https://doi.org/10.17221/1/2020-PPS

Assessing BactoMix 5 efficacy for clubroot control in naturally infested soil

Kaire Loit, Riinu Kiiker, Britt Puidet, Liina Soonvald, Marian Põldmets, Marika Mänd*

Chair of Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia

**Corresponding author: riinu.kiiker@emu.ee*

Citation: Loit K., Kiiker R., Puidet P., Soonvald L., Põldmets M., Mänd M. (2021): Assessing BactoMix 5 efficacy for clubroot control in naturally infested soil. Plant Protect. Sci., 57: 14–20.

Abstract: The cultivation of cruciferous crops is threatened by extensive yield losses caused by the soil-borne pathogen *Plasmodiophora brassicae* Woronin, 1877. The objective of the study was to assess the potential of the bacterial product BactoMix 5 for the control of clubroot on a naturally infested soil in growth chamber trials using a *P. brassicae*-specific qPCR methodology. The results did not show a significant decrease in the *P. brassicae* in the soil nor a reduction of the disease symptoms on the plants. The native soil microbiota may have exhibited an antagonistic activity against the bacterial species from BactoMix 5 and evoked the poor effect of the product. Therefore, potential biological control agents should be tested with native soil microbiota and the regional production should be advanced to increase the product efficacy in the environment.

Keywords: *Plasmodiophora brassicae*; *Brassica*; biocontrol; biostimulant

The causal agent of clubroot disease, *Plasmodiophora brassicae* (Woronin) is a serious soil-borne pathogen of cruciferous crops worldwide (Dixon 2009; Hwang et al. 2012). It is especially dangerous due its ability to survive in the soil for up to 20 years as resting spores (Wallenhammar 1996).

So far, only precautionary methods, such as applying a longer crop rotation, growing more resistant cultivars, liming the field before planting and disinfecting the equipment have been implemented to control clubroot disease epidemics (reviewed by Říčařová et al. 2016). There are currently no synthetic plant protection products in use to effectively control the clubroot disease of brassica crops (CABI 2019). The fungicide Altima 500 SC (a.i. fluazinam, Kurowski et al. 2009) and Ranman (a.i. cyazofamid, Peng et al. 2014) have shown efficacy in controlling clubroot when the pathogen inoculum level was low (Peng et al. 2014). Also, due to the side effects on non-target organisms and the environment, soil-drench treatments with chemical fungicides are not standardly used in European agricultural practice.

The biological control of the clubroot disease is a more environmentally friendly and sustainable alternative for the implementation in integrated pest management (IPM) strategies, compared to using synthetic fungicides (Kumar 2012). The biological control includes the ability of microbiological antagonists to inhibit plant pathogens and pests, favour the growth and development of the plant, enhance the resilience, inactivate toxins and strengthen the plants' ability to compete for nutrients (Pal & Mcspadden Gardener 2006). There are numerous biological control agents (BCAs) used against pathogens,

Supported by the Republic of Estonia Ministry of Education and Research, Institutional Research, Project No. IUT36-2 and by the Development Fund of Estonian University of Life Sciences, Project No. PM170156PKTK.

https://doi.org/10.17221/1/2020-PPS

weeds, arthropods and nematodes, and most of them are designed to suppress soil-borne pathogens (Vinale et al. 2008; Singh 2014). BCAs can inhibit the spread of a pathogen *in vitro*, but can also survive and multiply in the field (O'Brien 2017). One drawback of a biocontrol is the inconsistency in the disease suppression by a BCA (O'Brien 2017). Therefore, many manufacturers have simultaneously used multiple BCAs in their products (Xu et al. 2011).

BactoMix 5 is a combination of different BCAs that each serve a purpose for the benefit of the host plant. For instance, *Pseudomonas aurantiaca* primarily inhibits the pathogen development (Mehnaz 2013), *Brevibacillus* sp. produces antifungal compounds, and favours plant growth (Walker et al. 1998; Panda et al. 2014), and some strains of *Bacillus subtilis* have shown a positive impact on suppressing the soil-borne pathogen *Plasmodiophora brassicae* Woronin, 1877 (Lahlali et al. 2013).

To our knowledge, no studies have assessed the effect of BCAs in a naturally infested soil under a controlled environment. The objective of this study was to assess the potential of the commercially available bacterial product BactoMix 5 against the clubroot disease-causing pathogen *P. brassicae* in a naturally infested soil. We hypothesised that the bioproduct BactoMix 5 suppresses *P. brassicae*, and, therefore, can be applied within a *P. brassicae* biocontrol strategy.

MATERIAL AND METHODS

Pot experiment. Soil naturally infested with *P. brassicae* was collected on the 23rd of October 2017 from the post-harvest organic cabbage and turnip farming experimental plots at the Rõhu research station (58°21'58.4" N 26°39'55.1" E). Composite bulk soil samples were collected, using a soil auger, from the topsoil layer (0–20 cm) in each experimental plot (each soil sample consisted of 20 random piercings from each experimental plot). The soil type was Leptosol (IUSS WG WRB 2015) with a pH of 5.83. The soil was maintained at 4 °C until the beginning of the pot experiment, which was conducted from the 19th of March 2018 until the 25th of May 2018. The five treatments included a control treatment (C), 1 mL of BactoMix 5 applied once throughout the growing season (T1), 2 mL of BactoMix 5 applied once throughout the growing season (T2), 1 mL of BactoMix 5 applied twice throughout the growing season (T3), 2 mL of BactoMix 5 applied twice throughout the growing season (T4) (Table 1), each with six replications. To determine the inoculum level of the pathogen at the beginning of the experiment, DNA was extracted from the homogenised soil before starting the pot experiment. In each 2 L pot, two *Brassica rapa* var. *pekinensis* cv. Granaat (Chinese cabbage) seeds were planted, and one plant was left to grow after sprouting. For the inoculation treatments, the bacterial product BactoMix 5 (which consists of the biologically active microorganism species *Bacillus subtilis* V-845 D and V-843 D, *B. megaterium*, *Pseudomonas aurantiaca* and *Brevibacillus* sp.) was used.

The plants were grown in a growth chamber with a 16 h light period (22 °C) and 8 h of darkness (15 °C). The relative humidity during germination was 70%, whereas it was set to 60% during the plant growth period. The plants were watered regularly with tap water. At the end of the five-week growing period, all Chinese cabbage plants were uprooted, and the clubroot infection was determined on a 0–3 scale according to Kuginuki et al. (1999). The remaining soil was dried and maintained at room temperature until the DNA extraction.

Quantification of *Plasmodiophora brassicae* via qPCR. Two 0.2 g soil samples were collected from each replication and pooled together. The samples were homogenised by bead beating with MixerMill MM400 (Retsch, Germany) using three metal balls added to the tubes. The DNA was extracted using a DNeasy PowerSoil Kit (Qiagen, USA), following the manufacturer´s protocol. The concentration of the DNA was measured using a NanoDrop 2000 (Thermo Fisher Scientific, USA) spectrophotometer. The acquired DNA was maintained at –20 °C until further analysis.

The qPCR reactions were performed using the *P. brassicae* 18S rRNA-specific primers TC1F (5'TGG-TCGAACTTCATTAAATTTGGGCTCTT- 3') and

Table 1. Applied treatments used in the study

Treatment symbol	Treatment	Treatment timing	
		1st day	20th day
C	untreated soil		
T1	I*	×	
T2	II**	×	
T3	I*	×	×
T4	II**	×	×

*1 mL of BactoMix 5 + 100 mL of distilled water; **2 mL of BactoMix 5 + 100 mL of distilled water; × – applied treatment

https://doi.org/10.17221/1/2020-PPS

RTPcR1a (5' TCAGCACCGTTTCCGGCTGCTA-AGGC- 3') (Cao et al. 2014). All the samples were amplified in three technical replicates using a reaction mix in a 20 µL volume consisting of 1 µL of the sample DNA (at 10 ng/µL), each of the primers in a final concentration of 0.3 µM, 4 µL of a 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Estonia) and sterile distilled water. The amplification was performed on a qPCR cycler Rotor Gene Q (Qiagen, USA). The PCR setup for the amplification was as follows: 50 °C for 2 min [with the UDG (Uracil-DNA glycosylase) enzyme]; 95 °C for 12 min; and 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 seconds. Immediately after the amplification, a melting curve analysis was performed by increasing the temperature from 70 to 95 °C (0.35 °C every 3 s), with a continuous fluorescence recording to confirm the specificity of the amplification. A template control without the DNA was included in every qPCR run. In each assay, a sterilised field-collected soil (autoclaved at 121 °C for 20 min) was used as the negative control, and the DNA extracted from the resting spores in the clubroot galls (Castlebury et al. 1994) was used as the positive control, both originating from the experimental site.

A standard calibration curve was prepared to assess the number of gene copies in the samples (Figure 1). The standard calibration curve prepared with the sterile distilled water consisted of a 10-fold serial dilution series of the genomic DNA of the *P. brassicae* extracted from the resting spores in the clubroot galls (Castlebury et al. 1994), ranging from 1×10^6 to 1×10^2 gene copies/µL.

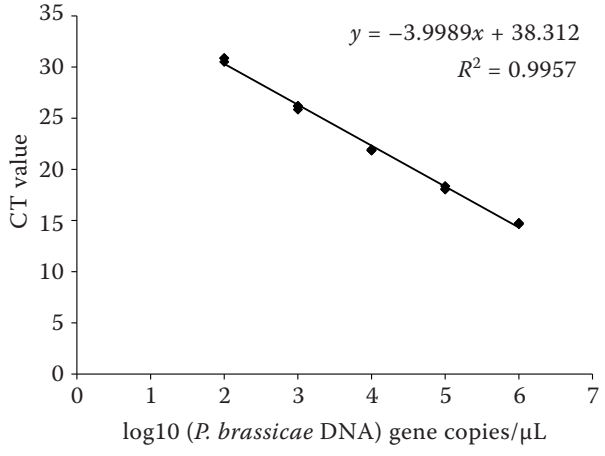

Figure 1. Standard calibration curve of the qPCR assays with a tenfold serially diluted *P. brassicae* 18S rRNA gene (106 to 102 copies/µL)

CT – the number of cycles required for the fluorescent signal to cross the threshold

qPCR data quality estimation and statistical analyses. The qPCR analysing program LinRegPCR (version 2013.0) was used to assess the efficacy of the individual replications (Ruijter et al. 2009). The mean efficacy of the amplification was calculated for each replication. The samples were analysed using an outlier detection, where the technical replicates with an individual efficacy aberration of > 5% from the mean efficacy were excluded. Thus, two T1 and T3 replications, and one T2 replication, were excluded from the further analysis. The 18S rRNA gene copy numbers of the tested pathogen were determined after quality control by comparing the results of the amplification with the standard curve. The DNA quantification results were converted to *P. rassicae* 18S rRNA gene copies per 1 g of the original soil sample.

The data analysis was performed with STATISTICA (version 64), using Kruskal-Wallis ANOVA multiple comparisons between the independent groups of the samples. The *P*-value was set to $P < 0.05$.

RESULTS

A reproducible linear standard curve of *P. brassicae* DNA was generated from 1×10^6 to 1×10^2 gene copies/µL (corresponding to 2.75×10^4 pg to 2.75 g DNA). Thus, the detection limit of the qPCR assay was regarded as 100 gene copies/µL (Figure 1). A good PCR amplification efficiency (E = 1.908) of the standard curve was confirmed. The mean amplification efficacy of our qPCR assay was calculated based on three technical replicates of each replication. In ideal conditions, the amount of DNA is duplicated after each qPCR amplification cycle (E = 2). The mean amplification efficacies of *P. brassicae* DNA in the individual replications were between 1.826 and 1.945.

In the pot experiment, all the Chinese cabbage plants from each treatment were visually assessed as having severe symptoms of clubroot disease infection. The mean amount of the pathogen DNA in the soil was calculated before starting the pot experiment (O), for each treatment (T1, T2, T3, T4) and for the untreated control (C) (Table 2). Before starting the pot experiment (O), the mean amount of *P. brassicae* was 412 146.4 gene copies/g soil (SE = 65 989.8). When taking the infestation level of the pathogen at the beginning of the experiment into account,

https://doi.org/10.17221/1/2020-PPS

Table 2. Mean amount of *Plasmodiophora brassicae* genomic DNA copies/g in the soil detected for each treatment

Treatment	Mean *P. brassicae* DNA gene copies	SE
O	412 146.4	65 989.8
C	426 874.1	238 473.7
T1	98 822.4	46 131.3
T2	65 321.3	21 435.3
T3	301 465.9	204 319.7
T4	172 065.7	75 373.5

O – before starting the experiment; C – control; T1 – 1 mL of BactoMix 5; T2 – 2 mL of BactoMix 5; T3 – 1 mL of BactoMix 5 applied twice during the plant's growth; T4 – 2 mL of BactoMix 5 applied twice during the plant's growth

the mean amount of the pathogen was reduced in all of the treatments at the end of the experiment (76.0% in T1; 84.2% in T2; 26.9% in T3; 58.3% in T4). Conversely, a slight increase (3.6%) in the mean pathogen amount occurred in the untreated control.

Among the treatments, the highest pathogen concentration was detected in the T3 treatment, where BactoMix 5 was applied twice; and the lowest concentration was detected in the T2 treatment, where BactoMix 5 was applied once (Figure 2). However, the four treatments (T1, T2, T3, T4) amended with BactoMix 5 were not statistically different from the untreated control (Kruskal-Wallis test: $H_{4,25}$ = 2.004, P = 0.735, Figure 2).

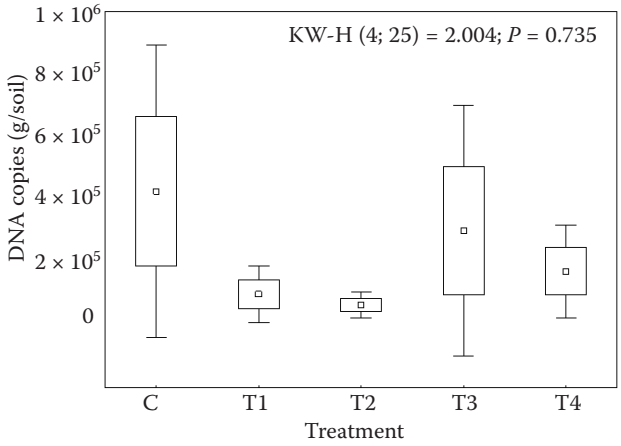

Figure 2. The mean concentration of the *Plasmodiophora brassicae* genomic DNA (DNA copies/g) detected in the soil for each treatment

C – control; T1 – 1 mL of BactoMix 5; T2 – 2 mL of BactoMix 5; T3 – 1 mL of BactoMix 5 applied twice during the plant's growth; T4 – 2 mL of BactoMix 5 applied twice during the plant's growth

DISCUSSION

The aim of the study was to assess the efficacy of the bacterial product BactoMix 5 against the clubroot disease pathogen *P. brassicae* in a naturally infested soil. Soil-borne pathogens are difficult to control due to the dynamic and inconsistent environment of the rhizosphere (Handelsman & Stabb 1996). Our study showed no reduction in the amount of the clubroot disease pathogen *P. brassicae* in the soil, nor any decrease in the disease symptoms on the treated plants compared to those of the control treatment.

The quantitative detection of plant pathogens is an innovative approach in controlling the pathogens of cruciferous plants. Several protocols have been successfully designed to determine the quantity of *P. brassicae* from the soil (Rennie et al. 2011; Wallenhammar et al. 2012; Almquist 2016). The results can be implemented by growers to improve agronomic practices and to keep longer crop rotations between cruciferous crops if necessary. Thus, minimising the spread of the clubroot disease on cruciferous crops.

A generally accepted infection rate to emerge for clubroot symptoms is 1 000 resting spores per 1 g of dry soil (Faggian & Strelkov 2009). However, the threshold could be even lower, depending on the soil type and environmental conditions (Wallenhammar et al. 2012). Growers can use this threshold to decide on the suitability of a field for growing cruciferous crops. Currently, there are no similar experiments conducted in Estonia or the Baltic region that would help to determine these thresholds and develop guidelines for growing cruciferous crops. This study provides the first step in establishing these guidelines.

According to a comprehensive reasearch from Sweden, 325 000 *P. brassicae* DNA gene copies per 1 g of soil is considered as the threshold where even resistant oilseed rape cultivars are not recommended for cultivation (Wallenhammar et al. 2016). At the beginning of our experiment, the mean concentration of *P. brassicae* DNA was 412 000 gene copies per 1 g of soil, a high infection rate. Therefore, growing cruciferous crops in that field is not recommended for several years due to the threat of a *P. brassicae* infection.

According to information from the distributing company, Scandagra (SCANDAGRA 2019), BactoMix 5 helps to promote the soil biological activity, improve the plant health and inhibit plant pathogens.

https://doi.org/10.17221/1/2020-PPS

However, in this study, the inhibiting effect of the biological product BactoMix 5 on the soil-borne pathogen *P. brassicae* was not confirmed. Interestingly, the amount of *P. brassicae* was found to be greater in the treatments, where the soil was treated twice with BactoMix 5 during the plant growth (T3 and 4) compared to the treatments, where the soil was treated only once in the beginning of the experiment (T1 and T2). These results concur with Einhorn et al. (1991), who suggested that antagonistic bacteria may have a stimulating effect on the germination of *P. brassicae* resting spores. However, in several studies, the reducing effect of *Bacillus* sp. on *P. brassicae* has been confirmed (Lahlali et al. 2013; Gao & Xu 2014; Peng et al. 2014; He et al. 2019). The contradictory results may be explained by the antagonistic effect of certain microorganisms. The rhizosphere is a complex environment, and, therefore, it is difficult to predict the effect that biological products have on the plants, as well as on the microorganisms. Furthermore, the efficacy of a product not only depends on the active ingredients, but also the substrate that it is used on (Lengai & Muthomi 2018). Also, the adjuvants in the bioproducts may increase the efficacy in some specific formulations (Todero et al. 2018).

The purpose of combining BCAs in one product is either to confer the additive or synergistic effects on the target organisms (Guetsky et al. 2002). However, Xu et al. (2011) studied the efficacy of biocontrol products having combined BCAs, and their work indicated that the BCAs in these products are more likely to antagonistically interact rather than additively or synergistically.

Several BCA products are on the market, but information about the product efficacy is only available through brochures produced by the distributing companies. The decisions made by farmers could be financially costly if the products applied to the fields do not deliver the desired service. Therefore, testing potential BCAs in advance, in natural soil conditions with native microbiota, should be a task of future research studies to see if BCAs are able to survive within the competitive rhizosphere. It is also important to optimise the concentrations of the products, in order to maximise the efficiency of the product use. Moreover, it is necessary to study the effect of BCA products on different commercial cultivars, and in both conventional and organic cultivation systems, while considering other factors like the soil type and the climatic conditions (O'Brien 2017). Furthermore, several studies have shown the low efficacy of BCAs under high disease pressure, indicating that they should be used rather as components of an integrated disease management system (Donald et al. 2004). Used in combination with agricultural practices, such as longer crop rotation (Wallenhammar 1996), soil amendment (Dixon & Page 1998) and growing disease-resistant cultivars (Donald & Porter 2009), BCAs could help reduce the pathogen load in the soil and allow the biological products to reach their efficacy potential.

CONCLUSION

The biological control of clubroot disease is gaining more attention, as current control measures are satisfactory. This study did not confirm the inhibiting effect of the biological product BactoMix 5 on *P. brassicae* in a naturally infested soil. Assessing the pathogen rate from the soil using highly sensitive qPCR methods allows for more precise cultivation plans and the application of appropriate control measures, as the pathogen *P. brassicae* is a long-term resident in the soil.

Acknowledgement: The authors are grateful to Dr Reet Karise for valuable comments on the manuscript and Jonathan Willow for the language editing.

REFERENCES

Almquist C. (2016): Monitoring important soil-borne plant pathogens in Swedish crop production using real-time PCR. [PhD Thesis]. Upsala, Swedish University of Agricultural Sciences: 78.

CABI (2019): Invasive Species Compendium. Datasheet: *Plasmodiophora brassicae* (club root). Available at https://www.cabi.org/isc/datasheet/41865 (accesed March 28, 2019).

Cao T., Rennie D.C., Manolii V.P., Hwang S.F., Falak I., Strelkov S.E. (2014): Quantifying resistance to *Plasmodiophora brassicae* in Brassica hosts. Plant Pathology, 63: 715–726.

Castlebury, L.A., Maddox J.V., Glawe D.A. (1994): A technique for the extraction and purification of viable *Plasmodiophora brassicae* resting spores from host root tissue. Mycologia, 86: 458–460.

Dixon G.R., Page L.V. (1998): Calcium and nitrogen eliciting alterations to growth and reproduction of *Plasmodiophora brassicae* (clubroot). Acta Horticulturae, 459: 343–349.

Dixon G.R. (2009): The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. Journal of Plant Growth Regulation, 28: 194–202.

https://doi.org/10.17221/1/2020-PPS

Donald C., Lawrence J.M., Porter I. (2004): Influence of particle size and application method on the efficacy of calcium cyanamide for control of clubroot of vegetable brassicas. Crop Protection, 23: 297–303.

Donald C., Porter I. (2009): Integrated control of clubroot. Journal of Plant Growth Regulation, 28: 289–303.

Einhorn G., Bochow H., Huber J., Krebs B. (1991): Methodological studies on the detection of antagonists of the clubroot pathogen *Plasmodiophora brassicae* Wor. Archive für Phytopathologie und Pflanzenschutz, 27: 205–208.

Faggian R., Strelkov S.E. (2009): Detection and measurement of *Plasmodiophora brassicae*. Journal of Plant Growth Regulation, 28: 282–288.

Gao Y., Xu, G. (2014): Development of an effective nonchemical method against *Plasmodiophora brassicae* on Chinese cabbage. International Journal of Agronomy, 2014: 1–5.

Guetsky R., Shtienberg D., Elad Y., Fischer E., Dinoor A. (2002): Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology, 92: 976–985.

Handelsman J., Stabb E.V. (1996): Biocontrol of soilborne plant pathogens. The Plant Cell, 8: 1855–1869.

He P., Cui W., Munir S., He P., Li X., Wu Y., Yang X., Tang P., He Y. (2019): *Plasmodiophora brassicae* root hair interaction and control by *Bacillus subtilis* XF-1 in Chinese cabbage. Biological Control, 28: 56–63.

Hwang S., Strelkov S.E., Feng J., Gossen B. D., Howard R.J. (2012): *Plasmodiophora brassicae*: a review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop. Molecular Plant Pathology, 13: 105–113.

Kuginuki Y., Hiroaki Y., Hirai M. (1999): Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. spp. *pekinensis*). European Journal of Plant Pathology, 105: 327–332.

Kumar S. (2012): Biopesticides: a need for food and environmental safety. Journal of Biofertilizers and Biopesticides, 3: e107. doi: 10.4172/2155-6202.1000e107

Kurowski T.P., Majchrzak B., Kowalska E. (2009): The effectiveness of the biological control of clubroot (*Plasmodiophora brassicae*) in Brassicaceae plants. Phytopathologia, 52: 5–12.

Lahlali R., Peng G., Gossen B.D., McGregor L., Yu F.Q., Hynes R.K., Hwang S.F., McDonald M.R., Boyetchko S.M. (2013): Evidence that the biofungicide Serenade (*Bacillus subtilis*) suppresses clubroot on canola via antibiosis and induced host resistance. Phytopathology, 103: 245–254.

Lengai G., Muthomi J. (2018): Biopesticides and their role in sustainable agricultural production. Journal of Biosciences and Medicines, 6: 7–41.

Mehnaz S. (2013): Secondary metabolites of *Pseudomonas aurantiaca* and their role in plant growth promotion. In: Arora N.K. (ed.): Plant Microbe Symbiosis: Fundamentals and Advances. New Delhi, Springer: 373–393.

O'Brien P. (2017): Biological control of plant diseases. Australasian Plant Pathology, 46: 293–304.

Pal K.K., McSpadden Gardener B. (2006): Biological control of plant pathogens. The Plant Health Instructor. doi: 10.1094/PHI-A-2006-1117-02

Panda A.K , Bisht S.S., DeMondal S., Senthil Kumar N., Gurusubramanian G., Panigrahi A.K. (2014): *Brevibacillus* as a biological tool: a short review. Antonie Van Leeuwenhoek, 105: 623–639.

Peng G., Lahlali R., Hwang S.F., Pageau D., Hynes R.K., Mc-Donald M.R., Gossen B.D., Strelkov S.E. (2014): Crop rotation, cultivar resistance, and fungicides/biofungicides for managing clubroot (*Plasmodiophora brassicae*) on canola. Canadian Journal of Plant Pathology, 36: 99–112.

Rennie D.C, Manolii V.P., Cao T., Hwang S.F., Howard R.J., Strelkov S.E. (2011): Direct evidence of surface infestation of seeds and tubers by *Plasmodiophora brassicae* and quantification of spore load. Plant Pathology, 60: 811–819.

Říčařová V., Kazda J., Singh K., Ryšánek P. (2016): Clubroot caused by *Plasmodiophora brassicae* Wor.: a review of emerging serious disease of oilseed rape in the Czech Republic. Plant Protection Science, 52: 71–86.

Ruijter J.M., Ramakers C., Hoogaars W.M. H., Karlen Y., Bakker O., van den Hoff M.J.B., Moorman A.F.M. (2009): Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Research, 37: e45. doi: 10.1093/nar/gkp045

SCANDAGRA (2019): BactoMix 5. Available at https:// https://scandagra.ee/tooted/bactomix-5/ (accessed March 20, 2019).

Singh H.B. (2014): Management of plant pathogens with microorganisms. Proceedings of the Indian National Science Academy, 2: 443–454.

Todero I., Confortin T.C., Luft L., Brun T., Ugalde G.A., de Almeida T.C., Arnemann J.A., Zabot G.L., Mazutti M.A. (2018): Formulation of a bioherbicide with metabolites from *Phoma* sp. Scientia Horticulturae, 241: 285–292.

Vinale F., Sivasithamparam K., Ghisalberti E., Marra R., Barbetti M.J., Li H., Woo S., Lorito M. (2008): A novel role for The *Trichoderma*–plant interaction *Trichoderma* secondary metabolites in the interactions with plants. Physiological and Molecular Plant Pathology, 72: 80–86.

Walker R., Powell A.A., Seddon B. (1998): *Bacillus* isolates from the spermosphere of peas and dwarf French beans with antifungal activity against *Botrytis cinerea* and *Pythium* species. Journal of Applied Microbiology, 84: 794–801.

Wallenhammar A.C. (1996): Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central

https://doi.org/10.17221/1/2020-PPS

Sweden and factors influencing soil infestation levels. Plant Pathology, 45: 710–719.

Wallenhammar A., Almquist C., Söderström M., Jonsson A. (2012): In-field distribution of *Plasmodiophora brassicae* measured using quantitative real-time PCR. Plant Pathology, 61: 16–28.

Wallenhammar A.C., Gunnarson A., Hansson F., Jonsson A. (2016): Quantification of *Plasmodiophora brassicae* using a DNA-based soil test facilitates sustainable oilseed rape production. Plants (Basel), 5:21. doi: 10.3390/plants5020021

Xu X.-M., Jeffries P., Pautasso M., Jeger M.J. (2011): Combined use of biocontrol agents to manage plant diseases in theory and practice. Phytopathology, 101: 1024–1031.

Received: January 1, 2020
Accepted: October 10, 2020
Published online: November 30, 2020