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Potato Late Blight Control with a Botanical Product and Reduced Copper Applications

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Abstract

Potato late blight (PLB), caused by the pathogenic oomycete *Phytophthora infestans*, can cause extensive economic damage. Copper is used to combat PLB mainly in organic production. However, its reduction or elimination as a fungicide is a priority in Europe, and it is already banned in some countries. Alternative control strategies, including botanicals, could potentially reduce copper and control PLB. We investigated the application of *Frangula alnus* bark, reduced copper applications, and *F. alnus'* sequential use with reduced copper applications in field and laboratory experiments. Different dosages and preparations influenced *F. alnus* efficacy and the quantity of its posited active ingredients. Through in vitro and in planta experiments, we investigated whether *F. alnus* directly or indirectly controlled PLB. A bacterium (*Erwinia* spp.), originating from the *F. alnus* extract, colonized the media and caused most of the direct inhibition in vitro, but filtering out microorganisms had no effect on the extract's efficacy in planta. The contribution of extract-associated microorganisms to PLB control is unclear and requires additional experimentation to assess. The measured anthraquinones likely contributed to the effect of *F. alnus.* During 4 years of field experiments, the reduced copper and *F. alnus* treatments decreased disease severity in four and in three years, respectively, compared with the water control. No differences in disease severity or yield were observed between full and reduced copper treatments. Potato variety more consistently drove differences in total and marketable yields compared with the treatment. The yield was relatively stable within each year, suggesting that the treatment effect on yield is intertwined with the timing of disease development and environmental conditions.

Keywords: alternative control strategies, *Erwinia*, *Frangula alnus*, oomycete, *Phytophthora infestans*, plant protection, potato late blight

In many countries, including Switzerland, organic potato production still relies on the use of copper to combat potato late blight (PLB) caused by the oomycete *Phytophthora infestans* (La Torre et al. 2018). Copper, however, is a heavy metal that has long-term consequences for soil health and toxicity (Shabbir et al. 2020) and is already banned as a plant protection product by some countries, including Denmark, Estonia, Finland, the Netherlands, and Sweden (Tamm et al. 2022). In recent decades, copper reduction in organic potato production has become a priority (Tamm et al. 2022), and much research has been focused on alternative plant protection products, including the application of microorganisms or natural products (Bangemann et al. 2014; Dorn et al. 2007). PLB is a polycyclic pathogen that typically requires multiple fungicide treatments within a growing season to control it, resulting in organic potato production receiving one of the highest fungicide and copper applications of all arable crops (Andrivon et al. 2018; Katsoulas et al. 2020). Therefore, copper reduction is linked to the development of alternative strategies for controlling PLB.

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Copper applications can be reduced through the deployment of resistant varieties, but potato breeding requires a long-time horizon of at least 10 to 16 years (Keijzer et al. 2022; Pandey et al. 2023), which could be shortened to 7 to 13 years with the implementation of marker-assisted selection and genomic-estimated breeding values of complex traits (Slater et al. 2014). However, previous potato breeding efforts show that it is difficult to combine late blight resistance, tuber yield, starch content, and other characteristics (Reslow et al. 2022), and only small genetic gains have been shown in newly released cultivars in Europe (Ortiz et al. 2022). Furthermore, the use of a single resistance gene is not a sustainable strategy, as *P. infestans* strains can evolve to overcome qualitative resistance (Gilroy et al. 2011). Genome-editing techniques could facilitate the development of resistant varieties, but the public, particularly organic producers and consumers, do not accept genetically modified organisms' use in potato breeding (Pacifico and Paris 2016). Therefore, adopting lower copper application levels and pairing them with an alternative product can help reduce copper application levels on a shorter time scale.

Previous experiments with botanicals demonstrate efficacy against oomycetes (Dagostin et al. 2010; Forrer et al. 2017; Mulholland et al. 2017; Taillis et al. 2022), and buckthorn alder (*Frangula alnus*) bark extract, from material that has been dried and finely ground, is among the botanicals that show potential to combat PLB (Forrer et al. 2017). However, further optimization of the preparation and dosage is needed to enable the economic feasibility of the treatment.

The goal of this work was to determine the efficacy of the extract of dried and ground *F. alnus* bark, gain information about its accumulative effects, evaluate its sequential use with reduced copper treatments, and optimize its usage. To determine the efficacy of different *F. alnus* preparations in the field, we carried out field experiments over four growing seasons to test various preparations and dosages. In addition, we conducted lab experiments, including in planta and in vitro assays, and analytical chemistry to gain more information about the nature of the active compounds, treatment dosage and timing, and applicability to practice. Overall, this work highlights the possibility of further reducing copper allowances and applying different treatments sequentially.

Materials and Methods

Field experimental setup

From 2019 to 2022, four potato field experiments were conducted at Agroscope in Zurich Reckenholz, Switzerland. A split-plot design was used in the experimental fields with six replications in 2019 and 2020 and five replications in 2021 and 2022. Each plot included two subplots: one subplot with two rows of the variety Agria and another subplot with two rows of the variety Victoria. 'Agria' and 'Victoria' are common varieties used in Swiss potato production, including organic production (Swisspatat 2024). These varieties are classified as moderately susceptible to late blight according to the recommended Swiss Variety List of potatoes (Schwaerzel et al. 2019). Varieties with moderate susceptibility are recommended for the evaluation of low-risk products, which are expected to be less effective than high-risk products (Evenhuis 2021). Each subplot was 7.5 m^2 and contained two rows that were each 75 cm wide and 5 m long. In each row, the 15 planted tubers were spaced 33 cm apart. The placement of 'Agria' and 'Victoria' subplots within the plots was randomly assigned. In the four rows between the plots, two rows of a more resistant variety ('Panda' in 2019 and 2020, 'Jelly' in 2021, and 'Innovator' in 2022) were planted along the long plot edge. The more resistant variety was changed in different years because of availability. The highly susceptible variety, Bintje, was planted between the two rows of the more resistant variety (see Supplementary Fig. S1 for more information on field layout) to act as spreader rows. Treatments were randomly assigned to plots within each block.

For all field experiments, certified seeds were used, and the fields were managed according to the European and Mediterranean Plant Protection Organization guidelines (EPPO 2021) for conducting fungicide efficiency experiments against *P. infestans* on potatoes. Briefly, it provides standards on how to conduct trials to evaluate fungicide efficacy against *P. infestans*, including trial conditions, layout, treatment application, and disease assessment. The only deviations from these guidelines were regarding plot length and variety choice. Although a longer plot (8 m) is prescribed for yield evaluations, a shorter plot length (5 m) was used because of field size constraints. Additionally, the varieties used in this study are considered moderately susceptible to PLB to better mimic conditions in organic potato production (Schwaerzel et al. 2019). In every year except 2022, PLB occurred naturally in the field experiments. In 2022, untreated leaves from the susceptible variety Bintje that had been infected naturally in a nearby untreated field were laid in the 'Bintje' spreader rows to promote the infection within the experiment. Haulms of 'Bintje' spreader rows were destroyed when 5 to 15% of the leaves were diseased. More details on the experimental setup and agronomic conditions can be found in the supplementary material (Supplementary Tables S1 and S2; Supplementary Fig. S2).

Field experiment treatments

All experiments contained a water control, a copper full-dose (0.4 kg/ha) treatment, a copper reduced-dose (0.2 kg/ha) treatment, and one or more *F. alnus* treatments consisting of different dosages or preparations (Table 1). The dosage of copper was chosen based on a schedule with 10 spray applications that result in a total application of 4 kg/ha per year, the maximum copper amount allowed in Switzerland. The number of treatments increased through the years as more information about the dosages and extraction methods became available through laboratory experiments. Starting from the field experiment's second season, treatments with a reduced *F. alnus* dosage were added. Additionally, an *F. alnus* treatment with a lyophilization step during its extraction (see the "Preparation of *F. alnus* solution" section for details on preparation) was included in the third and fourth seasons of the field experiment. For copper treatments, Kocide Opti (Bayer Agrar Schweiz AG, Basel, Switzerland) was used, which contains copper hydroxide in the form of a water-dispersible granule. The water control (treatment 1) was sprayed with the same amount of water as the treated plots during the applications. Treatment number 9 consisted of a water treatment for the first four applications before switching to the reduced copper dose, and it served as a control for treatment number 8. Treatment 8 included four initial *F. alnus* treatments followed by six copper treatments.

Preparation of *F. alnus* solution

For treatments 4, 5, and 6 (Table 1), dried and milled bark of *F. alnus* (Faulbaumrinde PhEur, Dixa AG, St. Gallen, Switzerland, country of origin Poland) was suspended in tap water and stirred at room temperature (see Supplementary Table S2 for water volumes). In 2019 and 2020, the *F. alnus* solution was stirred for 2 h as previously published (Forrer et al. 2017). The *F. alnus* treatments used in 2021 and 2022 were prepared with a reduced stirring time of 30 min because a laboratory experiment revealed that the efficacy was unchanged between stirring times (see section below on extraction procedure experiments for details). After stirring, the solution was filtered through a 0.2-mm cheesecloth and then used within 2 h for the treatments in the field. For treatment 7, the extraction method of *F. alnus* was modified according to a previously described procedure (Massana-Codina 2020). In short, powder-ground bark of *F. alnus* was extracted at 20% wt/vol using aqueous extraction in nanopure water by stirring for 3 h at room temperature. The resulting extract was then centrifuged (4,000 rpm, 10 min, 20°C), and the supernatant was filtered through a 9-cm-diameter 589/3 cellulose filter (Schleicher and Schuell, Dassel, Germany) using a vacuum. The aqueous solution was then freeze-dried (FD). This modified procedure will be referred to as the low-dose, FD treatment (Table 1). The fine powder obtained was stored in a sealed box at 10°C in darkness. Before field applications, the amount of the fine powder that corresponds to the original bark weight of the low-dose

Table 1. Treatments and dosages applied for potato field experiments conducted in the years 2019 to 2022[a]

Years	Treatment number	Treatment	Treatment amount per ha
2019–2022	1	Water control	NA
2019–2022	2	Copper full dose	0.4 kg[b]
2019–2022	3	Copper reduced dose	0.2 kg
2019–2021	4	*Frangula alnus* full dose	40 kg[c]
2020–2022	5	*Frangula alnus* medium dose	25 kg
2021–2022	6	*Frangula alnus* low dose	2.5 kg
2021–2022	7	*Frangula alnus* low dose (FD)	0.5 kg[d]
2021–2022	8	4× *Frangula alnus* low dose, followed by 6× reduced-dose copper	2.5 kg (*F. alnus*)/0.2 kg (copper)
2022	9	4× no treatment (water), followed by 6× reduced-dose copper	0.2 kg

[a] Dosages in the "Treatment amount per ha" column show the amount sprayed per ha for each of the 10 sprayings. Each treatment was mixed with tap water based on a total spray volume of 450 liters/ha for the first four applications. To account for the growing canopy, the amount of water was increased for subsequent applications to 700 liters/ha in 2019 to 2021 and 630 liters/ha in 2022. The full dosage of the copper treatment, 0.4 kg/ha, applied 10 times equals the maximum allowed dosage in Switzerland. FD = freeze-dried; NA = not applicable.

[b] The copper weight refers to the copper amount in the product Kocide Opti, which is 30% copper wt/wt in the form of copper hydroxide.

[c] The *Frangula alnus* weight refers to the dried ground bark used to make the extract.

[d] The 0.5 kg of extracted FD material is the product of 2.5 kg of *F. alnus* ground bark that was used as a starting material. Therefore, the same amount of original material was used in both low-dose treatments (treatments 6 and 7).

treatment (i.e., 20% of the original weight) was suspended in tap water and stirred for 30 min in the same manner as the other *F. alnus* treatments. All treatments were then directly used in the field following their preparation.

Field experiment spray application

Dosages during applications were consistent throughout the season (Table 1), although spraying volume increased to accommodate the growing plants (Supplementary Table S2). The first application was carried out according to the recommendation of the Swiss decision support system PhytoPRE (https:/www.phytopre.ch) (Cao et al. 1997; Steenblock and Forrer 2002). The system considers late blight occurrence (date, location, and distance) and growth stage of the crop as well as weather conditions favorable for the development of *P. infestans*. All subsequent applications were performed in a weekly routine and were generally applied in the morning unless rain postponed the spraying times. The applications were shifted according to the weather forecast to try to avoid application on days with rainfall, and treatments were aimed to be applied 1 day in advance when rain was forecasted (see Supplementary Fig. S2 for meteorological conditions during each season and on treatment days). For some applications, spraying on days with rainfall was unavoidable. For those occurrences, we attempted to postpone the treatment until after precipitation or ensured that the spray coating could dry before rainfall. A total of 10 applications was made each year (see Supplementary Table S2).

In 2019 and 2020, the applications were conducted with a plot sprayer (Schachtner Gerätetechnik, Ludwigsburg, Germany) equipped with a 1.5-m spray-boom and three IDK compact air induction nozzles (IDK yellow, IDK 90-02 C, Lechler GmbH, Metzingen, Germany) at 2.2 bar. In 2021 and 2022, the treatments were applied with a tractor (Fendt F 345 GT, AGCO GmbH, Neuhausen, Switzerland) with a 3-m spray-boom and six IDK nozzles (IDK red, IDK 120-04 C). For all years, a spraying volume of 450 liters/ha was used for the first four applications. To cover the entire plant canopy once the plants reached the growth stage defined by the Biologische Bundesanstalt, Bundessortenamt, and Chemical Industry (BBCH) 31 to 35, the six top-down nozzles were complemented with five drop-leg nozzles (Lechler tongue nozzles, FT 140-02), and a larger spraying volume was used: 630 liters/ha in 2019, 2020, and 2021 and 700 liters/ha in 2022. In 2020, because of extremely wet soil conditions, the fifth treatment was applied with a knapsack sprayer (Birchmeier, Stetten, Switzerland) outfitted with a 3-m spray-boom and six IDK nozzles (IDK brown, IDK 120-05 C, 1.9 bar). The spraying pressure was adjusted according to the equipment used.

Data collection from field experiments

The infection severity was estimated based on the area of infected plant tissue within each variety subplot (Forbes et al. 2014). In June and the beginning of July, more frequent observations were performed (two times per week) to better assess the early development of the disease, whereas later in the season (mid- and late July), the assessments were made weekly. Potatoes were harvested and yield was calculated from the two internal rows of each plot. The potatoes were sorted into four size classes (<42, 42 to 55, 55 to 70, and >70 mm). The middle two size classes (42 to 55 and 55 to 70 mm) constitute the marketable potatoes and were weighed to calculate the marketable yield. During the harvest, the tubers were examined for tuber blight. Additionally, 40 to 50 tubers per treatment were then examined for signs of tuber blight 1 month after harvest. For plots in which fewer than 50 tubers were harvested, all tubers were examined, and the minimum number examined per subplot was 40 tubers.

Climate chamber and laboratory experiments

Laboratory experiments were concurrently conducted with field experiments to better understand the extraction procedure, dosages, spraying time, and mechanism of *P. infestans* control with *F. alnus*. The climate chamber experiments consisted of detached leaf assays and mycelium growth experiments as well as the chemical analyses of the *F. alnus* solutions.

Extraction procedure and application timing experiment on detached leaves

Detached leaves of the susceptible variety Bintje were used to compare the efficacy of *F. alnus* after 30 versus 120 min of extraction. A 4% concentration of *F. alnus* was prepared in the same manner as the field experiment explained previously. The aqueous solution of *F. alnus* was stirred for 30 min in addition to the treatment stirred for 120 min.

Within this experiment, the timing of the *F. alnus* application in relation to *P. infestans* inoculation was also tested. The three treatment levels consisted of applications (i) 1 day before *P. infestans* inoculation, (ii) 2 days before *P. infestans* inoculation, or (iii) both 2 days and 1 day before *P. infestans* inoculation. A control treatment composed of deionized water was included, and it was sprayed according to the same regime. A gravity feed spray gun with cup (DeVilbiss, Bournemouth, U.K.) was used to apply 3 ml of the treatments to each terminal leaf at a pressure of 1 bar. All treatments included eight detached leaves that were each placed separately on a 7- × 7-cm square of wire mesh that had been laid over a moistened filter paper in individual round dishes with a diameter of 125 mm and height of 34 mm (Semadeni AG, Ostermundigen, Switzerland). The dishes were closed with a lid and were set at a slight angle to allow for the water to collect at the bottom, and they were randomly distributed in a blocked design within a climate chamber. The experiment was run at 18°C and a 16:8 h day/night schedule of which 2 h consisted of a gradual reduction or increase of light. The humidity was 85% at night and 75% during the day.

A *P. infestans* polyspore isolate (polyspore isolate no: 18-001) collected in 2018 from untreated 'Bintje' leaves in Reckenholz, Zurich, was used for this and all laboratory and climate chamber experiments presented in this study. Its simple sequence repeat genotype (Martin et al. 2019) had been categorized to "other" (D. Cooke, *personal communication*), and it is an A1 mating type. The sporangia inoculation solution was prepared by scraping mycelia from a 2-week-old rye agar plate and diluting the sample to a sporangia concentration of 5×10^5 per ml. The spore preparation was incubated at 4°C for 2 h in the dark before inoculation to promote the release of zoospores. Each detached leaf consisted of the terminal leaflet and two first primary leaflets. The terminal leaflets were inoculated with *P. infestans* two times whereas each primary leaflet was inoculated once, totaling four inoculation points across the three leaflets (see Supplementary Fig. S3). A volume of 30 µl of the *P. infestans* sporangia preparation was used for each inoculation point. The humidity within the climate chamber was increased to 100% for 48 h following the *P. infestans* inoculation, during which the climate chamber was kept dark. After the 48-h period, the previous day/night (16:8 h) schedule and humidity settings (day: 85% and night: 75%) were reinstated. The percentage of *P. infestans*-diseased leaf area was visually assessed and estimated 7 days after infection.

F. alnus dosage experiment

Detached leaf assays were carried out in the same manner as described previously to test different application amounts of *F. alnus* and to determine its half-maximal inhibitory concentration (IC_{50}). Previous studies had used 4% *F. alnus* extracts, which corresponded to approximately 40 kg/ha (Forrer et al. 2017; Krebs et al. 2006). To obtain a more economically feasible *F. alnus* treatment, reduced *F. alnus* treatment amounts were tested in laboratory experiments to assess the efficacy of lower application amounts in a controlled environment. A five-point dilution series of *F. alnus* was used for the treatment amounts, ranging from 250 kg/ha to 0.025 kg/ha. To connect the laboratory dosages to relevant values for the field, we assumed that there are 40,000 plants/ha and each plant has 55 compound leaves. Therefore, for the highest dose, 250 kg/ha, a preparation of 11.364 g of *F. alnus* dried and ground bark was mixed with 300 ml of tap water. From this solution, a serial dilution was made for each dosage.

For this experiment, detached leaves of the variety Bintje were used. To obtain the treatment with the highest concentration, 4% *F. alnus* bark was extracted in tap water with stirring for 30 min and then strained with a 0.2-mm cheesecloth. From this concentration, the subsequent four treatments were serially diluted using tap water. The detached leaves were sprayed as described above with 3 ml of the treatment per leaf at 1 bar until the treatment was well distributed on both sides of the leaf. For the negative control, tap water was applied directly to the detached leaves.

Each treatment included 12 detached leaves that were randomly distributed according to a block design within the climate chamber. The climate chamber conditions and *P. infestans* strain used were the same as those used for the extraction procedure and spray timing experiment. The leaves were sprayed 2 days before artificial infection with *P. infestans*, which was applied as four drops of 30 µl of sporangia solution, including two drops on the terminal leaf and one drop on each of the first two primary leaflets. The sporangia was prepared in the same manner as in the experiment described previously, and the sporangia concentration used was 1×10^5 per ml. The disease severity was visually assessed by estimating the surface area of *P. infestans* infection on each detached leaf at 7 days after infection. The IC_{50} was calculated from this experiment using the four-parameter logistic regression model from the XLSTAT software (Lumivero, Denver, CO, U.S.A.). For the model, disease severity data were the dependent variables, and the extract concentration was the explanatory variable.

In vitro mycelial growth experiment

In this experiment, *F. alnus*'s direct control on *P. infestans*' mycelial growth was tested in vitro by applying the same treatments used in the field (treatment numbers 1 to 7, Table 1) on rye agar (200 g untreated rye seed boiled and strained with a normal kitchen strainer, 5 g D-glucose, and 20 g agar in 1,000 ml of water). *F. alnus* powder was prepared in the same manner as described above with a 30-min extraction step, but the tap water was autoclaved before the aqueous extraction step. Because we observed that a characteristic bacterial culture would grow on rye agar from *F. alnus* extractions, each of the *F. alnus* treatments were applied with and without an additional filtration step to eliminate the direct influence of microorganisms associated with the plant extracts. The bacterium was later identified to be an *Erwinia* spp. from sequencing the 16S ribosomal RNA gene (GenBank accession PV124383; see Supplementary Materials for details). Subsequent experiments revealed that the *Erwinia* bacterium could be cultured from multiple batches of *F. alnus*. Three batches were tested, and all three yielded an *Erwinia* spp. bacterium (see Supplementary Materials for additional information). The extent of the bacterium's co-occurrence with *F. alnus* bark is not known.

For the filtered solutions, two supplementary filtration steps were made that included (i) a vacuum filtration step using a paper filter (Schleicher & Schuell AG, 8714 Feldbach ZH) and (ii) a manual filtration step using a 20-ml Luer-Lok syringe (BD Plastipak, Becton Dickinson GmbH, Germany) equipped with a 0.2-µm filter (Minisart, Sartorius Stedim Biotech, Germany). Following filtration, the solution was plated to verify that the bacteria that grew on nonfiltered *F. alnus* had been removed.

A 5-mm-diameter *P. infestans* mycelial plug from a freshly cultivated colony (within 10 to 14 days) was placed upside down in the center of each Petri dish (9 cm diameter; Greiner Bio-one, Switzerland). Six concentric wells were constructed 15 mm from the dishes' center and were filled with 25 µl of the test substance, of which only one was used on each Petri dish. Each test substance was repeated in eight dishes. Any dishes that showed contamination were later excluded from the analysis except for the *Erwinia* spp., which grew in the treatments of the nonfiltered aqueous extractions of *F. alnus*. A second iteration was run with a subset of the treatments, including the negative control (autoclaved deionized water), copper (full), *Frangula* low, and *Frangula* low (filtered). After 7 days, the mycelial growth of *P. infestans* from the original plug was quantified as the percentage of mycelial coverage on each dish. For the measurements, the whole plate area was quantified with Image J (Schneider et al. 2012), and a modified ImageJ macro (Laflamme et al. 2016) was used to measure the area of a defined color threshold in pixels (see Supplementary Materials). The experiments were normalized based on a comparison between each treatment's mycelial growth to its negative water control within each experiment and then combined for statistical analysis. The difference between the mycelial coverage in the treatment and the negative water control was used to determine the treatments' growth inhibition.

Detached leaf assay with filtered and nonfiltered *F. alnus* extracts

Because bacterial growth was observed following plating *F. alnus* solutions on rye agar (see the in vitro experiment described above and Supplementary Materials for more information), detached leaf assays were run to determine whether filtered and nonfiltered *F. alnus* extracts differed in their efficacy in controlling *P. infestans*. Only the high dosage of *F. alnus* from the field experiment was included in the experiment with a positive control (copper) and negative control (autoclaved deionized water). A filtered bacterial-free extraction of high-dosage *F. alnus* was prepared as described in the in vitro experiment, and the same protocols for the detached leaf setup were followed as described in the detached leaf experiments focusing on dosage and *F. alnus* extraction and application timing. The assay was conducted two times with replicates of 10 leaves per treatment in each assay.

Leaves of the Bintje variety were placed individually in a Petri dish (125 mm, Semadeni AG, Switzerland) on a 7 × 7 plastic mesh lined with paper towels, and 8 ml of tap water was added to the bottom of each Petri dish to keep the leaves fresh. The upper side of the leaf was sprayed with 1.5 ml of the prepared treatments at 1 bar and incubated in a climate chamber at 18°C with 14 h day light and 75% humidity followed by 10 h darkness and 85% humidity. One day after treatment application, the leaves were infected at four points with a 30 µl *P. infestans* sporangia suspension. The spore suspension was prepared as described above with a final concentration of 1.60×10^5 sporangia per ml for the first assay (assay A) and 1.75×10^5 sporangia per ml for the second assay (assay B). One week after inoculation, the percentage of the infected leaf area was visually assessed as described above.

Chemical profiling and anthraquinone quantification from *F. alnus* bark

To investigate the chemical profile and determine the putative active compounds that may be involved in the *F. alnus* extracts' control of *P. infestans* in the field, freshly prepared extracts used in the field experiments were immediately frozen (within 30 min) at −20°C for later analysis. In total, five preparations of the medium dosage, low dosage and low FD dosage from two applications in 2021 and three applications in 2022 were analyzed. Additionally, the preparations from the aqueous extraction time experiment were profiled.

All preparations were thawed, filtered through a 22-µm filter and directly injected on a Vanquish Horizon ultra-high-performance liquid chromatography (UHPLC) system with charged aerosol detection (CAD) (ThermoFisher, Waltham, MA, U.S.A.). The UHPLC-CAD system also included a degasser, a mixing pump, an autosampler, and a diode array detector. Samples were separated on an Atlantis BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters, Milford, MA, U.S.A.) using a mobile phase consisting of nanopure water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). To obtain a chromatographic profile of the extract, a separation was performed using a step gradient of 5 to 40% B in 30 min, from 40 to 100% in 5 min, and isocratic at 100% for 3 min at a flow rate of 0.3 ml/min. To quantify frangulins and emodin, the separation was performed using a step gradient from 15 to 30% B in 4 min, isocratic at 30% B for 13 min, from 40 to 100% in 10 min, and held at 100% B for 4 min at a flow rate of 0.3 ml/min. Detection was performed at 210, 254, 280, 350 and 435 nm, and the charged aerosol detector conditions were the following: evaporator temperature set at 35°C and the data collection rate set at 10 Hz.

Statistical analysis

All data analyses were conducted in R (version 4.4.0) (R Core Team 2023). The area under the disease progress curve (AUDPC) was calculated using the R package 'agricolae' (de Mendiburu 2023). For field experiments, disease severity based on the AUDPC and marketable and total yields were compared separately for each year using linear mixed models with the experimental blocks as a random factor and variety, treatment, and their interaction as fixed factors using the R package 'nlme' (Pinheiro et al. 2023). The residual values were checked for normality and homoscedasticity using Q-Q and scale-location plots, respectively. To better fit the assumption of normality, the yield data were logarithmically transformed. Pairwise comparisons between treatments were performed using the estimated marginal means, and Tukey's honest significant difference tests were used to adjust *P* values in the 'emmeans' package (Lenth et al. 2024). The compact letter display function in R package 'multcomp' was used to display differences between groups (Hothorn et al. 2008). The measurements of compounds extracted from the *F. alnus* preparations were compared using linear mixed models as above, and these data were logarithmically transformed before analysis to account for the deviation from normality.

The climate chamber experiment on extraction duration and spraying regime was analyzed with the Kruskal-Wallis rank sum test to explore the differences between *F. alnus* and control treatments as well as pairwise differences within the experiment. To assess the effects of the *F. alnus*'s extraction time, treatment application regime, and their interaction, they were included in a zero-inflated negative binomial model using the 'glmmTMB' package (Brooks et al. 2017) with experimental blocks as random factors. All other laboratory experiments, including the detached leaf tests focusing on effects of *F. alnus* dosage and *F. alnus* filtration as well as the mycelia growth inhibition tests were analyzed with Kruskal-Wallis tests, followed by Conover's all-pairs rank comparison tests using the R package 'PMCMRplus' (Pohlert 2018) with the Benjamini and Hochberg adjustment for multiple comparisons (Benjamini and Hochberg 1995).

Results

Field experiments

The 2021 potato growing season was the most conducive for disease development followed by 2020. Accordingly, disease severity was highest in 2021, followed by 2020 and 2019, and in 2022 there was very little PLB (Fig. 1). In all years, the treatment had a significant effect on the AUDPC, whereas variety had a significant effect on the AUDPC in years 2019 and 2021, and no interaction between the treatments and variety was found in any of the years (Table 2). The *F. alnus* treatments showed significantly reduced AUDPCs compared with the water control (Fig. 2) except in 2021 when high amounts of precipitation led to increased disease pressure (Supplementary Fig. S2; Supplementary Table S1). In the field, the reduced copper did not significantly differ from the full copper treatment. The marketable and total yields showed significant variety effects in all four years but only significant treatment effects in 2019 and 2020. Compared with the copper treatments, the 'Victoria' plots treated with the high dosage of *F. alnus* showed

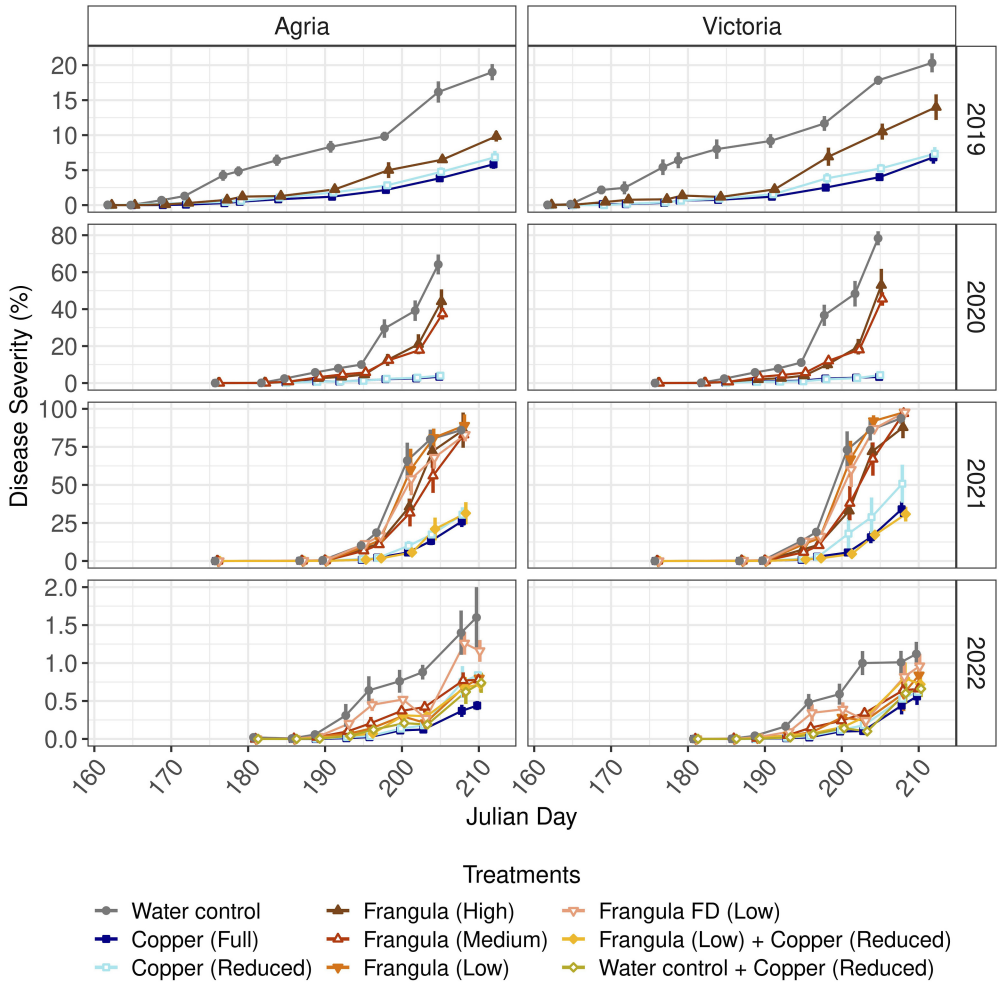

Fig. 1. Disease severity of potato late blight epidemics in two varieties from field experiments between 2019 and 2022. Treatments are described in Table 1. The *y*-axis scale changes according to the year, showing the high variation in disease severity across years. Bars indicate standard error of the means. FD = freeze-dried.

a trend of reduced yields in 2019 and 2020, which was found to be significant in marketable yield in 2019 and total yield in 2020 (Fig. 3). The cultivar Agria had no yield reduction nor did its medium dosage treatment when applied in 2020. In 2021 and 2022, the yields were not different among the treatments within each variety. Treatment and variety interactions did not have a significant effect on either marketable or total yield (Table 2).

At the time of harvest, tubers infected with late blight were found only in 2021 (0 to 4 infected tubers per treatment and variety were found in the 240 to 250 tubers analyzed per treatment and variety). There was no difference in the number of infected tubers across the treatments ($\chi^2 = 10.88$, df = 7, $P = 0.144$) or varieties ($\chi^2 = 2.15$, df = 1, $P = 0.142$). In all years, no tuber blight was found in the 3,941 tubers examined 1 month after harvest from all treatments.

F. alnus extraction duration experiment on detached leaves

The detached 'Bintje' leaves that had been treated with *F. alnus* were significantly less infected with *P. infestans* compared with the negative water controls ($\chi^2 = 38.08$, df = 5, $P < 0.001$). There was no difference between *F. alnus* treatments when the extraction time was 30 min or 120 min based on pairwise comparisons (Fig. 4). Between the *F. alnus* treatments within the experiment, neither the treatment timing (1 day, 2 days, and both 1 and 2 days before inoculation) ($\chi^2 = 2.62$, df = 2, $P < 0.270$) nor the extraction time ($\chi^2 = 0.312$, df = 1, $P < 0.576$) affected the infection rate. There was also no interaction between treatment timing and the *F. alnus* extraction time ($\chi^2 = 2.44$, df = 2, $P < 0.295$). Furthermore, the UHPLC-CAD profile of the *F. alnus* treatments did not show major differences in extracted compounds between the different extraction times, although quantities of compounds extracted differed (see section below on the chemical analysis of *F. alnus* for more details). Because no difference was found in treatment efficacy based on extraction time, the extraction time for *F. alnus* treatments in the field experiments was reduced to 30 min starting in 2021.

F. alnus dosage experiment on detached leaves

Based on detached leaf assays, the Kruskal-Wallis test revealed a difference among *F. alnus* extracts and resulting disease severity ($\chi^2 = 33.98$, df = 5, $P < 0.001$). Dosages less than 2.5 kg/ha (Fig. 5) were significantly less effective than the dosages that were equal to or greater than 2.5 kg/ha. There were no significant differences in disease severity among the highest three dosages (2.5, 25, and 250 kg/ha). The disease severity found in the treatments of the lowest two dosages (0.025 and 0.25 kg/ha) did not differ from the water control. The IC_{50}, indicating the dosage at which *F. alnus* inhibits half of the *P. infestans* infection that it is maximally capable of, was found to be 1.253 kg/ha. At 2.5 kg/ha, 69.4% of the maximum inhibitory potential of *P. infestans* was reached based on this dosage experiment.

In vitro mycelial growth experiment

The mycelia of *P. infestans* showed significantly different levels of growth inhibition depending on the treatment ($\chi^2 = 88.35$, df = 10, $P < 0.001$).

Table 2. Analysis of variance table of the linear mixed-effects models of experimental treatment, variety, and their interaction on disease pressure and marketable and total yields from field trials between 2019 and 2022

Response variable[a]	Year	Factor	df (df1, df2)	*F*-value	*P*-value
AUDPC	2019	Treatment	3, 35	140.82	<0.001[b]
		Variety	1, 35	7.33	0.010[b]
		Treatment × variety	3, 35	1.47	0.239
	2020	Treatment	4, 45	63.43	<0.001[b]
		Variety	1, 45	0.76	0.389
		Treatment × variety	4, 45	0.69	0.605
	2021	Treatment	7, 60	40.44	<0.001[b]
		Variety	1, 60	3.46	0.068
		Treatment × variety	7, 60	0.34	0.931
	2022	Treatment	7, 60	28.68	<0.001[b]
		Variety	1, 60	7.08	0.010[b]
		Treatment × variety	7, 60	0.83	0.565
Marketable yield	2019	Treatment	3, 35	4.99	0.006[b]
		Variety	1, 35	5.58	0.024[b]
		Treatment × variety	3, 35	1.41	0.257
	2020	Treatment	4, 45	3.65	0.012[b]
		Variety	1, 45	11.77	0.001[b]
		Treatment × variety	4, 45	1.17	0.333
	2021	Treatment	7, 60	0.53	0.810
		Variety	1, 60	64.83	<0.001[b]
		Treatment × variety	7, 60	0.83	0.568
	2022	Treatment	7, 60	1.44	0.206
		Variety	1, 60	10.78	0.002[b]
		Treatment × variety	7, 60	0.24	0.975
Total yield	2019	Treatment	3, 35	3.62	0.023[b]
		Variety	1, 35	69.89	<0.001[b]
		Treatment × variety	3, 35	1.13	0.351
	2020	Treatment	4, 45	6.46	<0.001[b]
		Variety	1, 45	18.83	<0.001[b]
		Treatment × variety	4, 45	1.25	0.303
	2021	Treatment	7, 60	0.98	0.452
		Variety	1, 60	12.45	<0.001[b]
		Treatment × variety	7, 60	0.62	0.739
	2022	Treatment	7, 60	1.31	0.263
		Variety	1, 60	15.40	0.001[b]
		Treatment × variety	7, 60	0.11	0.998

[a] AUDPC = area under the disease progress curve.
[b] Significant after correction for multiple comparisons.

There were no differences in mycelial growth on plates that received the autoclaved deionized water control, the filtered *F. alnus* low treatment, and both filtered and nonfiltered *F. alnus* FD treatments (Fig. 6). For the high, medium, and low *F. alnus* treatments that had been prepared by aqueous extraction, there was significantly greater mycelial inhibition compared with their respective filtered treatments of the same dosages. The nonfiltered medium and high *F. alnus* treatments that resulted in the growth of the *Erwinia* spp. inhibited mycelial growth more than the filtered treatments of the same dosages (Fig. 7). For filtered treatments prepared with an aqueous extraction, the low *F. alnus* dosage showed decreased efficacy compared with the medium and high *F. alnus* treatments. The full copper treatment showed an increase in mycelial inhibition compared with the reduced copper treatment but no difference from the medium and high nonfiltered *F. alnus* treatment where bacterial colonization of the *Erwinia* spp. was observed.

Detached leaf assay with filtered *F. alnus*

A detached leaf assay (assay A) was run to test the difference between the filtered and nonfiltered *F. alnus* treatments that had been prepared with an aqueous extraction method. The assay was then run a second time (assay B) to determine the consistency of the results. There were differences among treatments in both assay A ($\chi^2 = 29.133$, df = 3, $P = 0.001$) and assay B ($\chi^2 = 35.648$, df = 3, $P = 0.001$). The autoclaved deionized water control showed significantly higher rates of disease severity in both assays (Fig. 8), whereas the copper treatment yielded a significantly lower disease severity than the *F. alnus* treatments in assay A. In assay B, copper and *F. alnus* treatments showed either low or no *P. infestans* infections, and the treatments were no different from each other.

Chemical analysis of *F. alnus* extracts

The UHPLC-CAD profile of the different dosages of the aqueous suspension of *F. alnus* (medium and low dosage) showed a similar chemical composition, differing only on the concentration of the extracted compounds. However, the FD low dosage showed that the filtering and freeze-drying step concentrates the extract on polar compounds, thus reducing the relative quantity of semipolar and apolar compounds (i.e., anthraquinones). Indeed, the quantification of the main constitutive anthraquinones differed among *F. alnus* dosage amounts (Fig. 9, frangulin A: $F_{(2,8)} = 299.190$, $P < 0.001$, frangulin B: $F_{(2,8)} = 205.947$, $P < 0.001$, emodin: $F_{(2,8)} = 20.928$, $P < 0.001$). As expected, the medium dosage consistently yielded more anthraquinones than the low dosage. The medium dosage included 10 times more ground *F. alnus* bark than the low dosage, but it proportionally yielded more frangulin A and frangulin B (17 to 18 times more) than the low dosage, and proportionally less emodin (only five times more). The low dosages with the lyophilization step (FD low) also yielded comparatively lower compounds than both aqueous extractions (2.5 times less than the low dosage). Although the duration of the aqueous extraction did not change the efficacy of the *F. alnus* treatments (Fig. 4), the 30-min extraction appeared to yield more frangulin A and frangulin B than the 120-min extraction but similar levels of emodin (Supplementary Fig. S4). Based on two

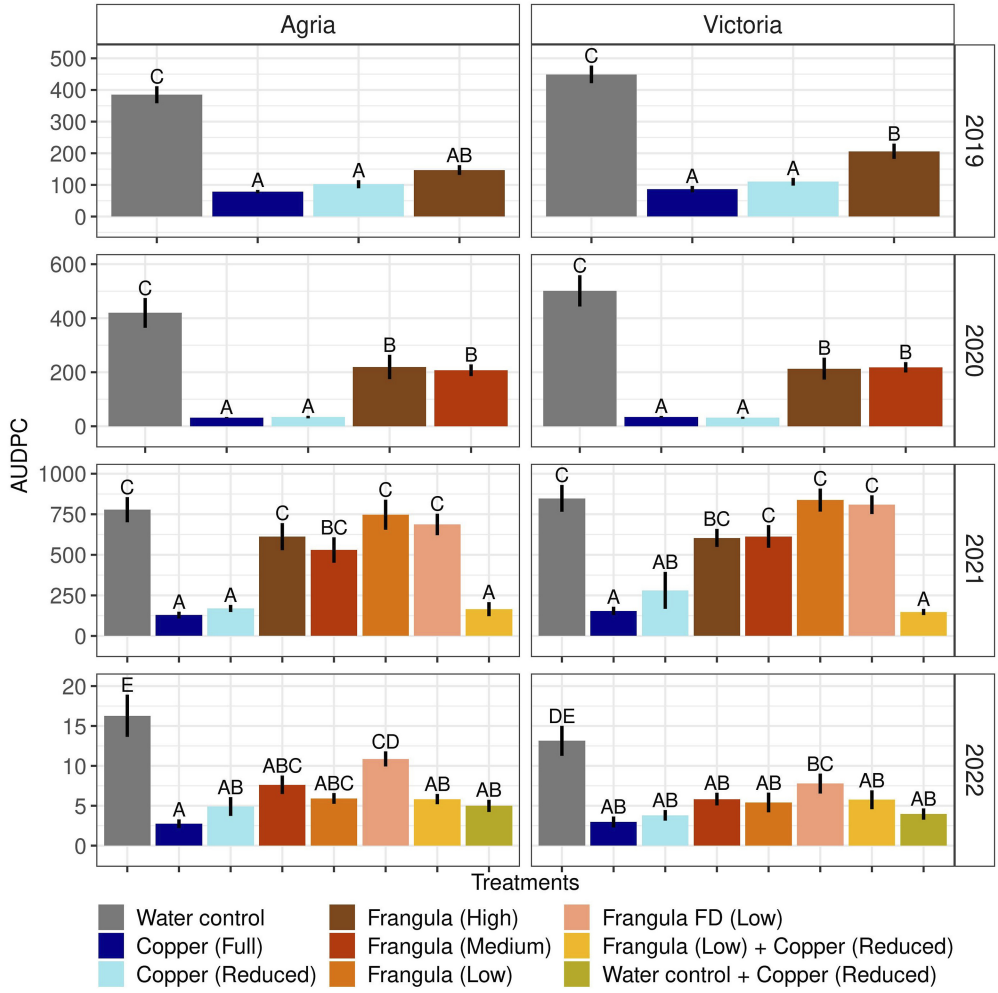

Fig. 2. Area under the disease progress curve (AUDPC) of potato late blight in two varieties from field experiments between 2019 and 2022, showing treatment by color as described in Table 1. The scale of the *y*-axis varies according to the year, reflecting the substantial variation in disease severity, particularly from 2021 to 2022. Treatments that do not share grouping letters were shown to be significantly different from each other. Bars indicate the standard error of the mean. FD = freeze-dried.

measurements of the *F. alnus* preparation following both extraction periods, the 30-min extraction yielded on average four times more frangulin A (100.4 µg/ml versus 23.5 µg/ml) and almost three times more frangulin B (52.7 µg/ml versus 18.2 µg/ml) than the 120-min extraction.

Discussion

P. infestans is difficult to control because of its ability to evolve and evade host resistance (Leesutthiphonchai et al. 2018). In countries that still authorize copper as a plant protection product, it effectively controls PLB on organic farms owing to its multisite mode of action (La Torre et al. 2018; Tamm et al. 2022). However, because its residues persist and can detrimentally affect soil organisms, countries that still allow its use seek avenues to reduce it (Möhring et al. 2020; Silva et al. 2022). In 12 European countries, 56% of producers already use half of the allowable amount of copper (Tamm et al. 2022), showing that the reduction is feasible and potentially could be more broadly adopted.

Our research contributes additional evidence that shows using only half of the allowable copper (2 kg/ha/year) compared with the full allowable amount in Switzerland (4 kg/ha/year) can result in similar levels of disease control and potato yield. Previous work also showed that reduced copper did not tend to affect yield, which was rather driven by year, site, variety, and disease pressure (Bangemann et al. 2014; González-Jiménez et al. 2023; Wiik 2014). In our experiments, variety influenced marketable and total yield more than treatment. In 2021, the increased disease pressure reduced the yield for both varieties, especially for 'Victoria'. The 'Agria' plots in our study had a lower yield reduction (35%) than the average yield reduction estimated across organic production (57%) in 2021 compared with yields in 2019, 2020, and 2022 (Bio Suisse 2022). On the other hand, the yields in our 'Victoria' plots were reduced by 63%. These varietal differences in yield reduction underscore the role that potato variety selection has in combating PLB's damage; the yield differences between varieties were much greater than any treatment differences in our study.

Treatment had a limited effect on yield and no effect on tuber infections, which may have been because of the relatively late start of the epidemic or low disease pressure in three of the four years (Wiik 2014). The plot size of the experiment was smaller than recommended to measure yield differences (EPPO 2021), which may also play a role in the outcome. The disease severity as measured by AUDPC, on the other hand, fluctuated to a much greater degree among treatments. In the years with low to moderate disease pressure in the field experiments, including 2019, 2020, and 2022, the

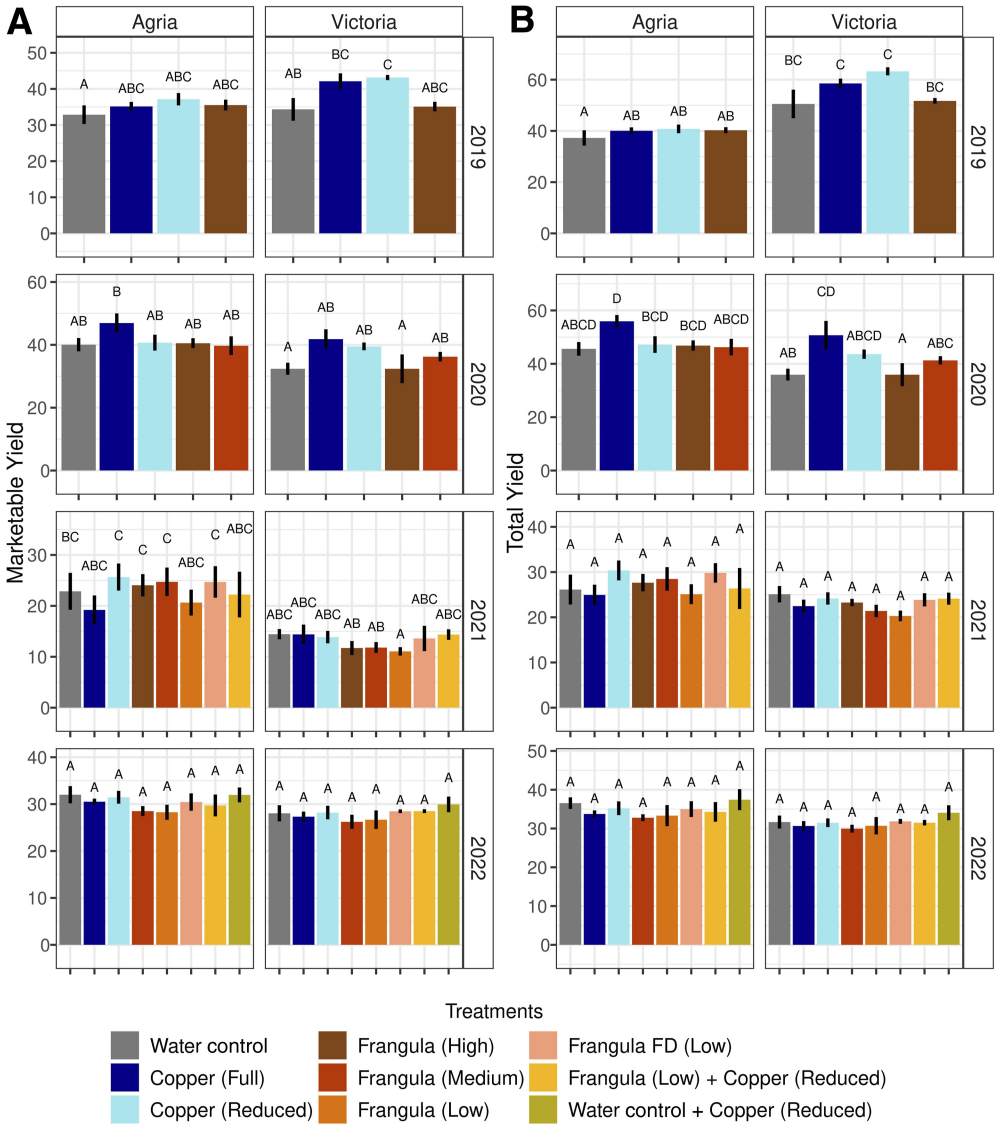

Fig. 3. Yields from potato late blight field experiments between 2019 and 2022, including **A,** marketable yield (tons per ha) of tubers in two varieties (in size classes: 42 to 55 mm and 55 to 70 mm) and **B,** total yield (tons per ha) of tubers in two varieties of all size classes. Treatments are shown by color and described in Table 1. Bars indicate the standard error of the mean. FD = freeze-dried.

F. alnus–treated plots had lower disease severity compared with the water control. The reduction from the *F. alnus* medium and high dosages in the 2020 field experiment confirmed results from former field studies ran in 2010 to 2012 (Forrer et al. 2017). In these experiments, there was no difference in PLB disease severity between a 4% *F. alnus* solution and a reduced copper treatment (0.3 kg/ha per

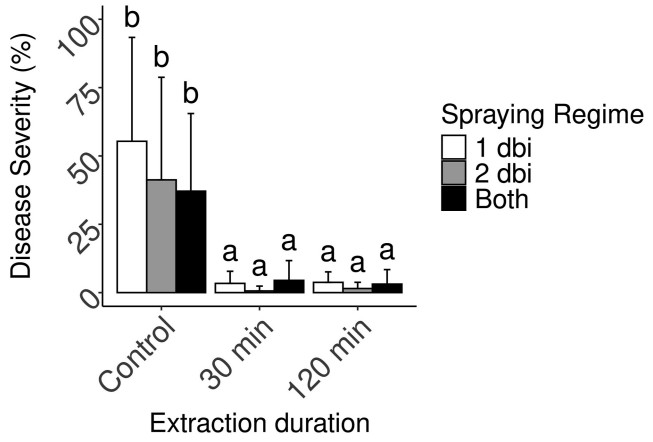

Fig. 4. Disease severity on detached leaves when sprayed with *Frangula alnus* that had been extracted in an aqueous solution for 30 or 120 min compared with a negative control treatment of tap water. The treatments included spraying regimes consisting of 1 day before, 2 days before, and both 1 and 2 days before inoculation (dbi) of *Phytophthora infestans*. Bars indicate the standard error of the mean.

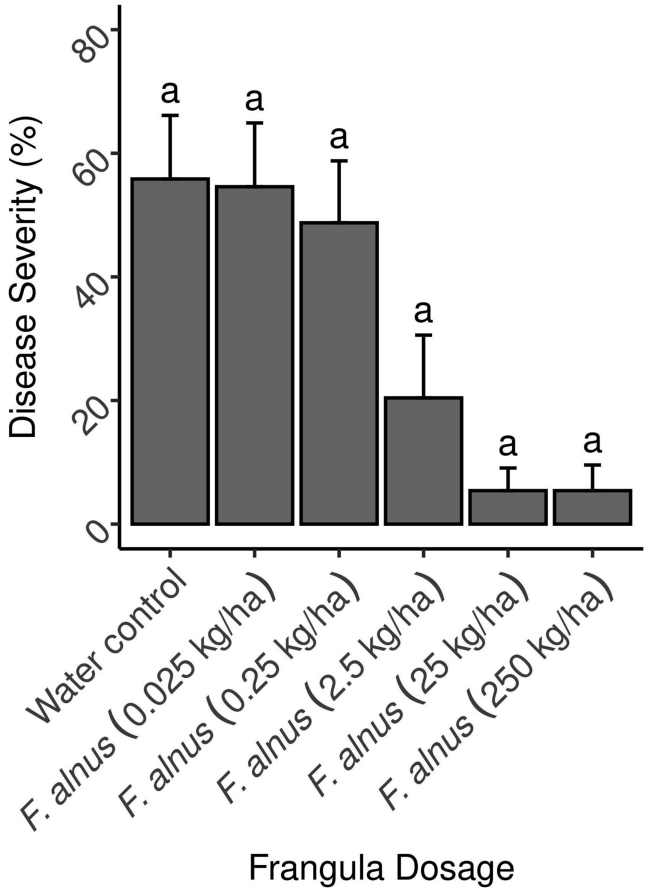

Fig. 5. Disease severity of detached leaves when sprayed with different dosages of *Frangula alnus* compared with a negative control treatment of tap water shown with the standard error of the mean. The 25 kg/ha dosage corresponds to the medium dosage from the 2020 to 2022 field experiments, and 2.5 kg/ha is the same dosage applied to the low *F. alnus* treatment in the field (Table 1).

treatment applied eight times), and the *F. alnus* treatment reduced disease severity by 37% based on the AUDPC.

During the last 2 years of our experiments, we no longer included the *F. alnus* high-dosage treatment because of the lack of economic feasibility. Instead, a low dose, consisting of 2.5 kg/ha was included, which would be in the economic range of copper and fungicide treatments (430 to 580 CHF per season/ha). The low dosage treatment can inhibit 69.4% of the pathogen compared with the maximal inhibitory concentration of *F. alnus* based on the laboratory-based dosage experiment. During a high disease pressure year such as 2021, however, the inhibition is not effective at reducing disease severity in the field based on the AUDPC. Because the *F. alnus* extraction has not undergone any subsequent formulation, its leaching may also have contributed to its inability to inhibit *P. infestans*. Nonetheless, it reduced disease severity based on the AUDPC in 2022 along with all *F. alnus* dosages, although it was a year with minimal PLB pressure.

In 2021, only copper-containing treatments showed a significant reduction in PLB, including one treatment (treatment 8) that switched from low dosage *F. alnus* to copper treatments on the fifth spraying application, resulting in less copper use overall. This sequential treatment, during which an *F. alnus* solution was first sprayed until disease pressure was elevated, followed by a switch to a low-dose copper treatment midway through the season, resulted in a 70% reduction in copper application compared with the full dosage (i.e., the maximum application allowed in Switzerland per year with 10 applications). Therefore, delayed application of reduced dosages (treatments 8 and 9) could help producers reach targets of reduced copper applications. Further work is needed to demonstrate whether the *F. alnus* gives an added benefit or copper's delayed application is sufficiently protective without any early *F. alnus* treatments. However, *F. alnus* clearly reduces *P. infestans* infection as

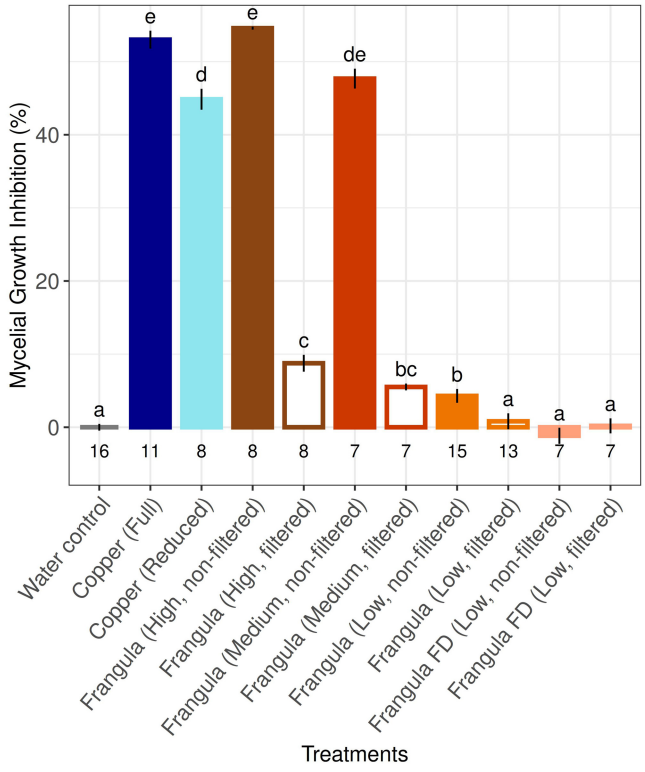

Fig. 6. Mycelial growth inhibition in vitro experiments on rye agar. Measurements of the mycelial area of all treatments were compared with the negative water control that had received autoclaved water. The reduction of mycelial area shows the growth inhibition owing to the direct efficacy of the treatment on *Phytophthora infestans*. Treatment descriptions are found in Table 1. The numbers below the *x*-axis represent the number of plates included in each treatment. Bars indicate the standard error of the mean. FD = freeze-dried.

demonstrated through the field studies in years of low to moderate PLB severity and reinforced by our laboratory experiments. Previous work has focused on mixed treatments that are sprayed on the same date (Liljeroth et al. 2010; Stridh et al. 2022), and there is less information about sequentially used treatments.

The laboratory studies provided a controlled environment to gain further information about the mode of action and effect of different spraying regimes and application dosages. The in vitro work was performed to understand if *F. alnus* directly inhibits *P. infestans* mycelial growth. When the nonfiltered *F. alnus* aqueous extractions were plated, the *Erwinia* spp. associated with the *F. alnus* bark established on the plate and directly inhibited or competed with *P. infestans*. The bacterium did not grow from the *F. alnus* extracted through lyophilization (treatment 7, low dose, FD), neither on the filtered extracts nor on the copper and water control plates. The effect of the bacterium originating from the *F. alnus* bark on *P. infestans* in the in vitro experiment also suggests that botanical applications may act not only through the chemical compounds from the plant but, depending on the extraction method, potentially also through their microbiota. The microbiota of plants can provide protection against pathogens (Vogel et al. 2021), and perhaps the application of botanical plant protection products could also transfer protective microbial constituents through targeted microbiome transplants (Chock et al. 2021). However, it is unclear to what extent the

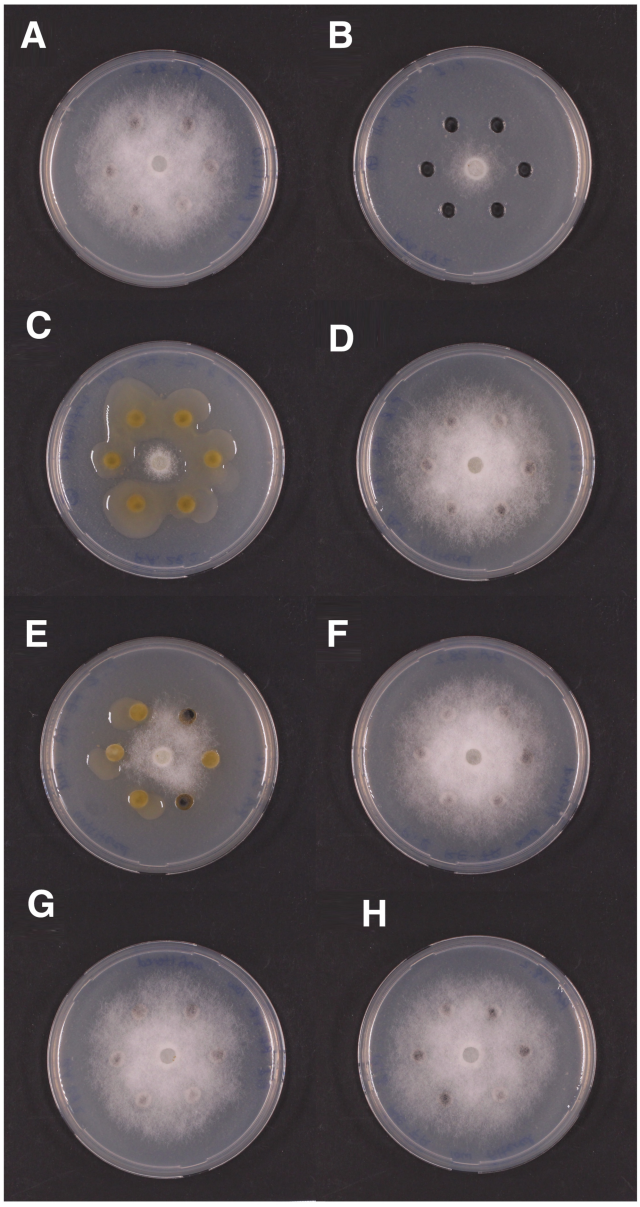

Fig. 7. Pictures from in vitro assays to assess treatment inhibition of *Phytophthora infestans* mycelial growth. Treatments shown include **A,** negative water control; **B,** copper (full) dosage; **C,** *Frangula alnus* high dosage, nonfiltered; **D,** *F. alnus* high dosage, filtered; **E,** *F. alnus* medium dosage, nonfiltered; **F,** *F. alnus* medium dosage, filtered; **G,** *F. alnus* low dosage, nonfiltered; and **H,** *F. alnus* low dosage, filtered. The bacterium associated with the *F. alnus* bark that colonized the plates in treatments shown in panels C and E was identified to be an *Erwinia* spp.

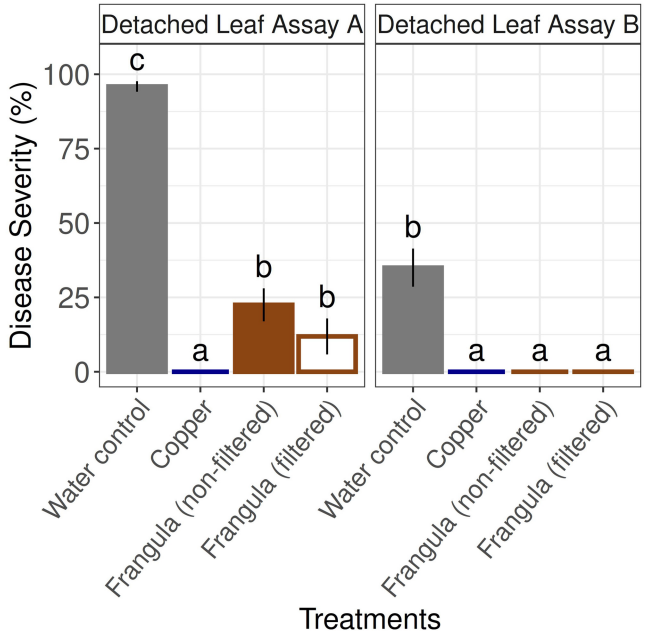

Fig. 8. Two assays (assay A and assay B) were run on detached leaves in which *Frangula alnus* that had been filtered was compared with *F. alnus* that had not been filtered. The high dosage of *F. alnus* was used for this experiment. Copper was included as a positive control. Bars indicate the standard error of the mean.

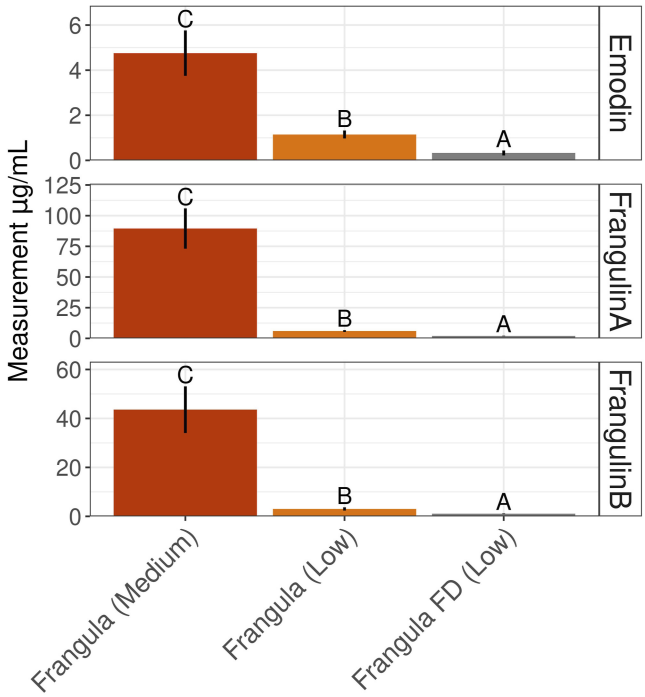

Fig. 9. Chromatographic measurements of compounds extracted from *Frangula alnus* preparations that were associated with potato late blight reductions. The medium dosage corresponds to 25 g/ha and the low dosage to 2.5 g/ha from the ground bark. The freeze-dried (FD) extraction included a lyophilization step whereas the other extractions were stirred at room temperature in tap water for 30 min. Bars indicate the standard error of the mean.

microbiota of *F. alnus* treatment actually establishes on the potato plant, and further work would need to directly assess the colonization ability of a treatment's microbiota. Additionally, it is also unclear how widespread *Erwinia* spp. co-occurs in *F. alnus* bark. Previous work, in which *F. alnus* extracts were plated, mentioned no bacterial colonization (Forrer et al. 2017). Nonetheless, the possible transmission of microbiota could potentially influence a botanical treatment's effect and should be considered in future studies.

To disentangle the PLB mycelial inhibition from the bacterium and *F. alnus*'s chemical compounds, the extracts from all treatments were added to the in vitro assays either after filtration to eliminate the bacterium or directly without filtration. These in vitro results suggest that the chemical compounds themselves provided minimal direct inhibition to *P. infestans* mycelial growth compared with the bacterium associated with the bark, which appears to provide most of the direct in vitro inhibition. These findings corroborate previous results that showed *F. alnus* only slightly inhibited mycelial growth (Forrer et al. 2017). However, it was found that *F. alnus* can inhibit sporangial germination of *P. infestans* (Forrer et al. 2017). Indeed, several anthraquinones and their analogs have been shown to possess antifungal activity against several phytopathogens (Barilli et al. 2022; Choi et al. 2004; Kim et al. 2004), and the presence of these compounds in the *F. alnus* extracts might account for decreased *P. infestans* through direct mechanism such as decreased sporangial germination and slightly decreased mycelial growth.

Unlike in vitro tests, both filtered and unfiltered *F. alnus* treatments resulted in significantly reduced *P. infestans* disease severity on detached leaves. The filtered *F. alnus*, which had a much lower direct effect on *P. infestans* in vitro, showed as much efficacy as the nonfiltered extraction in detached leaf assays, pointing to the possible involvement of induced resistance (Forrer et al. 2017). Anthraquinones have also been shown to induce resistance in plants (Konstantinidou-Doltsinis and Schmit 1998), and other chemical families present in the *F. alnus* extracts, such as polyphenols, have been implicated in defense induction (Gillmeister et al. 2019). In *Vitis vinifera*, the application of *F. alnus* bark extract induces the production of the phytoalexins piceid, resveratrol, and pterostilbene and protects against infection of the oomycete *Plasmopara viticola* (Godard et al. 2009). Plant elicitation can complement direct antifungal activity if deployed in the right situations to avoid reduced yields (Heil et al. 2000); therefore, genotypic and environmental variables should be evaluated before applying elicitors because these factors can influence their success (Walters et al. 2013). For example, previous research with *F. alnus* showed a varietal difference in yield (Forrer et al. 2017), which may be because of the way in which a potato genotype responds to an elicitor (Bruce 2014) and may also account for the varietal differences we see in yield in our field experiments.

Current obstacles to adoption include the cost of the material, effort required for the preparations, and stability of the extraction. To further develop an effective and potentially cost-effective treatment, more information about the compounds involved could better elucidate the mode of action. Frangulin A, frangulin B, and emodin were found in the *F. alnus* extract, but it is not clear which chemicals are responsible for the direct or indirect inhibition. Singularly testing each compound would help decipher how they individually and cumulatively control PLB. These steps could help determine whether a stable formulation can be achieved to obtain cost-effective treatment. Therefore, many steps are still required (i.e., an understanding of kinetic degradation of the active compounds, stability in solution and on foliage, and optimal formulations) to achieve a viable PLB spraying regime that incorporates *F. alnus* or its components.

Our work suggests that copper application amount could be substantially reduced based on the low-dose copper treatment's results that showed no yield differences from the full-dose treatment. Potentially, a low-dose copper treatment could be supplemented with alternative products, such as *F. alnus*, but it is not yet clear if the botanical gives added value. Further testing of sequential deployments of different treatments (i.e., switching from an alternative treatment, such as a botanical or another plant elicitor, to copper) can also help contribute to overall copper reduction. The success of such strategies would be dependent on the response of the variety to plant elicitation, and the timing of the epidemic may also very much impact their success. Reductions in copper application combined with the deployment of resistant varieties (Kessel et al. 2018) can help achieve goals of reduced pesticide targets (Purnhagen et al. 2021; Silva et al. 2022). Owing to the importance of potato production in Europe, a combination of strategies is necessary to combat PLB.

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Literature Cited

Andrivon, D., Bardin, M., Bertrand, C., Brun, L., Daire, X., Fabre, F., Gary, C., Montarry, J., Nicot, P., Reignault, P., Tamm, L., and Savini, I. 2018. Can organic agriculture cope without copper for disease control? Synthesis of the collective scientific assessment report. INRA, France. https://www.inrae.fr/sites/default/files/pdf/can-organic-agriculture-cope-without-copper-for-disease-control-synthesis-of-the-collective-scientific-assessment-report-1.pdf

Bangemann, L.-W., Westphal, A., Zwerger, P., Sieling, K., and Kage, H. 2014. Copper reducing strategies for late blight (*Phytophthora infestans*) control in organic potato (*Solanum tuberosum*) production. J. Plant Dis. Prot. 121:105-116.

Barilli, E., Agudo, F. J., Masi, M., Nocera, P., Evidente, A., and Rubiales, D. 2022. Anthraquinones and their analogues as potential biocontrol agents of rust and powdery mildew diseases of field crops. Pest Manag. Sci. 78:3489-3497.

Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Stat. Method 57:289-300.

Bio Suisse. 2022. Market reflections organic potatoes (in German). https://www.bioaktuell.ch/fileadmin/documents/ba/Markt/Kartoffeln/2022_09_Marktspiegel_Kartoffeln.pdf (accessed 7 October 2024).

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., and Bolker, B. M. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9:378-400.

Bruce, T. J. A. 2014. Variation in plant responsiveness to defense elicitors caused by genotype and environment. Front. Plant Sci. 5:349.

Cao, K. Q., Ruckstuhl, M., and Forrer, H. R. 1997. Crucial weather conditions for *Phytophthora infestans*: A reliable tool for improved control of potato late blight? Pages 85-90 in: PAGV-Special Report. Proceedings of the Workshop on the European Network for Development of an Integrated Control Strategy of Potato Late Blight, vol. 1. E. Bouma and H. Schepers, eds. Research Station for Arable Farming and Field Production of Vegetables, Lelystad, the Netherlands.

Chock, M. K., Hoyt, B. K., and Amend, A. S. 2021. Mycobiome transplant increases resistance to *Austropuccinia psidii* in an endangered Hawaiian plant. Phytobiomes J. 5:326-334.

Choi, G. J., Lee, S.-W., Jang, K. S., Kim, J.-S., Cho, K. Y., and Kim, J.-C. 2004. Effects of chrysophanol, parietin, and nepodin of *Rumex crispus* on barley and cucumber powdery mildews. Crop Prot. 23:1215-1221.

Dagostin, S., Formolo, T., Giovannini, O., Pertot, I., and Schmitt, A. 2010. *Salvia officinalis* extract can protect grapevine against *Plasmopara viticola*. Plant Dis. 94:575-580.

de Mendiburu, F. 2023. agricolae: Statistical procedures for agricultural research. R package version 1.3-6. https://cran.r-project.org/web/packages/agricolae/index.html

Dorn, B., Musa, T., Krebs, H., Fried, P. M., and Forrer, H. R. 2007. Control of late blight in organic potato production: Evaluation of copper-free preparations under field, growth chamber and laboratory conditions. Eur. J. Plant Pathol. 119:217-240.

EPPO. 2021. PP 1/002 (5) *Phytophthora infestans* on potato. EPPO Bull. 51:79-82.

Evenhuis, A. 2021. Efficacy to Control Potato Late Blight by Applying Biological Crop Protection Products: EuroBlight Field Experiment AGV8005. Wageningen Plant Research, Wageningen, the Netherlands.

Forbes, G., Perez, W., and Andrade-Piedra, J. L. 2014. Field Assessment of Resistance in Potato to *Phytophthora infestans*: International Cooperators Guide. International Potato Center, La Molina, Peru.

Forrer, H.-R., Vogelgsang, S., and Musa, T. 2017. Botanicals and phosphonate show potential to replace copper for control of potato late blight. J. Fungi 3:65.

Gillmeister, M., Ballert, S., Raschke, A., Geistlinger, J., Kabrodt, K., Baltruschat, H., Deising, H. B., and Schellenberg, I. 2019. Polyphenols from *Rheum* roots inhibit growth of fungal and oomycete phytopathogens and induce plant disease resistance. Plant Dis. 103:1674-1684.

Gilroy, E. M., Breen, S., Whisson, S. C., Squires, J., Hein, I., Kaczmarek, M., Turnbull, D., Boevink, P. C., Lokossou, A., Cano, L. M., Morales, J., Avrova, A. O., Pritchard, L., Randall, E., Lees, A., Govers, F., van West, P., Kamoun, S., Vleeshouwers, V. G. A. A., Cooke, D. E. L., and Birch, P. R. J. 2011. Presence/absence, differential expression and sequence polymorphisms between *PiAVR2* and *PiAVR2-like* in *Phytophthora infestans* determine virulence on *R2* plants. New Phytol. 191:763-776.

Godard, S., Slacanin, I., Viret, O., and Gindro, K. 2009. Induction of defence mechanisms in grapevine leaves by emodin- and anthraquinone-rich plant extracts and their conferred resistance to downy mildew. Plant Physiol. Biochem. 47:827-837.

González-Jiménez, J., Andersson, B., Wiik, L., and Zhan, J. 2023. Modelling potato yield losses caused by *Phytophthora infestans*: Aspects of disease growth rate, infection time and temperature under climate change. Field Crops Res. 299:108977.

Heil, M., Hilpert, A., Kaiser, W., and Linsenmair, K. E. 2000. Reduced growth and seed set following chemical induction of pathogen defence: Does systemic acquired resistance (SAR) incur allocation costs? J. Ecol. 88:645-654.

Hothorn, T., Bretz, F., and Westfall, P. 2008. Simultaneous inference in general parametric models. Biom. J. 50:346-363.

Katsoulas, N., Løes, A.-K., Andrivon, D., Cirvilleri, G., de Cara, M., Kir, A., Knebl, L., Malińska, K., Oudshoorn, F. W., Willer, H., and Schmutz, U. 2020. Current use of copper, mineral oils and sulphur for plant protection in organic horticultural crops across 10 European countries. Org. Agric. 10:159-171.

Keijzer, P., van Bueren, E. T. L., Engelen, C. J. M., and Hutten, R. C. B. 2022. Breeding late blight resistant potatoes for organic farming—A collaborative model of participatory plant breeding: The Bioimpuls project. Potato Res. 65:349-377.

Kessel, G. J. T., Mullins, E., Evenhuis, A., Stellingwerf, J., Cortes, V. O., Phelan, S., van den Bosch, T., Förch, M. G., Goedhart, P., van der Voet, H., and Lotz, L. A. P. 2018. Development and validation of IPM strategies for the cultivation of cisgenically modified late blight resistant potato. Eur. J. Agron. 96:146-155.

Kim, J.-C., Choi, G. J., Lee, S.-W., Kim, J.-S., Chung, K. Y., and Cho, K. Y. 2004. Screening extracts of *Achyranthes japonica* and *Rumex crispus* for activity against various plant pathogenic fungi and control of powdery mildew. Pest Manag. Sci. 60:803-808.

Konstantinidou-Doltsinis, S., and Schmit, A. 1998. Impact of treatment with plant extracts from *Reynoutria sachalinensis* (F. Schmidt) Nakai on intensity of powdery mildew severity and yield in cucumber under high disease pressure. Crop Prot. 17:649-656.

Krebs, H., Dorn, B., and Forrer, H. R. 2006. Control of late blight of potato with medicinal plant suspensions. Agric. Res. 13:16-21 (in German).

Laflamme, B., Middleton, M., Lo, T., Desveaux, D., and Guttman, D. S. 2016. Image-based quantification of plant immunity and disease. Mol. Plant-Microbe Interact. 29:919-924.

La Torre, A. L., Iovino, V., and Caradonia, F. 2018. Copper in plant protection: Current situation and prospects. Phytopathol. Mediterr. 57:201-236.

Leesutthiphonchai, W., Vu, A. L., Ah-Fong, A. M. V., and Judelson, H. S. 2018. How does *Phytophthora infestans* evade control efforts? Modern insight into the late blight disease. Phytopathology 108:916-924.

Lenth, R. V., Banfai, B., Bolker, B., Buerkner, P., Giné-Vázquez, I., Hervé, H., Jung, M., Love, J., Miguez, F., Piaskowski, J., Riebl, H., and Singmann, H. 2024. emmeans: Estimated marginal means, aka least-squares means. R Package Version 1.10.1. https://cran.r-project.org/web/packages/emmeans/emmeans.pdf

Liljeroth, E., Bengtsson, T., Wiik, L., and Andreasson, E. 2010. Induced resistance in potato to *Phytophthora infestans*—Effects of BABA in greenhouse and field tests with different potato varieties. Eur. J. Plant Pathol. 127:171-183.

Martin, F. N., Zhang, Y., Cooke, D. E. L., Coffey, M. D., Grünwald, N. J., and Fry, W. E. 2019. Insights into evolving global populations of *Phytophthora infestans* via new complementary mtDNA haplotype markers and nuclear SSRs. PLoS One 14:e0208606.

Massana-Codina, J. 2020. Epidemiological insights, cultivar resistance and natural products to control black dot (*Colletotrichum coccodes*) and silver scurf (*Helminthosporium solani*) on potato (*Solanum tuberosum*) tubers. PhD dissertation. University of Geneva, Geneva, Switzerland.

Möhring, N., Ingold, K., Kudsk, P., Martin-Laurent, F., Niggli, U., Siegrist, M., Studer, B., Walter, A., and Finger, R. 2020. Pathways for advancing pesticide policies. Nat. Food 1:535-540.

Mulholland, D. A., Thuerig, B., Langat, M. K., Tamm, L., Nawrot, D. A., James, E. E., Qayyum, M., Shen, D., Ennis, K., Jones, A., Hokkanen, H., Demidova, N., Izotov, D., and Schärer, H. J. 2017. Efficacy of extracts from eight economically important forestry species against grapevine downy mildew (*Plasmopara viticola*) and identification of active constituents. Crop Prot. 102:104-109.

Ortiz, R., Reslow, F., Cuevas, J., and Crossa, J. 2022. Genetic gains in potato breeding as measured by field testing of cultivars released during the last 200 years in the Nordic Region of Europe. J. Agric. Sci. 160:310-316.

Pacifico, D., and Paris, R. 2016. Effect of organic potato farming on human and environmental health and benefits from new plant breeding techniques. Is it only a matter of public acceptance? Sustainability 8:1054.

Pandey, J., Scheuring, D. C., Koym, J. W., Endelman, J. B., and Vales, M. I. 2023. Genomic selection and genome-wide association studies in tetraploid chipping potatoes. Plant Genome 16:e20297.

Pinheiro, J., Bates, D., and R Core Team 2023. nlme: Linear and nonlinear mixed effects models. R package version 3.1-163. https://cran.r-project.org/web/packages/nlme/index.html

Pohlert, T. 2018. PMCMRplus: Calculate pairwise multiple comparisons of mean rank sums extended. Version 1.4.2. https://cran.r-project.org/web/packages/PMCMRplus/index.html

Purnhagen, K. P., Clemens, S., Eriksson, D., Fresco, L. O., Tosun, J., Qaim, M., Visser, R. G. F., Weber, A. P. M., Wesseler, J. H. H., and Zilberman, D. 2021. Europe's farm to fork strategy and its commitment to biotechnology and organic farming: Conflicting or complementary goals? Trends Plant Sci. 26:600-606.

R Core Team. 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reslow, F., Carlson-Nilsson, U., Crossa, J., Cuevas, J., and Ortiz, R. 2022. Public potato breeding progress for the Nordic Region of Europe: Evidence from multisite testing of selected breeding clones and available released cultivars. Acta Agric. Scand. - B Soil Plant Sci. 72:553-562.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9:671-675.

Schwaerzel, R., Torche, J.-M., Ballmer, T., Bussereau, F., Grabenweger, G., Dupuis, B., Riot, G., Tallant, M., and Vetterli, C. M. 2019. Schweizerische Sortenliste für Kartoffeln 2019. Agrar. Schweiz. 9:1-6 (in German).

Shabbir, Z., Sardar, A., Shabbir, A., Abbas, G., Shamshad, S., Khalid, S., Natasha, Murtaza, G., Dumat, C., and Shahid, M. 2020. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. Chemosphere 259:127436.

Silva, V., Yang, X., Fleskens, L., Ritsema, C. J., and Geissen, V. 2022. Environmental and human health at risk – Scenarios to achieve the Farm to Fork 50% pesticide reduction goals. Environ. Int. 165:107296.

Slater, A. T., Cogan, N. O. I., Hayes, B. J., Schultz, L., Dale, M. F. B., Bryan, G. J., and Forster, J. W. 2014. Improving breeding efficiency in potato using molecular and quantitative genetics. Theor. Appl. Genet. 127:2279-2292.

Steenblock, T., and Forrer, H.-R. 2002. Phytopre+2000: A plot specific decision support system on the internet. Agric. Res. 9:207-214 (in German).

Stridh, L. J., Mostafanezhad, H., Andersen, C. B., Odilbekov, F., Grenville-Briggs, L., Lankinen, Å., and Liljeroth, E. 2022. Reduced efficacy of biocontrol agents and plant resistance inducers against potato early blight from greenhouse to field. J. Plant Dis. Prot. 129:923-938.

Swisspatat. 2024. Statistical information 2024: Potato cultivation and potato utilization (in German and French). https://www.kartoffel.ch/de/Medien/statistik.html

Taillis, D., Pébarthé-Courrouilh, A., Lepeltier, É., Petit, E., Palos-Pinto, A., Gabaston, J., Mérillon, J.-M., Richard, T., and Cluzet, S. 2022. A grapevine by-product extract enriched in oligomerised stilbenes to control downy mildews: Focus on its modes of action towards *Plasmopara viticola*. OENO One 56:55-68.

Tamm, L., Thuerig, B., Apostolov, S., Blogg, H., Borgo, E., Corneo, P. E., Fittje, S., de Palma, M., Donko, A., Experton, C., Alcázar Marín, É., Morell Pérez, Á., Pertot, I., Rasmussen, A., Steinshamn, H., Vetemaa, A., Willer, W., and Herforth-Rahmé, J. 2022. Use of copper-based fungicides in organic agriculture in twelve European countries. Agronomy 12:673.

Vogel, C. M., Potthoff, D. B., Schäfer, M., Barandun, N., and Vorholt, J. A. 2021. Protective role of the *Arabidopsis* leaf microbiota against a bacterial pathogen. Nat. Microbiol. 6:1537-1548.

Walters, D. R., Ratsep, J., and Havis, N. D. 2013. Controlling crop diseases using induced resistance: Challenges for the future. J. Exp. Bot. 64: 1263-1280.

Wiik, L. 2014. Potato late blight and tuber yield: Results from 30 years of field trials. Potato Res. 57:77-98.