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Combination of Potassium Phosphite and Reduced Doses of Fungicides Encourages Protection against *Phytophthora infestans* in Potatoes

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Abstract: Late blight caused by the oomycete *Phytophthora infestans* is considered the biggest threat to potato farming worldwide. For susceptible cultivars, the disease is often managed by frequent applications of fungicides to reduce yield loss. The use of bio-based compounds that interfere with biologically active systems is an innovative strategy for improving disease management. In the present work, the control of *P. infestans* infection on potatoes by potassium phosphite (KPhi) combined with recommended and reduced doses of active ingredients (Ais) from different fungicides was evaluated. The protective effects of different combinations were initially assessed in vivo and subsequently compared with a greenhouse screening. The active ingredients cyazofamid (CFD) and mancozeb (MCB), used at recommended and reduced doses, were less effective at reducing *P. infestans* infections than when combined with KPhi. In greenhouse trials, CFD, mandipropamid (MPD) and MCB at recommended doses were the most effective treatments when combined with KPhi; meanwhile, the combination of KPhi with azoxystrobin (AZ), benthiavalicarb-isopropyl/mancozeb (ISO/MCB), and CFD at reduced doses exhibited strong protective activity compared to other similar combinations. This decreased the severity of infection by *P. infestans* up to ~89%. Greenhouse experiments also demonstrated that a combination of KPhi and CFD at both doses caused the highest reduction in disease severity (up to ~90%) within 35 days of infection. In microplot experiments, KPhi delayed the progression of late blight in susceptible potato varieties; therefore, in the combined treatments AUDPC values were significantly lower than those obtained after applications with CFD doses, providing sufficient protection against late blight. Our data suggest that optimizing the formulation with addition of KPhi could result in a lower recommended dose. This would result in a reduction of the active compounds of the fungicides in potato farming. Furthermore, the impact of KPhi on late blight development makes it a potential component for incorporation into an integrated pest management system.

Keywords: field experiment; fungicide active ingredients; late blight; *Phytophthora infestans*; potassium phosphite

1. Introduction

Potatoes (*Solanum tuberosum* L.) are one of the most important non-grain commodities in the world. It is estimated that global production of potatoes has exceeded 368 million tons since 2018, according to the Food and Agriculture Organization (FAO) [1]. However, potato varieties vary in their susceptibility to a number of pathogens that can affect both quality and yield. One of the most serious diseases affecting potato production is late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. Under favorable humidity and moderate temperatures, the disease can lead to significant economic loss in the field within 7–10 days if left uncontrolled [2,3]. Since oomycetes, including the *Phytophthora* species, have higher evolutionary potential in response to changing environments compared to true fungi [4,5], the durability of genetic resistance has often been limited to conventional breeding programs. There is increasing concern about the overuse of synthetic fungicides because of their detriment to human health and environmental consequences. However, under controlled procedures, safe and efficient application of fungicides are used while considering their economic benefits and toxicity [6]. In locations where late-blight-susceptible or moderately resistant cultivars are planted, frequent fungicide applications are required as the predominant means of disease control. Development of fungicide resistance in populations of *P. infestans* can be another major problem when these fungicides are used for a long period of time [7]. Thus, the repetitive use of fungicides with the same mode of action should be limited, as this does not lead to sustainable management of the disease. In this scenario, the need for economic and more environmentally sustainable alternative methods to control late blight is evident.

The development and exploitation of management strategies such as induced resistance has been proposed in various pathosystems as an alternative sustainable strategy in disease control [8,9]. Green leaf volatiles (GLVs), biosurfactants, and plant extracts induce a wide range of defense responses to reduce potato late blight [10–12]. Other immunity inducers such as thiamine, β-aminobutyric acid (BABA), thiadiazole-7-carbo thiocacid S-methyl ester, and phosphite (Phi) have been shown to exhibit enhanced resistance to many fungal diseases [13,14]. For instance, several studies have shown that BABA enhances resistance to potato late blight in the greenhouse and under field conditions [15–17]. However, it may be necessary to combine BABA with other fungicide treatments when there is insufficient evidence of protection under field conditions [16]. Similarly, phosphite-based compounds have been described as having high anti-oomycete activity. Amongst them, potassium phosphite (KPhi) has high potential for controlling *P. infestans* due to its direct and indirect effects. KPhi has a direct inhibitory effect on mycelial growth and can alter or reduce metabolism [18–20]. It also causes indirect effects by stimulating plant defense mechanisms, inducing hypersensitivity reactions (HRs) and aggregating phenylpropanoid biosynthetic enzymes to ultimately inhibit the development of late blight [14,20–23]. Although phosphite-based compounds can enhance plant defense responses, applications of additional fungicides are occasionally necessary to provide control of recalcitrant pathogens [20].

In agriculture, phosphites are known as fertilizers and systemic fungicides [24–26]. Indeed, phosphites (inorganic salts of phosphorous acid) are extensively available either as a superior source of plant phosphorus nutrition (P) or as plant defense activators that are translocated in both xylem and phloem to prevent pathogen spread over a wide range of hosts [27–29]. Due to the low risk to human health and the environment, phosphite salts are widely used against late blight in some developing countries as potential alternatives to conventional fungicides [30]. According to field studies from a number of tropical countries, phosphites provide a relatively constant control efficacy, comparable to conventional contact fungicides such as chlorothalonil and mancozeb, across locations [30]. Therefore, since phosphites are rather inexpensive, eco-friendly, and systemically mobile chemicals, they might be considered as key factors in the control of *Phytophthora* infection in potato cultivation [28,31]. Moreover, phosphite-based compounds have a very low impact on the environment due to their very low toxicity, according to EFSA [32].

Monopotassium salts of phosphorous acid known as potassium phosphite (Kphi) are the most common phosphite. In the literature, there are only a few reports available on foliar applications of Kphi against *P. infestans*. Foliar applications of potato plants with phosphite considerably reduced *P. infestans* infection in greenhouse and field experiments [33]; however, the efficacy of phosphites compared to the commonly used active substances was not investigated. Kphi may stimulate a strong and rapid reaction in potato against the pathogen infection through activation of various defense responses including pathogenesis-related enzymes, antioxidant enzyme activities, and defense biochemical compounds [23]. In another study, Mayton et al. [34] applied KPhi alone or in combination with chlorothalonil, leading to late blight suppression. Phosphite-based compounds have also been shown to confer increased protection against potato late blight when applied in combination with a fungicide containing chlorothalonil [35,36]. Similarly, in large-scale field trials, foliar treatments of KPhi in combination with lower fungicide doses achieved efficient protection against potato late blight, compared to fungicides used at recommended higher doses [37]. Overall, alternative control strategies, including nontoxic substances, biocontrol agents, and alternative disease management methods (such as fungicide spraying) have gained interest due to their potential for reducing the amount of chemicals that are damaging to the environment and human health [38,39].

With this aim, it is necessary to evaluate if the combined use of phosphorus-based compounds and fungicide formulations containing different active ingredients (AIs) can reduce the total amount of application dosage used throughout the cropping season. Therefore, greenhouse and microplot trials were undertaken to investigate the control of potato late blight by KPhi alone, and in combination with widely used active ingredients.

2. Materials and Methods

2.1. Plant and Pathogen Materials

For foliar assays, potato tubers (cv. Bintje) were planted in pots (4 L) comprising standard commercial potting substrate (NPK 14-16-18, pH 5–6.5, organic-dry matters: 25–20%) and maintained under optimal greenhouse conditions at 18 °C and 16 h photoperiod. *P. infestans* genotype EU-13 grown on rye-B agar (supplemented with 0.005% b-sitosterol) (Merck, Darmstadt, Germany) was subcultured on V8-juice medium and incubated at 18 °C under fluorescent light with a 16 h photoperiod for seven days [40]. Sporangial suspensions were prepared by scraping the culture surface as previously described by Najdabbasi et al. [40]. To release zoospores, newly formed sporangia were chilled at 4 °C for 2 h before being used as inoculum. Numbers of sporangia were quantified with a compound microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany) using a bright-line hemocytometer and adjusted to a final concentration of 5×10^4 sporangia mL^{-1} using sterile distilled water (SDW) with 0.01% Tween 80 (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Experimental Design and Treatments

Experiments used the late-blight-susceptible potato cv. Bintje. Spray applications of active ingredients from different fungicides (produced by BASF SE, Ludwigshafen, Germany), either alone or combined with KPhi Trafos K® (Tradecorp, Madrid, Spain), were performed at the recommended doses at high dose (HD), or at reduced dose (RD) of 70% of their recommended dosage rates (Table 1). Altogether, twenty-two treatments comprising different combinations of AIs-KPhi were applied in our experimental design (Table 1). In the greenhouse, eight-week-old potato plants were treated by using a spraying cabinet (XR Teejet nozzle 11001 and spray volume of 300 L/ha) with varying doses of ingredients, and then challenged with sporangial suspension of *P. infestans*. Potato plants were sprayed with water only as a negative control.

Table 1. Product combinations applied to potato plants in this study.

Active Ingredient (AI) *	Commercial Product	Treatment
504 g/kg potassium phosphite	Trafos K	24 L/ha [2]
160 g/L cyazofamide	Ranman Top	0.5 L/ha
	Ranman Top [1]	0.35 L/h
160 g/L cyazofamide + 504 g/kg potassium phosphite	Ranman Top + Trafos K	0.5 L/ha + 24 L/ha
	Ranman Top [1] + Trafos K	0.35 L/ha + 24 L/ha
250 g/L mandipropamid	Revus	0.6 L/ha
	Revus [1]	0.42 L/h
250 g/L mandipropamid + 504 g/kg potassium phosphite	Revus + Trafos K	0.6 L/ha + 24 L/ha
	Revus [1] + Trafos K	0.42 L/ha + 24 L/ha
700 g/kg mancozeb	Dithane WG	2 K/ha
	Dithane WG [1]	1.4 K/ha
700 g/kg mancozeb + 504 g/kg potassium phosphite	Dithane WG + Trafos K	2 K/ha + 24 L/ha
	Dithane WG [1] + Trafos K	1.4 K/ha + 24 L/ha
250 g/L azoxystrobin	Amistar	0.25 L/ha
	Amistar [1]	0.17 L/ha
250 g/L azoxystrobin + 504 g/kg potassium phosphite	Amistar + Trafos K	0.25 L/ha + 24 L/ha
	Amistar [1]+ Trafos K	0.17 L/ha + 24 L/ha
17.5 g/kg benthiavalicarb-isopropyl, 700 g/kg mancozeb	Valbon	1.6 kg/ha
	Valbon [1]	1.11 kg/ha
17.5 g/kg benthiavalicarb-isopropyl, 700 g/kg mancozeb + 504 g/kg potassium phosphite	Valbon + Trafos K	1.6 kg/ha + 24 L/ha
	Valbon [1] + Trafos K	1.11 kg/ha + 24 L/ha
Untreated control	-	-

* Commonly used abbreviations are used in the text to refer to a shortened form of active ingredients. KPhi: potassium phosphite; cyazofamid: CFD; mandipropamid: MPD; mancozeb: MCB; azoxystrobin: AZ; benthiavalicarb-isopropyl, mancozeb: ISO/MCB. [1] Reduced dose as 70% of their recommended dosage rates. [2] To improve the stability of compound on potato leaves, splitting the applied dose of potassium phosphite to 3 L/ha was contemplated with the spray intervals in the microplot trial.

2.3. Assessment of Late Blight In Vivo

A detached leaflet bioassay was performed to evaluate the efficacy of KPhi, alone and in combination with different doses of each AI, against *P. infestans* on potato leaves, using the method described by Najdabbasi et al. [35]. Leaves of eight-week-old plants were cut one day after foliar application of KPhi and fungicides, and immediately positioned on the abaxial surface on foams inside the plastic trays (60 cm × 50 cm × 20 cm). Three compound leaves consisting of fifteen leaflets were collected at random per treatment. For each replicate, a single 20 µL droplet of 5×10^4 sporangia mL^{-1} was placed at the center of each leaflet and incubated at 18 °C with relative humidity (RH) of 80% for seven days. Typical disease symptoms on leaves were scored using a 1–9 grading scale [41] and subsequently transformed into disease severity index (DSI) on a percentage basis according to the following Formula (1):

$$\text{DSI}\ (\%) = \frac{\sum_{i=1}^{9} \text{class}_i \ \times \#\ \text{leaflets in class}_i}{\text{total number of leaflets}\ \times\ \text{maximum class}} \times 100 \quad (1)$$

The experiment was repeated twice and each treatment was applied on three plants.

2.4. Assessment of Late Blight in the Greenhouse

A greenhouse trial was conducted to examine the potential protective effects of KPhi in combination with various AIs on foliar blight development. One day after the last application, plants were inoculated with sporangial suspension of *P. infestans* (5×10^4 sporangia mL^{-1}) using a handheld sprayer (MS-1H, 48 fl. oz.) and kept in darkness at >90% RH for 24 h. Plants were then moved to the greenhouse with growing conditions of 18–20 °C, ~80% RH and 16 h photoperiod. Ten days after inoculation, disease incidence was individually assessed for each plant. The percentage leaf area affected was recorded using an arbitrary rating scale of 0 to 4, where 0 = no leaf lesions, 1 = a few scattered spots per composed leaf, 2 = around 10%, 3 = up to 25%, and 4 = 25–50% of leaf area infected. Extent of disease severity was also evaluated over time by recording disease severity at 5-day intervals until 35 days after inoculation. These data were combined into a single value using the area under disease progress curve (AUDPC) for each treatment, according to the following Equation (2):

$$\text{AUDPC} = \sum \frac{\sum(x_i + x_{i+1})}{2} \times (t_{i+1} - t_i) \quad (2)$$

where X_i and X_{i+1} represent percentage of disease severity in the evaluations of *i* and (*i* + 1); t_i and t_{i+1} represent time at the *i*th and (*i*th + 1) observations [42]. The experiment included four replications for each treatment.

2.5. Microplot Trial

To further evaluate the potential benefit of integrating control strategy against late blight, combinations of KPhi with a selected AI that showed DSI less than 50% against *P. infestans* in the greenhouse were examined in a microplot trial under multivariable field conditions. The experiment was conducted at the experimental farm of Ghent University, Belgium during the growing season of 2020. Ten-week-old potato plants grown in 3 m wide and 12 m long plots were treated with varying product combinations using AKZO sprayer (XR Teejet nozzle 11003) in a spray volume of 300 L/ha, 24 h before challenge inoculation with *P. infestans*. There was a blank space of 1.5 m between plots in order to be able to treat the plots. The following treatments were considered for foliage application with the spray intervals according to manufacturer's recommendations: (i) a mixture of standard recommended dose of the AI and Kphi; (ii) a mixture of reduced dose of the AI and Kphi; (iii) standard recommended dose; (iv) reduced dose; and (v) KPhi alone. Fungicides at the standard recommended doses were applied according to manufacturer's recommendations. After the last treatment, plants of each plot were inoculated with sporangial suspensions of *P. infestans* (5×10^4 sporangia mL^{-1}), prepared as mentioned above, by spraying in the late afternoon. To increase the incidence of *Phytophthora* infection, plants were inoculated twice, with a five-day interval between the first and second inoculation. Humidity level was elevated by spraying potato plants with water 1 h before and 10 h after inoculation. At 10 days post-inoculation, visual inspections were performed at the appearance of first symptoms for a duration of two months with 7 days intervals. The intensity of foliage blight was rated by estimating the affected percentage leaf area of all leaves according to James [43] with some modifications: 0 = no disease observed; 1 = a few scattered plants blighted; 2 = up to 10 spots per plant formed; 3 = about 50 spots per plant formed; 4 = nearly every leaflet infected, but plants retained in normal form; 5 = every plant affected and about 50% of leaf area destroyed; 6 = about 75% of leaf area destroyed; 7 = only a few leaves on plants remained, with no effect on stems; 8 = all leaves and stems dead. Rating scores were then converted to DSI according to the following Formula (3):

$$\text{DSI}\ (\%) = \frac{\sum_{i=1}^{9} \text{class}_i \ \times \# \text{ plants in class}_i}{\text{total number of plants } \times \text{ maximum class}} \times 100 \quad (3)$$

The overall percentage was used to calculate AUDPC, according to the above-mentioned formula. Microplots consisted of a single row enclosed by plant borders, and each row having 10 potato plants (cv. Bintje). Each treatment was randomly allocated within each block and replicated four times.

2.6. Statistical Analyses

All experiments were performed as randomized complete block designs (RCBD). Accordingly, the data are shown as means ± standard deviations (SD) and represented in box and whisker plot format. Since the data met the normality assumption (Shapiro–Wilk test) and homoscedasticity assumption (Levene's test) for a parametric hypothesis test, an ANOVA was performed to determine the effect of different treatments using "R" software package (version 2.15.3). In case there were significant differences (p-value < 0.05) between each independent group, a post hoc Tukey test was performed to see which treatments differed.

3. Results

3.1. Assessment of Late Blight In Vivo

The efficacy of KPhi alone and in combination with various AIs against the highly pathogenic *P. infestans* (EU-13 genotype) was examined on potato leaves (cv. Bintje) using a detached leaflet bioassay. Seven days after inoculation, most treatments were able to reduce lesion development on detached leaflets compared to the untreated control (Figure 1). Application of KPhi combined with AIs used at both standard and reduced doses resulted in a significant reduction in disease severity of up to 100% compared to the control leaves (Figure 1). The combinations of a reduced dose of MPD with KPhi and AZ at the recommended dose with KPhi led to the complete control of late blight infection. Although control of late blight was less efficient with the application of CFD or MCB at standard and reduced doses alone, a considerable disease reduction was observed when KPhi was combined with these two AIs, compared to other treatments. Contrarily, the highest reduction in disease index was found after the application of the ISO/MCB at both applied doses, but its control efficacy decreased when combined with KPhi. Overall, the most effective treatments included combinations of KPhi and MPD at both standard and reduced doses, showing ~1.7% and 0% DSI, respectively.

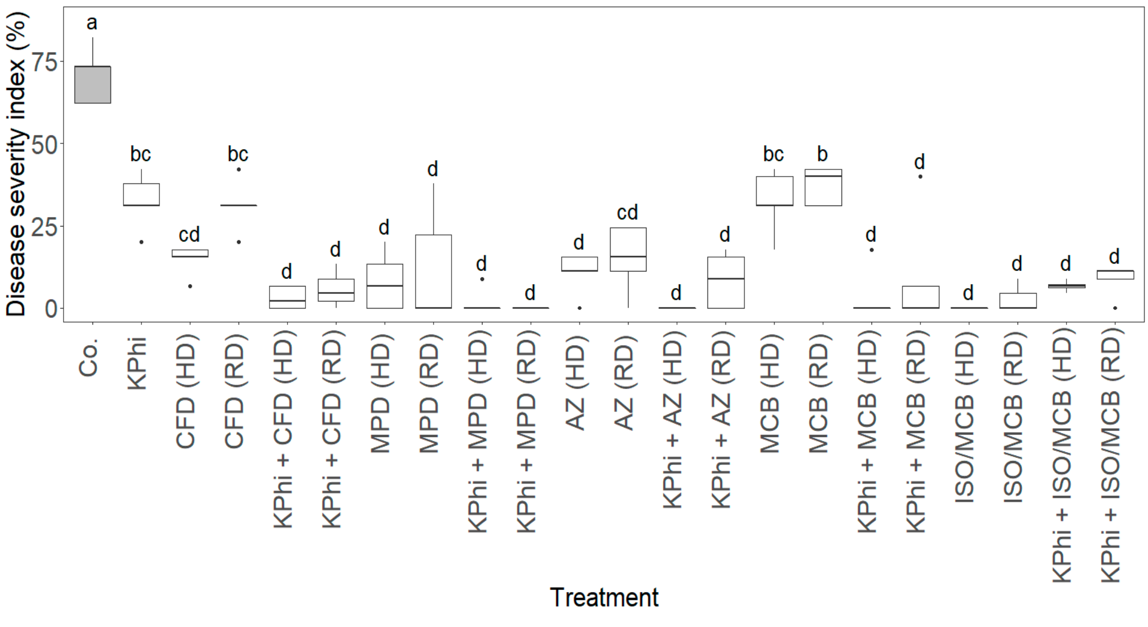

Figure 1. Late blight disease severity index (%) on detached potato leaflets treated with different combinations of potassium phosphite (KPhi) and active ingredients (AIs). CFD: cyazofamid; MPD: mandipropamid; MCB: mancozeb; AZ: azoxystrobin; ISO/MCB: benthiavalicarb-isopropyl, mancozeb; Co: control treatment with water. Treatments separated by plus indicate combinations. HD: recommended high dose; RD: reduced dose as 70% of its recommended dosage rate. Boxplots provide visual representation of the median, quartiles, maximum, and minimum of the data set. Boxplots with the same letters do not differ significantly according to a Tukey test (p-value < 0.05).

3.2. Assessment of Late Blight in Greenhouse

The suppression of late blight by KPhi in combination with standard and reduced doses of AIs was assessed on whole (potato) plants grown in the greenhouse one day after foliar application. Each treatment, with the exception of those containing MCB at reduced doses, showed a significant (p-value < 0.05) reduction in disease severity index compared to the untreated control (Figure 2). Ten days after inoculation, most AIs-KPhi combinations applied at their recommended doses resulted in significantly lower DSI compared to plants treated with only AIs at the recommended doses. CFD, MPD, and MCB at the HD showed the lowest DSI scores when combined with KPhi, and inhibited the development of late blight by up to 100% (0% disease severity index). In contrast, combinations of Kphi with reduced doses of AZ, ISO/MCB, and CFD presented strong protective activity compared to other similar combinations, and reduced DSI up to ~89%. When combined with KPhi, no significant differences (p-value > 0.05) in DSI were observed between HDs and RDs of AZ, ISO/MCB, and CFD. Both on its own and combined with KPhi, MCB showed significantly higher control of late blight at HDs compared with RDs. Reduced doses of MCB, alone and in combination with Kphi, showed the highest DSI of all treatments (~72%) and were not significantly more effective than the untreated control. Similarly, MPD was more effective at HDs with a significant reduction in disease suppression at RDs in combination with KPhi compared to other treatments containing MPD.

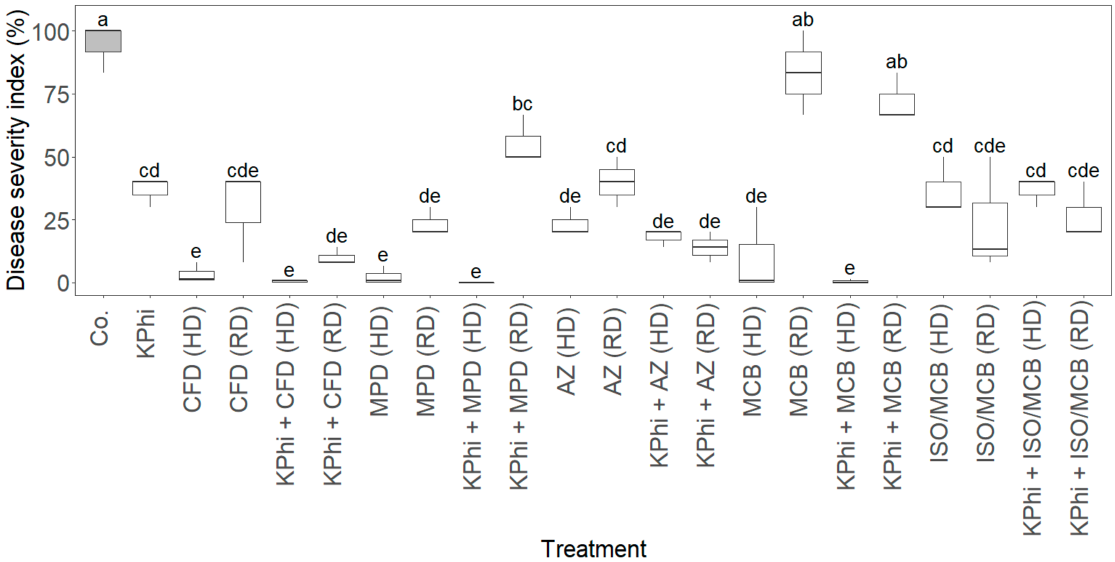

Figure 2. Late blight disease severity index (%) on whole potato plants in greenhouse experiments treated with different combinations of potassium phosphite (KPhi) and active ingredients (AIs). CFD: cyazofamid; MPD: mandipropamid; MCB: mancozeb; AZ: azoxystrobin; ISO/MCB: benthiavalicarb-isopropyl, mancozeb; Co: control treatment with water. Treatments separated by a plus indicate combinations. HD: recommended high dose; RD: reduced dose as 70% of its recommended dosage rate. Boxplots provide visual representation of the median, quartiles, maximum, and minimum of the data set. Boxplots with the same letters do not differ significantly according to a Tukey test (p-value < 0.05).

With the exception of MCB at RDs, all AIs significantly (p-value < 0.05) reduced late blight progress over time compared to the control, according to AUDPC values (Figure 3). We found a clear dose-response effect for CFD, MPD, and MCB active ingredients where the HDs had greater effects than others against late blight and differed from their reduced doses, according to the AUDPC values. The AUDPC values across CFD, MCB, AZ, and ISO/MCB applied in combination with KPhi were generally lower than the AUDPC with these four fungicides applied alone. The reduction in AUDPC by KPhi was most pronounced when combined with the recommended doses of CFD, MPD, and MCB. Altogether, AZ and ISO/MCB at the recommended full doses were moderately effective against *P. infestans*—even in combination with KPhi—compared to untreated control during 5 weeks of screening, and showed an average AUDPC value of ~90% and ~114%, respectively. CFD disclosed the highest effectiveness of all treatments tested within 35 days after infection (but not significantly more effective). Plants treated with CFD in combination with KPhi showed DSI up to 99% lower than untreated control, and were selected for a microplot trial to assess their efficacy on late blight control under field conditions.

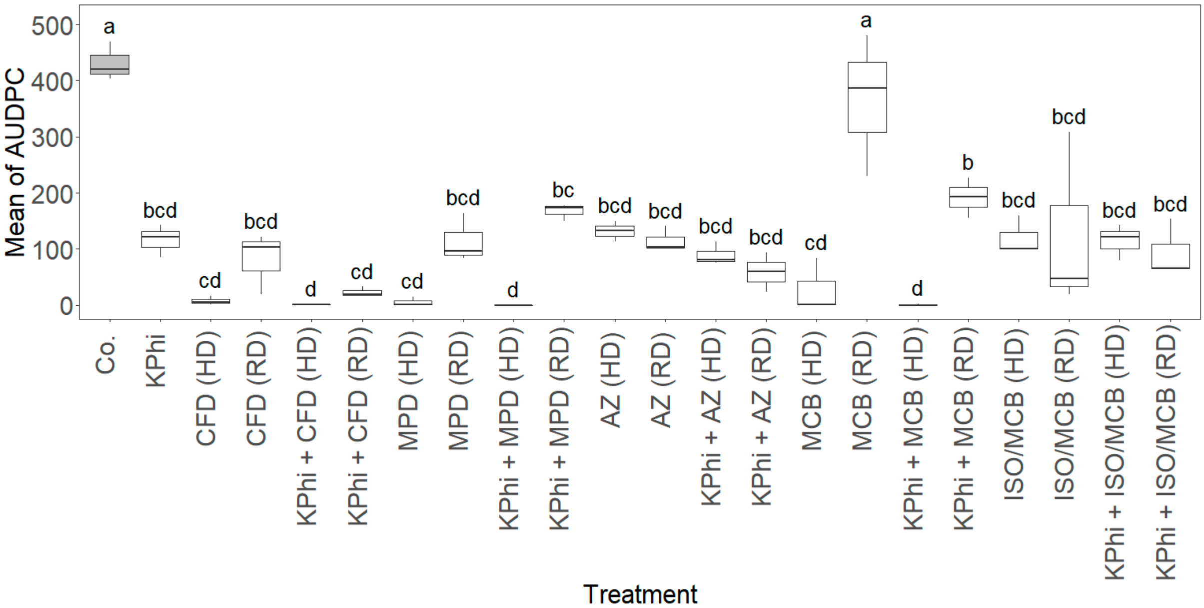

Figure 3. Area under disease progress curve (AUDPC) values in response to different combinations of potassium phosphite (KPhi) and doses of active ingredients (AIs) on potato plants artificially infected by *P. infestans* in the greenhouse. CFD: cyazofamid; MPD: mandipropamid; MCB: mancozeb; AZ: azoxystrobin; ISO/MCB: benthiavalicarb-isopropyl, mancozeb; Co: control treatment with water. Treatments separated by a plus indicate combinations. Values are calculated using disease severity index (DSI) ratings at five-day intervals for 35 days post-inoculation. HD: recommended high dose; RD: reduced dose as 70% of its recommended dosage rate. Boxplots provide visual representation of the median, quartiles, maximum, and minimum of the data set. Boxplots with the same letters do not differ significantly, according to a Tukey test (p-value < 0.05).

3.3. Microplot Trial

A microplot field trial was performed to assess control of late blight by KPhi combined with the selected active ingredient, cyazofamid. Potato plants were treated with CFD at recommended and reduced doses, alone and in combination with KPhi. The incidence of foliage blight was monitored 10, 17, 24, 31, 38, 45, and 52 days after inoculation. CFD at the reduced dose did not have a significant effect on late blight development in terms of suppressing AUDPC values, even if disease severity was relatively low (Figure 4). Moreover, there seemed to be a positive dose-response effect for CFD since a higher AUDPC value was achieved with its reduced dose. For CFD + Kphi treatments, the mean AUDPC was lower at the recommended dose compared to the reduced dose, but this did not show significance. CFD + Kphi at the recommended dose gave the lowest mean AUDPC value and combined analysis of data showed significant differences (p-value < 0.05) compared to the untreated control. Furthermore, Kphi + CFD treatments were significantly lower (p-value < 0.05) when compared to CFD only at the same dose. Additionally, CFD + Kphi at a reduced dose (RD) was comparable to the control achieved by the higher recommended dose (HD) of cyazofamid on its own. However, these treatments were not significantly different to the untreated control despite lower mean AUDPC. Overall, due to hot and dry climate conditions during July and August, with an average temperature of 24 °C and relative humidity of 65–70%, the infection level on field-grown plants was much lower than on plants grown in the greenhouse. Consequently, although significant protective effects were attained after KPhi treatment combined with different doses of CFD, the cautions and limitations related to the data need to be kept in mind.

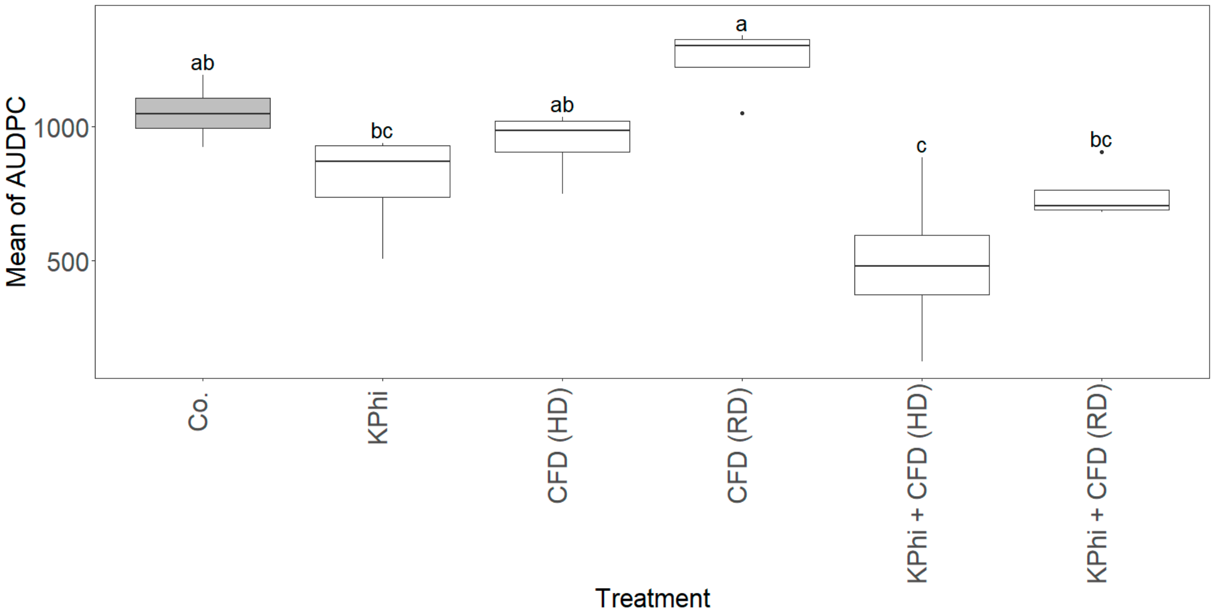

Figure 4. Area under disease progress curve (AUDPC) values in response to different combinations of potassium phosphite (KPhi) and cyazofamid (CFD) at both standard and reduced doses on potato plants artificially infected by *P. infestans* in the field. Co: control treatment with water. Treatments separated by a plus indicate combinations. Values are calculated using disease severity ratings at seven-day intervals for 52 days post-inoculation. HD: recommended high dose; RD: reduced dose as 70% of its recommended dosage rate. Boxplots provide visual representation of the median, quartiles, maximum, and minimum of the data set. Boxplots with the same letters do not differ significantly, according to a Tukey test (p-value < 0.05).

4. Discussion

Late blight is one of the most devastating diseases in potato production; therefore, intensive and repeated fungicide applications are frequently needed to control the disease. Continuous application of synthetic fungicides is in conflict with a sustainable approach to controlling late blight. For this reason, there is a need to develop alternative control strategies against late blight to reduce long-term environment-related health concerns. This study is the first demonstration of improved control of *P. infestans* EU-13 (late blight) on potatoes when active ingredients (AIs) from different fungicides are combined with the inorganic salt KPhi in greenhouse and field conditions. The aggressiveness of EU-13 was demonstrated in our previous studies [12,40] where, among the *P. infestans* genotypes tested, EU-13 showed the highest rate of aggressiveness and sporulation intensity on both potato tubers and leaves.

In the first part of this study, whole-plant bioassays were employed together with detached leaflet bioassays to support our screening results from leaflet assessments implemented in growth chambers. The reduction in green leaf tissues in untreated plants infected by *P. infestans* was considerably higher than in treated leaves in vivo and in greenhouse experiments. Cyazofamid (CFD), mandipropamid (MPD), and mancozeb (MCB) at the recommended doses were the most effective treatments when combined with KPhi, and significantly inhibited the severity of late blight disease 10 days after inoculation. Despite a lower mean disease severity in the detached leaf bioassays (~70%) in comparison with the greenhouse experiments (~94%) on untreated plants, for most cases the results of detached leaflet bioassays were consistent with greenhouse observations in suppression of late blight lesions. This suggests that results derived from in vivo bioassays can be generalized to greenhouse assays. Detached leaf assay is a fast, cost-effective and high-throughput screening approach which is less dependent on space limitations compared to the greenhouse or field screening methods [44,45]. In this context, Foolad et al. [46] revealed correspondence relationship between detached leaf results and those from field and greenhouse screenings for late blight resistance. Furthermore, detached leaflet bioassays which were efficiently used for *Phytophthora* blight assessments were comparable to natural conditions when testing the anti-oomycidal activity of plant-derived compounds against potato late blight [40]. This technique has been widely used for laboratory evaluations of potato foliage susceptibility to late blight in several studies [12,47–49], demonstrating reliability of the detached leaflet assay. However, losing the inoculum source is a particular risk in the case of detached leaflet or leaf disk bioassays [50].

The current study shows that even under environmental conditions conducive to rapid infection of *P. infestans*, disease development was strongly suppressed by certain combinations of treatments in a controlled setting. In greenhouse trials, the combination of KPhi with a reduced dose of CFD significantly reduced *Phytophthora* infection, and protected potato plants over time. Disease progression in potato plants inoculated with *P. infestans* was reduced by ~95% in the greenhouse at 35 days post-inoculation. However, no significant difference in AUDPC values was observed when applying the combination of KPhi and both doses of CFD. There was also a clear relationship between the applied doses of CFD, MPD, and MCB combined with KPhi and disease progress on potato plants. Application of these combinations at least one day before the pathogen infection was necessary to provide maximum protection and preserve its performance. Interestingly, it has been shown that although azoxystrobin (AZ) is highly effective in the control of potato early blight caused by *Alternaria solani* when applied pre- and post-infection [51,52], the current study showed that application of the full dose of this AI combined with KPhi was moderately effective against *P. infestans*. Several studies have shown that disease progression of potato late blight is successfully reduced by azoxystrobin over time [5,53,54]. MCB at the reduced dose + KPhi showed the least effectiveness against *P. infestans* on potato plants screened for more than a month, despite reducing disease severity by up to ~86% in detached leaf bioassays. On the contrary, a few days after inoculation, there was observable physiological decay in untreated potato leaves, such as small, light-to-dark-green, and round-to-irregularly shaped lesions, underlying the transition from biotrophic to necrotrophic growth of *P. infestans* in infected tissues [55]. Evaluation of the protective efficacy of compounds against *P. infestans* was demonstrated to be essentially dependent on experimental conditions, including application time before the pathogen inoculation; initial inoculum density of the pathogen; and the visual screening method applied to measure disease severity within the timescale specified [40].

Analysis of microplot trials showed that, the average AUDPC value with application of KPhi alone was lower than the average AUDPC with the application of CFD at the recommended dose. All combined treatments were superior to the fungicide treatments at both doses applied at 5-day intervals (Figure 4). Foliar treatment with the mixture of KPhi solution and reduced dose of CFD was found to be effective at controlling late blight compared to the recommended full dose. In other words, under field conditions and with the highly susceptible cultivar Bintje, the reduced dose of a conventional active ingredient combined with KPhi exhibited good disease suppression, based on AUDPC and final disease severity, in comparison to other treatments applied. While all treatments resulted in a lower disease level than the untreated control plants, the high and low doses of CFD combined with KPhi tended to be better at suppressing late blight, resulting in the lowest disease intensity. This indicates that the fungicide dose could be reduced with no significant increase in foliage blight for at least two months after infection. The effectiveness of KPhi at controlling potato tuber blight has also been demonstrated in several other studies [37,56–58]. Phosphite could also provide consistent control against pink rot, caused by *P. erythroseptica*, even when applied several hours after infection [59,60]. The potential benefits of this compound could lead to more sustainable control strategies in practical potato cultivation, leading to potential cost savings, with up to a 70% reduction in fungicide use during the growing season. However, under favorable weather and crop conditions for *P. infestans*, early and widespread outbreak of late blight can be always expected in potato cultivation.

Results of the current research strengthen the idea that both KPhi-based formulation and conventional AIs provide comparable efficacy for controlling late blight. In our study, under field conditions, the efficacy of KPhi alone was even better than CFD at both standard and reduced doses alone, according to the AUDPC values. The reports of Kromann et al. [30] revealed that KPhi was able to control late blight on potato foliage similarly to the conventional contact fungicides chlorothalonil and mancozeb applied at the same doses. The current study found KPhi and reduced doses of AIs were able to slow progression of late blight on plants, suggesting KPhi could be practically used in potato farming. However, when KPhi was combined with the recommended dose of CFD in the field, the highest level of disease suppression was observed. Very few studies have been conducted on the application of phosphite treatments to control late blight. For instance, Wang-Pruski et al. [35] showed that the combined treatment of phosphite with a protectant fungicide containing chlorothalonil provided the best late blight suppression in the field, followed by treatment with chlorothalonil alone. Efficacy of a phosphite-based spray program has also been examined, where the application of phosphite combined with the fungicide fosetyl-aluminum caused enhanced foliar protection against *P. infestans* with the possibility of controlling tuber blight [33]. According to this evidence, combining KPhi with reduced AI application can improve effective protection to *Phytophthora* infection, resulting in reduced disease development on potato plants. However, cultivar susceptibility/resistance is an important factor to be considered in this context. Varying interactions of different potato cultivars to phosphite application have been reported in earlier studies [35,58]. Liljeroth et al. [37] showed that the influence of phosphite treatment against late blight on the partially resistant cultivars was relatively better than on susceptible potato cultivars, which indicated lower AUDPC values. On the contrary, a meta-analysis using field studies and different combinations of potato varieties revealed that there was no clear correlation between the resistance level of potato varieties used and efficacy of phosphite [30].

Potassium phosphite is characterized by a high rate of phloem mobility and is readily translocated within the plants in a systemic manner [36]. This compound has both direct and indirect mechanisms of action involved in disease control. The direct mechanism of action is associated with the inhibition of the pathogen's zoospore production and spore germination [61–63], while the indirect mechanism of action is related to activation of plant defense responses such as induced resistance (IR) which is more useful for suppressing the pathogen than the direct mechanism [31,61]. Given this background, Lobato et al. [57] evaluated that the beneficial effect of foliar applications of KPhi in disease protection to field-grown potatoes resulted in post-harvest tubers with a reduced susceptibility to *P. infestans*, *Fusarium solani*, and *Erwinia carotovora* infections, demonstrating the induction of a systemic defense response. However, our study did not explicitly demonstrate the defense mechanisms activated by potassium phosphite in potato plants. Besides the potential inhibitory impact on disease development, there are many proven beneficial effects of phosphites on crops [64–66]. A study conducted by Tambascio et al. [67] showed that the application of KPhi to seed tubers resulted in early plant growth by reducing the period between planting and emergence, and increasing leaf area, dry-matter, and mycorrhizal colonization in potatoes under field conditions. Furthermore, using histomorphological and PCR-based molecular assays, Oyarburo et al. [68] showed that tolerance to UV-B stress was markedly potentiated by KPhi pre-treatment in potato leaves. Taken together, this comprehensive set of data suggests that the hypothesis of the integration of phosphite treatments into current management strategies can be implemented in potato farming systems.

5. Conclusions

Our results from in vivo, greenhouse, and field experiments strongly indicate that synergy between phosphite and fungicide active ingredients could play an important protective role against *Phytophthora* infection, even for susceptible cultivars such as Bintje. Indeed, phosphites could boost the inhibitory activity of one or both doses of fungicides more than when they are applied alone. Such disease reduction could be attributable to induced resistance, which can considerably reduce the number of fungicides needed for effective late blight control. Moreover, the risk of developing resistance to fungicides in the pathogen population can be reduced by combining different mechanisms of action into disease management strategies. If slower disease progression could be achieved, e.g., compared to fungicide application, there would be significant economic benefits for both the local economy and farmers simultaneously.

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