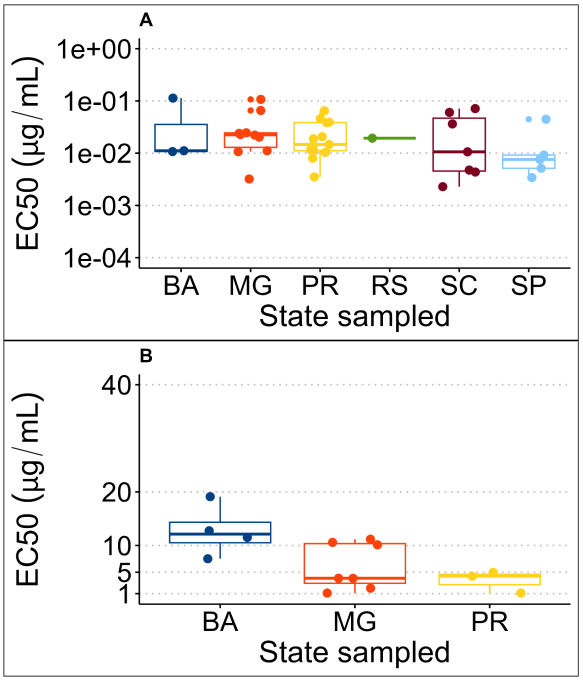


Figure 6. Box plot of EC50 values estimated by mycelial growth of *Phytophthora infestans* (A), and sporulation on leaf disc (B) separated by state of origin of each isolate assessed regarding their sensitivity to zoxamide. State acronyms: BA = Bahia, MG = Minas Gerais, PR = Paraná, RS = Rio Grande do Sul, SC = Santa Catarina, and SP = São Paulo.

Sensitivity to oxathiapiprolin. *P. infestans* isolates were assessed regarding their sensitivity to oxathiapiprolin by sporangia germination (n = 58), mycelial growth (n = 49), and sporulation on leaf disc (n = 40). All isolates assessed were from potato plants.

The models used to estimate the EC_{50} were LL2.5, LL.4, and LL.3 for sporangia germination, mycelial growth, and sporulation on leaf disc, respectively. The EC_{50} estimated for sporangia germination ranged from $8.21x10^{-7}$ to $2.67x10^{-2}$ µg/mL, with a median of $3.41x10^{-4}$ µg/mL. For mycelial growth, the EC_{50} estimated ranged between $3.47x10^{-5}$ and $8.05x10^{-4}$ µg/mL, and the median was $2.88x10^{-4}$ µg/mL. For sporulation on leaf disc, the EC_{50} was estimated between $5.61x10^{-4}$ to $6.52x10^{-2}$ µg/mL, and the median was $1.43x10^{-2}$ µg/mL (Figure 7, 8).

The Kruskal-Wallis rank sum test was not significant for the EC_{50} values of the isolates obtained from different states of origin. The sporangia germination (p-value = 0.023), mycelial growth (p-value = 0.004), and sporulation on leaf disc (p-value = 0.011) (Figure 9).

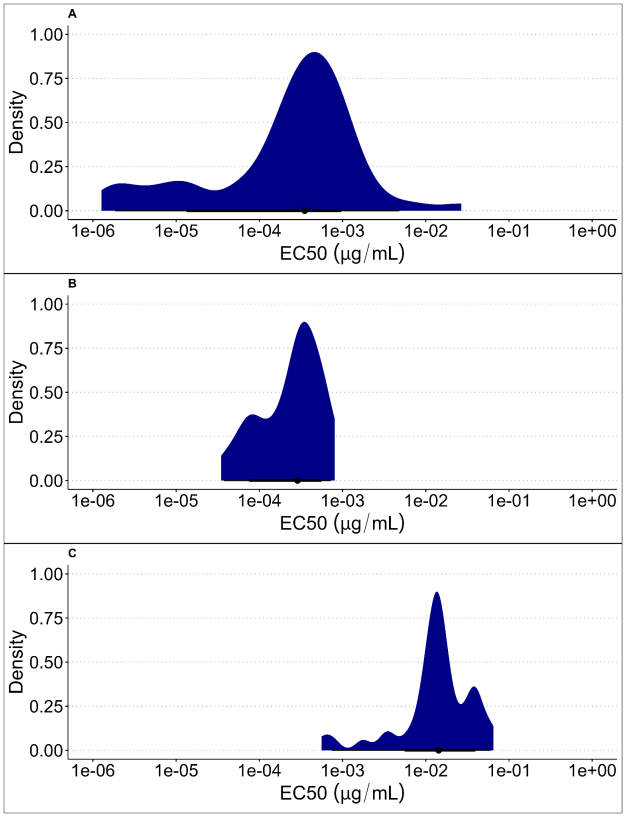


Figure 7. Density distribution of EC_{50} values for oxathiapiprolin. A. Density of EC_{50} values estimated by *Phytophthora infestans* sporangia germination assay; B. Density of EC_{50} values estimated by mycelial growth; C. Density of EC_{50} values estimated by sporulation on leaf disc.

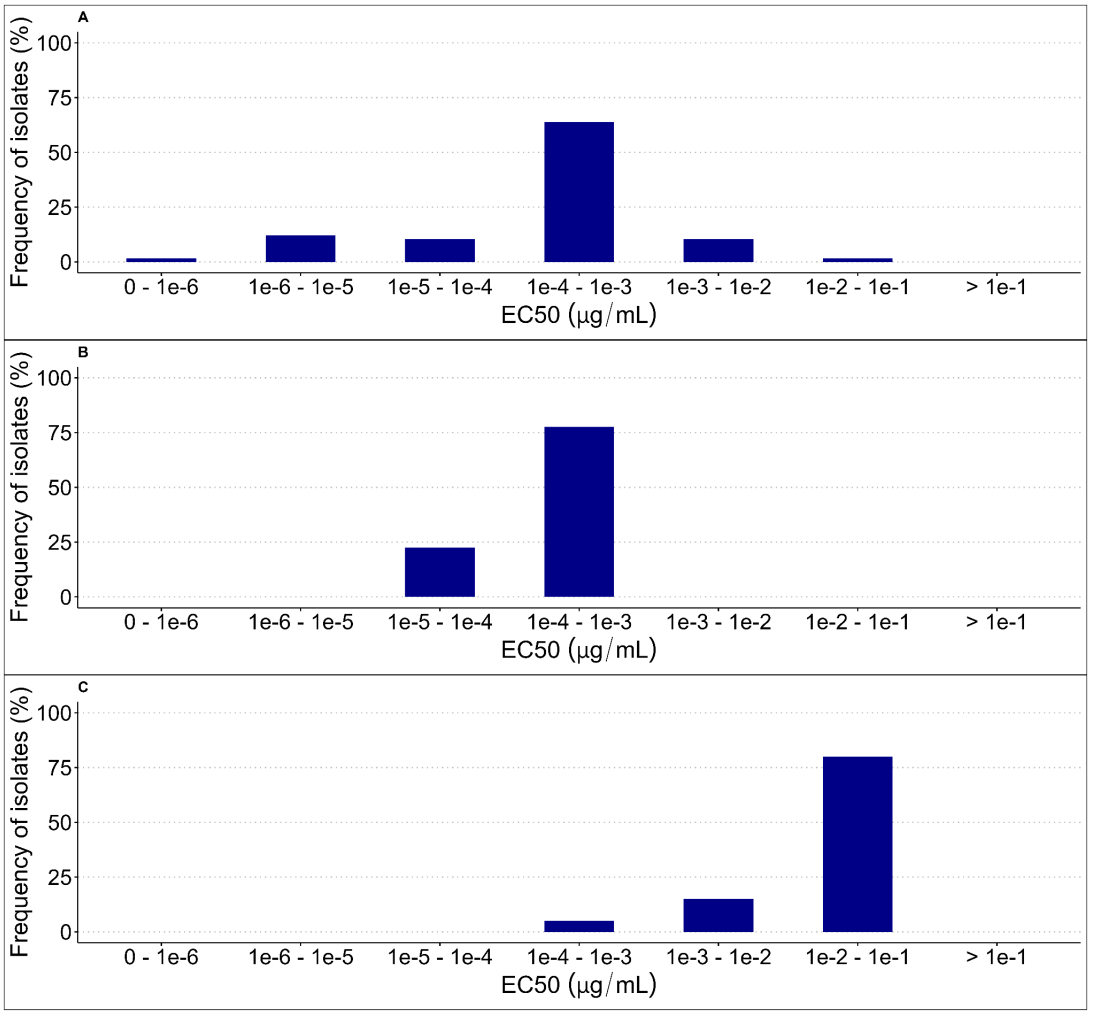


Figure 8. Frequency distribution of EC_{50} values for oxathiapiprolin. A. Frequency of EC_{50} value intervals assessed by sporangia germination; B. Frequency of EC_{50} value intervals assessed by mycelial growth; and C. Frequency of EC_{50} value intervals assessed by sporulation on leaf disc.

Table 3. Description of the isolates from the population of *Phytophthora infestans* from Brazil. Geographic origin (Municipality), year of sample (Year), host of origin (Host), class of sensitivity to mefenoxam and mandipropamid, and EC_{50} values estimated to oxathiapiprolin and zoxamide to the assays assessed.

Isolate	Municipality	Year	Host	Mefenoxam	Mandipropamid	Oxathiapiprolin			Zoxamide	
						Germination	Mycelial growth	Sporulation	Mycelial growth	Sporulation
Pi1	Viçosa - MG	2020	Potato	-	-	-	-	-	-	-
Pi2	Patrocínio - MG	2020	Potato	-	-	-	-	-	-	-
Pi3	Perdizes - MG	2020	Potato	-	-	-	-	-	-	-
Pi4	Perdizes - MG	2020	Potato	-	-	-	-	-	-	-
Pi5	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi6	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi7	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi8	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi9	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi10	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi11	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi12	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi13	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi14	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi15	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi16	São Gotardo - MG	2020	Potato	-	-	-	-	-	2.28x10-02	-
Pi17	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi18	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi19	São Gotardo - MG	2020	Potato	-	-	-	-	-	-	-
Pi20	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi21	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-

Pi22	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi23	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi24	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi25	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi26	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi27	Rio Paranaíba - MG	2020	Potato	I	S	-	-	-	3.21x10-03	-
Pi28	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi29	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi30	Viçosa - MG	2020	Tomato	-	-	-	-	-	-	-
Pi31	Rio Paranaíba - MG	2020	Potato	-	-	-	-	-	-	-
Pi32	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	-
Pi33	Ipuiúna - MG	2020	Potato	-	-	-	-	-	-	-
Pi34	Espírito Santo do Dourado - MG	2020	Potato	-	-	-	-	-	-	-
Pi35	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	-
Pi36	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	-
Pi37	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	-
Pi38	Camanducaia - MG	2020	Potato	S	S	-	-	-	1.11x10-02	-
Pi39	Ipuiúna - MG	2020	Potato	-	-	-	-	-	-	-
Pi40	Ipuiúna - MG	2020	Potato	R	S	-	-	-	2.46x10-02	-
Pi41	Espírito Santo do Dourado - MG	2020	Potato	-	-	-	-	-	2.03x10-02	-
Pi42	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	-
Pi43	Ipuiúna - MG	2020	Potato	-	-	-	-	-	-	-
Pi44	Espírito Santo do Dourado - MG	2020	Potato	-	-	-	-	-	2.40x10-02	-

Pi45	Espírito Santo do Dourado - MG	2020	Potato	S	S	-	-	-	2.22x10-02	2.03x10+00
Pi46	Ipuiúna - MG	2020	Potato	-	-	-	-	-	-	-
Pi47	Espírito Santo do Dourado - MG	2020	Potato	-	-	-	-	-	-	-
Pi48	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	-
Pi49	São Francisco de Paula - RS	2021	Potato	-	-	1.09x10-03	4.67x10-04	3.95x10-02	-	-
Pi50	São Francisco de Paula - RS	2021	Potato	-	-	-	-	-	-	-
Pi51	São Francisco de Paula - RS	2021	Potato	-	-	-	-	-	-	-
Pi52	São Francisco de Paula - RS	2021	Potato	-	-	3.51x10-04	6.91x10-04	1.65x10-02	-	-
Pi53	Palmas - PR	2021	Potato	-	-	-	5.17x10-04	1.32x10-02	-	-
Pi54	São Francisco de Paula - RS	2021	Potato	-	-	3.20x10-04	2.88x10-04	1.31x10-02	-	-
Pi55	Guarapuava - PR	2021	Potato	I	S	2.02x10-06	7.32x10-04	1.35x10-02	3.52x10-03	4.27x10+00
Pi56	São Francisco de Paula - RS	2021	Potato	-	-	5.70x10-04	2.18x10-04	-	-	-
Pi57	São Francisco de Paula - RS	2021	Potato	-	-	3.68x10-04	2.58x10-04	-	-	-
Pi58	São Francisco de Paula - RS	2021	Potato	-	-	1.11x10-04	3.71x10-04	1.44x10-02	-	-
Pi59	Ipuiúna - MG	2020	Potato	-	-	-	-	-	-	-
Pi60	Água Doce - SC	2021	Potato	-	-	2.65x10-04	-	-	7.14x10-02	-
Pi61	Água Doce - SC	2021	Potato	-	-	1.69x10-04	-	1.46x10-02	-	-
Pi62	Guarapuava - PR	2021	Potato	-	-	3.30x10-04	2.75x10-04	1.03x10-02	1.38x10-02	1.12x10+00
Pi63	São Francisco de Paula - RS	2021	Potato	-	-	5.02x10-04	1.30x10-04	-	-	-
Pi64	São Francisco de Paula - RS	2021	Potato	-	-	9.64x10-04	6.02x10-04	5.96x10-02	-	-
Pi65	São Francisco de Paula - RS	2021	Potato	-	-	9.53x10-04	3.62x10-04	1.58x10-02	-	-
Pi66	Guarapuava - PR	2021	Potato	-	-	-	-	-	-	-
Pi67	São Francisco de Paula - RS	2021	Potato	S	II	6.18x10-05	6.45x10-04	1.02x10-02	1.94x10-02	-
Pi68	São Francisco de Paula - RS	2021	Potato	-	-	7.31x10-04	3.29x10-04	-	-	-

Pi69	São Francisco de Paula - RS	2021	Potato	-	-	1.80x10-04	2.75x10-04	-	-	-
Pi70	Guarapuava - PR	2021	Potato	I	II	9.38x10-04	1.93x10-04	-	3.92x10-02	-
Pi71	Palmas - PR	2021	Potato	-	-	-	-	3.14x10-02	-	-
Pi72	Palmas - PR	2021	Potato	-	-	-	-	3.14x10-02	-	-
Pi73	Água Doce - SC	2021	Potato	I	S	-	-	1.32x10-02	1.06x10-02	-
Pi74	Palmas - PR	2021	Potato	I	S	-	-	-	-	-
Pi75	Calmon - SC	2021	Potato	-	-	6.47x10-04	-	3.80x10-03	-	-
Pi76	Guarapuava - PR	2021	Potato	I	S	4.34x10-04	5.59x10-04	1.56x10-02	4.60x10-02	-
Pi77	Guarapuava - PR	2021	Potato	-	-	-	3.06x10-04	7.57x10-03	1.47x10-02	-
Pi78	Ipuiúna - MG	2020	Potato	-	-	-	-	-	6.57x10-02	1.12x10+00
Pi79	Bom repouso - MG	2020	Potato	-	-	-	-	-	-	-
Pi80	São José dos Ausentes - RS	2021	Potato	-	-	4.75x10-04	-	1.41x10-02	-	-
Pi81	Guarapuava - PR	2021	Potato	-	-	-	-	-	1.16x10-02	-
Pi82	Camanducaia - MG	2020	Potato	S	S	-	-	-	1.07x10-01	1.12x10+01
Pi83	Camanducaia - MG	2020	Potato	S	S	-	-	-	1.07x10-02	3.86x10+00
Pi84	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	1.06x10+01
Pi85	Palmas - PR	2021	Potato	-	-	6.33x10-05	-	6.52x10-02	-	-
Pi86	Guarapuava - PR	2021	Potato	R	S	8.62x10-04	5.41x10-04	7.57x10-04	2.08x10-02	4.98x10+00
Pi87	Guarapuava - PR	2021	Potato	-	-	6.90x10-04	5.84x10-04	-	-	-
Pi88	Água Doce - SC	2021	Potato	I	S	4.61x10-04	-	8.69x10-03	4.35x10-03	-
Pi89	Água Doce - SC	2021	Potato	-	-	2.47x10-04	-	1.46x10-02	3.63x10-02	-
Pi90	Tatuí - SP	2021	Potato	I	-	1.42x10-05	-	-	-	-
Pi91	Tatuí - SP	2021	Potato	-	-	-	-	-	-	-
Pi92	Tatuí - SP	2021	Potato	-	-	-	-	-	-	-
Pi93	Guarapuava - PR	2021	Potato	-	-	3.27x10-04	2.95x10-04	4.47x10-02	1.89x10-02	-

Pi94	Mucugê - BA	2021	Potato	S	II	8.21x10-07	7.38x10-05	1.01x10-02	1.11x10-02	1.27x10+01
Pi95	Itapetininga - SP	2021	Potato	I	S	2.57x10-06	-	-	5.13x10-03	-
Pi96	Mucugê - BA	2021	Potato	S	S	1.66x10-05	1.90x10-04	1.17x10-02	-	-
Pi97	Tatuí - SP	2021	Potato	I	S	6.46x10-06	-	-	9.22x10-03	-
Pi98	Tatuí - SP	2021	Potato	R	S	8.41x10-06	-	-	3.40x10-03	-
Pi99	Palmas - PR	2021	Potato	-	-	-	-	-	-	-
Pi100	Mucugê - BA	2021	Potato	-	-	-	1.13x10-04	-	-	-
Pi101	Tatuí - SP	2021	Potato	I	S	-	-	-	7.60x10-03	-
Pi102	Calmon - SC	2021	Potato	S	S	2.17x10-04	-	1.07x10-02	2.28x10-03	-
Pi103	Mucugê - BA	2021	Potato	S	S	-	-	-	-	-
Pi104	Água Doce - SC	2021	Potato	I	II	8.14x10-04	-	-	4.75x10-03	-
Pi105	Água Doce - SC	2021	Potato	-	-	-	-	-	-	-
Pi106	Água Doce - SC	2021	Potato	-	-	2.74x10-03	-	-	-	-
Pi107	Mucugê - BA	2021	Potato	S	II	-	-	1.76x10-03	1.07x10-02	1.91x10+01
Pi108	Santa Maria de Jetibá - ES	2021	Tomato	I	S	-	-	-	-	-
Pi109	Afonso Cláudio - ES	2021	Tomato	-	-	-	-	-	-	-
Pi110	Afonso Cláudio - ES	2021	Tomato	-	-	-	-	-	-	-
Pi111	Afonso Cláudio - ES	2021	Tomato	-	-	-	-	-	-	-
Pi112	Coimbra - MG	2021	Tomato	-	-	-	-	-	-	-
Pi113	Palmas - PR	2021	Potato	-	-	1.67x10-03	-	2.56x10-02	-	-
Pi114	Palmas - PR	2021	Potato	-	S	4.51x10-06	-	1.98x10-02	3.85x10-02	-
Pi115	São Francisco de Paula - RS	2021	Potato	-	-	1.92x10-04	3.41x10-04	2.03x10-02	-	-
Pi116	Água Doce - SC	2021	Potato	I	S	2.67x10-02	-	4.06x10-02	-	-
Pi117	Palmas - PR	2021	Potato	-	-	-	-	1.10x10-02	-	-
Pi118	Palmas - PR	2021	Potato	S	S	-	-	5.61x10-04	7.99x10-03	-

Pi119	Viçosa - MG	2022	Tomato	R	-	-	-	-	-	-
Pi120	Viçosa - MG	2022	Tomato	-	-	-	-	-	-	-
Pi121	Palmas - PR	2021	Potato	I	-	1.50x10-04	4.44x10-04	-	1.03x10-02	-
Pi122	São Francisco de Paula - RS	2022	Potato	-	-	-	-	-	-	-
Pi123	Viçosa - MG	2023	Tomato	-	-	-	-	-	-	-
Pi124	Espírito Santo do Dourado - MG	2022	Potato	S	-	-	-	-	-	-
Pi125	Espírito Santo do Dourado - MG	2022	Potato	R	-	-	-	-	-	-
Pi126	Calmon - SC	2022	Potato	-	-	-	-	-	-	-
Pi127	Cajuri - MG	2023	Tomato	-	-	-	-	-	-	-
Pi128	Viçosa - MG	2023	Tomato	I	-	-	-	-	-	-
Pi129	Castelo - ES	2024	Tomato	-	S	-	-	-	-	-
Pi130	Castelo - ES	2024	Tomato	-	-	-	-	-	-	-
Pi131	Santa Maria de Jetibá - ES	2024	Tomato	-	-	-	-	-	-	-
Pi132	Domingos Martins - ES	2024	Tomato	-	S	-	-	-	-	-
Pi133	Castelo - ES	2024	Tomato	-	-	-	-	-	-	-
Pi134	Santa Maria de Jetibá - ES	2024	Tomato	-	II	-	-	-	-	-
Pi135	Santa Maria de Jetibá - ES	2024	Tomato	-	-	-	-	-	-	-
Pi136	Santa Maria de Jetibá - ES	2024	Tomato	-	II	-	-	-	-	-
Pi137	Santa Maria de Jetibá - ES	2024	Tomato	-	-	-	-	-	-	-
Pi138	Castelo - ES	2024	Tomato	-	S	-	-	-	-	-
Pi139	Castelo - ES	2024	Tomato	-	-	-	-	-	-	-
Pi140	Domingos Martins - ES	2024	Tomato	-	-	-	-	-	-	-
Pi141	Mucugê - BA	2021	Potato	-	S	-	-	-	-	1.15x10+01
Pi142	Ponta Grossa - PR	2021	Potato	-	S	1.68x10-04	-	-	1.12x10-02	-

Pi143	Viçosa - MG	2024	Tomato	-	-	-	-	-	6.48x10-02	-
Pi144	Ponta Grossa - PR	2021	Potato	-	-	1.34x10-05	-	-	-	-
Pi145	Camanducaia - MG	2020	Potato	-	-	-	-	-	-	1.01x10+01
Pi146	Mucugê - BA	2021	Potato	-	-	-	7.55x10-05	3.15x10-03	-	7.52x10+00
Pi147	São Francisco de Paula - RS	2021	Potato	-	-	3.58x10-04	3.12x10-04	1.58x10-02	-	-
Pi148	São José dos Ausentes - RS	2021	Potato	-	-	-	2.84x10-04	-	-	-
Pi149	São Francisco de Paula - RS	2021	Potato	-	-	-	3.75x10-04	1.91x10-02	-	-
Pi150	São José dos Ausentes - RS	2021	Potato	-	-	8.51x10-04	-	-	-	-
Pi151	Guarapuava - PR	2021	Potato	-	-	-	1.85x10-04	4.10x10-02	-	-
Pi152	Guarapuava - PR	2021	Potato	-	-	6.32x10-04	4.94x10-04	-	-	-
Pi153	Palmas - PR	2021	Potato	-	-	7.02x10-03	3.48x10-05	-	-	-
Pi154	Palmas - PR	2021	Potato	-	-	5.03x10-04	3.59x10-04	1.25x10-02	-	-
Pi155	Palmas - PR	2021	Potato	-	-	9.54x10-04	-	3.72x10-02	-	-
Pi156	Mucugê - BA	2021	Potato	-	-	1.82x10-03	9.17x10-05	-	-	-
Pi157	Mucugê - BA	2021	Potato	-	-	1.13x10-03	7.58x10-05	-	-	-
Pi158	Mucugê - BA	2021	Potato	-	-	-	9.65x10-05	-	-	-
Pi159	Mucugê - BA	2021	Potato	-	-	4.30x10-05	7.86x10-05	-	-	-
Pi160	Mucugê - BA	2021	Potato	-	-	1.73x10-06	-	-	-	-
Pi161	Mucugê - BA	2021	Potato	-	-	4.26x10-04	-	-	-	-
Pi162	Mucugê - BA	2021	Potato	-	-	1.11x10-04	-	-	-	-
Pi163	Tatuí - SP	2021	Potato	-	-	2.37x10-04	-	-	-	-
Pi164	Tatuí - SP	2021	Potato	-	-	4.09x10-04	6.55x10-05	-	-	-
Pi165	Tatuí - SP	2021	Potato	-	-	2.53x10-04	3.48x10-05	-	-	-

*S = sensitive; I = intermediate; and R = resistant to mefenoxam.

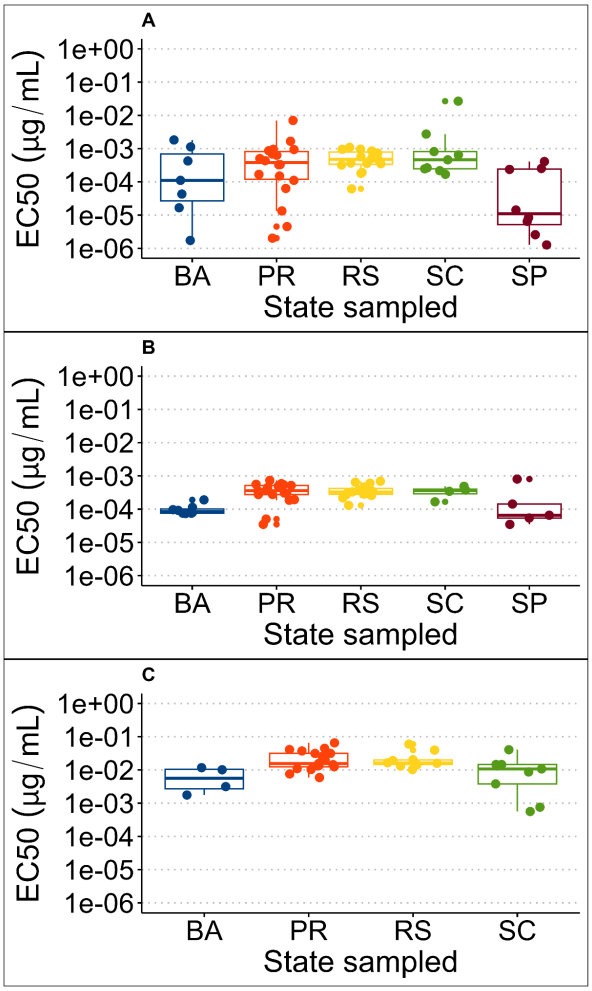


Figure 9. Box plot of EC_{50} values estimated by sporangia germination *Phytophthora infestans* (A), mycelial growth (B), and sporulation on leaf disc (C) separated by state of origin of each isolate assessed regarding their sensitivity to oxathiapiprolin. State acronyms of BA = Bahia, PR = Paraná, RS = Rio Grande do Sul, SC = Santa Catarina, and SP = São Paulo.

Oomycides efficacy under field conditions

Climate conditions

The maximum and minimum temperatures during the field experiments for oxathiapiprolin and zoxamide on potato varied between 10 and 21 °C and the average relative humidity was 68 to 92 % (Figures 10A and 10B). In the oxathiapiprolin efficacy trial the highest precipitation event was 13 mm recorded at 81 days after planting (Figure 10A), and in the experiment to assess the efficacy of zoxamide the precipitation ranged between 0.2 and 19 mm (Figure 10B), the highest precipitation occurred 69 days after planting. During the tomato field experiment, the temperature (maximum and minimum) varied between 19 and 28 °C, the average relative humidity was 72 to 96%, and no precipitation was recorded (Figure 10C).

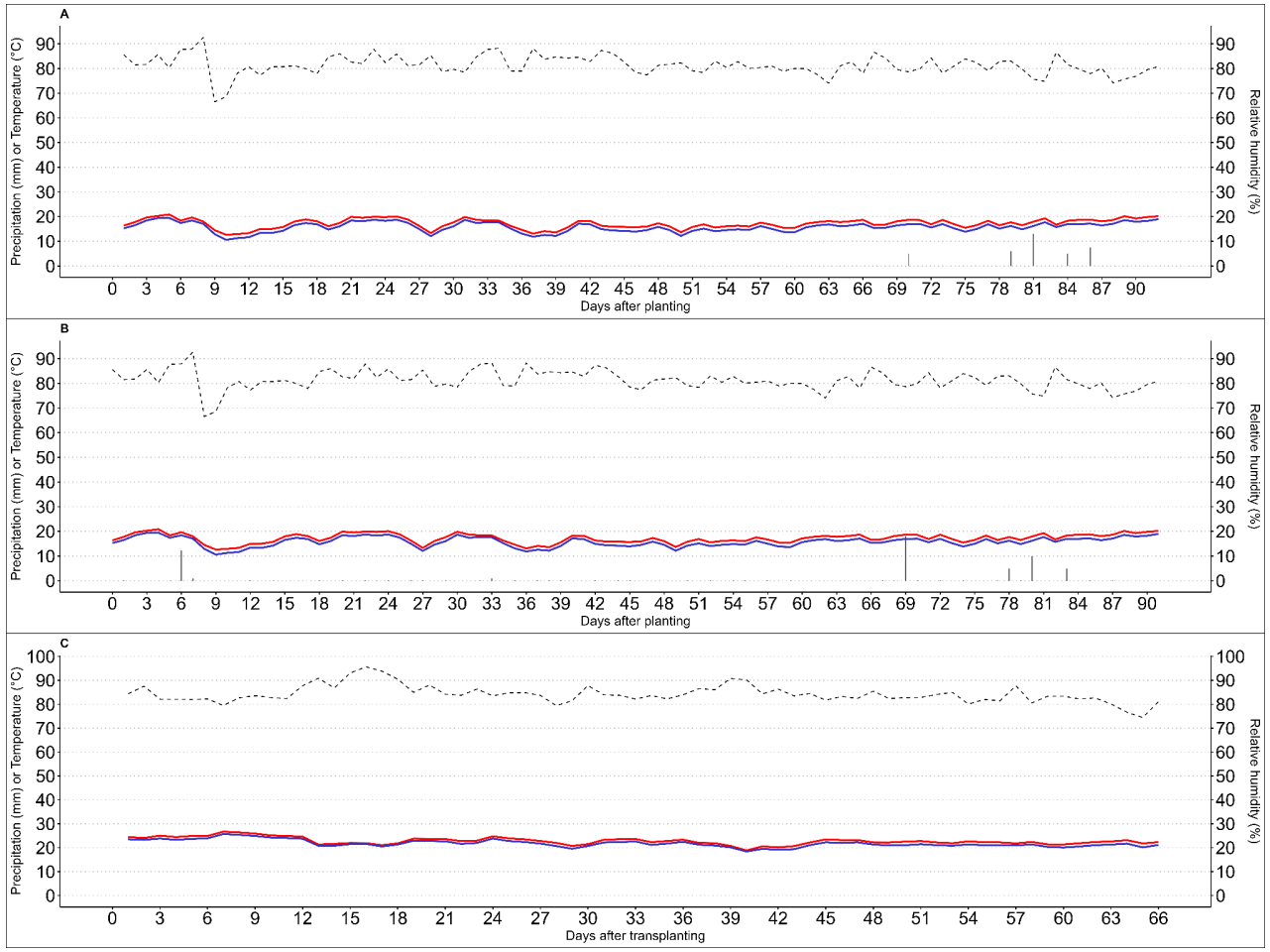


Figure 10. Meteorological variables recorded during field trial. A. Potato trial to assess the field efficacy of oxathiapiprolin in controlling late blight caused by *Phytophthora infestans*; B. Potato trial to evaluate the field efficacy of zoxamide; and C. Tomato field trial to evaluate the efficacy of oxathiapiprolin and zoxamide. A dotted black line represents the average relative humidity (%), vertical bars indicate the rainfall precipitation, and solid red and blue lines represent the maximum and minimum average temperatures.

Potato trial

Zoxamide field efficacy

In the experiment to test the field efficacy of zoxamide, the AUDPC of treatment 1 was 2166.084, treatment 2 was 1934.478, and treatment 3 was 1283.350 (Figure 11). The coefficient of variation was 2.88 %. According to the Shapiro-Wilk test at 5 % significance, the residuals can be considered normal (p-value = 0.525). The variances can be considered homogeneous (p-value = 0.662), as indicated by the O'Neill and Mathews test at 5 % significance. The AUDPC means differed among each other according to the Tukey test.

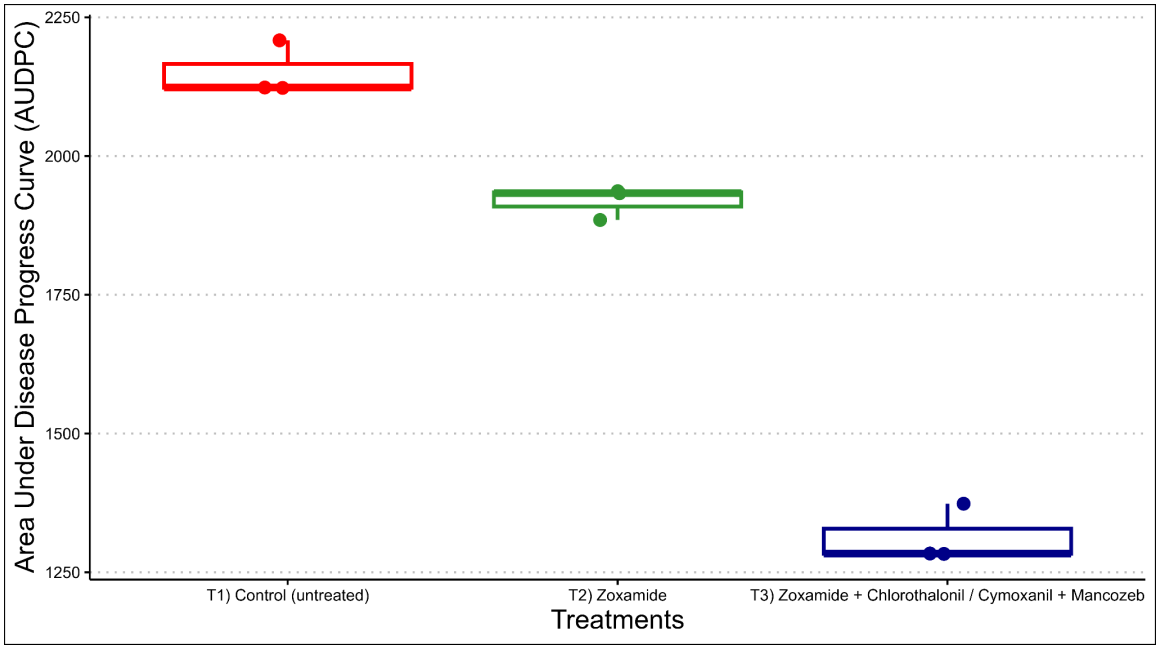


Figure 11. Boxplot of data distribution of the Area Under Disease Progress Curve (AUDPC) a field experiment to evaluate the efficacy of zoxamide in potato late blight control. The colors represent the epidemic treatments: red - 1) Control (untreated), green - 2) Zoxamide, and blue - 3). Zoxamide + Chlorothalonil/Cymoxanil + Mancozeb.

The late blight progress curves were plotted (Figure 6) and the epidemics were best fitted to the Logistic model (R^2 = 0.70 - 0.96; RSE = 0.90 - 2.15; CCC = 0.83 - 0.98) (Table 2) (Figure 12). The Gompertz, Exponential, and Monomolecular models did not fit the epidemics adequately and presented high predicted errors. The rates of the epidemics (T1, T2, and T3) were 0.40 to 0.53, with standard error (SE) from 0.017 to 0.041 (Figure 8A). The paired comparison of rates and rates SE by t-test indicates that the rates of epidemic T1 and T2 (p-value = 0.185), T1 and T3 (p-value =0.106), and T2 and T3 (p-value = 0.737) can be different.

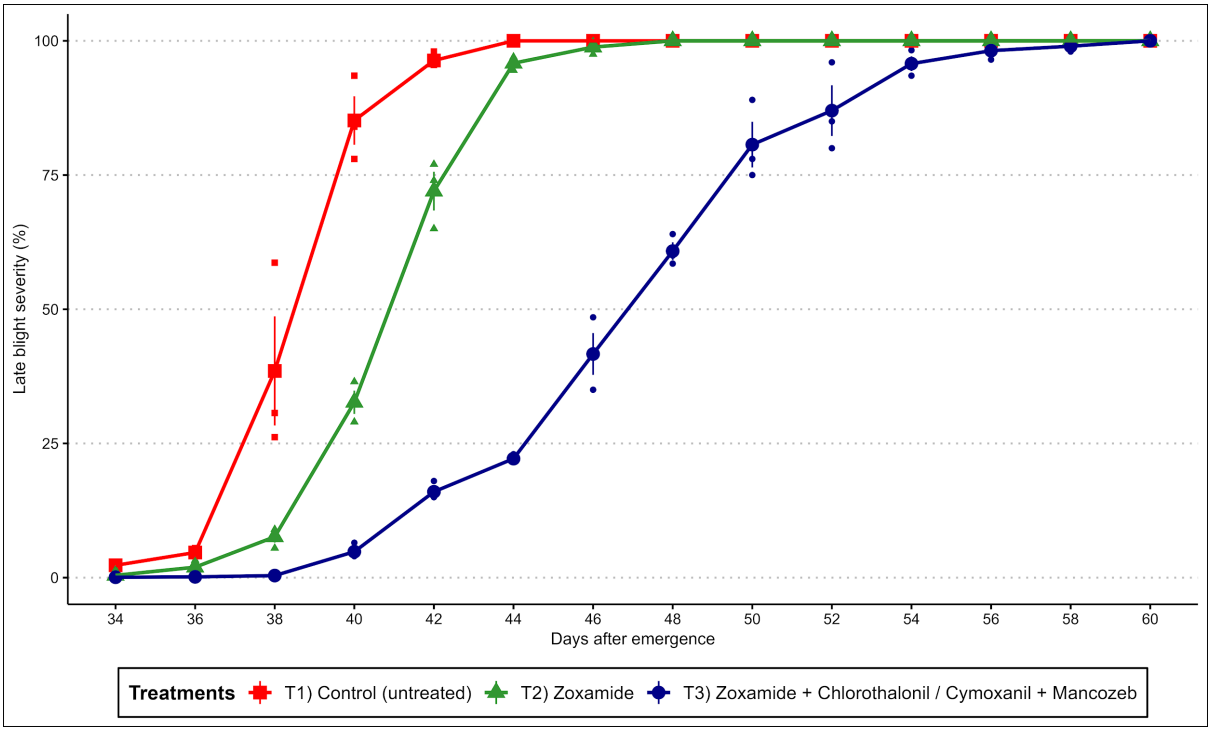


Figure 12. Disease progress curves of a field experiment to evaluate the efficacy of zoxamide in potato late blight control. The lines indicate the progress of epidemics, small shapes (circles, triangles, and squares) are the late blight severity registered for the three blocks, and big shapes are the late blight severity mean of the blocks. The colors represent the epidemic treatments: red - 1) Control (untreated), green - 2) Zoxamide, and blue - 3). Zoxamide + Chlorothalonil/Cymoxanil + Mancozeb.

Table 2. Parameters values of Logistic model fitted for epidemic treatments of a field experiment to evaluate the efficacy of zoxamide in potato blight control.

Treatment	Model	R^2	RSE	CCC	r	y_0
T1) Control (untreated)	Logistic	0.706	2.155	0.828	0.404	$4.16x10^{-07}$
T2) Zoxamide	Logistic	0.826	1.948	0.905	0.515	$7.46x10^{-10}$
T3) Zoxamide + Chlorothalonil / Cymoxanil + Mancozeb	Logistic	0.959	0.905	0.979	0.530	$1.28x10^{-11}$

R^2 = R-squared; RSE = Residual Standard Error; CCC = Lin's Concordance Correlation Coefficient; r = rate of the epidemic; y_0 = initial amount of disease.

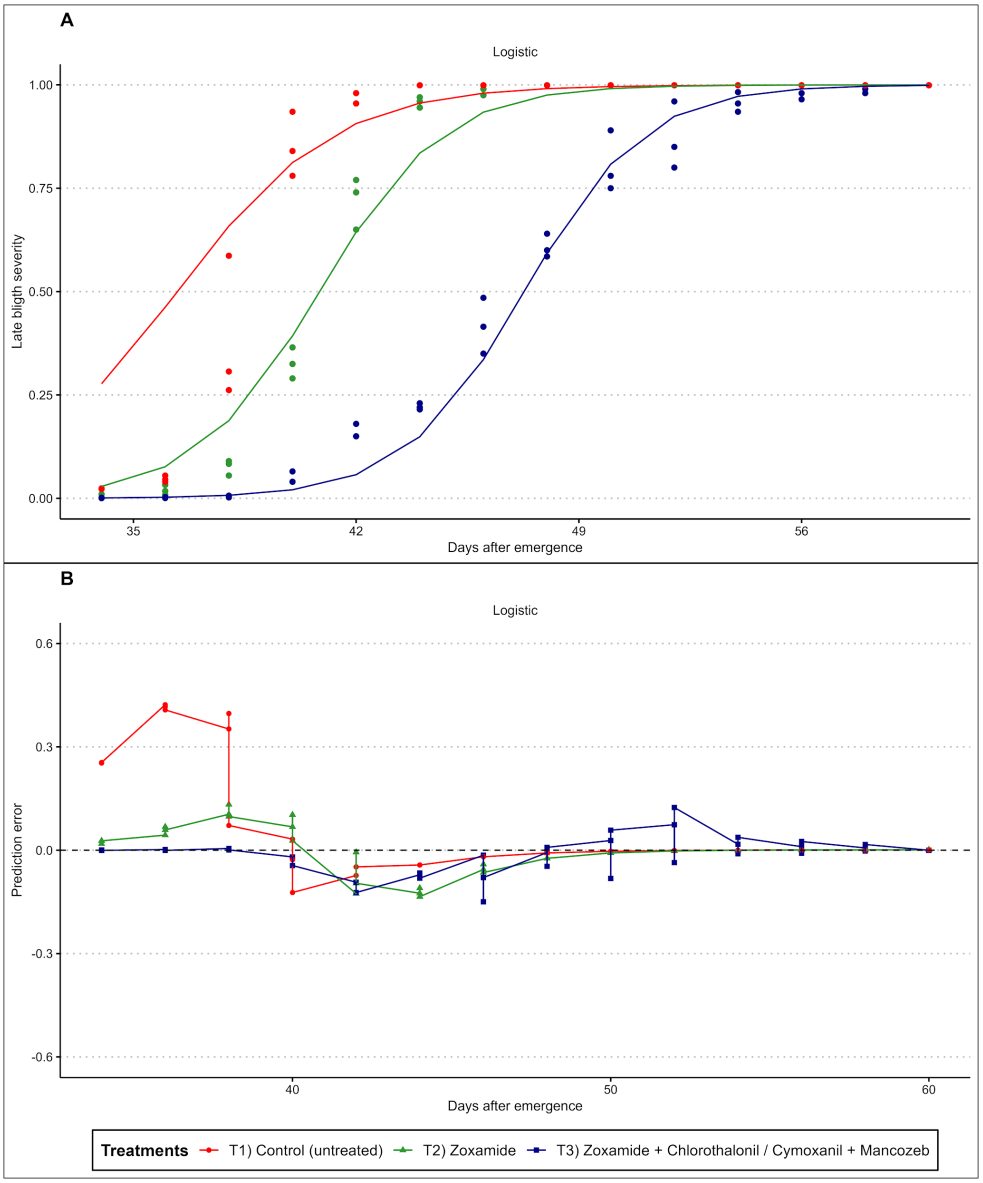


Figure 13. A. Disease progress curves fitted by a Logistic model. The lines indicate the model fitting. B. Prediction error by the models fitted to the disease progress curves. The shapes (circles, triangles, and squares) indicate the data (A. Late blight severity; B. Error predicted). The colors represent the epidemic treatments: red - 1) Control (untreated), green - 2) Zoxamide, and blue- 3). Zoxamide + Chlorothalonil/Cymoxanil + Mancozeb.

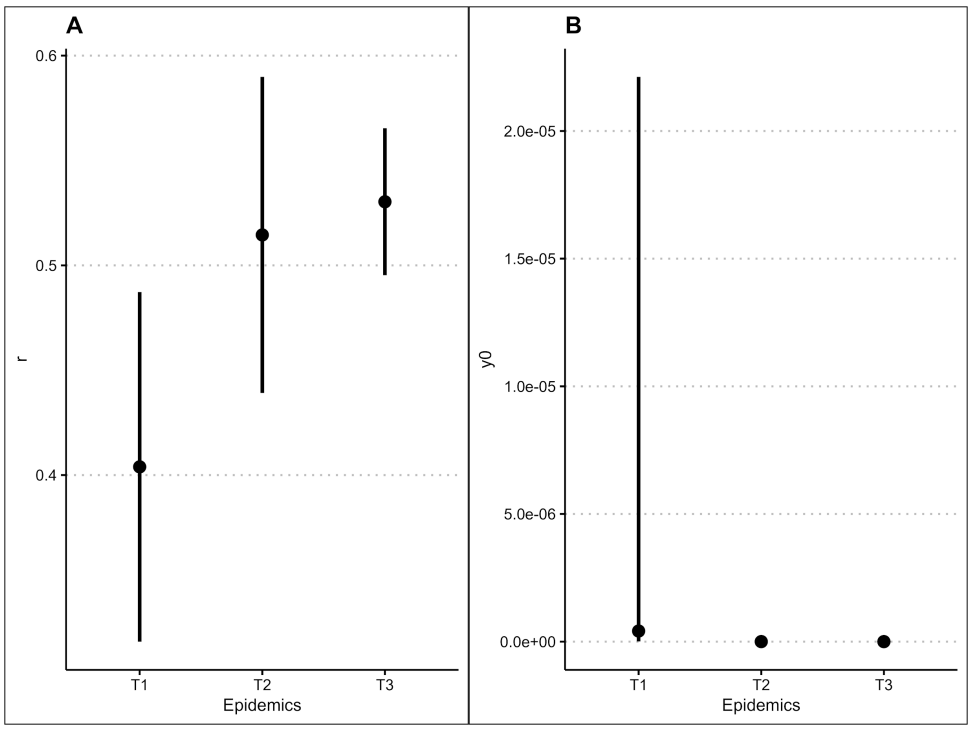


Figure 14. A. Estimated infection rates (r) and B. initial amount of disease (y_0) by a Logistic model fitted to the progress curves of three epidemics of a field experiment to evaluate the efficacy of zoxamide to control potato late blight. The bars represent the 95% confidence interval.

Oxathiapiprolin field efficacy

In the experiment to test the field efficacy of oxathiapiprolin the AUDPC of treatment 1, 2, and 3 were, 1854.202, 1069.889, and 349.748, respectively (Figure 15). According to the analysis of variance analysis, the coefficient of variation was 9.68 %; in the Shapiro-Wilk test at 5 % significance the residuals can be considered normal (p-value = 0.095); the O'Neill and Mathews test at 5 % significance, the variances can be considered homogeneous (p-value = 0.561), and in the Tukey test at 5 % significance, the AUDPC treatment means differed among them.

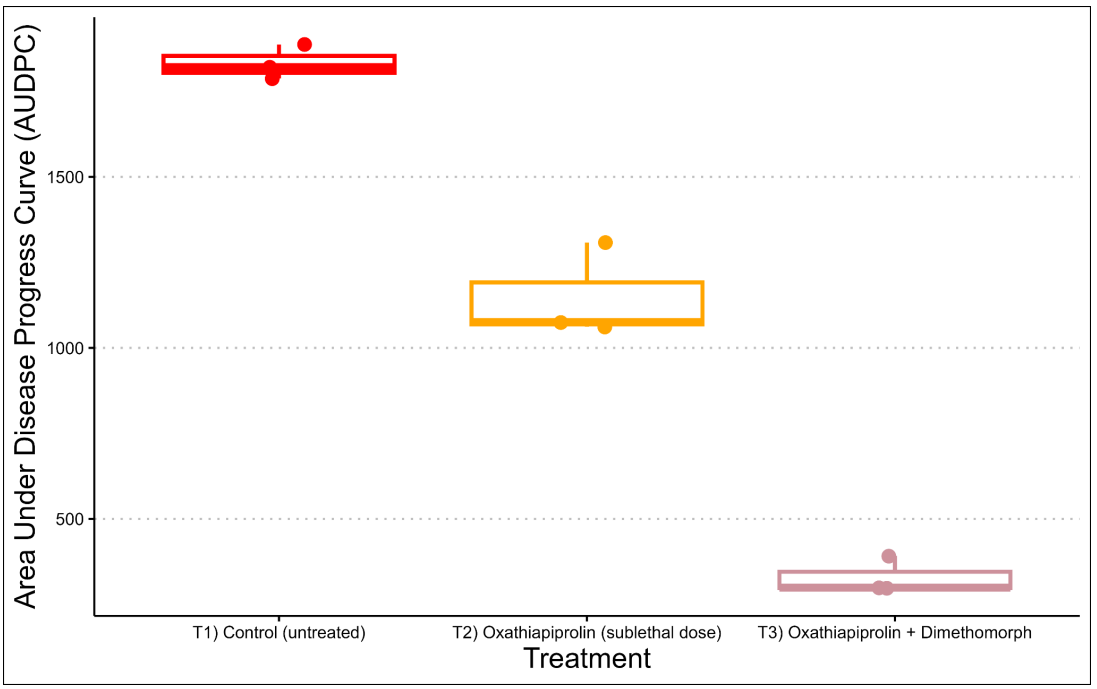


Figure 15. Boxplot of data distribution of the area under disease progress curve (AUDPC) of a field experiment to evaluate the efficacy of oxathiapiprolin in potato blight control. The colors represent the epidemic treatments: red - 1) Control (untreated), orange - 2) Oxathiapiprolin (sublethal dose), and pink - 3) Oxathiapiprolin + Dimethomorph.

The late blight progress curves were plotted (Figure 16) and the epidemics were fitted to the Logistic and Gompertz models (Table 3). The exponential and monomolecular models were inadequate to fit the epidemics. The Logistic model best described the DPCs of T1 and T2 (R^2 = 0.94 and 0.92; RSE = 1.39 and 1.25; CCC = 0.97 and 0.96). The DPC of T3 was best described by the Gompertz model (R^2 = 0.94; RSE = 0.20; CCC = 0.97).

Considering the fitting model (Figure 17A) and predicted errors (Figure 17B), the Logistic model was selected for all epidemics to do t-statistic for rate (slope) and initial amount of disease (intercept). The rates of the epidemics (T1, T2, and T3) were between 0.31 and 0.57, with standard error (SE) from 0.018 to 0.020 (Figure 18A). The initial inoculum was $8.50x10^{-13}$ to $3.12x10^{-09}$ (Figure 18B). The paired comparison of rates and rates SE by t-test indicates that the rates of epidemic T1 and T2 (p-value = 0.033), T1 and T3 (p-value = 0.011) can be different, and T2 and T3 (p-value = 0.050) can not be considered different.

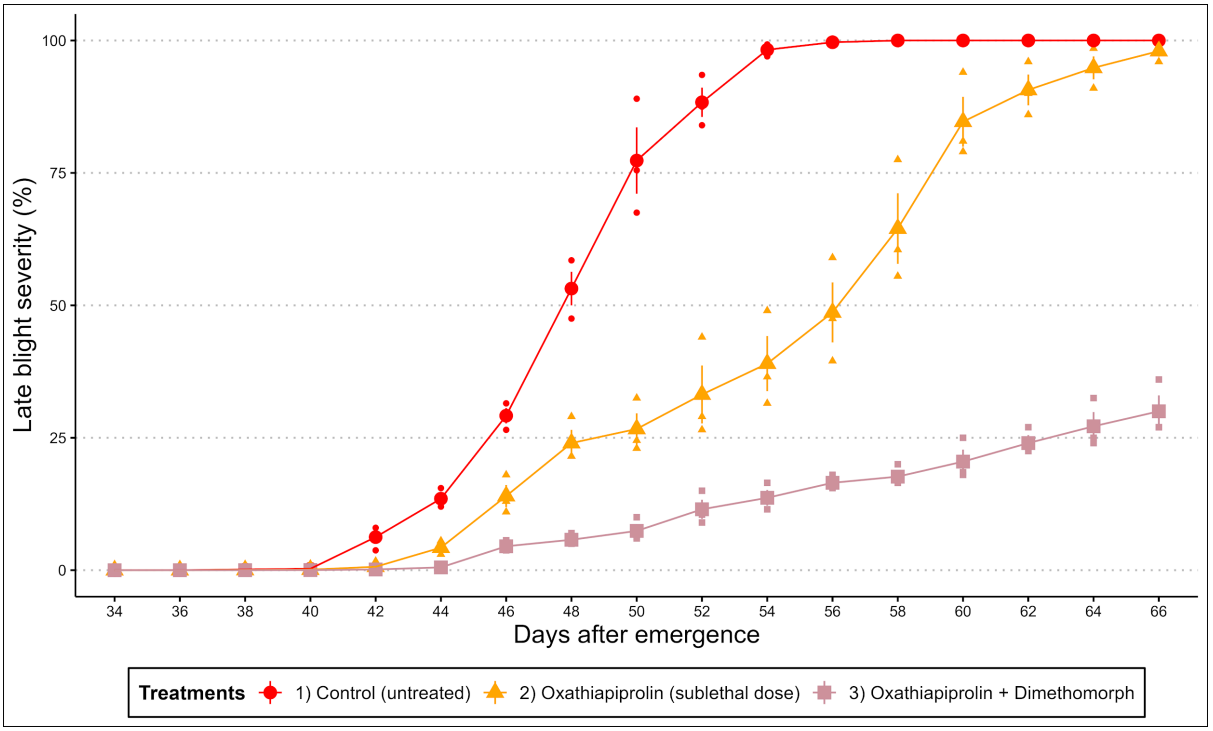


Figure 16. Disease progress curves of a field experiment to evaluate the efficacy of oxathiapiprolin in potato blight control. The lines indicate the progress of epidemics, small shapes (circles, triangles, and squares) are the late blight severity registered for the three blocks, and big shapes are the late blight severity mean of the blocks. The colors represent the epidemic treatments: red - 1) Control (untreated), orange - 2) Oxathiapiprolin (sublethal dose), and pink - 3) Oxathiapiprolin + Dimethomorph.

Table 3. Parameter estimates of Logistic and Gompertz models fitted for epidemic treatments in a field experiment to evaluate the efficacy of oxathiapiprolin in potato late blight control.

Treatment	Model	R^2	RSE	CCC	r	y_0
T1) Control (untreated)	Logistic	0.94	1.39	0.97	0.57	$8.50x10^{-13}$
T1) Control (untreated)	Gompertz	0.93	0.98	0.96	0.36	0.00
T2) Oxathiapiprolin (sublethal dose)	Logistic	0.92	1.25	0.96	0.43	$6.38x10^{-11}$
T2) Oxathiapiprolin (sublethal dose)	Gompertz	0.90	0.64	0.95	0.19	0.00
T3) Oxathiapiprolin + Dimethomorph	Logistic	0.84	1.34	0.91	0.31	$3.12x10^{-09}$
T3) Oxathiapiprolin + Dimethomorph	Gompertz	0.94	0.20	0.97	0.07	$8.23x10^{-56}$

R^2 = R-squared; RSE = Residual Standard Error; CCC = Lin's Concordance Correlation Coefficient; r = rate of the epidemic; y_0 = initial amount of disease

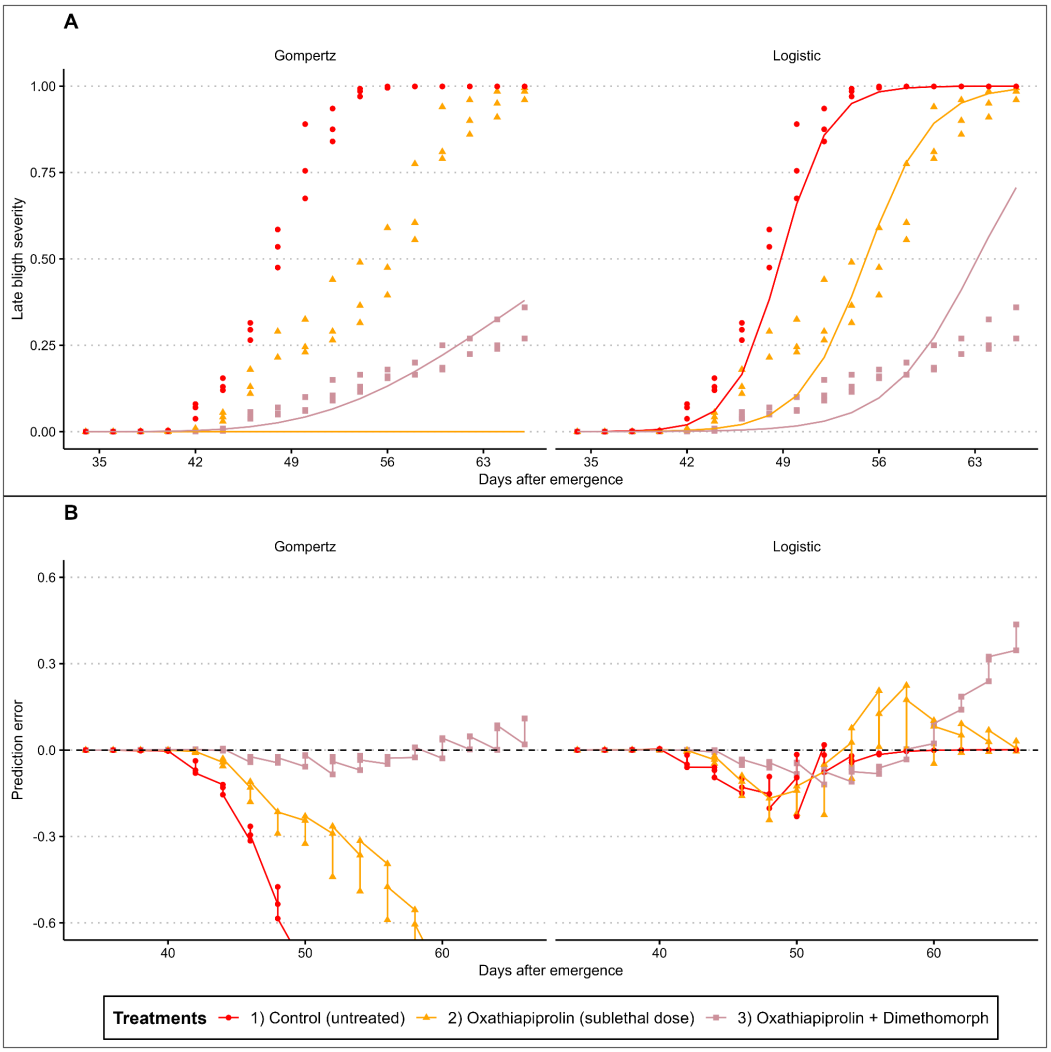


Figure 17. A. Disease progress curves fitted by Logistic and Gompertz models. The lines indicate the model fitting. B. Prediction error by the models fitted to the disease progress curves. The shapes (circles, triangles, and squares) indicate the data (A. Late blight severity; B. Error predicted). The colors represent the epidemic treatments: red - 1) Control (untreated), orange - 2) Oxathiapiprolin (sublethal dose), and pink - 3) Oxathiapiprolin + Dimethomorph.

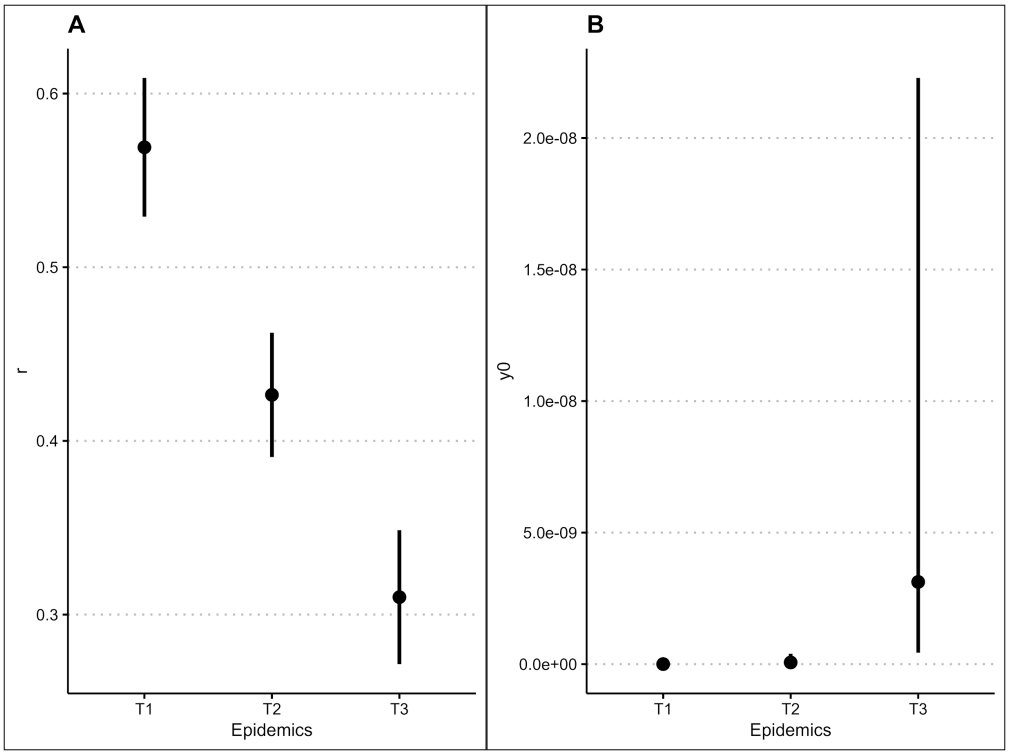


Figure 18. A. Estimated infection rates (r) and B. initial amount of disease (y_0) by a Logistic model fitted to the progress curves of three epidemics of a field experiment to evaluate the efficacy of oxathiapiprolin to control potato late blight. The bars represent the 95% confidence interval.

Tomato survey

In the experiment to test the field efficacy of oxathiapiprolin and zoxamide to control tomato late blight, the AUDPC was 2188.6, 1023.7, 457.2, 1985.9, and 1590.8 to treatments 1 to 5, respectively (Figure 19). The coefficient of variation was 6.83 %. The residues can be considered normal (p-value = 0.609) due to the Shapiro-Wilk test with 5 % of significance. The variances can be regarded as normal (p-value = 0.476) according to the oneillmathews test with 5 % of significance. According to the Tukey test, the average AUDPC of treatments 1 and 4 do not differ and the average AUDPC of the other treatments differ from them.

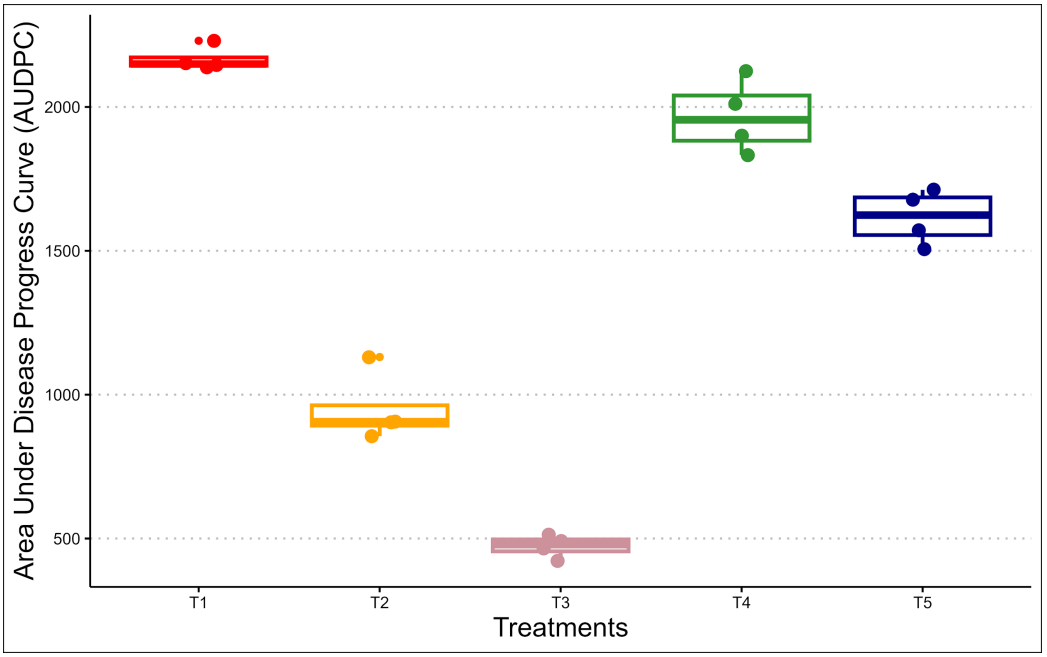


Figure 19. Boxplot of data distribution of the Area Under Disease Progress Curve (AUDPC) of a field experiment to evaluate the efficacy of zoxamide and oxathiapiprolin in tomato blight control. The colors represent the epidemic treatments: red - 1) Control (untreated), orange - 2) Oxathiapiprolin (sublethal dose), pink - 3) Oxathiapiprolin + Dimethomorph, green - 4) Zoxamide, and blue - 5) Zoxamide + Chlorothalonil / Cymoxanil + Mancozeb.

The late blight progress curves were plotted (Figure 20) and the epidemics were fitted to the Gompertz model (Table 4) (Figure 21). The exponential and monomolecular models were inadequate to fit the epidemics. The Gompertz model best described the DPCs (R^2 = 0.90 - 0.96; RSE = 0.20 - 0.58; CCC = 0.95 - 0.98).

The rates of the epidemics (T1, T2, T3, T4, and T5) were between 0.06 and 0.16, with standard error (SE) from 0 to 0.01 (Figure 22A). The initial amount of disease was $1.84x10^{-305}$ to $3.61x10^{-22}$ (Figure 22B). The paired comparison of rates and rates SE by t-test indicates that only the rates of epidemic T1 and T4 (p-value = 0.5536) can be considered different.

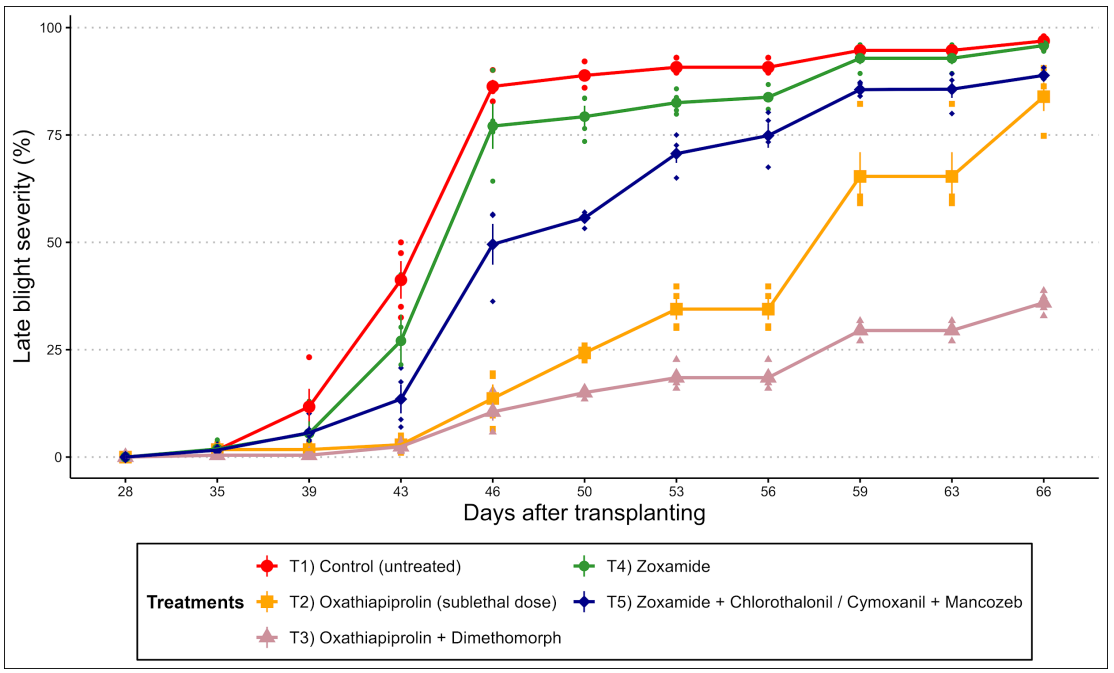


Figure 20. Disease progress curves of a field experiment to evaluate the efficacy of zoxamide and oxathiapiprolin in tomato late blight control. The lines indicate the progress of epidemics, small shapes (circles, triangles, and squares) are the late blight severity registered for the three blocks, and big shapes are the late blight severity mean of the blocks. The colors represent the epidemic treatments: red - 1) Control (untreated), orange - 2) Oxathiapiprolin (sublethal dose), pink - 3) Oxathiapiprolin + Dimethomorph, green - 4) Zoxamide, and blue - 5) Zoxamide + Chlorothalonil / Cymoxanil + Mancozeb.

Table 4. Parameter estimates of the Gompertz model fitted for epidemic treatments in a field experiment to evaluate the efficacy of zoxamide and oxathiapiprolin in tomato late blight control.

Treatment	Model	R^2	RSE	CCC	r	y_0
T1) Control (untreated)	Gompertz	0.911	0.580	0.953	0.159	$1.09x10^{-289}$
T2) Oxathiapiprolin (sublethal dose)	Gompertz	0.908	0.366	0.952	0.099	$1.32x10^{-78}$
T3) Oxathiapiprolin + Dimethomorph	Gompertz	0.922	0.208	0.959	0.061	$3.61x10^{-22}$
T4) Zoxamide	Gompertz	0.910	0.556	0.953	0.152	$1.84x10^{-305}$
T5) Zoxamide + Chlorothalonil / Cymoxanil + Mancozeb	Gompertz	0.962	0.285	0.981	0.123	$3.51x10^{-128}$

R^2 = R-squared; RSE = Residual Standard Error; CCC = Lin's Concordance Correlation Coefficient; r = rate of the epidemic; y_0 = initial amount of disease.

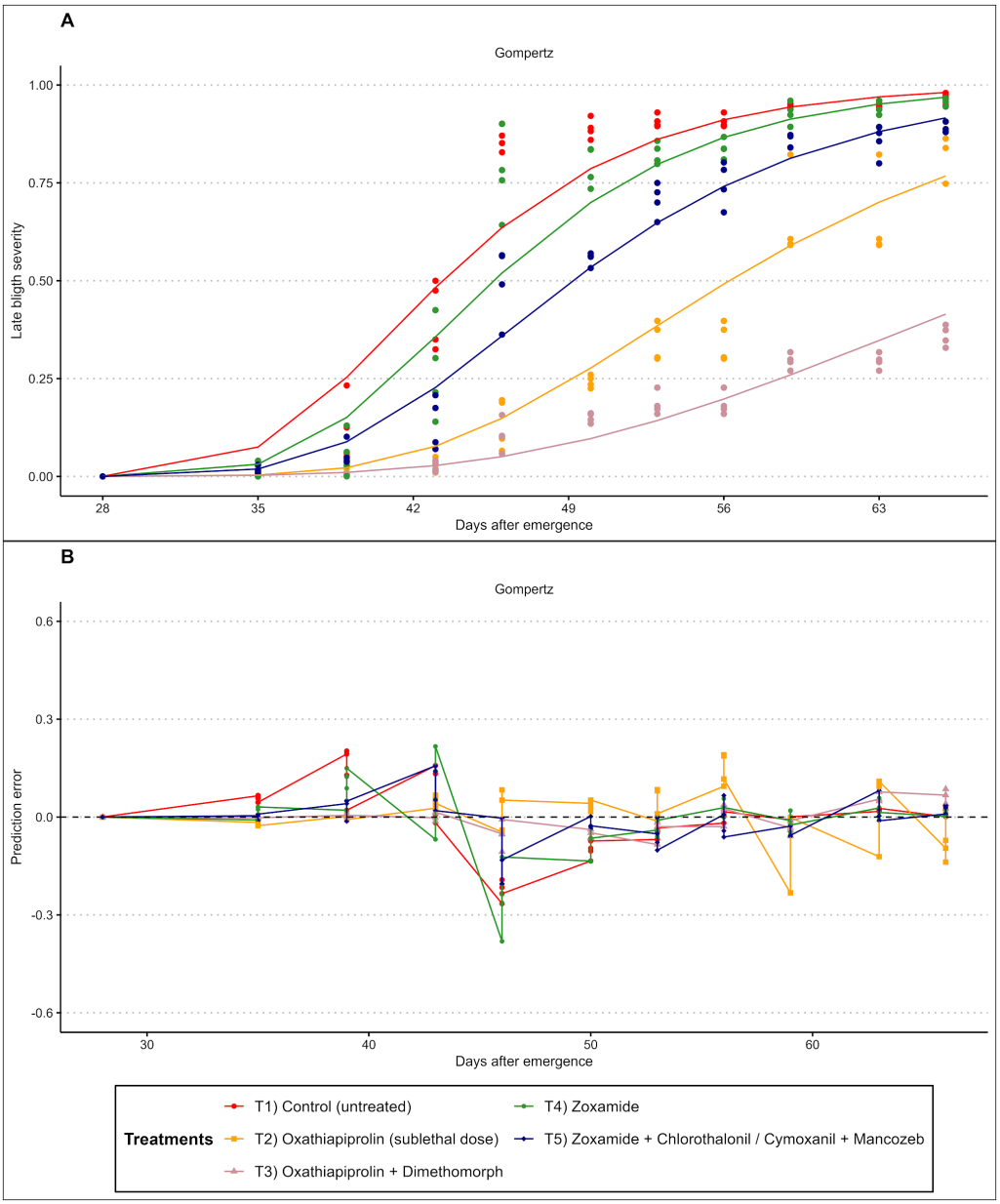


Figure 21. A. Disease progress curves fitted by Logistic and Gompertz models. The lines indicate the model fitting. B. Prediction error by the models fitted to the disease progress curves in tomato crop. The shapes (circles, triangles, and squares) indicate the data (A. Late blight severity; B. Error predicted). The colors represent the epidemic treatments: red - 1) Control (untreated), orange - 2) Oxathiapiprolin (sublethal dose), and pink - 3) Oxathiapiprolin + Dimethomorph.

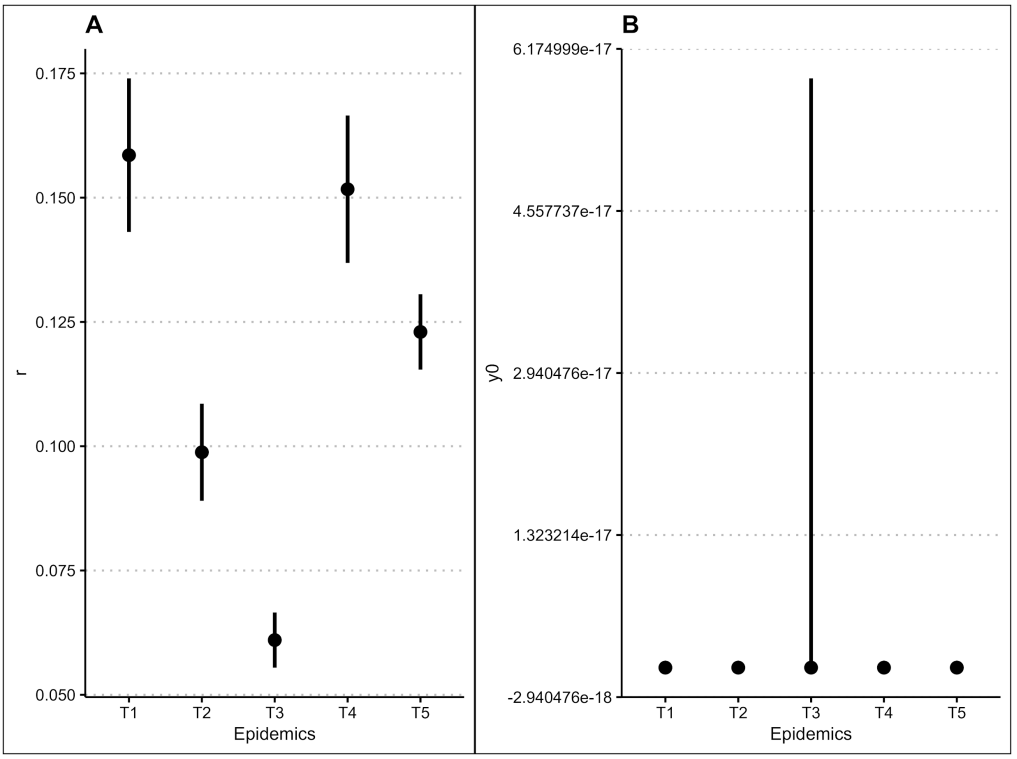


Figure 22. A. Estimated infection rates (r) and B. initial inoculum (y_0) by a Logistic model fitted to the progress curves of three epidemics of a field experiment to evaluate the efficacy of oxathiapiprolin to control potato late blight. The bars represent the confidence interval of 95 %.

Yield

In the trial field efficacy of oxathiapiprolin, the yield means of treatments 1 to 3 were respectively 32.91, 30.27, and 44.99 (t/ha) (Figure 23A). The coefficient of variation was 32.25 % and the ANOVA assumptions were met (p-value > 5 %). According to the Tukey test, the yield means can not be considered different.

Regarding the yield, in the experiment to test the field efficacy of zoxamide, the yield means of treatments 1 to 3 were respectively 24.40, 28.23, and 32.90 (t/ha) (Figure 23B). According to the variance analysis, the coefficient was 12.48 % and the ANOVA assumptions were met (p-value > 5 %). According to the Tukey test, the yield means can not be considered different.

Regarding the yield, in the experiment to test the field efficacy of oxathiapiprolin and zoxamide to tomato late blight control, the number of tomato fruits means of treatments 1 to 5 were respectively 0.47, 4.78, 6.47, 1.12, and 2.91 (t/ha) (Figure 23C). The coefficient of variation was 97.51 % and the ANOVA assumptions were met (normal residues (p-value = 0.455) and homogeneous variance (p-value = 0.897). According to the Tukey test, the yield of tomato means that treatments 1, 2, 4, and 5 were not significantly different, treatments 2

and 3 can not be considered different, and only treatment 3 can be considered different from treatments 1, 4, and 5.

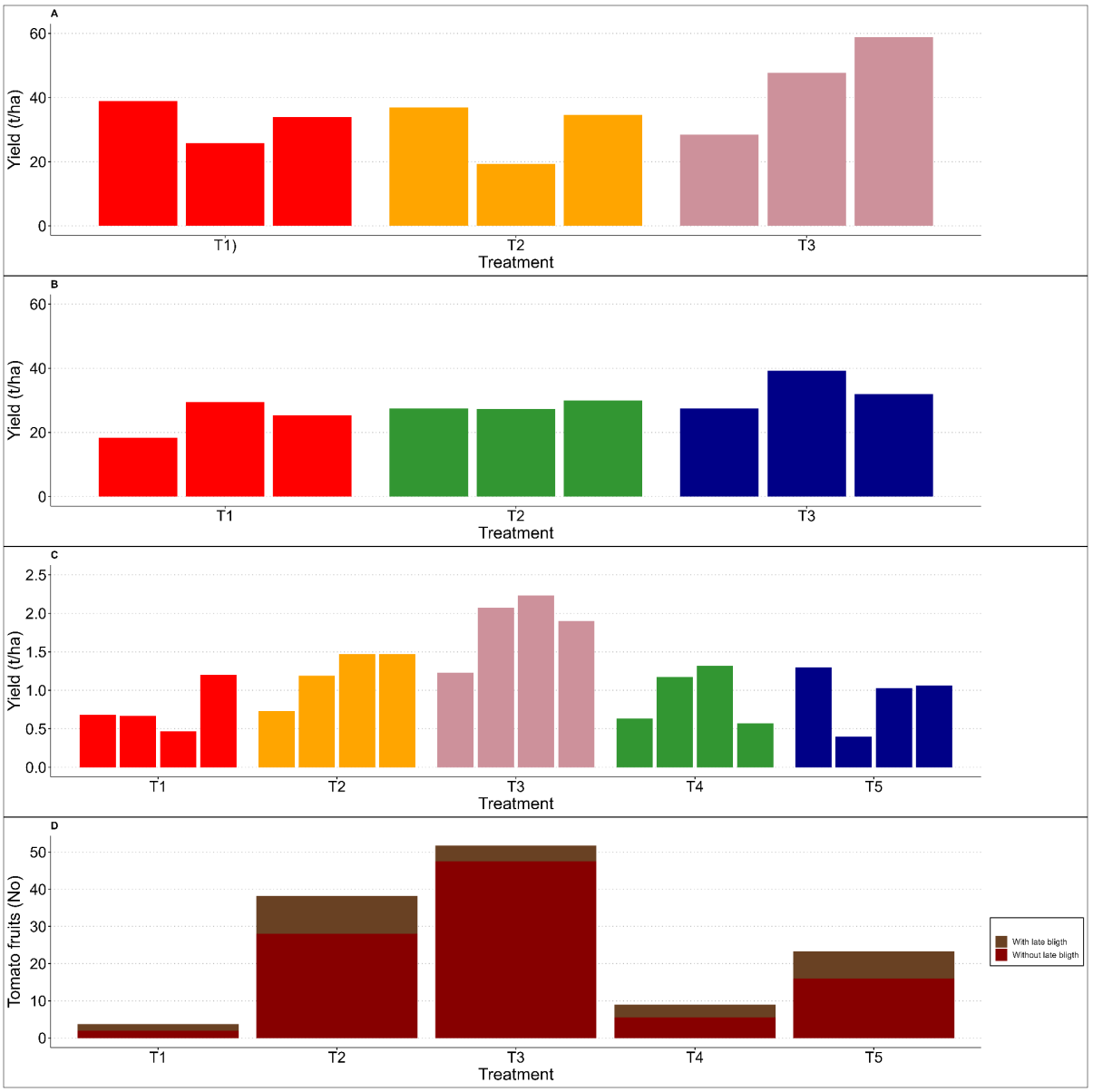


Figure 23. Potato and tomato yield of field experiments to evaluate the oxathiapiprolin or/and zoxamide efficacy. A. Yield (t/ha) from potato experiment to evaluate field efficacy and stability of oxathiapiprolin. B. Yield (t/ha) from potato experiment to evaluate field efficacy and stability of zoxamide. C. Yield (ton/ha) from tomato experiment to evaluate field efficacy and stability of zoxamide and oxathiapiprolin. D. Yield (number of fruits with and without late blight symptoms) from tomato experiment to evaluate field efficacy and stability of zoxamide. The color indicates treatments: red - control (untreated), orange - oxathiapiprolin (sublethal dose), pink - oxathiapiprolin + dimethomorph, green - zoxamide, blue - zoxamide + chlorothalonil / cymoxanil + mancozeb, brown - with late blight lesions, dark red - without late blight lesions.

Zoxamide pressurization assay

The zoxamide pressurization experiment on potato was done twice during the potato cycle, on days 38 and 48 after emergence, and one week after the second and third applications. In the first pressurization test, at dose zero lesions and sporulation were observed normally for leaves collected from the control (untreated) and zoxamide (T1 and T2) treatments, and a reduction of 50 % on the lesions and sporulation was observed for leaves on plots of zoxamide + chlorothalonil/ cymoxanil + mancozeb (T3). At dose 20 µg/mL lesions were formed but there was no sporulation for leaves of T1, and reduction from 50 to 100 % of lesions and sporulation for leaves of T2 and T3.

In the second pressurization test, leaves were collected only from blocks of T3, because the epidemics were advanced in the treatments T1 and T2. At dose 0, lesions and sporulation were observed normally, and at dose 20 µg/mL there was a reduction from 50 to 100 % of lesions and sporulation was observed.

Oxathiapiprolin pressurization assay

Samples, lesions on leaves, from the potato experimental plots were collected at 69 and 90 DAE. These dates correspond to the day immediately after with the second and the third sprays of oxathiapiprolin. In the first sampling all isolates obtained from the plots that received treatment 3 did not infect the leaf discs used as control (untreated) or the discs treated with the discriminatory dose. Inoculation of the isolates obtained from plants in the plot that received treatment 2 resulted in four lesions and sporulation on control but not in the discs with discriminatory dose. The isolates from control plots sporulated on control discs but not in discs treated with discriminatory doses. In the second sampling the isolates from treatment 3 did not sporulate on the control or in leaf discs treated with discriminatory dose. The isolates from other treatments did not sporulate on discs treated with discriminatory dose.

Zoxamide and oxathiapiprolin pressurization assay

Samples from tomato experimental plots were collected at 38, 50, and 65 DAT. The results observed were similar to all samplings. Isolates obtained from plots treated with oxathiapiprolin (sublethal dose) (T2) and oxathiapiprolin+dimethomorph (T3) were inoculated on leaves but there was no infection. On leaves inoculated with isolates obtained from plots treated with zoxamide treatment (T4) was observed a reduction on lesions and sporulation up to 50 % at dose 0, and no lesion nor sporulation was observed at dose 20 µg/mL.

Discussion

This study was conducted as part of a monitoring action of the sensitivity of the Brazilian population of *P. infestans* to mefenoxam and mandipropamid. Additionally, it generated new information regarding the sensitivity of this pathogen to zoxamide. This is also the first assessment of the sensitivity of *P. infestans* collected from BA, SP, PR, SC, and, RS states to oxathiapiprolin, and it also contributes to the knowledge about field efficacy and stability of the sensitivity to oxathiapiprolin and zoxamide. The sensitivity of *P. infestans* to these compounds was assessed by sporangia germination, mycelial growth, and sporulation on leaf disc assays. The sensitivity information generated in this study is valuable in delineating anti-resistance strategies for late blight management.

There was variable sensitivity to mefenoxam. Most isolates tested presented sensitivity intermediate to mefenoxam, and just five isolates were resistant to this compound. The occurrence of resistant isolates of the Brazilian *P. infestans* population was reported from 2003 onwards (Reis et al. 2003). Since then, mefenoxam-insensitive isolates of *P. infestans* have always been reported in previous studies, however, at each assessment time (study) there have been changes in the proportion of isolates in each class of sensitivity (Reis et al. 2005, 2006; Santana et al. 2013).

Among the isolates evaluated as sensitive to mefenoxam, most of them (n = 12) originated from potato while the minority originated from tomato (n = 2). Only isolates from potato were classified as intermediate. The resistant class was composed of two isolates from potato and three from tomato. The number of isolates evaluated originating from potato and tomato hosts is disparate, and an equal comparison of the proportion of sensitivity in each host is not possible. Even though a few isolates from tomato were assessed, three out of five were resistant to mefenoxam. The difference in the isolate sensitivity according to the host of origin was observed before in Brazil (Reis et al. 2005, 2006; Santana et al. 2013). However, the large difference in samples prevents further comparisons.

Given the occurrence of insensitive isolates in the Brazilian population throughout studies conducted in the past two decades, there is a considerable chance of the resistance to mefenoxam being fixed in the population and not being introgressed by new individuals (Reis et al. 2005). Historically, metalaxyl and mefenoxam (metalaxyl - M) are related to insensitivity problems worldwide (Davidse et al. 1981; Dowley and O'sullivan 1981; Pánek

et al. 2022; Reis et al. 2005; Santana et al. 2013). Metalaxyl was very effective in controlling late blight and has been used for several decades, but soon after it started to be used to manage potato and tomato late blight, resistant isolates were reported (Davidse et al. 1981; Dowley and O'sullivan 1981; Pánek et al. 2022). An enantiomer of metalaxyl (mefenoxam or metalaxyl - M) was developed to reduce the resistance and to allow the molecule to be used to control late blight; however resistant isolates keep being reported. The case of mefenoxam/metalaxyl-resistance illustrates the risk of resistance in populations of *P. infestans* and the concern about delineating anti-resistance strategies and monitoring the sensitivity of a pathogen population to a chemical molecule.

The development of resistance soon after the start of commercial use of an active ingredient suggests that resistant genotypes preexist in the population (Gisi and Cohen 1996). The lack of a metalaxyl sensitivity study before its commercial use does not allow the testing of this hypothesis. Studies about the sensitivity to metalaxyl of the *P. infestans* population from wild *Solanum* in Mexico contribute to understanding the status of the population previous to the commercial use of the compound. Resistant metalaxyl isolates of *P. infestans* collected from wild species of potato were identified in low frequencies in the 1983 and 1984 seasons, supporting the hypothesis that phenylamide resistance naturally occurred in the population of *P. infestans* (Matuszak et al. 1994). Additionally, the high range of sensitivity values reported in the Toluca Valley, Mexico population over seasons can be explained by sexual reproduction, which makes difficult the fixation of resistant genotypes (Grünwald et al. 2006; Matuszak et al. 1994). Populations with the least variation in sensitivity indicate predominance of asexual reproduction (clonal population) and is easier for resistant genotypes to be selected and fixed (Matuszak et al. 1994).

The fact that the Brazilian population of *P. infestans* is predominantly clonal (Reis et al. 2003, 2006; Oliveira 2010) supports the hypothesis that resistance to mefenoxam is fixed. Thus, there is risk of resistance selection in Brazil. This insensitivity to mefenoxam limits the use of this compound in potato and tomato late blight management.

Most isolates tested for the sensitivity to mandipropamid were sensitive. Only four presented intermediate sensitivity to this compound and all isolates were sensitive to 10 µg/mL of mandipropamid. The last monitoring population to mandipropamid occurred in 2010, the EC_{50} of the oomycide for isolates studied ranged from 0.05 to 1.4 µg/mL, and no insensitive isolates were detected (Oliveira 2010).

Mandipropamid was made commercially available around 2006 (Lamberth et al. 2006). This single-site oomycete was classified as low to medium resistance risk (Fungicide

Resistance Action Committee 2019) and has high activity against spore germination, good translaminar movement, and is more effective than the other carboxylic acid amide molecules, as dimethomorph and iprovalicarb, to late blight control (Cohen and Gisi 2007) (Cohen and Gisi 2007). An insensitive isolate of *P. infestans* to mandipropamid was recently related in a Swedish field (Ericsson 2024). The isolate was able to sporulate at 10 µg/ml, but no single-point mutation was connected to this resistance phenotype (Ericsson 2024). The large period of commercial use of this molecule without insensitivity related to *P. infestans* can be explained by correct anti-resistance strategies adopted until then or by recessive characteristics of resistance.

According to our results, the Brazilian population of *P. infestans* remains sensitive to mandipropamid. This compound today is one of the best chemicals available to control potato and tomato late blight and delaying the insensitivity is key to avoiding yield losses in potato and tomato fields. The monitoring of sensitivity must be done to identify a population change, avoid the spread of resistant genotypes, and the fixation of the resistance allele in the population.

When regarding the sensitivity of the Brazilian *P. infestans* population to zoxamide, the results show that all isolates were sensitive. Studies with European and Chinese populations of *P. infestans* and *P. capsici* also demonstrated the sensitivity of these oomycete pathogens to zoxamide. The EC_{50} values of zoxamide for these populations were < 100 µg/L and between 2.3 x 10^{-2} to 3.23 x 10^{-1} µg/mL on *in vitro* assays to *P. infestans* and *P. capsici*, respectively, and the mean EC_{50} ranged from 5 to 88 µg/L on *in vivo* assays with leaf discs inoculated with *P. infestans* (Bi et al. 2011; Cooke et al. 2001). The EC_{50} values reported in the present study for the Brazilian population of *P. infestans* are according to what have been described for other *Phytophthora* species.

The sensitivity to zoxamide was assessed by mycelial growth and sporulation on leaf disc and there was large variation in the EC_{50} values according to the methodologies used. This variation was also observed before in populations of *P. infestans* from Europe (Cooke et al. 2001). European populations of *P. infestans* show minimum inhibitory concentration (MIC) values from 0.5 to > 20 mg/L, with mean EC_{50} values of zoxamide ranging from 5 to 88 µg/L on *in vivo* assay with leaf discs, and < 100 µg/L for *in vitro* assay with mycelial growth (Cooke et al. 2001). The authors explain the lack of correlation in the results of *in vivo* and *in vitro* assays due to variations in population sensitivity, non-systematicity of the compound and deficit of uniformity when spraying the product on leaf discs, and dependence of physiological stage of leaf to sporulation of the pathogen (Cooke et al. 2001). Additionally

to these explanations, the variation in sensitivity assessed by the two methodologies used can be due to different effects of the compound on pathogen life stages.

Zoxamide is a single-site oomycide targeting the β-tubulin and affecting the cellular division. The interruption of cellular division most likely affect germination, inhibits pathogen germ-tube elongation, pathogen penetration in host tissue (Bi et al. 2011; Young and Slawecki 2001), inhibits further development of hyphae, and prevents the production of sporangiophores and sporangia (Cooke et al. 2001). Even though serial steps after germination are affected by zoxamide, in each step the pathogen may be affected at different levels and sensitivity can be variable. The fact that the compound is not fully systemic can also lead to variation in pathogen sensitivity due to lack of uniform distribution on plants.

The population of *P. infestans* from the Brazilian states of BA, SP, PR, SC, and RS were sensitive to oxathiapiprolin in all methodologies. The sensitivity to oxathiapiprolin was assessed by inspecting its effect on different life stages of the pathogen: germination of sporangia, mycelial growth, and sporulation. Oxathiapiprolin had a damaging effect in the three major stages of the *P. infestans* life cycle, consequently, interfering in the host-pathogen interaction and disease progress. The effects on the life cycle are the reduction or delay of the pathogen penetration, colonization, and reproduction. With the pathogen reproduction affected, the initial source of inoculum and the epidemic progress will be lower.

The results observed in this study agree with those observed in a Brazilian population of MG state, also sensitive to oxathiapiprolin in the three methodologies evaluated (Guimarães unpublished). Overall, there is high sensitivity of the population of *P. infestans* to oxathiapiprolin, and the EC_{50} values estimated are in the same range of values reported in studies with other oomycete populations worldwide. The EC_{50} values of oxathiapiprolin estimated for *Pseudoperonospora cubensis* (n = 77) in detached leaves in China varied from 2.4×10^{-5} to 9.63×10^{-4} µg/ml (Miao et al. 2018), to *Plasmopara viticola* in leaf disc in Europe (n = 495) varied from 1.0×10^{-3} to 2.64×10^{-2} µg/ml (Mboup et al. 2021) and in Italy (n = 29) the range was from 4×10^{-5} to over 4×10^{-1} mg/L (Massi et al. 2022), and to *Bremia lactucae* in lettuce plants (n = 26) in the USA, the values ranged from 1.2×10^{-4} to 3.3×10^{-3} mg/L (Olaya et al. 2019). Using a considerable number of isolates of different species of *Phytophthora*, such as *P. capsici* (n = 25) (Ji and Csinos 2015), (n = 175) (Miao et al. 2016), *P. citrophthora* (n = 62), *P. syringae* (n = 71), *P. nicotianae* (n = 31) (Gray et al. 2018), (n = 20)(Qu et al. 2016), *P. cinnamoni* (n = 71) (Belisle et al. 2019), and *P. colocasiae* (n = 97) (Wang et al. 2022), the estimated mean EC_{50} values were, respectively, 1.0×10^{-3} µg/mL, 5.6×10^{-4} µg/mL, 3.0×10^{-4} µg/mL, 1.0×10^{-4} µg/mL, 5.0×10^{-4} µg/mL, 1.0×10^{-3} µg/mL, 4.0 x

10^{-4} µg/mL, and 5.3 x 10^{-4} µg/mL. The estimated EC_{50} values in the current study are of similar magnitude to those reported above. For most oomycetes assayed so far worldwide, the EC_{50} values of oxathiapiprolin are in the order of 10^{-4} µg/mL, except for *Plasmopara viticola*, which are usually higher. However, regardless of the exact estimated value, several studies corroborate that oxathiapiprolin is effective at very low doses (Pasteris et al. 2019).

The oxathiapiprolin efficacy at very low doses is complemented by its effect in several stages of the life cycle of the pathogens. The compound inhibits zoospore release, zoospore germination, and sporangia germination (Pasteris et al. 2019; Salas et al. 2015). Reduction in mycelial growth and damage in the formation of lesions and spores were reported (Pasteris et al. 2019). Additionally, zoospores treated with oxathiapiprolin swam slower and encyst faster than untreated zoospores (Pasteris et al. 2019). Similar effects of oxathiapiprolin were reported for *P. cubensis* such as inhibition of zoospore release, cytospore germination, formation and expansion of lesions, sporangiophore development, and sporangial production (Cohen 2015). In the present study, the three methodologies adopted to assess sensitivity represent the stages of germination, host colonization, and reproduction of *P. infestans*. The low EC_{50} values observed in our study support the evidence that oxathiapiprolin negatively affects multiple stages of the pathogen life cycle.

A thorough comparison of the results reported here with those of other studies could not be done with exactly the three methodologies due to the lack of correlation among the methods used. Oxathiapiprolin acts on multiple stages, but there is variation in relation to degree of interference on the stages according to the pathogen population. Aiming to minimize the effect of methodology on sensitivities studies, a combination of methods should be adopted for more detailed knowledge about how different individuals respond to the application of oxathiapiprolin. Furthermore, agreeing upon specific methods for large-scale monitoring programs may help standardize procedures and compare results.

Despite the low EC_{50} values observed, the monitoring of *P. infestans* sensitivity to oxathiapiprolin should be done frequently to avoid loss of effectiveness of the product. Two facts contribute to the necessity of special attention on monitoring the sensitivity of this active ingredient: the high risk of resistance development (Hermann and Stenzel 2019) in pathogen populations and the clonal structure observed in the current Brazilian population of *P. infestans* (Guimarães unpublished). The fact of oxathiapiprolin is a single site oomycide increase the risk of resistance development, a single point mutation can be enough to a resistance genotype exist in the population. In addition, in clonal populations is easier to fix an resistant allele, and the emergence of resistance occur.

Reports of field resistance to oxathiapiprolin were related to *P. viticola* and *P. colocasiae* (Mboup et al. 2021; Massi et al. 2022; Wang et al. 2022). The point mutation G699V in PcoORP1 conferred resistance to oxathiapiprolin in *P. colocasiae* (Wang et al. 2022) and mutations N837I, L863W, and G770V in PvORP1 were associated with resistance in *P. viticola* (Mboup et al. 2021; Massi et al. 2022). Recently, an isolate fo *P. infestans* from a Swedish field was reported to be resistant to oxathiapiprolin (Ericsson 2024). Although the resistance phenotype was not connected to SNPs at the ORP1 gene, the isolate of the EU_43 genotype sporulated in a potato leaf disc treated with oxathiapiprolin at 1 µg/mL (Ericsson 2024). Lower sensitivity of *P. capsici* isolates was also reported. In one study the authors classified one isolate as moderately sensitive to oxathiapiprolin because the average of the EC_{50} values (8.1 x 10^{-4} µg/mL) was between the highest and second highest doses tested (7.5 x 10^{-4} and 1.0 x 10^{-3} µg/mL). In another study, the authors classified the isolate as resistant, growing more than 75 % at the discriminatory dose and 80 % relative to the control (Siegenthaler and Hansen 2021). Additionally, in another study, one isolate grew more than 90 % relative to the control colony diameter and was classified as insensitive to oxathiapiprolin (Parada-Rojas and Quesada-Ocampo 2022). Even though oxathiapiprolin insensitivity and resistance have been reported, the product remains effective. However, management changes combined with the monitoring of resistance are actions needed to minimize the impact on field and provide longevity to oxathiapiprolin (Siegenthaler and Hansen 2021).

The efficacy of zoxamide and oxathiapiprolin to control potato and tomato blight evaluated under field conditions presented differences. In the potato trials, the efficacy of zoxamide and oxathiapiprolin was evaluated in two independent experiments. Zoxamide in a mixture with chlorothalonil and alternated with cymoxanil and mancozeb resulted in good control of potato late blight. The compound applied solo differed from the control (untreated) but the infection rate was higher than other treatments. Zoxamide should be combined with other compounds to improve late blight control. Also, the compound should be applied as preventive, before the start of the epidemic due to its mode of action. being in cellular division, in the first stages of the pathogen infection. In addition, zoxamide can be an alternative molecule to resistance management.

In the potato trial to evaluate the efficacy of oxathiapiprolin, a mixture of oxathiapiprolin and dimethomorph resulted in good control of the late blight. The AUDPC and infection rate of this treatment were lowest. The higher efficacy of the mixture may be due to two reasons: first, the treatment with oxathiapiprolin solo was in a subdose, and

second, the dimethomorph contributes to improve the oxathiapiprolin efficacy. The second hypothesis seems more appropriate to increase late blight control and as an anti-resistant strategy.

In the field trial to assess the efficacy of zoxamide and oxathiapiprolin on tomato blight, the treatments with oxathiapiprolin were the most effective, and the oxathiapiprolin in the mixture with dimethomorph was the best treatment, with the lowest AUDPC and infection rate. Zoxamide solo had a high AUDPC and infection rate and did not differ from the control. These results corroborate the recommendation of zoxamide and oxathiapiprolin application combined with other compounds for better efficacy and resistance management.

The population of *P. infestans* from Brazil remains formed by individuals sensitive, intermediate, and resistant to mefenoxam. The sensitivity to mandipropamid observed is according to the EC_{50} values reported before (Oliveira 2010). The population of the pathogen is sensitive to oxathiapiprolin and zoxamide, and the sensitivity continues after potato and tomato growing seasons. The population sensitivity monitoring is crucial to keep the efficacy of the oomycides to late blight control and delay the resistance emergence in Brazil.

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General Conclusions

The current *P. infestans* population from Brazil is different from the previous population described before. At the present time, the population is composed by individuals of: (i) A1, A2, and self-fertile mating types; (ii) Ia and Ib mitochondrial DNA haplotypes; (iii) three clonal lineages (EU_2_A1, EU_37_A2, and SA_1_A2, the new one); and (iv) sensitive, intermediately insensitive, and insensitive to mefenoxam. Also, the current population is structured into three groups, by SSR genotypes.

The presence of A2 mating type associated with the SA_1_A2 SSR genotype in tomato fields was described for the first time in Brazil. These results and phenotypic differences observed between isolates from potato and tomato hosts suggest a major change in the population of *P. infestans* related to this host. This signal is supported by the Bayesian phylogeny analysis.

Regarding sensitivity to mefenoxam of the current *P. infestans* population in Brazil, sensitive, intermediately sensitive, and resistant were detected. There was a shift in the sensitivity to mandipropamid with some isolates intermediately insensitive. The population is sensitive to zoxamide and oxathiapiprolin and the sensitivity was stable after three applications in potato and tomato field trials. Under field conditions, the treatments of zoxamide + chlorothalonil + cymoxanil + mancozeb and oxathiapiprolin + dimethomorph presented the best results in the management of potato and tomato late blight.

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