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Compatible biological and chemical control systems for *Rhizoctonia solani* in potato*

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Abstract

A series of chemical and biological control agents were tested for compatibility with the *Rhizoctonia*-specific biocontrol fungus *Verticillium biguttatum* aimed at designing novel control strategies for black scurf (*Rhizoctonia solani*) and other tuber diseases in potato. The efficacy of chemicals, alone and in combination with *V. biguttatum* was tested in *in vitro* assays on nutrient agar plates, in bio-assays with minitubers and in the field. Generally, there were both antagonistic, neutral and additive interactions with *V. biguttatum* among the combinations tested; there were no indications for synergistic interactions. Broad-spectrum fungicides (azoxystrobin, chlorothalonil, thiabendazole) were fungitoxic to *V. biguttatum* as shown in *in vitro* assays, and hampered black scurf control by *V. biguttatum* in bio-assays. Oomycete-specific chemicals (cymoxanil and propamocarb) and various biocontrol strains (*Gliocladium* spp., *Pseudomonas* spp. and *Trichoderma* spp.) did not interfere with the growth of *V. biguttatum* on agar nutrient plates and did not affect black scurf control by *V. biguttatum* in co-applied treatments in the minituber bio-assay. *Rhizoctonia*-specific (pencycuron, flutalonil) fungicides co-applied with *V. biguttatum* showed additive effects on black scurf control. When combinations of *V. biguttatum* and cymoxanil or propamocarb were applied to immature potato tubers at green crop lifting, a reduction of both black scurf and *Pythium*- or *Phytophthora*-incited tuber rot was observed at harvest. In conclusion, the biocontrol fungus *V. biguttatum* is compatible with selected chemical control systems and may improve control efficacy in combination with *Rhizoctonia*-specific fungicides or may extend control spectrum in combination with Oomycete-specific fungicides.

Introduction

Verticillium biguttatum Gams is a specific antagonist of the plant pathogenic fungus *Rhizoctonia solani* Kühn (Van den Boogert et al., 1989) and it has potential to control black scurf disease in potato, caused by *R. solani* AG-3 (Jager et al., 1991) and stunt disease in barley, caused by *R. solani* AG-8 (Morris et al., 1995). Application opportunities of *V. biguttatum* to control black scurf in potato are diverse, ranging from seed tuber treatment at planting to post-harvest treatment of progeny tubers at winter storage (Jager and Velvis, 1988). A recently developed haulm destruction method, called 'Green Crop Lifting' (GCL), may open new perspectives for pre-harvest treatment of progeny tubers at their most susceptible stage to black scurf (Mulder et al., 1992). GCL implies (i) the mechanical destruction and removal of the aerial plant parts, (ii) the lifting of the progeny tubers from soil and the remaining plant and (iii) the deposition of tubers on a new soil bed and covering by soil. In fact GCL results in the early

*In this article fungicides and antagonists have been used aimed at controlling potato tuber diseases. None of these fungicides has been registered for the application in Green Crop Harvesting and, except for scientific goals, Plant Research International does not carry any responsibility for commercial exploitation of the control methods described in this article.

separation of the tubers from other plant parts, which is known to inhibit or delay black scurf development (Dijst et al., 1986; Mulder et al., 1992). Since progeny tubers are shortly freed from surrounding soil during the lifting, they can be treated with *V. biguttatum* spores at this strategic moment when tuber-borne sclerotia of *R. solani* start to develop. Moreover, the soil conditions at the time of GCL match well with temperature and moisture requirements for effective black scurf control by *V. biguttatum* (Van den Boogert et al., 1994).

To take full advantage of this GCL, current and novel control systems were tested for compatibility and efficacy in post-harvest diseases control. Positive effects could be expected of combined applications with *V. biguttatum*, but negative interactions may occur as well. Compatible interactions between (synthetic) fungicides and biological agents (such as antagonistic micro-organisms) may result in additive control of each single agent as reported for *Coniothyrium minitans* and (reduced) fungicide application (Budge and Whipps, 2001), *Talaromyces flavus* and potato seed piece fungicides (Fravel et al., 1985), and *Metarhizium anisopliae* and selected fungicides and insecticides (Moorhouse et al., 1992). Synergy between fungicides and *Trichoderma* spp. has also been reported (Harman et al., 1996). Synergy may be expected when two effective but independent mechanisms are involved in pathogen interaction: cell wall lytic enzymes produced from *Talaromyces harzianum* enhance the sensitivity of the target pathogen to fungitoxic compounds due to (partial) cell wall digestion (Di Pietro et al., 1993). Incompatible interactions with fungicides may result in reduced disease control by the biological control agent. To overcome incompatibility, mutants of *Trichoderma* spp. have been selected successfully for fungicide resistance and similar or enhanced antagonistic properties (Papavizas et al., 1982; Ahmad and Baker, 1988).

The purpose of the work described here was to evaluate the compatibility of the mycoparasite *V. biguttatum* with specific and broad-spectrum fungicides and antagonistic micro-organisms aimed at designing efficient biological and integrated disease control in potato cultivation.

Materials and methods

Culture media

Water agar (WA) was prepared from 12 g agar (Oxoid No. 3) in 1 l deionized water. Malt extract peptone agar (MPA) was prepared with 15.0 g malt extract (Oxoid L39), 1.0 g special peptone (Oxoid L72) and 12 g agar per litre deionized water. MPA-10 or MPA-1 contained 10% or 1% of the malt extract and peptone concentration, respectively. Malt peptone broth (MP) was used for liquid cultures. Malt mannitol yeast agar (MMYA) comprised 15 g malt extract, 5 g mannitol (Sigma M-4125), 2.5 g yeast extract (Oxoid L21) and 12 g agar per litre. Tripticase Soy Agar (TSA) or TS broth (TS) were prepared according to manufacturer's directions (BBL 11043). The media were autoclaved at 120 °C for 20 min.

Micro-organisms and inoculum preparation

The micro-organisms used (Table 1) were isolated from potato tubers in the Netherlands. Cultures were maintained on MPA (fungi) or on TSA (bacteria)

Table 1. Details of organisms used

Species	Strains	Properties	Origin
Gliocladium nigrovirens	IPO-1815	Antagonistic to *Erwinia chrysanthemi*	Potato tuber, The Netherlands
G. roseum	IPO-1813	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
Gliocladium sp.	IPO-M171	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
Pseudomonas putida[*]	WCS 358	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
Pseudomonas sp.[*]	WCS 371	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
Pseudomonas sp.[*]	WCS 402	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
Rhizoctonia solani AG-3	IPO-3R41	Pathogenic to potato	Potato tuber, The Netherlands
Trichoderma hamatum	IVT10	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
T. harzianum	IPO-1812	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
T. viride	IPO-1811	Antagonistic to *E. chrysanthemi*	Potato tuber, The Netherlands
Verticillium biguttatum	M73 (CBS 228.80)	Antagonistic to *R. solani*	Potato tuber, The Netherlands

[*]Strains kindly provided by Dr. P.A.H.M. Bakker, Department of Plant Ecology and Evolutionary Biology, University of Utrecht, the Netherlands.

in darkness at 20 °C. Conidial suspensions of sporulating fungi were obtained by rinsing the agar surfaces of 10–14-day-old colonies with sterile tap water. The crude conidial suspensions were passed through three layers of sterile cheese-cloth, then washed in three changes of sterile tap water, and centrifuged each time for 10 min at $8000 \times g$. Bacterial inoculum was prepared from crude TSA cultures after 3 days of incubation. Concentrations in cell suspensions were assessed with a Fuchs–Rosenthal haemocytometer and adjusted to desired levels with tap water or TS for conidia and bacterial cells, respectively. Mass-production of *V. biguttatum* conidia for field experiments was performed in 15 cm diam. Petri dishes containing 50 ml MMYA. Inoculum for *in vitro* tests consisted of 3 mm diam. discs taken from the margins of actively growing colonies *of R. solani* and *V. biguttatum* on MPA. Perlite-borne sclerotia of *R. solani*, used in bio-assays, were grown on MP-drenched perlite, according to Van den Boogert and Velvis (1992).

Fungicides

The fungicides used and product information are listed in Table 2. For *in vitro* tests, highly concentrated solutions were prepared in 70% ethanol and further diluted to desired concentrations in deionized water. The highest concentration of ethanol in the dilution series never exceeded 1%, for which an appropriate control was used to verify undesired side-effects of the solvent. Fungicide solutions used in bio-assays and field tests were directly dissolved in tap water according to user's guidelines.

In vitro *tests*

Rhizoctonia solani and *V. biguttatum* agar discs were transferred from an active colony to fresh MPA supplemented with various concentrations of fungicides (Table 2). Agar plates were incubated at 20 °C in darkness, and radial growth was recorded by measuring the colony diameter at 2-day intervals. In addition to growth, the germination of *V. biguttatum* conidia, and the number and length of the germ tubes were recorded on fungicide-amended MPA-10. For that purpose 0.5 ml of a conidial suspension adjusted to 1.2×10^6 spores ml^{-1} was evenly dispersed onto MPA-10 using a Drigalski spatula to obtain densities of about 100 conidia mm^{-2} agar surface. After 48 h of incubation at 20 °C, 100 non-clustered conidia were observed and rated for germination, tube length and number of germ tubes per conidium. The fungicide concentrations tested were 0.0, 0.5, 1.0, 5.0, 25, 50, 100 and 1000 µg active ingredient (ai) ml^{-1}.

Bacterial and fungal biological agents listed in Table 1 were also tested for compatibility with *V. biguttatum* on MPA-10 plates. Cell suspensions of *V. biguttatum* with each of co-inoculated biological agents were prepared at densities of 1.2×10^6 cells ml^{-1} and mixed up at equal volumes. Aliquots of 0.5 ml of the mixed suspension were spread onto MPA-10 to obtain densities of about 200 conidia mm^{-2} agar surface. After 48 h of incubation at 20 °C, 100 non-clustered *V. biguttatum* conidia were observed for germination.

The fungicide concentration required to limit colony growth, germination, germ tube number or length to 50% of the control (ED_{50}) was calculated for each fungicide–isolate combination using Probit link function (Genstat 5, release 2.2), based on the means of three replicates.

Bio-assays in a minituber system

Black scurf was allowed to develop on immature mini potato tubers sandwiched between two layers of soil. The MTS consists of plastic containers (12 cm in diam.; 11 cm height) filled with 250 g dry

Table 2. Details of fungicide dosages used in field and lab trials

Fungicide (trade mark; manufacturer)	Target	Recommended dose (N)	
		Formulation l or kg ha^{-1}	Active ingredient kg ha^{-1}
Azoxystrobin (Amistar; Syngenta)	Non-specific	1.0 l	0.20
Chlorothalonil (Daconil 2787; Aventis)	Non-specific	2.0 kg	1.50
Cymoxanil (Curzate 50; Dupont)	Oomycete-specific	0.40 kg	0.20
Flutolanil (Monarch; Aventis)	*Rhizoctonia*-specific	5.0 l	2.25
Pencycuron (Monceren; Bayer)	*Rhizoctonia*-specific	10.0 l	2.50
Propamocarb (Previcur N; Aventis)	Oomycete-specific	2.0 l	1.44

soil equivalents supplemented with 125 sclerotia produced on perlite. Additional soil amendment with fresh potato stem/stolon pieces (ca. 1 cm length) at 1% (w/w) favoured black scurf formation on the minitubers. A standard sandy loam soil (pH-KCl 7.3, organic matter 3.7%) from 'de Eng' near Wageningen was used throughout MTS assays. Before use, the soil was passed through a 3 mm sieve and moisture content was adjusted to 50% water holding capacity.

Minitubers were obtained from *in vitro* propagated potato plants (cv. Bintje) grown in pasteurized (1 : 1) perlite potting soil mixture. The minitubers achieved the right size (0.8–1.2 cm diam.) and maturity following a 6–8 weeks period of cultivation under controlled conditions (temperature 16–18 °C, relative humidity 90% and daily light periods of 16 h at 30 000 lux). After harvest, the minitubers were freed of adhering soil by washing in tap water and kept moist until use for 2 days at maximum. Stem and stolon pieces were obtained from 6 to 12-week-old potato plants (cv. Bintje) which were pre-grown in potting soil in the greenhouse.

Biological agents and fungicides were applied by dipping minitubers in cell suspensions alone or in mixed suspensions with fungicides. The fungicide concentrations tested ranged from 0.008 to 5.0 times the recommended dosage for single field application (Table 2). The experimental design was a randomized complete block with four replicates; suspension liquid without control agent served as treatment-control.

Field tests

The field tests were conducted on marine soils of the experimental farms Kollumerwaard (KW) in Munnekezijl, Oostwaardhoeve (OW) in Slootdorp and De Waag (BE) in Creil, and sandy soil of Kooijenburg (KB) in AA en Hunze, the Netherlands. The potato crop was grown according to current farmer's practice for seed tuber production, including haulm destruction. Three months after planting, the potato haulm was destructed according to GCL guidelines (Mulder et al., 1992). Each plot accommodated 320 potato plants in four parallel rows of 20 m, each with 0.75 m between rows. The progeny tubers were treated during tuber lifting by spraying the control agent in 300 l tap water per ha. The treatments were cymoxanil and propamocarb at recommended dosages (Table 2), *V. biguttatum* at 1.5×10^{12} spores ha^{-1} alone and in combination with one of the fungicides; tap water alone served as treatment-control. The experimental design was a randomized complete block with four replicates. After a 3 week maturation period, 100 progeny tubers per plot were randomly dug up by hand and rated for black scurf incidence and severity.

Disease rating and statistical analysis

Black scurf disease was visually rated for the presence of tuber-borne sclerotia and expressed as a black scurf index (SI) ranging from non-contaminated (SI = 0) to severely contaminated tubers (SI = 100), according to Van den Boogert and Jager (1984). Data on SI were subjected to analysis of variance (ANOVA) after arcsin transformation and treatment means were compared to the appropriate controls by least significant difference at $P = 0.05$ ($LSD_{5\%}$).

Results

In vitro *tests on tolerance of* V. biguttatum *to fungicides and biological agents*

In vitro tests on fungal growth and developmental behaviour ED_{50} indicated that there were significant differences between *R. solani* and mycoparasite in respect to (in-)sensitivity to the fungicides tested (Table 3). For example, *R. solani* exhibited extreme sensitivity to pencycuron and flutolanil, as indicated by relatively low values of ED_{50}, whereas *V. biguttatum* was very tolerant to these *Rhizoctonia*-specific fungicides. Other fungicides, like propamocarb, appeared similarly ineffective in reducing radial growth of both *R. solani* and *V. biguttatum* up to a concentration of 1000 $\mu g\ ml^{-1}$. Colony growth and spore germination may differ in sensitivity to fungicidal compounds: for instance chlorothalonil strongly decreased germination of *V. biguttatum*, but reduced its radial growth to a limited extent.

Approximately half of *V. biguttatum* conidia germinated within 24 h on MPA-10, whether these conidia were dispersed on the medium alone or mixed with conidia of *Trichoderma* or *Gliocladium* species. The germination rate of *Trichoderma* and *Gliocladium* conidia in mixed inoculations was 100%. On WA, *V. biguttatum* conidia failed to germinate, both alone or in mixtures with *Trichoderma* or *Gliocladium* conidia, which themselves germinated profusely. Less than 1% of *V. biguttatum* conidia co-inoculated onto MPA-10 with *Pseudomonas* spp. germinated within 24 h of incubation.

Table 3. Concentrations of active ingredient of fungicides in MPA which causes a 50% reduction (ED_{50}) in radial growth extension by *R. solani* and radial growth extension, conidial germinability, germ tube number and length by *V. biguttatum*

Fungicides (ai)	ED_{50} ($\mu g\,ml^{-1}$)				
	R. solani growth	*V. biguttatum*			
		Growth	Germinability	Number	Length
Azoxystrobin	5.5[a]	0.2[a]	2.5	4.3[a]	1.7[a]
Chlorothalonil	8.5[a]	31.5[b]	<0.5	<0.5	<0.5
Cymoxanil	9.7[a]	27.4[b]	>1000	59.6[b]	38.1[b]
Flutolanil	0.61[b]	>1000	>1000	>1000	>1000
Pencycuron	0.05[c]	>1000	>1000	>1000	>1000
Propamocarb	>1000	>1000	>1000	>1000	>1000

> or < mean ED_{50} values exceed concentration range tested, respectively. Data f-ollowed by different letters in each column are significantly different at $P < 0.01$.

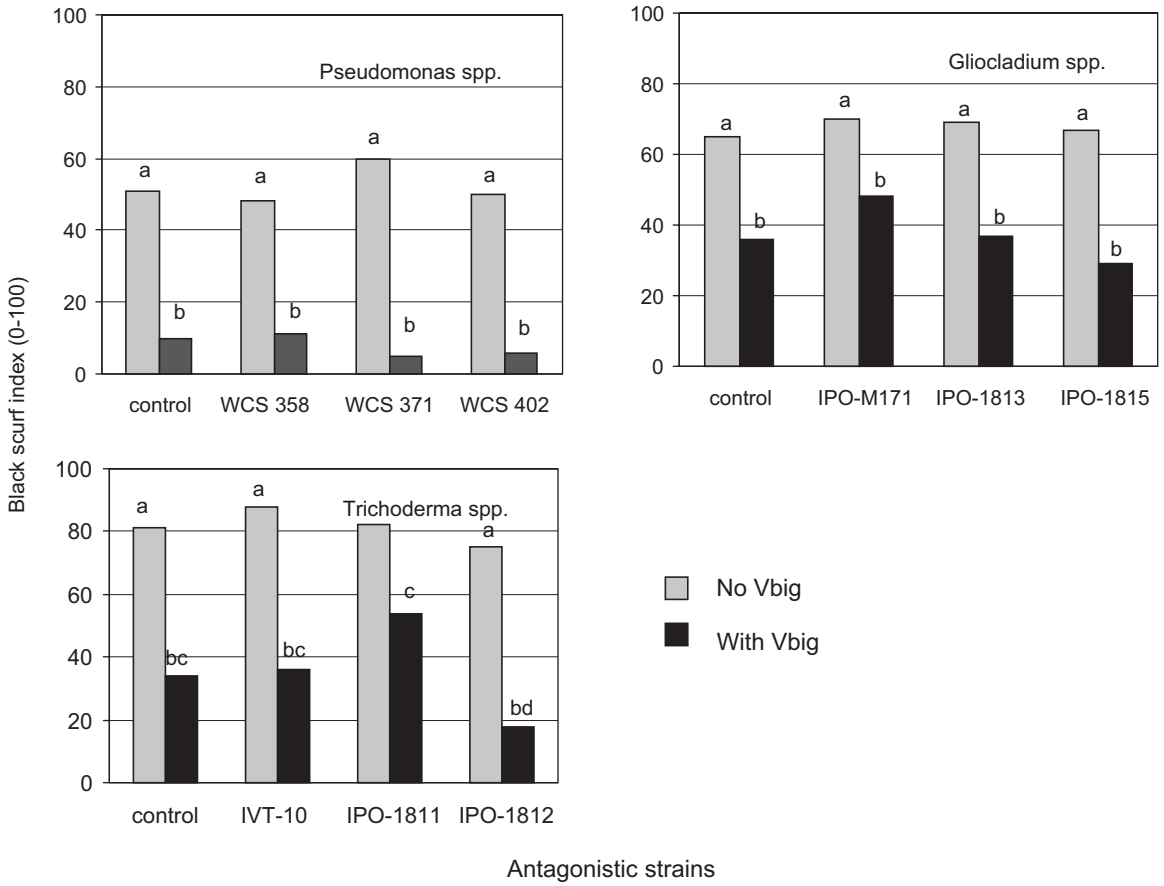

Figure 1. Black scurf development on minitubers in bio-assay, expressed as black scurf index (0–100), subjected to *Pseudomonas* spp. at 10^{10} cells ml^{-1}, *Gliocladium* spp. and *Trichoderma* spp. at 1×10^8 spores ml^{-1}, alone (no Vbig) and co-inoculated with *V. biguttatum* at 2×10^6 spores ml^{-1} (with Vbig). Different letters in each panel indicate significant differences at $P < 0.05$.

Bio-assays on compatibility with biological agents and fungicides

Biological agents

The first series of experiments were performed with conidial concentrations of 5×10^6 ml^{-1} at which *V. biguttatum* reduced black SI by ∼50%. Since there was no effect on black scurf control by *V. biguttatum*, the concentration of *Gliocladium* and *Trichoderma* strains was increased to 10^8 conidia ml^{-1}. Even at concentrations of co-inoculant fungi 20 times higher than of *V. biguttatum*, and at *Pseudomonas* concentrations of 4×10^{10} cells ml^{-1}, there was no effect on black scurf control by *V. biguttatum* (Figure 1).

Fungicides

The Oomycete-selective control agents cymoxanil and propamocarb did not affect black scurf development, nor hindered black scurf control by *V. biguttatum*, whereas the Rhizoctonia-fungicides flutolanil and pencycuron showed additive control of black scurf in a combined application (Figure 2).

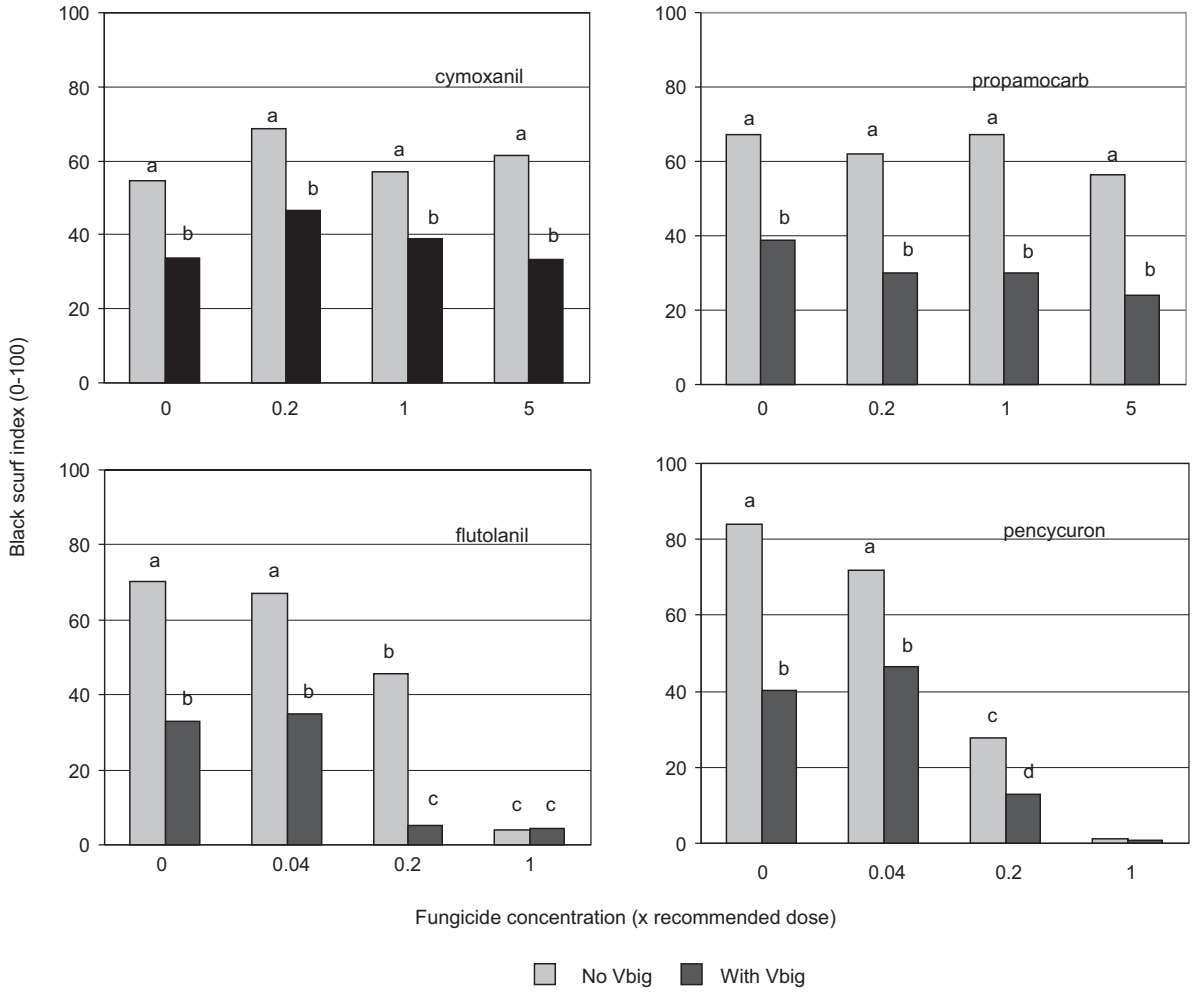

Figure 2. Black scurf development on minitubers in bio-assay, expressed as black scurf index (0–100), subjected to specific Oomycete (cymoxanil, propamocarb) and Rhizoctonia (flutolanil, pencycuron) fungicides at various concentrations (N = standard; see Table 2), alone (no Vbig) and co-applied with *V. biguttatum* (with Vbig) at 2×10^6 spores ml^{-1}. Different letters in each panel indicate significant differences at $P < 0.05$.

Table 4. Effect of *V. biguttatum* tuber inoculation alone and in combination with fungicides cymoxanil or propamocarb on black scurf disease in field-grown potato crops as applied in green crop lifting at the experimental farms located in Kooijenburg (KB), Oostwaardhoeve (OW), Kollumerwaard (KW) and Van Bemmelenhoeve (BE), the Netherlands

Tuber treatments	Black scurf index (0–100)					
	KB-92	OW-92	KW-94	KW-95	BE-95	BE-96
Control (water)	13.5[a]	29.6[a]	37.8[a]	12.3[a]	15.6[a]	36.4[a]
V. biguttatum	4.9[b]	17.5[b]	9.5[b]	0.2[b]	0.1[b]	7.4[b]
+ cymoxanil	nd	19.8[b]	10.1[b]	1.1[b]	nd	nd
+ propamocarb	9.1[ab]	17.8[b]	13.5[b]	nd	1.0[b]	7.2[b]

Data in each column followed by different letters are significantly different at $P < 0.01$; nd means not determined.

Field experiments with selected control agents

Verticillium biguttatum controlled black scurf disease in commercial seed potato growing as illustrated in six naturally infested fields. The field data presented in Table 4 also confirmed that mixed application with Oomycete fungicides did not affect biological control by *V. biguttatum*. Propamocarb and cymoxanil were quite effective at recommended dosages and reduced tuber rot by *Pythium* or *Phytophthora* species by 70% and 29%, respectively, in experimental field OW-92. Tuber rot was not present in the other fields listed in Table 4.

Discussion

A series of potential control agents was tested for compatibility with the biocontrol fungus *V. biguttatum* on agar, in tuber-based bio-assays and in the field. Here we present examples of compatible and incompatible combinations of biological and chemical control systems. It has been shown that *V. biguttatum* can partially replace *Rhizoctonia*-specific fungicides or may extend the control spectrum of other fungicides or biological agents. The synthetic fungicides flutolanil and pencycuron possess potential for simultaneous application with *V. biguttatum* for black scurf control, as shown in the minituber bio-assay. The synthetic fungicides propamocarb and cymoxanil possess potential for simultaneous application in disease control against both late blight (*Phytophthora infestans* and other Oomycete tuber pathogens) and black scurf. As expected, broad-spectrum synthetic fungicides, like chlorothalonil, thiabendazole and azoxystrobin, proved toxic to *V. biguttatum* in *in vitro* tests, depicting their incompatibility for simultaneous application with the mycoparasite. Biological control agents, like *Pseudomonas* spp., *Trichoderma* or *Gliocladium* spp., did not interfere with mycoparasitism by *V. biguttatum*, illustrating inter-species compatibility and their potential as co-inoculants for broad-spectrum control of tuber diseases. Even at 50-fold concentrations of co-inoculants, *V. biguttatum* is able to exhibit black scurf control.

The availability of compatible biological control alternatives opens new strategies for black scurf control in agricultural systems that rely on low input and reduced dependency of synthetic fungicides. In addition, the life-time of current *Rhizoctonia*-specific fungicides are expected to extend by alternating application with biological agents. Presented data on compatible control systems contribute to sustained disease control in potato crop against *R. solani* AG-3. Together with the biocontrol of *Erwinia* spp. by antagonistic *Pseudomonas* strains (Kastelein et al., 1994) and of cyst nematodes by *Hirsutella rhossiliencis* (Velvis and Kamp, 1995; 1996), data showed in the present study, on the control of *R. solani*, are a contribution to sustained control of diseases and pests affecting potato.

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References

Ahmad JS and Baker R (1988) Rhizosphere competence of benomyl-tolerant mutants of *Trichoderma* spp. Canadian Journal of Microbiology 34: 694–696

Budge SP and Whipps JM (2001) Potential for integrated control of *Sclerotinia sclerotiorum* glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. Phytopathology 91: 221–227

Dijst G, Bouman A, Mulder A and Roosjen J (1986) Effect of haulm destruction supplemented by cutting off roots on the incidence of black scurf and skin damage, flexibility of harvest period and yield of seed potatoes in field experiments. Netherlands Journal of Plant Pathology 92: 287–303

Di Pietro A, Lorito M, Hayes CK, Broadway RM and Harman GE (1993) Endochitinase from *Gliocladium virens*: Isolation, characterization, and synergistic antifungal activity in combination with gliotoxin. Phytopathology 83: 308–313

Fravel DR, Marois JJ, Dunn MT and Papavizas GC (1985) Compatibility of *Talaromyces flavus* with potato seed piece fungicides. Soil Biology Biochemistry 17: 163–166

Harman GE, Latorre B, Agosin E, San-Martin R, Riegel DG, Nielsen PA, Tronsmo A and Pearson RC (1996) Biological and integrated control of *Botrytis* bunch rot of grape using *Trichoderma* spp. Biological Control 7: 259–266

Jager G and Velvis H (1988) Inactivation of sclerotia of *Rhizoctonia solani* on potato tubers by *Verticillium biguttatum*, a soil-borne mycoparasite. Netherlands Journal of Plant-Pathology 94: 225–231

Jager G, Velvis H, Lamers JG, Mulder A and Roosjen J (1991) Control *of Rhizoctonia solani* in potato by biological, chemical and integrated measures. Potato-Research 34: 269–284

Kastelein P, Bouman A, Mulder A, Schepel E, Turkensteen LJ, de Vries PM and van Vuurde JWL (1994) Green crop harvesting and infestation of seed potato tubers with *Erwinia* spp. and perspectives for integrated control. In: Proceedings of 8th International Conference on Plant Pathogenic Bacteria, Versailles (pp 999–1004) INRA, Paris

Moorhouse ER, Gillespie AT, Sellers EK and Charnley AK (1992) Influence of fungicides and insecticides on the entomogenous fungus *Metarhizium anisopliae*, a pathogen of the vine weevil, *Otiorhynchus sulcatus*. Biocontrol, Science and Technology 2: 49–58

Morris RAC, Coley-Smith JR and Whipps JM (1995) The ability of the mycoparasite *Verticillium biguttatum* to infect *Rhizoctonia solani* and other plant pathogenic fungi. Mycological-Research 99: 997–1003

Mulder A, Turkensteen LJ and Bouman A (1992) Perspectives of green-crop-harvesting to control soil-borne and storage diseases of seed potatoes. Netherlands Journal of Plant Pathology 98(Suppl 2): 103–114

Papavizas GC, Lewis JA and Abd-El-Moity TH (1982) Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. Possible biological control of soilborne plant pathogens. Phytopathology 72: 126–132

Van den Boogert PHJF and Jager G (1984) Biological control of *Rhizoctonia solani* on potatoes by antagonists. 3. Inoculation of seed potatoes with different fungi. Netherlands Journal of Plant Pathology 90: 117–126

Van den Boogert PHJF, Reinartz H, Sjollema KA and Veenhuis M (1989) Microscopic observations of the mycoparasite *Verticillium biguttatum* with *Rhizoctonia solani* and other soil-borne fungi. Antonie van Leeuwenhoek 56: 161–174

Van den Boogert PHJF and Velvis H (1992) Population dynamics of the mycoparasite *Verticillium biguttatum* and its host *Rhizoctonia solani*. Soil Biology Biochemistry 24: 255–265

Van den Boogert PHJF, Kastelein P and Luttikholt AJG (1994) Green-crop-harvesting, a mechanical haulm destruction method with potential for disease control of tuber pathogens in potato. In: Martin TJ (ed) Seed Treatment: Progress and Prospects, BCPC Monograph No. 39 (pp 237–246) British Crop Protection Council, Farnham

Velvis H and Kamp P (1995) Infection of second stage juveniles of potato cyst nematodes by the nematophagous fungus *Hirsutella rhossiliensis* in Dutch potato fields. Nematologia 41: 617–627

Velvis H and Kamp P (1996) Suppression of potato cyst nematode root penetration by the endoparasitic nematophagous fungus *Hirsutella rhossiliensis*. European Journal of Plant Pathology 102: 115–122