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The effect of biological and chemical control agents on the health status of the very early potato cultivar Rosara

Bożena Cwalina-Ambroziak, Marta Maria Damszel, Małgorzata Głosek-Sobieraj*

Department of Entomology, Phytopathology and Moleculars Diagnostic, University of Warmia and Mazury in Olsztyn, ul. Michała Oczapowskiego 2, 10-719 Olsztyn

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Abstract: The external appearance and quality of table potatoes are determined, among other factors, by the health status of the plants during the growing season. Chemical control methods are often combined with biocontrol agents to effectively fight potato pathogens. Potatoes of the very early cultivar Rosara were grown in experimental plots. The plots were located in Tomaszkowo (NE Poland, 2007–2009). The experiment involved the following treatments: 1) biological control − mycorrhizal *Glomus* spp. inoculum was applied to the roots, − tubers were dressed and plants were sprayed with Polyversum three times during the growing season, 2) chemical control – at two-week intervals, plants were sprayed with the following fungicides: Infinito 687.5 SC and Tanos 50 WG, Valbon 72 WG and Tanos 50 WG. In the control treatment, potato plants were not protected against pathogens. During the growing season, the severity of late blight and early blight was evaluated on a nine-point scale. The composition of fungal communities colonising potato stems was analysed. The fungistatic properties of the fungicides used in the field experiment were evaluated in an *in vitro* test. The symptoms of infections caused by *Phytophthora infestans* and *Alternaria* spp. were significantly reduced in the treatment which used the integrated chemical and biological control. The least diverse fungal community was isolated from fungicide-treated plants. In the *in vitro* test, fungicides at all analysed concentrations inhibited the linear mycelial growth of selected pathogens.

Key words: diseases of potato, fungi, fungicides, mycorhizal inoculum, Polyversum

Introduction

Potatoes, including very early cultivars, are known for their high nutritional quality: they contain protein of high biological value, vitamin C and the B complex vitamins, minerals, and dietary fiber. Tubers are also a good source of nutritionally important antioxidants such as free and bound phenolic compounds, including chlorogenic acid and catechic acid (Im *et al.* 2008). The quality and yield of table potato tubers may be reduced by a bad forecrop, nutrient deficiency, and infections caused by pathogens that attack both the aboveground parts of potato plants and tubers (Möller *et al*. 2007; Bouws and Finckh 2008). Late blight (*Phytophthora infestans*) and early blight (*Alternaria solani, A. alternata*) are dangerous pathogens of vegetables of the family Solanaceae, including the very early and early potato cultivars (Haverkort *et al.* 2008). Since disease, symptoms appear at early development stages, chemical control programs should rely on prediction models and environmentally friendly plant protection practices aimed to reduce the fungicide dosage and extend intervals between applications (Andreua and Caldiz 2006). To reduce fungicide use, cultivars with improved resistance to pathogens are developed and healthy seed potatoes are planted (Olanya *et al.* 2006). Natural compounds, such as mannitol, citric acid, ascorbic acid, and kaolin, have proven effective against *P. infestans* and *A. solani* under greenhouse and field conditions (Haggag and El-Khair 2007).

Recent years have witnessed the increasing popularity of biological control agents as an alternative to fungicides (Glare *et al.* 2012). Plant extracts are safe and highly effective in controlling the development of bacterial pathogens and *P. infestans* (Gaufo *et al.* 2010). According to El-Mougy (El-Mougy 2009) a large reduction in early blight incidence was recorded in plants watered with 1% of carnation, caraway, and thyme essential oils. In a study by Chowdappa *et al.* (2013), isolates of *Bacillus subtilis* and *Trichoderma harzianum* successfully suppressed early and late blight. Li *et al.* (2008) used chitinase secreted by *Bacillus cereus* against Verticillium wilt of eggplants. *Gliocladium catenulatum,* the active ingredient of a biofungicide, has shown good control efficacy of seed- and soil-borne diseases caused by *Phytophthora* spp., *Alternaria* spp., and *Rhizoctonia* spp. in horticultural crops, and storage diseases caused by *Helminthosporium solani* and *Rhizoctonia solani* in potatoes (Niemi and Lahdenpera 2000). The application of *Chaetomium globosum* inhibited late blight infection in potatoes (Shanthiyaa *et al*. 2013).

The fungus-like organism *Pythium oligandrum* (Benhamou *et al*. 2012) destroys the cell walls of pathogens owing to the enzymatic activity of *P. oligandrum*. In recent years, this fungus-like organism has had great impor-

*Corresponding address:
marta.damszel@uwm.edu.pl

tance in plant protection (Horner *et al*. 2012). Ikeda *et al*. (2012) indicated that the mechanisms of mycoparasitism and induced resistance were responsible for the reduction of the incidence of black scurf in potato tubers caused by *P. oligandrum*. Early and rapid colonisation with mycorrhizal fungi *Glomus* spp., is of great importance in biological plant protection (Wehner *et al*. 2010). However, the share of mycorrhizal fungi in the inhibition of soil pathogen development by stimulating the biological activity in rhizosphere, as well as in the enhancement of plant resistance to infections with pathogens, is not sufficiently known (Cameron 2010; Cameron *et al*. 2013).

The objectives of this study were to evaluate the effect of chemical and biological control agents on the severity of late blight and early blight on potato plants of the very early cultivar Rosara, and on the composition of fungal communities colonising potato stems. The effectiveness of fungicides in inhibiting the growth of selected potato pathogens was estimated in *in vitro* tests.

Materials and Methods

Field experiment

A three-year field experiment was carried out in Tomaszkowo (53°73′13″N, 20°40′55″E), in 2007–2009, in a randomised block design, in four replications, on sandy soil which had the quality class IVb. Table potatoes of the very early cultivar Rosara (red-skinned tubers with a 12–13% starch content, cooking type B, good taste attributes) were grown. The experiment involved the following treatments:

- mycorrhizal *Glomus* spp. inoculum (Vaxi-Root, Mycoflor®) applied to the roots of five-week-old plants;
- tubers dressed with Polyversum WP (oospores of *Pythium oligandrum*, 10 g · kg^{-1} of tubers) and plants sprayed with Polyversum WP three times during the growing season (0.05% – 40 ml · $plant^{-1}$;
- plants sprayed at two-week intervals with Infinito 687.5 SC (625 g per l of propamocarb hydrochloride, 62.5 g per l of fluopicolide) and Tanos 50 WG (25% cymoxanil, 25% famoxat), according to the recommendations of the Institute of Plant Protection – National Research Institute in Poznań, Poland;
- plants sprayed at two-week intervals with Valbon 72 WG (bentiovalicarb – 1.75%, mancozeb – 70%, zinc complex with polymeric manganese dithiocarbamate containing 20% manganese and 2.5% zinc) and Tanos 50 WG.

In the control treatment, potato plants were not protected against pathogens.

During the growing season, two weeks from the last treatment, the severity of late blight and early blight was evaluated three times on all plants at the flowering stage to the beginning of yellowing, on a nine-point scale where: 1° – no symptoms, 2° – 0.1% infected leaves, 3° – 0.2–1%, 4° – 2–5%, 5° – 6–25%, 6° – 26–50%, 7° – 51–75%, 8° – 76–95%, 9° – 96–100% (most severe symptoms). The results were presented in the form of an infection index *Ii* in %.

$$Ii = \frac{\sum(a \times b) \times 100\%}{N \times I},$$

where: Σ ($a \times b$) – the sum of the products resulting from multiplying the number of the plants analysed and the Ninen scale degree, N – total number of plants analysed, I – the highest scale degree.

Isolation of fungi

Fungi collected from stems (one month before potato-lifting) were isolated at the laboratory. Stem segments collected from the lower part of potato plants (30 samples per treatment) were cut into 1 cm pieces. The samples were disinfected with 50% ethylene and 1% sodium hypochlorite, rinsed three times with sterile water, and transferred to the Potato Dextrose Agar (PDA) medium (five plates = five replications). After seven days of incubation at 22°C, fungal colonies were inoculated onto agar slants for microscopic identification.

In vitro test

In vitro tests were conducted to determine the mycelial growth of the following fungal pathogens: *A. alternata, Colletotrichum coccodes*, *Fusarium solani*, and *R. solani* on the PDA medium containing the fungicides: Infinito 687.5 SC, Valbon 72 WG, and Tanos 50 WG, recommended for late blight and early blight control, at a concentration of 1, 10, 100, and 1,000 mg · dm^{-3}. The diameters of fungal colonies were measured from the moment the medium had been thoroughly overgrown with fungal mycelium in the control treatment (plates with fungal inoculum on a medium containing no fertilisers). Then, the rate of colony growth was calculated.

Statistics

The results were verified statistically by an analysis of variance for a randomised block design (STATISTICA® 9.0 2009). The means were compared with the use of Duncan's test (significance level 0.05).

Weather conditions during the experimental period are presented in table 1. In the first two years of the study, temperatures in May and June were comparable with the long-term average, while July and August were warm. The growing season of 2007, in particular July, was very wet. The two subsequent growing seasons were characterized by moderate precipitation, and half of the rainfall occurred in August and June, respectively.

Results and Discussion

In 2007 and 2009 (with high precipitation in May–July which stimulated the growth of *P. infestans*), the highest infection intensity (over 60%) was observed on the unprotected plants. The weakest symptoms of late blight (about 35%) were observed on fungicide-sprayed potato plants in 2008. During the first and the last year of the experiment, the lowest values of the infection index were also noted in treatments with chemical control (Table 2). The

Table 1. Weather conditions (Meteorological Station in Tomaszkowo)

Month	The mean	10-days	Temperature [°C]				Rainfall [mm]			
			2007	2008	2009	the mean for 1961–95	2007	2008	2009	the mean for 1961–95
May	for 10 days	I	8.3	11.9	12.0		34.5	18.6	1.3	
		II	13.2	11.8	11.1		29.3	2.4	6.8	
		III	19.2	13.2	14.0		29.7	6.0	44.8	
	monthly	–	13.8	12.3	12.4	12.4	93.5	27.0	52.9	56.7
June	for 10 days	I	18.3	18.4	12.2		0.8	0	73.8	
		II	18.8	15.4	13.8		39.9	10.0	32.8	
		III	15.9	16.9	18.8		47.4	22.7	30.3	
	monthly	–	17.7	16.9	14.9	15.7	88.1	32.7	136.9	68.3
July	for 10 days	I	15.8	17.4	18.8		50.9	11.7	28.3	
		II	19.6	18.3	23.1		26.5	30.8	9.8	
		III	17.6	19.5	19.3		96.3	15.2	10.2	
	monthly	–	17.7	18.5	20.4	15.3	173.7	57.7	48.3	81.3
August	for 10 days	I	18.5	18.9	18.9		10.9	38.1	3.9	
		II	19.4	18.4	17.1		23.2	26.5	12.8	
		III	17.0	18.0	16.8		33.9	37.5	2.6	
	monthly	–	18.3	18.4	17.6	17.9	68.0	102.1	19.3	78.1
The mean/Total			16.9	16.5	16.3	15.3	423.3	219.5	257.4	284.4

Table 2. Infection of potatoes cv. Rosara by pathogens during the investigation (infection index *Ii* in %)

Treatments	*Phytophthora infestans*				*Alternaria alternata, A. solani*			
	2007	2008	2009	the mean	2007	2008	2009	the mean
The control	60.3b c*	53.6 e	65.3 a	59.7 a	26.2 cde	31.1 a	30.5 a	29.3 a
Polyversum	53.4 e	44.4 g	55.5 de	51.1 d	22.3 f–i	25.2 c–g	26.0 b–f	24.5 b
Mycorrhizal inoculum	57.2 cd	45.8 g	57.2 cd	53.4 c	22.8 d–i	24.4 c–h	26.3 bcd	24.4 b
Infinito 687,5 SC	40.3 h	34.3 i	42.4 gh	39.0 f	19.7 i	24.3 c–h	21.6 ghi	21.9 c
Valbon 72 WG	44.5 g	36.2 i	44.6 g	41.8 e	20.5 i	22.4 e–i	20.9 hi	21.3 c

*values followed by the same letters do not differ significantly

average values of the infection index were significantly higher in 2009 than in 2007 and 2008. The translaminar fungicide cymaxonil Curzate M-8 (8% cymoxanil + 64% mancozeb) with the contact Dithane M-45 (Kromann *et al*. 2008), mandipropamid (Jang *et al*. 2009) and the fenyloamide fungicide mefenoxam showed high efficacy in protecting potato leaves against *P. infestans* (Pomerantz *et al*. 2014). The last fungicide offered effective control of late blight as a result of blocking the transcription of RNA by inhibiting the activity of RNA polymerase.

In our study, in treatments with biocontrol agents, late blight severity was significantly reduced by Polversum in 2007, and by Polyversum in combination with a mycorrhizal inoculums *Glomus* spp. in 2008 and 2009, compared with unprotected plants. As shown by the average values of the infection index, infection rates were significantly lower in protected plants than in unprotected plants. Kurzawińska and Mazur (2009) demonstrated high efficacy of Polyversum used for tuber dressing and plant spraying in late blight control. The arbuscular mycorrhizal fungus (AMF – *Glomus intraradices, G. mosseae* reduced the populations of *Erwinia carotovora, P. infestans, Verticillium dahliae*), is a dangerous pathogen of the plant family Solanacea (Bharadwaj *et al*. 2008). Gallou *et al*. (2011) reported a reduction in the intensity of late blight on potato leaves in mycorrhizal potato plantlets. The authors explain this fact by the increased induction of salicylic acid and PR-genes in potatoes infected with *P. infestans*.

In the present experiment, the values of the infection index were 50% lower for early blight than for late blight. An infection index above 30% was noted in unprotected plants in 2008 and 2009 (a significant difference compared with all other treatments – Table 2). In 2007, weather conditions did not promote the spread of the disease, and early blight intensity levels were significantly lower in plants treated with fungicides and Polyversum, in comparison with the control treatment. An analysis of average infection rates over the entire experimental period revealed that the rates were significantly lower in treatments with chemical control and biological control in the form of Polyversum and a mycorrhizal inoculum. Boscalid + metiram were effective in early blight control (the first two applications of boscalid + metiram; spraying programs included 4–6 applications of fungicides) (Horsfield *et al*. 2010). Fairchild *et al*. (2013) indicated that in Idaho 57, 63, and 15% of the isolates of *Alternaria solani* and *A. alternata* in potato, were resistant to boscalid, to strobilurin, and to pyrimethanil, respectively. As demonstrated by Singh (2008) and Ferreira *et al*. (2014), Ridomil MZ and cupric fungicides provided the best control of late blight and early blight in potatoes. According to MacBean (2012), chlorotalonil is effective in protecting against these diseases (prevents germination and zoospore movement).

A more abundant community of filamentous fungi was isolated from potato stems in 2007 – 20 species. In 2008 and 2009 there were 14 and 15 species, respectively. Pathogenic fungi accounted for 72.3%, 83.3%, and 78.2% of all isolates, respectively. The predominant species was *C. coccodes* (Wallr.) Hughes (approx. 40.7% of all isolates), followed by fungi of the genus *Fusarium* [*F. avenaceum* (Fr.) Sacc., *F. culmorum* W.G. (Sm.) Sacc., *F. equiseti* Corda/ Sacc. and *F. oxysporum* Schlecht., *F. solani* (Mart.) Sacc. approx. 25.2%]. *Rhizoctonia solani* Kühn was less frequent (8.5%), and only single isolates of *A. alternata* (Fr.) Keissler, *B. cinerea* Pers. and *H. solani* Dur. et Mont were encountered (Fig. 1). A significant decrease in pathogen populations was noted in fungicide-treated plants (Table 3).

In a previous study Cwalina-Ambroziak (2012), the author isolated the above-mentioned pathogenic fungi from potato stems.

Antagonistic fungi [*Trichoderma hamatum* (Bon.) Bain] were isolated in low numbers, and only from potato stems treated with a mycorrhizal inoculum in 2008, and with a mycorrhiza and fungicides in 2009. Species of the or-

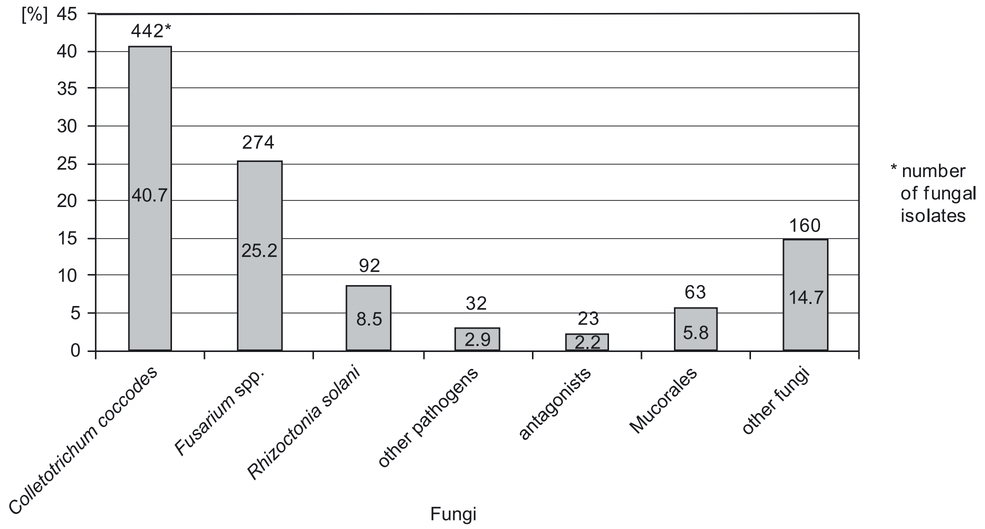

Fig. 1. Fungi isolated from the stems of potatoes cv. Rosara. Other pathogens were: *Acremonium strictum* W. Gams, *Cladosporium cladosporioides* Fres. De Vries, *Epicoccum* spp., *Endothia* spp., *Humicola brevis* Gillman and Abbott, *Penicilium* spp., *Sporotrichum olivaceum* Fries, and yeast-like fungi

Table 3. Fungi colonising potato stems (the mean number of isolates, 2007–2009)

Fungi	The control	Pol	Myc	Inf+Ta	Val+Ta
Colletotrichum coccodes	30.00 a	26.00 a	24.25 a	1.25 b	14.00 b
Fusarium spp.	16.00 a	18.00 a	16.75 a	10.25 b	7.5 b
Rhizoctonia solani	7.5 a	6.00 a	5.75 ab	0.75 c	3.00 bc
Other pathogens (*A. alternata*, *B. cinerea*, *H. solani*)	4.00 a	1.25 b	1.75 b	0.75 b	0.25 b
Antagonistic	0.0 b	0.0 b	2.50 a	1.50 ab	1.75 ab
Mucorales (*Mortierella alpina* Peyonel, *Mucor hiemalis* Wehmer, *Rhizopus* spp.)	4.00 ab	5.25 a	3.75 ab	1.00 c	1.75 bc

Pol – Polyversum; Myc – mycorrhizal inoculum; Inf+Ta – Infinito 687.5 SC+Tanos 50 WG; Val+Ta – Valbon 72 WG+Tanos 50 WG

Table 4. The effect of fungicides on the linear growth of some potato pathogens (diameter in mm)

Fungicides	Concentration [mg · dm^{-3}]	*Alternaria alternata*	*Colletotrichum coccodes*	*Fusarium solani*	*Rhizoctonia solani*
The control		90.0 a	90.0 a	90.0 a	90.0 a
Infinito 687.5 SC	1	74.6 b	81.7 b	84.8 b	90.0 a
	10	64.5 c	75.8 c	84.6 b	90.0 a
	100	60.9 d	75.6 c	81.4 bc	90.0 a
	1,000	56.3 ef	64.2 d	48.7 g	69.5 c
Valbon 72 WG	1	54.3 f	75.8 c	84.2 b	76.8 b
	10	37.6 g	65.3 d	78.4 cd	44.2 e
	100	20.9 h	32.0 f	54.3 f	10.0 f
	1,000	0.0 i	0.0 g	21.5 h	0.0 g
Tanos 50 WG	1	31.8 g	48.9 e	85.0 ab	57.0 d
	10	29.6 g	45.6 e	84.4 b	51.3 d
	100	25.6 g	42.4 e	76.5 d	41.3 e
	1,000	25.6 g	41.8 e	65.9 e	15.1 f

der *Mucorales* (*Mortierella alpina*, *Mucor hiemalis*, *Rhizopus* spp.) had a low share (5.8%) of the community. In a study by Patkowska (2006) the above-mentioned biopreparation inhibited the growth of many pathogenic fungi, such as: *A. alternata*, *B. cinerea*, *Fusarium* species, *R. solani*, *Sclerotinia sclerotiorum*. Begum and Lokesh (2008) reported a reduction in the growth rate of *A. alternata*, *F. solani*, and *R. solani* in response to carboxin and mancozeb. Yao *et al*. (2002) demonstrated that two vesicular-arbuscular mycorrhizal fungi; *Glomus etunicatum* and *G. intraradices*, reduced the extent of disease caused by *R. solani* on potato plants of susceptible varieties.

The three fungicides tested in our experiment significantly inhibited the linear mycelial growth of selected pathogenic fungi, with the exception of Infinito 687.5 SC which did not suppress the growth of *R. solani* when applied at a concentration of 1, 10, and 100 mg · dm^{-3} (Table 4). The inhibitory effect of the fungicides was proportional to the concentration applied. The fungicides Valbon 72 WG and Tanos 50 WG at the analysed concentrations, were more effective in inhibiting the growth of fungal colonies than Infinito 687.5 SC, except for *F. solani* treated with Tanos 50 WG at the highest concentration. Tanos 50 WG at a dose of 1 and 10 mg · dm^{-3} of the medium had a more inhibitory effect on *A. alternata* and *C. coccodes* than Valbon 72 WG at the same concentrations. However, Valbon 72 WG, added to the PDA medium at 100 and 1,000 mg · dm^{-3}, was characterized by the highest level of fungistatic activity against all fungal species – it completely suppressed the growth of *A. alternata*, *C. coccodes*, and *R. solani*.

Kurzawińska and Mazur (2009), conducted an *in vitro* test that showed that Polyversum significantly suppressed the mycelia growth of *P. infestans*. In an *in vitro* experiment, Burgieł *et al*. (2008) indicated an inhibitory effect of extracts from plants of the family Apiaceae on the growth pathogens of, among others, *A. alternata* and *Fusarium* spp. It was also found that Azoxystrobin and enestroburin had a strong inhibitory effect on the mycelial growth of *P. infestans* under in vitro conditions (Liu *et al*. 2014). In a study by Kapsa (2009), Infinito 687.5 SC, along with other tested fungicides, was highly effective in inhibiting the growth of *A. alternata* and *A. solani*. Based on laboratory tests, Wharton *et al*. (2012) indicated that 15% of the investigated isolates of *A. solani* in potato were resistant to boscalid. Prochloraz, carbendazim, and mancozeb were effective in inhibiting the mycelial growth of fungi of the genus *Fusarium* (Mamza *et al*. 2010). Carboxin and thiram inhibited the radial growth of *R. solani* (Stępniewska-Jarosz *et al*. 2008).

Conclusions

The weakest symptoms of infection caused by *P. infestans* and *Alternaria* spp. were observed in fungicide-treated plants. In treatments with biocontrol agents, late blight severity was most significantly reduced by Polyversum.

The abundance of pathogenic fungi colonising the stems of potato plants was reduced by fungicides, which was confirmed by the results of *in vitro* tests. The fungicides used in our experiment inhibited the linear mycelial growth of selected pathogenic fungi, and their fungistatic activity was proportional to the concentration applied.

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