Journal of General Plant Pathology (2020) 86:494–502
https://doi.org/10.1007/s10327-020-00953-z

DISEASE CONTROL

Management of *Phytophthora palmivora* disease in *Citrus reticulata* with chemical fungicides

N. M. Chi[1] · P. Q. Thu[1] · H. B. Nam[2] · D. Q. Quang[1] · L. V. Phong[2] · N. D. Van[2] · T. T. Trang[1] · T. T. Kien[3] · T. T. T. Tam[3] · B. Dell[4]

Received: 29 February 2020 / Accepted: 14 April 2020 / Published online: 15 September 2020

Abstract
Over 200,000 ha of citrus are grown in Vietnam, and many orchards have been impacted by Phytophthora disease, leading to tree decline and death. *Phytophthora palmivora* has recently emerged as a serious problem in mandarin (*Citrus reticulata*) orchards in northern Vietnam, and producers are looking for management solutions. Therefore, we evaluated the efficacy of a range of commercial fungicides and biological agents on the growth of *P. palmivora* in vitro and for *P. palmivora* disease management in 6-month-old *C. reticulata* seedlings and in a 2-year-old *C. reticulata* orchard. In the in vitro experiment, potassium phosphonate, metalaxyl-M, mancozeb, and *Trichoderma viride* strongly inhibited the growth of *P. palmivora*. For diseased seedlings, the percentage recovery of seedlings 50 days after being treated with metalaxyl-M was 70.9%, potassium phosphonate, 69.1%, and mancozeb 57.1%. The percentage recovery of mandarin trees declining from *P. palmivora* in the orchard 6 months after the first application of potassium phosphonate was 50.0%, metalaxyl-M 45.9%, and mancozeb 30.6%.

Keywords *Citrus reticulata* · Disease management · Fungicide · Mandarin · *Phytophthora palmivora* · Vietnam

Introduction

Total global citrus production exceeds 100 million tons/year, and oranges comprise about two thirds of the production (Anonymous 2016). In Vietnam, over one million tons of oranges (*Citrus sinensis*) and mandarins (*C. reticulata*) were traded in 2018 (Anonymous 2018). Oranges have been grown for a long time in the Mekong Delta (Thanh et al. 2018), and in recent decades, production of oranges and mandarins has expanded into northern Vietnam (Quang 2013; Tien 2013).

Root diseases and citrus gummosis cause substantial loss in production, and *Phytophthora* spp. are the most widespread causal agents (Graham and Feichtenberger 2015; Timmer et al. 2003). A number of species have been implicated, e.g., *P. nicotianae* and *P. palmivora* in Florida in the United States (Graham et al. 2003; Widmer et al. 1998), *P. nicotianae* and *P. cryptogea* in Tunisia (Boughalleb-M'hamdi et al. 2018), *P. nicotianae* in Thailand (Watanarojanaporn et al. 2011), and *P. palmivora* in Japan (Tashiro et al. 2012), Egypt (Ahmed et al. 2014), and Vietnam (Hung et al. 2015b). In northern Vietnam, *Phytophthora palmivora* is the main pathogen causing root rot disease in *Citrus reticulata* and *C. sinensis* in Quang Ninh Province (Chi et al. 2019).

Chemicals, live organisms (McGrath 2009), and changes in horticultural practices are among the wide range of methods used to mitigate diseases caused by *Phytophthora* spp. including. Metalaxyl-M, mancozeb, ethaboxam, mandipropamid, fluopicolide, benzethonium chloride, and potassium phosphonate have been used commercially to reduce or prevent disease (Graham 2011; Hao et al. 2019; Lawrence et al. 2017; Ramallo et al. 2019; Rolando et al. 2017; Vawdrey et al. 2004). *Trichoderma* spp. have been used to reduce *Phytophthora* diseases in fruit (de Oliveira et al.

All of the authors contributed equally to this work.

✉ N. M. Chi
nguyenminhchi@vafs.gov.vn

[1] Forest Protection Research Centre, Vietnamese Academy of Forest Sciences, Bac Tu Liem District, Hanoi, Vietnam

[2] Quang Ninh Department of Science and Technology, Ha Long City, Quang Ninh Province, Vietnam

[3] Thai Nguyen University of Agriculture and Forestry, Thai Nguyen city, Thai Nguyen Province, Vietnam

[4] School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia

2018; Sriwati et al. 2019), and three species of *Chaetomium* were used to reduce *P. palmivora* and *P. nicotianae* root rot in citrus (Hung et al. 2015b, a). Application of arbuscular mycorrhizal fungi (*Acaulospora tuberculata* and *Glomus etunicatum*) also reduced *P. nicotianae* root rot in citrus (Watanarojanaporn et al. 2011). Preventative measures to control *Phytophthora* root rot in citrus include enacting phytosanitary requirements for producing pathogen-free nursery trees in enclosed structures and propagation from indexed and certified pathogen-free sources (Graham and Feichtenberger 2015), reducing flooding in orchards (Chaudhary et al. 2016), and using resistant rootstocks (Lima et al. 2018; Mourão Filho et al. 2008; Widmer et al. 1998; Yan et al. 2017).

Thanh et al. (2004) noted the need for more research on *Phytophthora* species in citrus in Vietnam. The present study focused on developing practical solutions to control root rot disease caused by *P. palmivora* in mandarin (*C. reticulata*) in northern Vietnam and preventing tree decline and death.

Materials and methods

Evaluation of agents for inhibition of *P. palmivora* in vitro

Five fungicides and five biological agents were evaluated for their efficacy to inhibit the growth of *P. palmivora* (isolate QN729) compared to a water control (see Table 1 for the chemical and biological formulations). QN729 was isolated from diseased roots of mandarin in 2018 in An Sinh Ward, Dong Trieu District, Quang Ninh Province and was identified as *P. palmivora* based on the ITS sequence (MT113313) (Chi et al. 2019). Pathogenicity was previously tested by inoculating *Citrus sinensis* and *C. reticulata* seedlings, and QN729 was shown to be an aggressive isolate (Chi et al. 2019). The different treatments against *P. palmivora* were evaluated in vitro using the method of Singh and Tripathi (1999) with some modifications. Mycelial plugs (5 mm diameter) of QN729 were placed at three places on potato dextrose agar (PDA, Merck KGaA, Darmstadt, Germany) at equal distances from each other on the perimeter of each Petri dish. One 5-mm-diameter well was created in the center of each Petri dish, and 50 µl of the test solution was placed into each well (Table 1). Each treatment was applied to six replicate plates, and the experiment was done five times, for a total of 30 plates for each treatment and the control. Plates were placed into an incubator at 22 ℃ for 7 days, then the diameter of any inhibition zone of *P. palmivora* growth was measured across each well and classified as $D = 0$ cm (no inhibition); $0 \text{ cm} < D \leq 1.0$ cm (weak); $1.0 \text{ cm} < D \leq 2.0$ cm (average); $2.0 \text{ cm} < D \leq 3.0$ cm (strong); $D > 3.0$ cm (very strong).

Evaluation of agents for reducing *P. palmivora* disease in mandarin seedlings

Metalaxyl-M, potassium phosphonate, mancozeb, and *Trichoderma viride* had the greatest inhibitory effect against *P. palmivora* in vitro and were tested further against *P. palmivora* in 6-month-old *C. reticulata* seedlings. Seeds of a single family of *C. reticulata* were sown in sterilized substrate (8 parts soil, 2 parts compost) in containers, and seedlings were grown in a glasshouse for 6 months to a

Table 1 Formulations tested for control of *Phytophthora palmivora*

Active ingredient (a.i.)	Trade name	Concentration of a.i.	Concentration of a.i. used
Potassium phosphonate	Agri-fos 400 (Agrichem, Queensland, Australia)	400 g/l	10.00 ml/l
Fosetyl aluminium	Aliette 800WG (Bayer, Berlin, Germany)	800 g/kg	3.13 g/l
Chlorothalonil	Daconil 75WP (SDS Biothech K.K., Tokyo, Japan)	75%	3.00 g/l
Mancozeb	Fovathane 80WP (Nantong Baoye Chemical Co., Ltd, Jiangsu, China)	80%	3.13 g/l
Metalaxyl-M	Metaxyl 500WP (Vinasa Agri Tech J.S.C, Ho Chi Minh, Vietnam)	500 g/kg	6.25 g/l
Bacillus subtilis	*Bacillus subtilis* (FPRC, Hanoi, Vietnam)	2×10^{12} CFU/ml	0.25 ml/l
Trichoderma viride	Biobus 1.00WP (Agrilife, Andhra Pradesh, India)	1%	1.00 g/l
Bacillus sp. + *Trichoderma* sp. + *Pseudomonas* sp. + *Rhizobium* sp. + *Azotobacter* sp.	Earthcare with Sumagrow Inside (ESI, Wisconsin, USA)	2×10^{12} CFU/ml	1.25 ml/l
Cytosinpeptidemycin	Sat 4SL (Nambac Co., Ltd, Long An, Vietnam)	4%	0.94 ml/l
Pseudomonas fluorescens	TKS Pseudomonas (Thuykimsinh Co., Ltd, Dak Lak, Vietnam)	2×10^{9} CFU/g	0.10 g/l
Distilled water	Control		

height of 50–60 cm. Complete liquid fertilizer was applied every 15 days to promote healthy growth. To prepare the inoculum, a solid medium comprising 250 g of rice bran, 250 g boiled unhusked rice, 17.5 ml of vegetable juice broth, and 3 g $CaCO_3$ was placed in 500 ml Erlenmeyer flasks and autoclaved at 121 ℃ for 30 min. Agar plugs with mycelia of isolate QN729 were added to the cooled medium, and the flasks were incubated at 22 ℃. After 5 weeks, 2.0 g of the culture was placed on the surface of the potting medium (2/seedling) in each pot, the pots were briefly flooded with water, then the surface was covered with 1–2 cm of autoclaved soil. Each of the five treatments (control was sterile water) was applied with a hand sprayer to 15 6-month-old seedlings, 50–60 cm in height, and the experiment was done four times. At 40 days after inoculation with QN729, and again 15 days later, the treatments were sprayed on the foliage, stem and base of each seedling (200 ml/seedling) and also applied to the soil around the base (200 ml/seedling). Dosages are given in Table 1.

Symptoms on foliage were evaluated 40 days after inoculation, and foliage and root damage were assessed after 90 days. Disease severity was then classified from 0 to 4 using the methods of Chi et al. (2019) where 0 = healthy foliage/healthy roots; 1 = yellowing and sparse foliage/ < 25% of roots diseased; 2 = top of the branches wilting/25–50% roots diseased; 3 = plant completely wilted/51–75% roots diseased; 4 = plant dead/ > 75% roots diseased. These ratings were then used to calculate a disease index (described later in the disease analysis section). Representative samples were collected, the pathogen was reisolated, and hyphal and sporangial traits were examined with a light microscope to confirm *P. palmivora* was present.

Evaluation of agents for reducing *P. palmivora* disease in a mandarin orchard

The four treatments tested for control of *P. palmivora* on seedlings in the nursery were then evaluated against *P. palmivora* in a commercial mandarin orchard. Mandarin orchards in An Sinh Ward, Dong Trieu District, Quang Ninh Province were surveyed for disease incidence and disease index, and a 2-ha property with 625 trees/ha and about 40% disease incidence was selected (21151′03"N, 106516′05"E, 68 m a.s.l.) for the experiment. The orchard had previously hosted one rotation of citrus, and *P. palmivora* had been isolated from scions (15 trees) and rootstocks (45 trees) in this orchard (Chi et al. 2019) where mandarin was grafted onto pomelo (*C. maxima*) seedlings. The orchardist had chosen to use pomelo because it is alleged by producers to be "stronger" than mandarin, but it is known to be susceptible to *P. palmivora* (Hung et al. 2015b). The location has a mean annual temperature of 23.8 ℃, 1,600 h sunshine/year, and average rainfall of 1,850 mm/year. The terrain is flat, and the soil is a red-yellow ferralite > 50 cm in depth. The trees were trickle-irrigated daily using groundwater, the weeds were cut by hand, and the soil was cultivated monthly. The fertilizer schedule for the orchard was February, 30 kg of organic fertilizer + 500 g NPK/tree; May, 500 g NPK/tree; and August, 400 g NPK/tree. The presence of drainage ditches prevents flooding in the orchard during the wet season. The experiment was set up with three blocks, each contained five plots (500 m^2), and each plot had 30 trees. The treatments were randomly assigned to the plots. The plots were separated by buffer strips of at least 10 m. Trees in the plots were planted in 2017, 2.5–3.0 m tall and first produced fruit in 2019.

The fungicides and biopesticide were sprayed (electric sprayer, 2 l/tree) on foliage, branches, and trunk (including the rootstock), and they were also applied to the soil within 50 cm of the trunk (2 l/tree) (dosages: Table 1). The treatments were applied in the late afternoon, without rain. The four treatments were applied twice, 15 days apart, in April and repeated in July 2019. This regime was chosen based on government recommendations on the use of fungicides for preventing gummosis disease on citrus.

The trees were assessed twice: when the trial was established (April 2019) and after 6 months (October 2019). Foliage symptoms were used to assess trees in April and October. The level of disease was classified at five levels (0 to 4), where 0 = healthy plant; 1 = yellowing and sparse foliage; 2 = foliage wilting; 3 = plant completely wilted; 4 = plant dead.

Disease analysis

Using the results of symptom classification, the disease incidence (P%) for each treatment was determined as $P\% = (n/N) \times 100$, where n = number of diseased plants; N = total number of plants assessed.

The disease index (DI) value was calculated as $DI = (\Sigma n_i \times v_i)/N$, where v_i = class of disease severity; n_i = number of plants recorded in this class; N = total number of plants assessed. The DI was then classified according to the range in values: DI = 0 (no infection); $0 < DI \le 1$ (low); $1 < DI \le 2$ (medium); $2 < DI \le 3$ (high); $3 < DI \le 4$ (very high).

The level of disease inhibition (E) of each agent was calculated as:

$$E = \left(1 - \frac{C_a T_b}{C_b T_a}\right) \times 100,$$

where E = inhibitory effect (%); C_a = disease incidence/disease index of the control treatment at the start of the trial; T_a = disease incidence/disease index of the agent treatment at the start of the trial; C_b = disease incidence/disease index of the control treatment at the end of the trial; and T_b = disease incidence/disease index of the agent treatment at the end of the trial.

The recovery of trees (R%) of each agent was calculated as $R\% = [(Nb - Na)/Nb] \times 100$, where Nb = number of diseased plants at the time of application of treatments; Na = number of diseased plants after application of treatments.

Statistical analyses

Data were analysed using GenStat Release 12.1 software package (VSN International Ltd., Hemel Hempstead, UK). Analysis of variance (ANOVA) was used to test for significant effect of treatments or site factors, followed by Duncan's multiple range test for comparisons of means. Differences were considered to be significant at $P < 0.05$ or very significant at $P < 0.01$.

Results

Inhibition of *P. palmivora* in vitro

There was a significant effect (Fpr < 0.001) of chemical and biological treatments on *P. palmivora* growth in vitro (Table 2). The chemicals metalaxyl-M (Fig. 1a) and potassium phosphonate (Fig. 1b) very strongly inhibited growth, followed by mancozeb and *Trichoderma viride* (Fig. 1c) which strongly inhibited growth compared to the control (Fig. 1d). Chlorothalonil and cytosinpeptidemycin were weak inhibitors.

Table 2 Comparison of chemical and biological treatments for their inhibition of growth of *Phytophthora palmivora* in vitro

Treatment name	Mean diameter of inhibition (cm)	Inhibition
Metalaxyl-M	4.25 ± 0.08^{h}	Very strong
Potassium phosphonate	3.13 ± 0.06^{g}	Very strong
Mancozeb	2.86 ± 0.08^{f}	Strong
Trichoderma viride	2.12 ± 0.06^{e}	Strong
Pseudomonas fluorescens	1.68 ± 0.08^{d}	Average
Bacillus subtilis	1.52 ± 0.09^{cd}	Average
Fosetyl aluminium	1.42 ± 0.07^{c}	Average
Bacillus sp. + *Trichoderma* sp. + *Pseudomonas* sp. + *Rhizobium* sp. + *Azotobacter* sp.	1.35 ± 0.05^{c}	Average
Chlorothalonil	0.52 ± 0.03^{b}	Weak
Cytosinpeptidemycin	0.51 ± 0.06^{b}	Weak
Control (water)	0.00^{a}	None
LSD	0.19	
Fpr	< 0.001	

The alphabet lelters were automatically produced by Genstat software when we did the analysis of difference of means with $p = 0.05$ using Duncan test. If the means are significantly difference, it will show different letters (e.g a, b, c) next to the means. If the means are not significantly difference, it will show the same letters (eg. a, a). These results did not consider variations between chemicals in solubility and impact on inhibition

The pathogen was reisolated from roots, and all isolates (15 per treatment) on agar produced lumpy-branching hyphae with hyphal swellings. Sporangia were papillate and caducous with short pedicels up to 6 µm long. Sporangial shape varied from ellipsoid, ovoid, pyriform, obpyriform to near spherical. The characteristics were the same as described previously by Chi et al. (2019).

Reducing *P. palmivora* disease in *C. reticulata* seedlings

After 40 days of inoculation 90.0–93.3% of the *C. reticulata* seedlings were infected with a mild and homogeneous disease index (DI = 1.00–1.05). Seedlings with uniform symptoms of leaf yellowing (Fig. 2) were selected, ensuring homogeneity before application of inhibitory treatments.

After 50 days, the disease incidence (P%) and disease index (DI) differed significantly (Fpr < 0.001) between treatments (Table 3). Approximately 50% of the control seedlings were wilted compared to 6% of seedlings in the metalaxyl-M treatment. Plants treated with metalaxyl-M and potassium phosphonate had the lowest P% after 90 days with 26.7% and 28.3% of seedlings, respectively, followed by mancozeb (40.0%). The mean disease index of these fungicides was also small, ranging from 0.57 to 0.93. In comparison, the biofungicide *T. viride* was only mildly effective with P% of 60.0%. The untreated control had a disease incidence of 96.7% and disease index of 2.82.

Reducing *P. palmivora* disease in a mandarin orchard

Disease classification of the canopy before treatments were first applied indicated that the trees were infected mildly and homogeneously (DI = 0.50–0.51; disease incidence P% = 40.0–42.2%, the trees were yellowing and had sparse foliage, ensuring homogeneity for the experiment before application of treatments (Fig. 3a). The P% and DI for plants after 6 months differed significantly (Fpr < 0.001) among treatments (Table 4).

After 6 months, trees treated with potassium phosphonate or metalaxyl-M yielded the lowest P%, 21.1% and 22.2% of trees, respectively. The mean DI of these treatments decreased to 0.30 to 0.37. *T. viride* was only weakly effective with P% of 38.9% and DI of 0.77, the latter higher than before the treatment was applied (Fig. 3c). The P% and

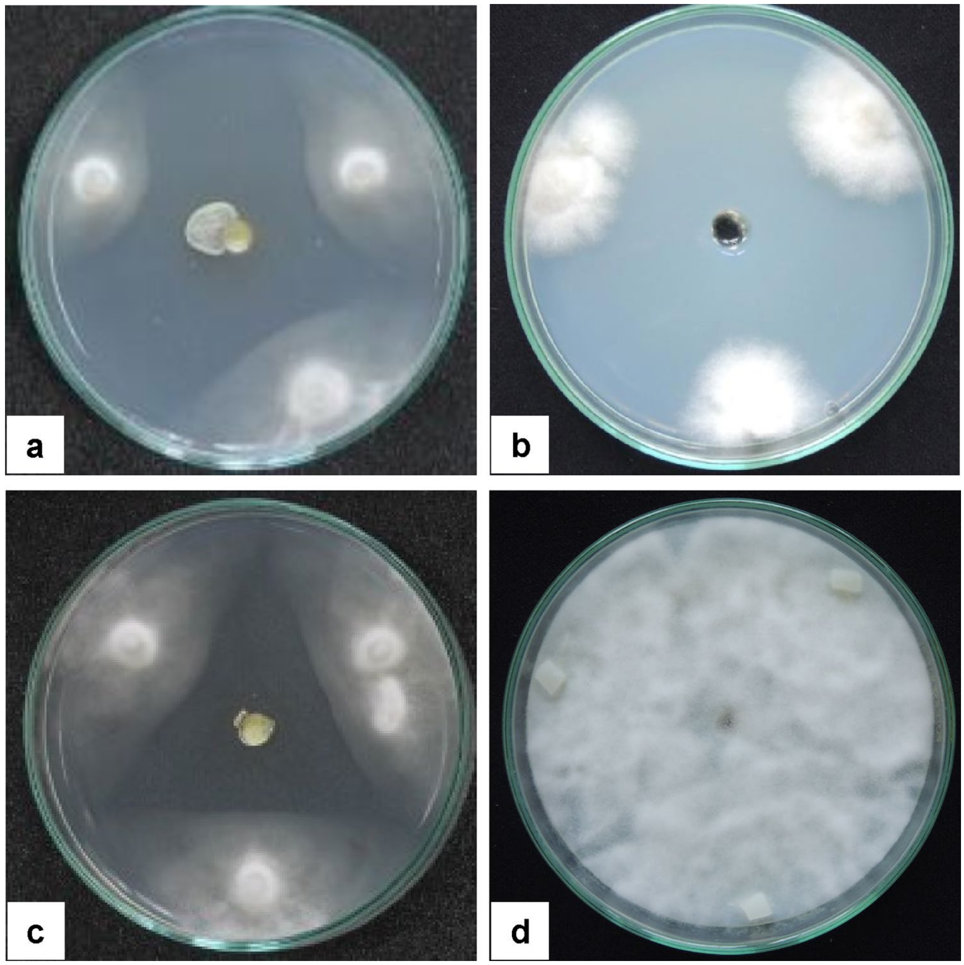

Fig. 1 Inhibition of *Phytophthora palmivora* on PDA by **a** metalaxyl-M; **b** potassium phosphonate; **c** *Trichoderma viride*; **d** control

DI in the control treatment increased significantly to 66.7% and 1.20, respectively (Fig. 3d). Disease symptoms were the most reduced by potassium phosphonate (Fig. 3b).

Discussion

This is the first study to compare the effectiveness of fungicides and biological agents against *P. palmivora,* the cause of Phytophthora disease in *C. reticulata* in Vietnam. The results showed that metalaxyl-M, potassium phosphonate, mancozeb, and *T. viride* strongly inhibited the growth of *P. palmivora* in vitro and markedly reduced disease in inoculated seedlings. Furthermore, potassium phosphonate and metalaxyl-M applied by spraying the canopy and soil led to the recovery about 50% of grafted mandarin trees in a commercial orchard. These findings provide an interim option for producers to better manage Phytophthora disease in Vietnam.

The fungicides identified in the present study with the greatest inhibitory effect against *P. palmivora* have been tested in numerous studies for their efficacy against a wide range of diseases in crops and trees. Potassium phosphonate has been widely used for decades to control root rot caused by *P. cinnamomi* in many different forest, plantation, and horticultural tree species (González et al. 2017). Potassium phosphonate has been used to control lemon brown rot caused by *P. citrophthora* both in vitro and in orchards. For example, it reduced by 40–60% the incidence of rot in fruit (Ramallo et al. 2019). Metalaxyl-M + mancozeb, fosetyl-Al, and phosphorous acid have been used to control Phytophthora crown and root rot caused by *P. palmivora* in apricot and cherry (Türkölmez and Derviş 2017). Metalaxyl-M and mancozeb inhibited mycelial growth of *Phytophthora* species in vitro (Rolando et al. 2017) and in pot trials (Reglinski et al. 2009), and they have been used for management of *Botryosphaeria* causing stem cankers in field-grown *Protea magnifica* (Denman et al. 2004). In our study, metalaxyl-M, potassium phosphonate, and mancozeb strongly inhibited the growth of *P. palmivora* in vitro. After screening of fungicides in vitro, it is important that field trials are established as efficacy of fungicides can be changed in field conditions for control of pathogens.

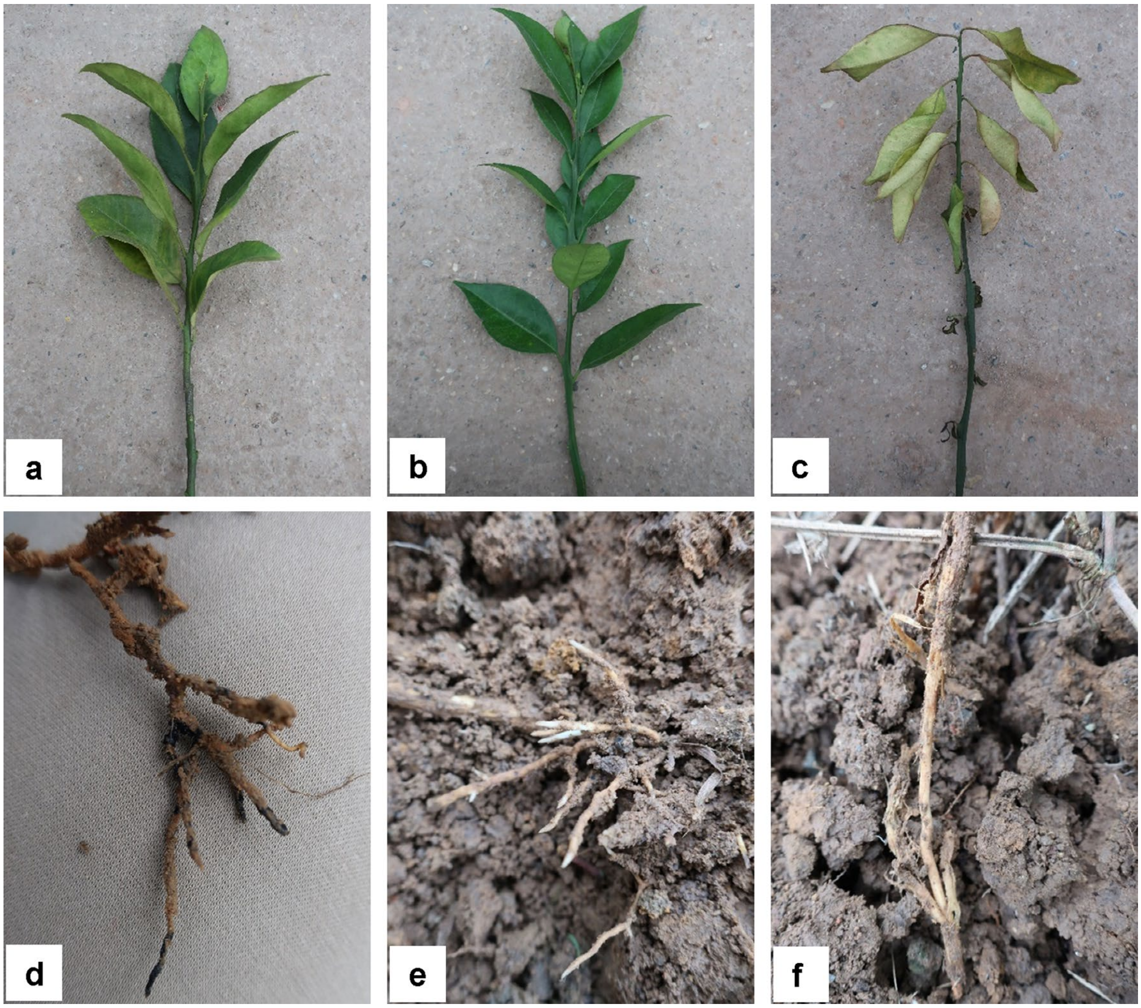

Fig. 2 Effect of *Phytophthora palmivora* infection on *Citrus reticulata* seedlings **a**, **d** above- and belowground at 40 days after inoculation; **b**, **c**, **e**, **f** 50 days after treatment: **b**, **e** diseased seedling after metalaxyl-M above- and belowground; **c**, **f** control above- and belowground

Table 3 Disease incidence (P%) and disease index (DI) for foliage on 6-month-old *Citrus reticulata* seedlings after various chemical or biological treatment

Treatment	Foliage at time of first application		Foliage 50 days after first application		Roots 50 days after first application		Disease inhibition (%)		Trees recovered (%)
	P%	DI	P%	DI	P%	DI	P%	DI	
Metalaxyl-M	91.7^a	1.02^a	26.7^a	0.55^a	26.7^a	0.58^a	72.9	80.2	70.9
Potassium phosphonate	91.7^a	1.02^a	28.3^a	0.57^a	28.3^a	0.60^a	71.2	79.6	69.1
Mancozeb	93.3^a	1.05^a	40.0^b	0.90^b	40.0^b	0.97^b	60.1	68.4	57.1
Trichoderma viride	91.7^a	1.03^a	60.0^c	1.52^c	60.0^c	1.55^c	39.1	47.3	34.6
Control	90.0^a	1.00^a	96.7^d	2.80^d	96.7^d	2.83^d	0.0	0.0	-
LSD	5.94	0.09	6.22	0.12	6.22	0.136			
Fpr	0.835	0.828	< 0.001	< 0.001	< 0.001	< 0.001			

The alphabet lelters were automatically produced by Genstat software when we did the analysis of difference of means with $p = 0.05$ using Duncan test. If the means are significantly difference, it will show different letters (e.g a, b, c) next to the means. If the means are not significantly difference, it will show the same letters (eg. a, a). The agents were sprayed to the seedling and applied to the soil (400 ml/seedling)

LSD least significant difference, *Fpr F*-probabilities for the analysis of variance

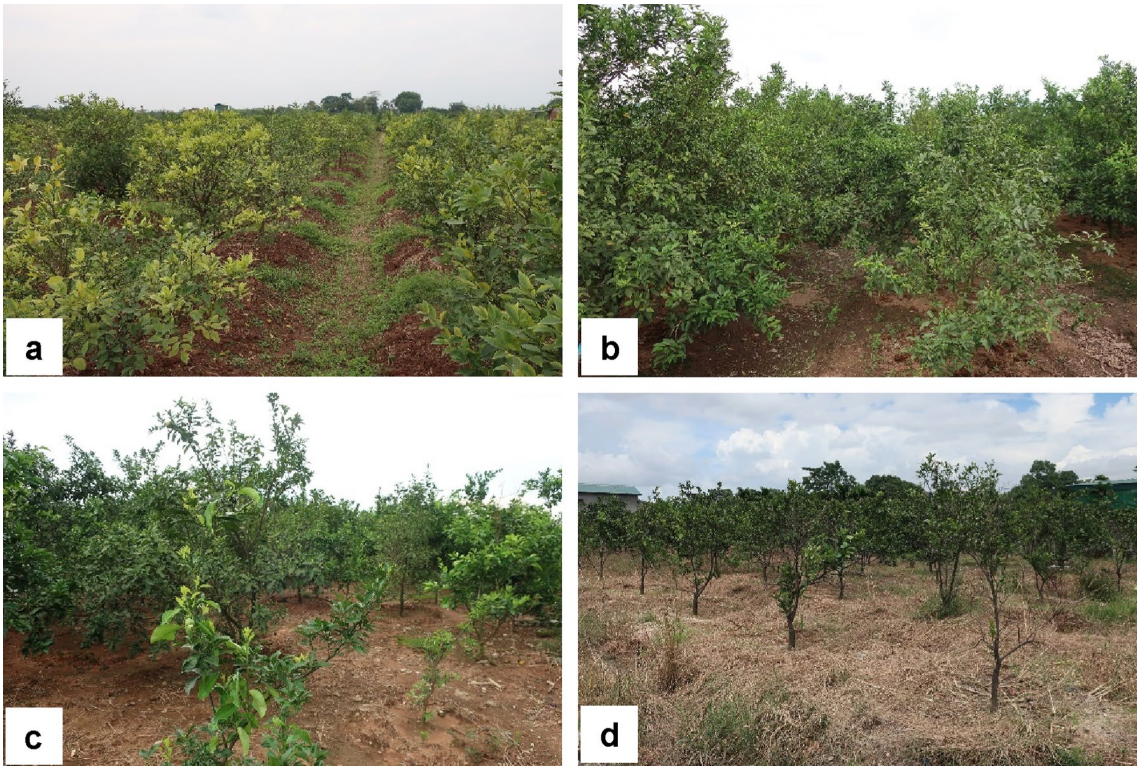

Fig. 3 Appearance of *Citrus reticulata* trees in commercial orchard after infection with *Phytophthora palmivora.* **a** When the trial was established, approximately 40% of the trees were yellow. **b** Six months after potassium phosphonate treatment, the trees were green, and 50% had recovered. **c** Six months after *Trichoderma viride* treatment, the trees had some new shoots, but only 5.4% had recovered. **d** Untreated control trees had yellow leaves, dead branches or loss of canopy, and symptoms were more severe than 6 months before

Table 4 *Phytophthora palmivora* disease incidence (P%) and disease index (DI) for foliage on *Citrus reticulata* trees in a commercial orchard at the time of application various chemical or biological treatments and 6 months after application

Treatment	Aboveground appearance of trees				Disease inhibition (%)		Trees recovered (%)
	At application		At 6 months				
	P%	DI	P%	DI	P%	DI	
Potassium phosphonate	42.2^{a}	0.51^{a}	21.1^{a}	0.30^{a}	70.0	75.5	50.0
Metalaxyl-M	41.1^{a}	0.51^{a}	22.2^{a}	0.37^{b}	67.6	70.1	45.9
Mancozeb	40.0^{a}	0.50^{a}	27.8^{b}	0.43^{c}	58.3	63.9	30.6
Trichoderma viride	41.1^{a}	0.51^{a}	38.9^{c}	0.77^{d}	43.2	37.5	5.4
Control	40.0^{a}	0.50^{a}	66.7^{d}	1.20^{e}	0.0	0.0	-
LSD	3.84	0.05	3.13	0.06			
Fpr	0.682	0.951	< 0.001	< 0.001			

The alphabet lelters were automatically produced by Genstat software when we did the analysis of difference of means with $p = 0.05$ using Duncan test. If the means are significantly difference, it will show different letters (e.g a, b, c) next to the means. If the means are not significantly difference, it will show the same letters (eg. a, a)

LSD least significant difference, *Fpr F*-probabilities for the analysis of variance

The same three fungicides were also effective in reducing symptoms of *P. palmivora* disease in nursery seedlings and in a commercial mandarin orchard. The disease incidence on 6-month-old inoculated seedlings was less than 40% following treatment, whereas the control seedlings were severely diseased (P% = 96.7%). In the commercial orchard, the trees showed symptoms of disease in the years before treatment (2017: P% = 8.0–10.0%; 2018: P% = 15.0–20.0%), and at the time of treatment, the trees were just coming into bearing age (Chi et al. 2019). The disease incidence of mandarin trees was reduced to less than 28% after treatment with potassium phosphonate, metalaxyl-M, and mancozeb, and the untreated trees in the control treatment remained severely diseased (66.7% disease incidence).

We showed that the biological agent *T. viride* had some potential to inhibit the growth of *P. palmivora* in vitro and reduce disease on seedlings and in mandarin trees. However, it was not as effective as potassium phosphonate, metalaxyl-M, or mancozeb. On our results so far, the putative biological control agents that were tested are not recommended for commercial use in mandarin orchards in northern Vietnam. So far, most commercial biological control agents apparently are ineffective in controlling Phytophthora in vivo.

In this study, we only tested a single concentration of each treatment, and therefore, we were unable to determine the most effective concentration of each treatment. Further research is required not only to determine the optimal concentrations for control of *P. palmivora* disease in *C. reticulata* in the field, but also to identify the number and timing of applications to optimize disease control.

As new fungicides are approved for use in Vietnam, they can be evaluated in local conditions. Recently, registration for the fungicides ethaboxam and mandipropamid has been requested, and two other fungicides, fluopicolide and oxathiapiprolin, have received full registration for the control of Phytophthora root rot in citrus in the United States (Hao et al. 2019).

Another factor to be considered is the mode of application of fungicides to citrus in Vietnam. *P. cinnamomi* root rot in avocado was controlled by spraying or drenching with phosphorous acid and a 20% solution of partially neutralised phosphorus acid injected into the trunks of 7-year-old diseased trees resulted in the rapid recovery of trees (Pegg et al. 1985). Trunk injections are now routinely being applied to many tree species. Holderness (1992) showed that trunk injection of potassium phosphonate gave control of *P. palmivora* pod rot comparable to that obtained with conventional metalaxyl sprays. Furthermore, potassium phosphonate foliar sprays gave poor control of both canker and pod rot and were less effective than either trunk injection or metalaxyl sprays.

More attention should be paid to hygiene, selection of rootstocks, orchard design, and management. Pomelo seedlings are routinely used as rootstock in northern Vietnam, but they are susceptible to *P. palmivora* and *P. nicotianae* (Hung et al. 2015b, a). The local mandarin in China (*C. reticulata*) called Guanggan is strongly resistant to *P. nicotianae* (Yan et al. 2017) and could be evaluated as a rootstock in Vietnam. Phytophthora disease is known to be more prevalent when citrus orchards are flooded (Chaudhary et al. 2016). The orchard used in our study was well drained, but many orchards in the region have water ponds between trees after monsoonal rain. Future work to develop sustainable integrated management strategies for local conditions will greatly benefit growers and the environment.

Acknowledgements This work was supported by the Department of Science and Technology of Quang Ninh Province, Vietnam under decree number 1756/QD-UBND dated 21/5/2018. We thank the numerous households for their support in collecting data, and Gondess Pty Ltd for assistance with travel for BD.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ahmed Y, D'Onghia AM, Ippolito A, Yaseen T (2014) First report of citrus root rot caused by *Phytophthora palmivora* in Egypt. Plant Dis 98:155

Anonymous (2016) Citrus fruit-fresh and processed. Statistical Bulletin 2016. Food and Agriculture Organization of the United Nations (FAO) Rome, Italy 2017, https://www.fao.org/3/a-i8092e.pdf. Accessed 26 Dec 2019

Anonymous (2018) Sản lượng cây có múi năm 2018 [Yield of citrus in 2018, in Vietnamese]. General Statistics Office of Viet Nam. https://www.gso.gov.vn/SLTK/Table.aspx?rxid=8f161760-9ba0-4c6d-8898-fdef1a92c072&px_db=06.+N%c3%b4ng%2c+l%c3%a2m+nghi%e1%bb%87p+v%c3%a0+th%e1%bb%a7y+s%e1%ba%a3n&px_type=PX&px_language=vi&px_tableid=06.+N%c3%b4ng%2c+l%c3%a2m+nghi%e1%bb%87p+v%c3%a0+th%e1%bb%a7y+s%e1%ba%a3n%5cV06.34.px&layout=tableViewLayout1. Accessed 2 Feb 2020

Boughalleb-M'hamdi N, Benfradj N, Migliorini D, Luchi N, Santini A (2018) *Phytophthora nicotianae* and *P. cryptogea* causing gummosis of citrus crops in Tunisia. Trop Plant Pathol 43:36–48

Chaudhary S, Kusakabe A, Melgar JC (2016) Phytophthora infection in flooded citrus trees reduces root hydraulic conductance more than under non-flooded condition. Sci Hort 202:107–110

Chi NM, Thu PQ, Hinh TX, Quan DT, Nam NV (2019) Fungal composition belonging Pythiaceae causing root rot disease on citrus in Quang Ninh Province. Vietnam Sci Tech J Agric Rural Dev 3:97–103

de Oliveira TAS, Blum LEB, Duarte EAA, Luz EDMN (2018) Control of *Phytophthora palmivora* on postharvest papaya with *Trichoderma asperellum*, *T. virens*, *T. harzianum* and *T. longibrachiatum*. Biosci J 34:1513–1521

Denman S, Crous PW, Sadie A, Wingfield MJ (2004) Evaluation of fungicides for the control of *Botryosphaeria protearum* on *Protea magnifica* in the Western Cape Province of South Africa. Australas Plant Pathol 33:97–102

González M, Caetano P, Sánchez ME (2017) Testing systemic fungicides for control of *Phytophthora* oak root disease. For Pathol 47:e12343

Graham JH (2011) Phosphite for control of Phytophthora diseases in citrus: model for management of *Phytophthora* species on forest trees? NZ J Forestry Sci 41S:S49–S56

Graham J, Feichtenberger E (2015) Citrus Phytophthora diseases: management challenges and successes. J Cit Pathol 2:iocv_journalcitruspathology_27203

Graham JH, Bright DB, McCoy CW (2003) *Phytophthora-Diaprepes* weevil complex: *Phytophthora* spp. relationship with citrus rootstocks. Plant Dis 87:85–90

Hao W, Gray MA, Förster H, Adaskaveg J (2019) Evaluation of new Oomycota fungicides for management of Phytophthora root rot of citrus in California. Plant Dis 103:619–628

Holderness M (1992) Comparison of metalaxyl/cuprous oxide sprays and potassium phosphonate as sprays and trunk injections for control of *Phytophthora palmivora* pod rot and canker of cocoa. Crop Protect 11:141–147

Hung PM, Wattanachai P, Kasem S, Poeaim S (2015a) Efficacy of *Chaetomium* species as biological control agents against *Phytophthora nicotianae* root rot in citrus. Mycobiology 43:288–296

Hung PM, Wattanachai P, Kasem S, Poeaim S (2015b) Biological control of *Phytophthora palmivora* causing root rot of pomelo using *Chaetomium* spp. Mycobiology 43:63–70

Lawrence SA, Armstrong CB, Patrick WM, Gerth ML (2017) High-throughput chemical screening identifies compounds that inhibit different stages of the *Phytophthora agathidicida* and *Phytophthora cinnamomi* life cycles. Front Microbiol 8:1340

Lima RPM, Máximo HJ, Merfa MV, Dalio RJD, Cristofani-Yaly M, Machado MA (2018) Genetic tools and strategies for citrus breeding aiming at resistant rootstocks to gummosis disease. Trop Plant Pathol 43:279–288

McGrath MT (2009) Fungicides and other chemical approaches for use in plant disease control. In: Schaechter M (ed) Encyclopedia of microbiology, 3rd edn. Academic Press, San Diego, pp 412–421

Mourão Filho FdAA, Pio R, Mendes BMJ, de Azevedo FA, Schinor EH, Entelmann FA, Alves ASR, Cantuarias-Avilés TE (2008) Evaluation of citrus somatic hybrids for tolerance to *Phytophthora nicotianae* and citrus tristeza virus. Sci Hort 115:301–308

Pegg KG, Whiley AW, Saranah JB, Glass RJ (1985) Control of Phytophthora root rot of avocado with phosphorus acid. Australas Plant Pathol 14:25–29

Quang NH (2013) The results of technically planting model trial of orange in Ba Be district. Infor Sci Tech Bac Kan Prov 2:6–7

Ramallo AC, Cerioni L, Olmedo GM, Volentini SI, Ramallo J, Rapisarda VA (2019) Control of Phytophthora brown rot of lemons by pre-and postharvest applications of potassium phosphite. Eur J Plant Pathol 154:975–982

Reglinski T, Spiers TM, Dick MA, Taylor JT, Gardner J (2009) Management of phytophthora root rot in radiata pine seedlings. Plant Pathol 58:723–730

Rolando CA, Dick MA, Gardner J, Bader MK-F, Williams NM (2017) Chemical control of two *Phytophthora* species infecting the canopy of Monterey pine (*Pinus radiata*). For Pathol 47:e12327

Singh J, Tripathi NN (1999) Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. Flavour Frag J 14:1–4

Sriwati R, Chamzurn T, Soesanto L, Munazhirah M (2019) Field application of Trichoderma suspension to control cacao pod rot (*Phytophthora palmivora*). Agrivita J Agric Sci 41:175–182

Tashiro N, Uematsu S, Ide Y, Matsuzaki M (2012) First report of *Phytophthora palmivora* as a causal pathogen of citrus brown rot in Japan. J Gen Plant Pathol 78:233–236

Thanh DVT, Vien NV, Drenth A (2004) Phytophthora diseases in Vietnam. In: Drenth A, Guest DI (eds) Diversity and management of Phytophthora in Southeast Asia. Australian Centre for International Agricultural Research, Canberra, pp 83–89

Thanh NN, Thu TA, Anh VTV, Loi NV, Guong VT (2018) Present situation of King mandarin technical cultivation in Tam Binh District, Vinh Long Province. J Vietnam Agric Sci Tech 4:38–44

Tien HD (2013) Situation of production, planning for development of oranges in Bac Kan Province. Infor Sci Tech Bac Kan Prov 2:8–10

Timmer LW, Garnsey SM, Broadbent P (2003) Diseases of citrus. In: Ploetz RC (ed) Diseases of tropical fruit crops. CABI, Wallingford, UK, pp 163–195

Türkölmez Ş, Derviş S (2017) Activity of metalaxyl-M + mancozeb, fosetyl-Al, and phosphorous acid against Phytophthora crown and root rot of apricot and cherry caused by *Phytophthora palmivora*. Plant Protect Sci 53:216–225

Vawdrey LL, Grice KE, Peterson RA, De Faveri J (2004) The use of metalaxyl and potassium phosphonate, mounds, and organic and plastic mulches, for the management of Phytophthora root rot of papaya in far northern Queensland. Australas Plant Pathol 33:103–107

Watanarojanaporn N, Boonkerd N, Wongkaew S, Prommanop P, Teaumroong N (2011) Selection of arbuscular mycorrhizal fungi for citrus growth promotion and *Phytophthora* suppression. Sci Hort 128:423–433

Widmer TL, Graham JH, Mitchell DJ (1998) Histological comparison of fibrous root infection of disease-tolerant and susceptible citrus hosts by *Phytophthora nicotianae* and *P. palmivora*. Phytopathology 88:389–395

Yan H, Zhong Y, Jiang B, Zhou B, Wu B, Zhong G (2017) Guanggan (*Citrus reticulata*) shows strong resistance to *Phytophthora nicotianae*. Sci Hort 225:141–149