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Evaluation of biocontrol preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves

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Abstract

The potential of biocontrol products and plant extracts for control of late blight on potato plants, caused by *Phytophthora infestans* was evaluated in detached leaf assays and on potted plants. Based on an initial screening of 22 preparations and plant extracts, the 10 most active treatments were selected for further investigation. In the detached leaf assays the commercial preparations Elot-Vis, Serenade and Trichodex, and plant extracts of *Rheum rhabarbarum* and *Solidago canadensis* showed a significant effect on the level of infestation by *P. infestans*. However, none of the treatments was as effective as copper. In the case of Serenade, the metabolites produced by its active micro-organism, *Bacillus subtilis*, were demonstrated to be the effective component of the formulation, and not the micro-organism itself. In order to take curative and protective modes of action into account, the test substances were applied 24 h before, or 90 min after inoculation with *P. infestans*. Generally, better effects were obtained when the applications were made 24 h before inoculation. For defining the optimum time of application, potted plants were treated 72 or 24 h before, and 1 and 24 h after inoculation with *P. infestans*. In these tests, Trichodex showed no activity, while Elot-Vis gave best results when applied 1 day before inoculation. Serenade and the extracts of *R. rhabarbarum* and *S. canadensis* (all at 5% concentration) however, were effective when applied up to 3 days before and just after inoculation with *P. infestans*. The results of the experiments on potted plants indicated direct effects on the pathogen for all agents except the extract of *S. canadensis*, but other mode of actions, e.g. induced resistance, could not be ruled out. None of the treatments had a curative effect.

Introduction

Late blight caused by *Phytophthora infestans* is one of the most important diseases affecting organic and conventional potato production world-wide. Under favourable environmental conditions for the pathogen the disease can spread very rapidly and cause severe crop losses. Protective sprays with copper fungicides are currently used to control the disease in most organic production systems. However, use of copper fungicides in organic farming has recently been restricted by the European Commission (European Commission, 2002) and may even be banned in future. Therefore, the development of alternative agents that can replace copper is very desirable. For some fungal pathogens, alternative, non-chemical control agents have already been developed (Copping, 1998; Fravel et al., 1998). These include micro-organisms and plant extracts. Their effect may be due to antibiosis, which means

the formation of specific or non-specific metabolites of microbial origin (Jackson, 1965), competition for nutrients (Elad and Chet, 1987) or indirect effects like induction of plant resistance (De Meyer et al., 1998; Ahmed et al., 2000; Ramamoorthy et al., 2001).

Efficacy of plant extracts against *P. infestans* has been demonstrated by several workers (e.g. Latten, 1994; Meinck, 1999; Neuhoff et al., 2002; Röhner et al., 2004). It has also been shown that microorganisms like *Pseudomonas* spp. and *Bacillus* spp. (Jongebloed et al., 1993; El-Sheikh et al., 2002; Daayf et al., 2003), *Trichoderma* species (Saikia and Azad, 1999; Etebarian et al., 2000;) and *Penicillium* (Garita et al., 1999) have a potential to control *Phytophthora* species. Apart from direct effects of plant extracts and biocontrol agents on the pathogen, effects on the host's metabolism may also play a role in limiting plant infestation by *P. infestans*. On potatoes, induced resistance against *P. infestans* caused by a non-pathogenic *Phytophthora cryptogea* has been described by Quintanilla (2002). In experiments conducted by Yamaguchi et al. (1992) tomato seedlings in soil pre-treated with *Fusarium oxysporum* showed reduced development of late blight following inoculation of *P. infestans* on the aerial parts. The author suggests that *F. oxysporum* can infect solanaceous crops symptomlessly, and induce a non-specific systemic resistance. Yan et al. (2002) demonstrated that plant growth promoting rhizobacteria (*Bacillus pumilus* and *Pseudomonas fluorescens*) can elicit systemic protection against late blight under greenhouse conditions.

The transfer of greenhouse results to the field is often difficult. Although Arora (2000), Jongebloed et al. (1993), Latten (1994) and Meinck (1999) achieved good results in greenhouse experiments, in the field none of the applied treatments was able to control *P. infestans* efficiently. Despite the positive approaches for the development of non-chemical control agents of *P. infestans* cited above, research in this field is still limited, and effective alternative treatments for late blight control are not available on the market. We report here the results of experiments for control of *P. infestans* with a number of alternative commercial preparations which are in development or already on the market and plant extracts with a known potential for control of phytopathogenic fungi. The activity of the treatments was tested on detached potato leaves. Control activity of selected agents was further characterised on potted potato plants. The results are discussed in relation to the possible mode of action of the respective agents.

Materials and methods

Preparation of pathogen inoculum

Phytophthora infestans was grown and maintained at 15 °C in Petri dishes (∅: 9 cm) on rye agar containing 0.005% β-sitosterol. Sporangial suspensions were prepared from 14-day-old cultures by flooding the plates with a sterile solution of 0.0125% Tween 80 in deionised water and gently scraping the mycelium with a spatula. The concentration of the resulting sporangial suspensions was determined using a Thoma hemocytometer and adjusted to the desired concentration. The suspensions were then incubated for 30–60 min at 5 °C to induce the release of zoospores.

Treatments

Leaves of *Rheum rhabarbarum*, *Helianthus annuus*, *Taraxacum officinale*, *Sambucus nigra* and leaves, stems and flowers of *Solidago canadensis*, *Artemisia vulgaris*, *Impatiens parviflora*, *Oenothera biennis* and *Urtica dioica* were collected in July/August 2001, immediately air-dried at 60 °C and milled with a SM 100 (Retsch, Germany). Cold water extracts (1%) were prepared immediately before application. They were obtained by stirring 0.5 g of the plant powder in 50 ml 0.0125% Tween 80 for 1 h, followed by separation from the residue with a Büchner funnel. Information on the tested commercial preparations is given in Table 1. The product Messenger and all microbial preparations were applied at a concentration of 1% (w/v) in deionised water, except for EM5 which was used at 0.2% (v/v). Elot-Vis was used at 10% (v/v) and Atempo at 0.5% (w/v).

Tests on detached leaves

Screening. Tubers of the highly susceptible potato cv. Secura were planted singly in 10 × 10 × 12 cm plastic pots in a commercial potting substrate. The pots were placed in a growth room at 17 °C and a 16/8 h day/night cycle. The fifth to seventh leaves of the main stems of 4-week-old plants were col-

Table 1. Commercial products tested

Products	Active organism/compound	Manufacturer/Distributor
Trichodex	*Trichoderma harzianum*	Makhteshim Chemical Works Ltd, Israel
Vitalin 1	*T. harzianum*	Vitalin, Germany
Vitalin 2	*T. harzianum*	Vitalin, Germany
Vitalin 3	*T. harzianum*	Vitalin, Germany
Vitalin 4	*T. harzianum*	Vitalin, Germany
Phytovit	*Bacillus subtilis*	Prophyta, Germany
BioPro	*B. subtilis*	Bio-Protect GmbH, Germany
Serenade	*B. subtilis*	AgraQuest Inc., USA
Sonata	*B. subtilis*	AgraQuest Inc., USA
FZB 55	*B. subtilis*	FZB Biotechnik, Germany
EM 5 (effective micro-organisms)	Mixture of micro-organisms	OLV-Shop, Germany
Rhizovit	*Streptomyces* spp.	Prophyta, Germany
Contans	*Coniothyrium minitans*	Prophyta, Germany
Elot-Vis	Plant extract	Prophyta GmbH, Germany
Messenger	Harpin protein	EDEN Bioscience corp., USA
Atempo	Copper oxychloride (750 g kg^{-1})	Neudorff, Germany

lected, and four leaves were placed together onto a steel wire mesh (18 × 18 cm) with soaked filter paper underneath in 20 × 20 × 5 cm plexi glass boxes covered with a translucent lid (Gerda GmbH, Schwelm, Germany). The preparations were either applied 24 h before, or 60 min after inoculation with *P. infestans*. In the first case, aimed at identifying protective activity and potential resistance inducing effects, the test preparations were sprayed on both surfaces of the leaves (2.5 ml per surface) using a chromatographic sprayer. In the second case, aimed at evaluating direct antifungal effects, the preparations were applied to the upper leaf surface only (2.5 ml per box). In both cases the leaves were inoculated with the pathogen by placing one droplet (2 μl) of the *P. infestans* suspension (concentration of sporangia before zoospore release: 5×10^5 ml^{-1}) on the upper side of the terminal leaflet at a distance of 1 cm from the mid vein. The boxes were then placed in an incubator with 15 °C and a day/night cycle of 16/8 h. After seven days of incubation, disease was rated using a scale from 0 to 4 (0, no lesion; 1, lesion between two veins; 2, lesion overgrowing the veins; 3, lesion growing over the mid vein; 4, (75% of the leaflet infected). Within the experiment three sets of treatments were tested three times using independent repetitions with copper and 0.0125% Tween 80 as control treatments.

Comparison of selected treatments. The experimental design was the same as in the screening described above except that instead of applying the pathogen inoculum as a droplet, 2.5 ml per box of a *P. infestans* suspension (concentration of sporangia before zoospore release: 1×10^5 ml^{-1}) were applied as a spray onto the upper leaf surfaces. Further, disease was rated by estimating the affected percentage leaf area (James, 1971) on the fifth to tenth day after inoculation. The obtained values were then used to calculate the area under the disease progress curve (AUDPC) (Kranz and Holz, 1993). The treatments were tested in three independent experiments with three boxes per treatment. The means of the AUDPC values were separated by the Student–Newman–Keuls test ($P < 0.05$). Statistical analysis was carried out with SAS 8.1 software for windows (SAS Institute Inc., 1989). For all experiments the homogeneity of variance was tested by the Levene test ($P < 0.1$).

Performance of preparations at different concentrations. The set-up of the experiment was as described above, except for the following modifications. A copper treatment and two control treatments (Tween 80 and water) were included. The preparations were applied at different concentrations, *R. rhabarbarum*, *S. canadensis*, Serenade and Trichodex at 0.1, 1 and 5% (w/v), and Elot-Vis at 1, 10 and 20% (v/v). Not all treatments

were applied before and after pathogen inoculation: *R. rhabarbarum* was only applied 90 min after, and *S. canadensis*, Trichodex and Elot-Vis only 24 h before *P. infestans*. Serenade was applied at both times. The experiment was carried out three times, and disease was rated by estimating the affected percentage leaf area (James, 1971) on the fifth, sixth and seventh day after inoculation. The AUDPC was determined and statistically analysed as described above.

Comparison of different Bacillus subtilis *products*. The three preparations Serenade, Sonata and FZB 55 containing *B. subtilis* as the active micro-organism, were applied at a concentration of 1% (w/v) both 24 h before and 60 min after inoculation with *P. infestans*. The leaf bioassays were carried out as described above except that the timing of treatments was 24 h before, or 1 h after pathogen inoculation. The experiment was carried out three times. Disease was rated by estimating the affected percentage leaf area (James, 1971) on the fifth, sixth and seventh day after inoculation. The AUDPC was determined and statistically analysed as described above.

Assessment of the activity of components of the Serenade formulation in relation to a B. subtilis *re-isolate*. *B. subtilis* was isolated by plating dilutions of the product Serenade onto tryptic soy agar (TSA; 0.17% casein peptone, 0.03% soy peptone, 0.05% sodium chloride, 0.025% dextrose, 0.025% dipotassium phosphate, 1.5% agar). A single colony was picked and sub-cultured. For preparation of inoculum the isolate was grown for 7 days on a rotary shaker (Infors, Multitron) at 100 rpm and 20 °C in 300 ml Erlenmeyer flasks with 100 ml liquid medium (0.24% meat extract, 0.24% peptone, 0.12% NaCl, 0.025% KH_2PO_4, 0.003% $MnSO_4 \cdot 4H_2O$). After centrifugation (15 min at $3000 \times g$) of the culture the supernatant was set aside, and the pellet was washed twice by re-suspending in water and centrifuging. The pellet thus obtained was re-suspended in water and, using a hemocytometer, the concentration of endospores was adjusted to 5×10^7 ml^{-1}, which is equivalent to the spore concentration in a 1% suspension of the commercial formulation of Serenade. Portions of 2.5 ml each of the supernatant, the spore suspension and, in addition, a supernatant prepared from Serenade (obtained by centrifuging 100 ml of a 1% suspension of Serenade in water) were employed in the assay on detached leaves as described above. The whole procedure, i.e. from culturing the isolate on the rotary shaker to performance of the leaf bioassay, was carried out three times.

In vitro *effect on mycelial growth of* P. infestans

Direct effects on mycelial growth of *P. infestans* were investigated for the plant extracts from *R. rhabarbarum* and *S. canadensis* and for the product Elot-Vis. For incorporation into the agar media, all extracts were prepared as described above but at double strength. Double strength concentrations of Elot-Vis were prepared by adding the respective amounts of the product to sterile distilled water. The solutions and plants extracts thus obtained were sterilized by filtration (Sartorius, 0.45 μm) and mixed (1:1) with double strength rye-sitosterol agar. Rye-sitosterol agar without amendment served as a control. The agar was poured into sterile Petri dishes and allowed to solidify. The plates were inoculated by placing a mycelial plug of a 14-day-old *P. infestans* culture in their centre and afterwards incubated at 15 °C in the dark. Length and width of the *P. infestans* colony were measured when mycelial growth in the controls had reached the edge of the plate. A total of 10 (*R. rhabarbarum*) or 20 plates (*S. canadensis* and Elot-Vis) was investigated.

Tests on potted plants

Potatoes (cv. Secura) were cultivated for four weeks as described above. Pots with plants having 2–4 stalks were moved to a growth room with 15 °C and a day/night cycle of 16/8 h and placed in translucent PVC-cylinders (diam: 25 cm, height: 60 cm). *Rheum rhabarbarum* extract was tested at a concentration of 5% (w/v), *Solidago canadensis* extract at 1 and 5% (w/v), Serenade at 1 and 5% (w/v), Trichodex at 1% (w/v) and Elot-Vis at 20% (v/v). These treatments (5 ml per pot) were applied with a chromatographic sprayer three or one day before, and 1 h or 1 day after pathogen inoculation. The controls (water for Serenade, Trichodex and Elot-Vis; 0.0125% Tween 80 for *R. rhabarbarum and S. canadensis*) and the copper treatment (Atempo at 0.5% w/v) were applied 3 days before pathogen inoculation. For inoculation with

P. infestans the plants were sprayed with 5 ml of a zoospore suspension (resulting from 1×10^5 sporangia ml^{-1}) per pot. After application of the preparations or pathogen inoculum, respectively, the pots were returned to the PVC cylinders in the growth room, watered from below, and the cylinders were covered with a 26 × 26 cm PVC sheet in order to obtain a water saturated atmosphere. Three pots were employed per treatment, and the experiment was carried out three times. Six days after inoculation with the pathogen disease was rated by estimating the affected percentage leaf area (James, 1971) of all leaves. The separation of means was done with the glm procedure (SAS Inc., 1989) by Student–Newman–Keuls test ($P < 0.05$) of the arcsine transformed data (Serenade, Trichodex, *S. canadensis*) or log 10 transformed data (Elot-Vis, *R. rhabarbarum*). The homogeneity of variance was tested by the Levene test ($P < 0.1$) described by Dufner et al. (1992).

Results

In the screening on detached leaves nine plant extracts and 13 commercial preparations were tested. The results obtained in the three independent experiments varied considerably. However, a certain degree of variability was also recorded for the copper treatment. Among both plant extracts and commercial products, for some treatments the affected leaf area was lower than 5%, e.g. *S. canadensis* and Vitalin 1 (Figure 1). Due to apparent promotion of the disease in at least one of the experiments, a negative effect was recorded for Messenger and some of the microbial products, but not for any of the plant extracts. Some of the treatments showed relatively low infestation with *P. infestans* both when applied 24 h before and 1 h after pathogen inoculation (e.g. *S. canadensis*, Serenade), while for others such a correlation was not recorded (e.g. *A. vulgaris*, Vitalin 2).

Based on these results, five plant extracts (*A. vulgaris*, *I. parviflora*, *R. rhabarbarum*, *S. canadensis*, *U. dioica*) and five commercial products (Elot-Vis, Serenade, Trichodex, Vitalin 1, Vitalin 2) were selected and re-tested in a second set of leaf assays involving application of the *P. infestans* inoculum by spraying instead of placement as a droplet. The application of the agents 24 h before the pathogen tended to be more effective than 1 h after the pathogen (Table 2). None of the tested preparations was as effective as te copper treatment. Of the agents applied 24 h before *P. infestans*

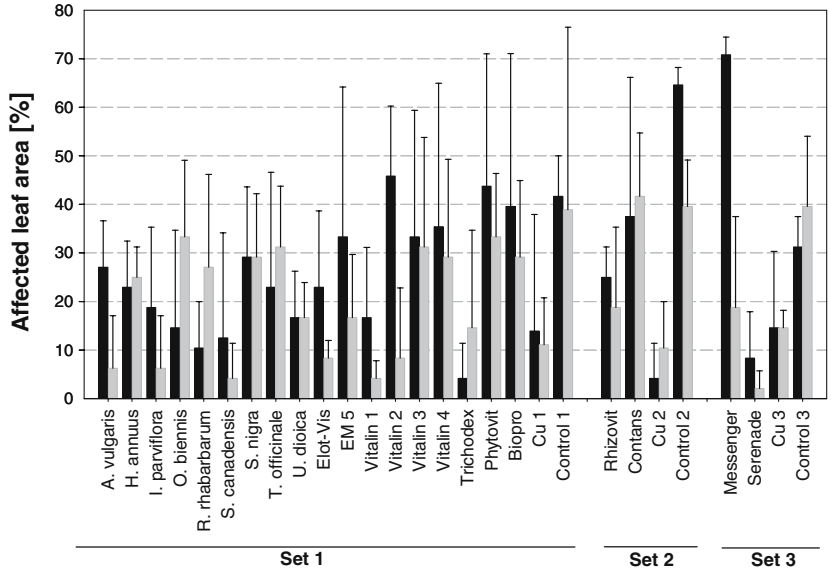

Figure 1. Effect of plant extracts and commercial preparations on disease symptoms on excised leaves caused by *P. infestans*. The agents were applied 24 h before (grey columns) or 1 h after (black columns) inoculation with *P. infestans*. Means and standard deviation (error bars) of three independent experiments with four leaves per box. The treatments were applied within three independent sets including one Cu and one 0.0125% Tween 80 control per set.

Table 2. Effect of different treatments on the development of *P. infestans* between the 5th and 10th day after inoculation on excised leaves

Treatment	Mean area under the disease progress curve (AUDPC)[a] (±SD)					
	Application 24 h before the pathogen			Application 90 min after the pathogen		
A. vulgaris	422.6	(±39.1)	ef[b]	469.4	(±7.68)	d
I. parviflora	395.8	(±41.8)	ef	453.5	(±17.5)	d
R. rhabarbarum	345.1	(±28.0)	de	382.3	(±11.0)	c
S. canadensis	251.0	(±49.6)	bc	430.2	(±48.0)	cd
U. dioica	446.5	(±8.48)	ef	476.0	(±18.2)	d
Elot-Vis	301.4	(±67.8)	cd	433.0	(±53.7)	cd
Serenade	192.7	(±18.2)	b	229.5	(±22.0)	b
Trichodex	308.3	(±61.4)	cd	444.4	(±24.7)	cd
Vitalin 1	451.4	(±11.0)	f	483.0	(±6.10)	d
Vitalin 2	424.3	(±45.5)	ef	487.2	(±2.62)	d
Cu	44.8	(±23.0)	a	152.8	(±50.2)	a
Control	444.1	(±38.6)	ef	476.0	(±11.8)	d

[a]Each treatment mean and standard deviations are calculated from three independent experiments with three replicates each.
[b]Means of the data within the column followed by the same letter are not significantly different following Student—Newman–Keuls test ($P < 0.05$).

inoculation, the best treatments were Serenade and the extract from *S. canadensis*, followed by Elot-Vis and Trichodex; *A. vulgaris*, *I. parviflora*, *U. dioica*, *R. rhabarbarum*, Vitalin 1 and Vitalin 2 did not show any significant effect compared to the control. When the treatments were applied one hour after inoculation with *P. infestans*, only Serenade and *R. rhabarbarum* had a significant effect.

The plant extracts and commercial products with a significant effect on *P. infestans* in the previous experiment (Table 2) were further tested on excised leaves at different concentrations (Table 3). A significant positive dose–response was observed in the case of Serenade applied 90 min after inoculation. A positive dose–response was also recorded for *R. rhabarbarum* (applied 90 min after inoculation) and Elot-Vis (applied 24 h before inoculation), but due to variability these were not statistically significant. Dose dependency for control of *P. infestans* was also observed for the lower concentrations of Serenade and Trichodex applied 24 h before inoculation. However, when these products were applied at the high concentration (5%), the disease symptoms were masked by symptoms of phytotoxicity. A clear lack of dose-dependency was recorded for *S. canadensis* applied 24 h before inoculation.

In order to test if other *B. subtilis* based products display activity against *P. infestans* similar to the commercial product Serenade, a separate test on excised leaves was performed. The products included were Serenade, Sonata and FZB55 and the copper standard. In this test, the three *B. subtilis* preparations differed in their ability to control *P. infestans* (Figure 2). The highest levels of control were obtained with the products Serenade and Sonata. However, the differences to the control were statistically significant only when these products were applied 1 h after inoculation. Application of FZB 55 had no significant effect on the development of *P. infestans*, irrespective of the time of application.

Taken together, the results indicated that the product Serenade has a higher activity against *P. infestans* than the other tested *B. subtilis*-products. This raised the question whether the higher activity was due to the active microbial ingredient or other components of the formulation. Therefore, the active microbial ingredient was re-isolated from the product and grown in liquid culture. After centrifugation of the culture, both the supernatant and the pelleted spores were tested in detached leaf bioassays in comparison to a suspension of Serenade and to the pellet obtained after centrifugation of the Serenade suspension. The supernatant of the Serenade suspension and the supernatant of the culture of the re-isolate were as active as the suspension of Serenade itself (Figure 3). This was the case for application both before and after inoculation with the pathogen. In contrast, the pelleted spores of the re-isolate did not show any effect.

Table 3. Effect of different concentrations of selected products and plant extracts on the development of *P. infestans* between the 5th and 7th day after inoculation on excised leaves

Treatment	Conc. [%]	Mean area under the disease progress curve (AUDPC)[a] (±SD)					
		Application 24 h before the pathogen			Application 90 min after the pathogen		
Elot-Vis	Control[b]	165.6	(±39.7)	a[c]			
	1	178.5	(±7.39)	a			
	10	118.8	(±60.1)	a			
	20	92.7	(±37.0)	a			
Serenade	Control	165.6	(±39.7)	a	169.1	(±30.5)	a
	0.1	167.0	(±15.1)	a	128.1	(±26.0)	a
	1	88.5	(±38.9)	b	50.7	(±35.6)	b
	5	158.3	(±31.1)	a	27.7	(±8.67)	b
Trichodex	Control	165.6	(±39.7)	ab			
	0.1	166.7	(±14.0)	ab			
	1	120.1	(±14.4)	b			
	5	183.0	(±11.5)	a			
R. rhabarbarum	Control				170.5	(±22.7)	a
	0.1				126.1	(±17.5)	ab
	1				90.4	(±35.0)	b
	5				58.1	(±44.3)	b
S. Canadensis	Control	188.2	(±1.59)	a			
	0.1	126.4	(±8.09)	b			
	1	142.7	(±17.2)	b			
	5	128.5	(±6.10)	b			
Cu	0.5	28.8	(±20.7)		29.2	(±23.0)	

[a]Each treatment mean and standard deviations are calculated from three independent experiments each with three replicates.
[b]Control: for Elot-Vis, Serenade and Trichodex deionised water, for *R. rhabarbarum* and *S. canadensis* 0.0125% Tween.
[c]Means of the data within the column of the same treatment followed by the same letter are not significantly different following Student—Newman–Keuls test ($P < 0.05$).

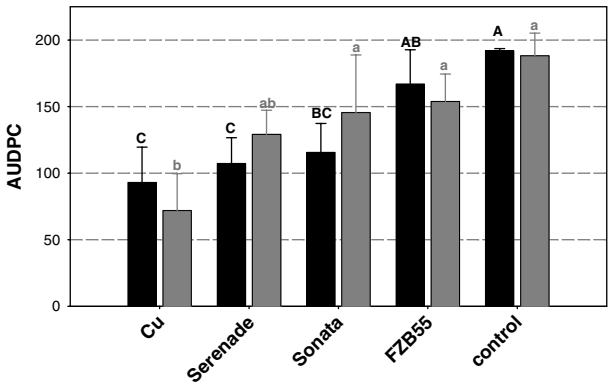

Figure 2. Effect of different *B. subtilis*-products on the development of *P. infestans* between the 5th and 7th day after inoculation on excised leaves (black columns: application of treatment 1 h after inoculation with the pathogen; grey columns: application of treatment 24 h before inoculation with the pathogen). Means of the AUDPC and standard deviations of three separate experiments each with three replicates. Means for each application followed by the same letter are not significantly different following Student—Newman–Keuls test ($P < 0.05$).

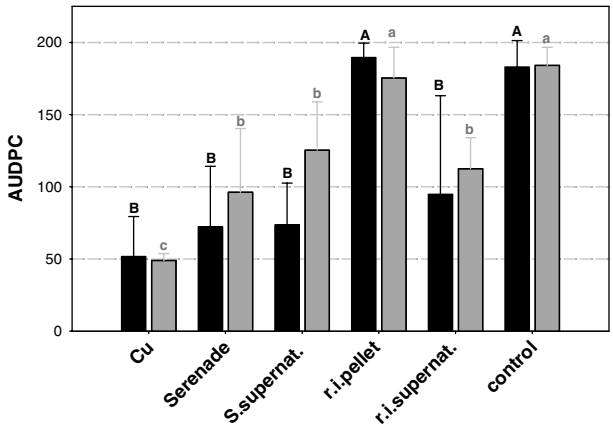

Figure 3. Effectiveness of Serenade and a culture of a re-isolate from the product against *P. infestans* between the 5th and 7th day after inoculation on excised leaves (black columns: application of treatment 1 h after inoculation with the pathogen; grey bar: application of treatment 24 h before inoculation with the pathogen). Means and standard deviations of three separate experiments each with three replicates. Means for each application followed by the same letter are not significantly different following Student—Newman–Keuls test ($P < 0.05$). S, Serenade; supernat., supernatant; r.i., re-isolate.

The *in vitro* assays (Figure 4) showed that water extracts of *S. canadensis* at the tested concentrations of 1 and 5% had a growth stimulating effect on mycelial growth of *P. infestans*. In contrast, a dose-dependent inhibition of mycelial growth was observed on agar medium containing the extract of *R. rhabarbarum* and Elot-Vis. At 1% the effect of the extract of *R. rhabarbarum* was stronger than inhibition by the same concentration of Elot-Vis. At the highest concentration tested (5%), both preparations restricted the growth of the mycelium completely.

Similar to the tests on excised leaves, most plant extracts and commercial products also provided control of *P. infestans* on potted potato plants. However, the level of control was in all cases significantly lower than after application of copper (Figure 5). For Serenade a clear relationship between dose and efficacy and between application time and efficacy could be established: application of the product 1 day after inoculation with the pathogen had no effect on *P. infestans*, whereas application at the time of pathogen inoculation resulted in the highest efficacy (Figure 5b). When

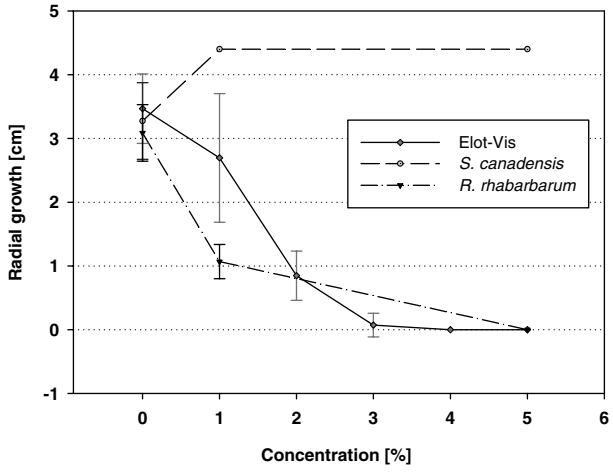

Figure 4. Mycelial growth of *P. infestans* on rye-sitosterol agar containing different concentrations of Elot-Vis or water extracts from *Solidago canadensis* and *Rheum rhabarbarum*. Means and standard deviations (error bars) of the radial growth on 10 (*Rheum rhabarbarum)* or 20 *(Solidago canadensis* and Elot-Vis) agar plates.

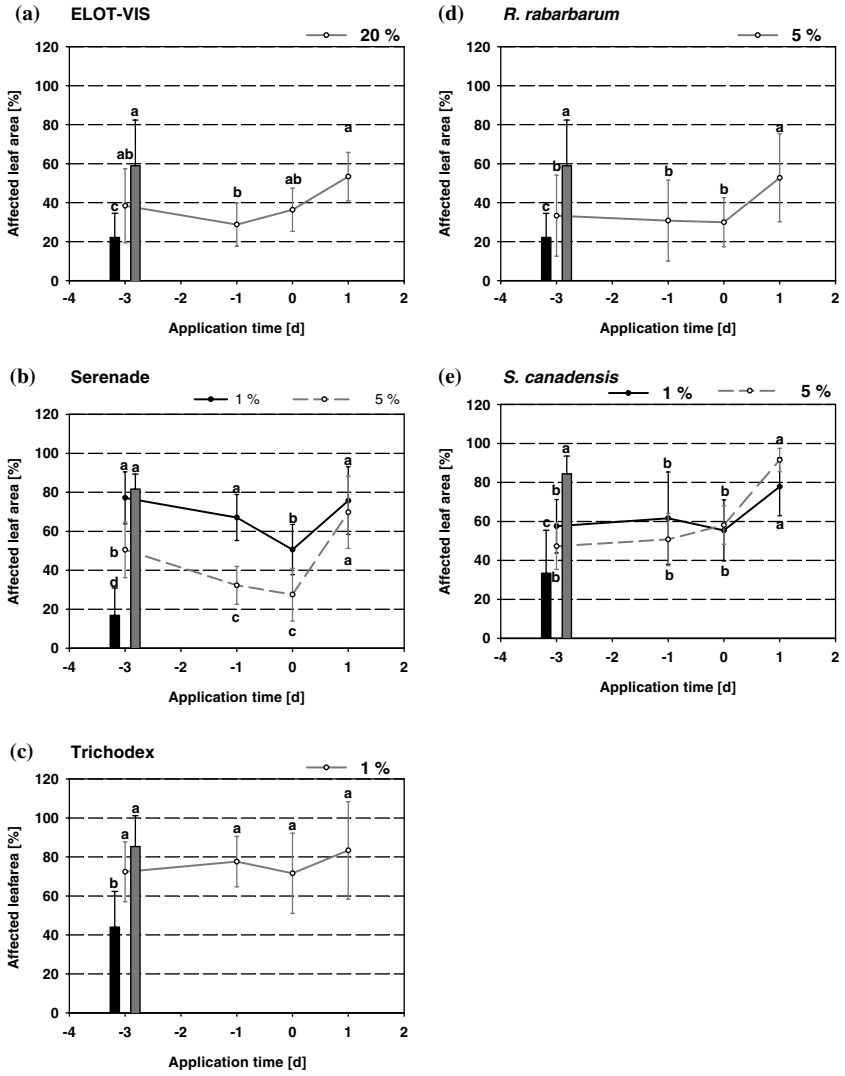

Figure 5. Effect of selected plant extracts and commercial products on disease symptoms caused by *P. infestans* on potted potato plants. The agents were applied at different intervals in relation to the time of inoculation with the pathogen (=0 d). The copper treatment (black columns) and the controls (water: grey bar in 5 a–c; Tween: grey bar in d–e) were applied 3 days before inoculation. Following concentrations were applied: Elot-Vis: 20%, Serenade: 1 and 5%, Trichodex: 1%, *R. rhababarum*: 5%, *S. canadensis*: 1 and 5%. Means and standard deviation (=error bars) of three separate experiments with three plants per treatment. Means for each application with the same letter are not significantly different followed by Student—Newman–Keuls test ($P < 0.05$) of the arcsine transformed data (Serenade, Trichodex, *S. canadensis*) or log 10 transformed data (Elot-Vis, *R. rhabarbarum*).

the product was applied before the pathogen, efficacy decreased gradually with increasing time between application of the product and inoculation with the pathogen. This tendency was obvious with both concentrations tested. In contrast, the plant extracts from *R. rhabarbarum* and *S. canadensis* also reduced infection with *P. infestans* significantly when applied up to 3 days before inoculation, and efficacy did not decrease with increasing time interval between treatment and infection. However, as with Serenade, no significant disease control was recorded when *R. rhabarbarum* and *S. canadensis* were applied 1 day after pathogen inoculation (Figure 5d, e). As already observed on detached leaves (Table 3), the efficacy of *S. canadensis* was not dependent on the concentration. On potted plants the products Elot-Vis and Trichodex did not show clear effects

(Figure 5a, c). Elot-Vis caused a statistically significant reduction of disease compared to the untreated control only when applied 1 day before pathogen inoculation.

Discussion

The results of this study demonstrate that plant extracts and microbial-based preparations can reduce leaf infestation on potato by *P. infestans* under controlled environmental conditions. Thus, the presented data correspond to results of other workers who investigated the potential of microorganisms and plant extracts for control of this pathogen (Latten 1994; Blaeser et al., 1998; Tadesse et al., 1998; Saika and Azad 1999; El-Sheikh et al., 2002; Daayf et al., 2003).

Using potted tomato plants, Latten (1994) studied the effects on *P. infestans* of 46 ethanolic plant extracts. Of these, 38 extracts, including those from *R. rhabarbarum* and *S. canadensis*, showed an effectiveness of at least 90%, and 15 extracts provided 100 % control of *P. infestans*. The results of our experiments with cold water extracts correspond to Latten's results, although with a range of 19–96% the efficacy was in some cases lower. This may be explained by the lower extract concentration of 1% employed here, compared to concentrations of 3–7% used by Latten. According to the information of the distributor (ProPhyta GmbH), Elot-Vis, an ethanolic plant extract marketed in Germany as a plant strengthening agent, has an efficacy of up to 40% against *P. infestans* on potatoes. This is in agreement with the results of the tests on detached leaves and potted plants in the present study.

The active ingredient of the product Messenger is harpin protein. This protein is involved in triggering the hypersensitive response of plants in incompatible interactions with phytopathogenic bacteria (Wei et al., 1992). Applications of Messenger have been reported to improve crop yield and to stimulate plant defence against damage by diseases and pests (Jones, 2001). Moreover, Li and Fan (1999) demonstrated a reduction of late blight in transgenic potato expressing harpin protein. The data obtained in our study, however, indicate an enhanced infestation of potato leaves with *P. infestans* after application of Messenger. However, the tests were carried out on detached leaves and not on whole plants, which might be a reason for this discrepancy.

The potential of *T. harzianum* and *Bacillus subtilis* as biocontrol agents of *Phytophthora* species was shown by Daayf et al. (2003), Etebarian et al. (2000), El-Sheikh et al. (2002) and Filippov and Kuznetsova (1994). In the screening tests performed in the present study, three of five *T. harzianum* products and the commercial product Serenade (*Bacillus subtilis*) proved to be the most effective microbial-based preparations. When the best treatments were compared in further experiments on detached leaves, only Trichodex and Serenade were effective. However, on potted plants Trichodex did not show any significant effect. In experiments on detached leaves with different *B. subtilis* preparations, the products Serenade and Sonata had a disease-reducing effect when applied 24 h before inoculation with the pathogen, whereas application of FZB 55 was not effective. These results indicate that products based on *T. harzianum* and *B. subtilis* have a potential for control of *P. infestans*. However, their efficacy may vary depending on the isolate and/or formulation of the respective product.

In order to take different modes of action, e.g. curative or protective, into account, in this study the test substances were applied 24 h before or 1 h after inoculation with *P. infestans*. Generally, better effects were obtained when applications were made 24 h before inoculation. This observation corresponds to results in the literature. Studying the efficacy of moss extracts, Tadesse et al. (1998) observed a better efficacy against *P. infestans* on tomato when the extracts were applied 2 days before the pathogen than applied at the same time. El Sheikh et al. (2002) state that protective treatments with antagonistic bacteria were more effective than curative treatments to control of *P. infestans* on potatoes. Similarly, XiuFen et al. (2001) found that the curative effect of the fermentation filtrate of *Xenorhabdus nematophilus* was lower than the protective effect.

In many cases, metabolites with antifungal or antibacterial properties have been reported to play an important role in the mode of action of microbial antagonists (Filippov and Kuznetsova, 1994a; Horvath et al., 1995; XiuFen et al., 2001). According to the product description of Serenade, its active microbial ingredient *B. subtilis* QST 713 produces three groups of lipopeptides that

together stop spores of plant pathogens from germinating, disrupt germ tube and mycelial growth and inhibit attachment of the plant pathogen to the leaf surface (Marrone, 2002). The results of our study strongly indicate that for Serenade the metabolites of *Bacillus subtilis* are the effective ingredients for controlling *P. infestans* and not the micro-organism itself. This result is in accordance with results of Scherm et al. (2004). In assays with potted plants a significant effect was obtained for *R. rhababarum*, *S. canadensis* and Serenade, and a weak effect for Elot-Vis when applied 1 h after inoculation. These effects can obviously be attributed to a direct effect of the treatments and are likely to be the result of a direct interaction between the pathogen and the control agent. Together with the inhibition of mycelial growth which we found in *in vitro* assays it has to be concluded that Elot-Vis and *R. rhababarum* have a direct effect on *P. infestans*. On the other hand, extracts from *S. canadensis* were found to be effective independent of the concentration used and did not show any inhibitory effects on mycelial growth of the pathogen. It is therefore likely that the activity of this extract is mainly based on effects on the metabolism of the host, similar to induced resistance.

The main aim of the present study was to provide a basis for the development of non-chemical treatments against *Phytophthora* blight in organic farming. Some alternative control agents were identified that should be tested in future field experiments. From the results obtained so far, it seems probable that their efficacy under field conditions may only reach in rare cases the efficacy of the copper fungicides currently used in organic farming. Additionally, these experiments give rise to the suspicion that the application window of the tested preparations is rather narrow. Therefore, for further studies on the field efficacy of the tested products, a combination with late blight forecasting models (Hijmans et al., 2000; Steenblock and Forrer, 2002) is essential. Furthermore, field studies should also consider potential tolerance inducing effects (Meinck, 1999). Additionally, the impact of harmful environmental effects (e.g. UV radiation) should be evaluated and formulations have to be optimised and/or developed to achieve more stable preparations.

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