European Journal of Plant Pathology **104:** 253–262, 1998.

Effect of moisture, inoculum production, and planting substrate on disease reaction of field bindweed (*Convolvulus arvensis* L.) to the fungal pathogen, *Phomopsis convolvulus*

S. Vogelgsang, A.K. Watson* and A. DiTommaso
Department of Plant Science, Macdonald Campus of McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec, Canada H9X 3V9 (Fax: 514 398-7897); * *Author for correspondence*

Accepted 2 December 1997

Key words: bioherbicide, biological weed control, controlled environment, granules, mycoherbicide, pre-emergence

Abstract

Phomopsis convolvulus Ormeno is being evaluated as a possible bioherbicide for field bindweed (*Convolvulus arvensis* L.) control. A granular barley formulation was applied pre-emergence onto the soil surface of pots containing pregerminated field bindweed seeds. Covering the pots with transparent plastic bags immediately after application increased disease incidence and resulted in up to 81% reduction in above-ground dry biomass, whereas a treatment of interrupted dew periods (8 h day^{-1}) for six days, resulted in only 56% biomass reduction. The size of container used for producing and for incubating the fungus granules had no significant effect on disease incidence and subsequent weed control of field bindweed. Likewise, no significant differences in efficacy were observed using inoculum that was milled once and then sieved or repeatedly milled and non-sieved. For early application dates, the use of two different planting substrates led to major differences in disease development. Pre-emergence application of inoculum on the surface of field collected soil on the same day that field bindweed seeds were planted resulted in an 81% mortality of seedlings emerging. In contrast, only 50% of emerging seedlings were killed when inoculum was applied on the surface of peat moss. Findings in this study indicate that moisture conditions and planting substrate may affect disease incidence and subsequent control of field bindweed by pre-emergence application of the selective fungal pathogen, *P. convolvulus*.

Introduction

The foliar pathogen, *Phomopsis convolvulus* Ormeno, selectively infects field bindweed (*Convolvulus arvensis* L.) and is currently being investigated as a possible bioherbicide against this serious perennial agricultural weed. Traditional control methods such as crop rotation, cultivation, and chemical herbicides have met with only limited success (Swan, 1980). In addition, reduced cultivation practices (Phillips et al., 1980) and variable susceptibility of field bindweed to several important herbicides (Whitworth and Muzik, 1967; DeGennaro and Weller, 1984; Kosinski and Weller, 1989; Yerkes and Weller 1996) have led to an increased prevalence of field bindweed. *Phomopsis convolvulus*, isolated in Québec, Canada, was first reported on field bindweed in 1988 (Ormeno-Nuñez et al., 1988a). Subsequently, studies on host specificity, conidia mass production, storage, and efficacy of foliar treatments have been carried out (Ormeno-Nuñez et al., 1988b; Morin et al., 1989a;b; 1990). Weed control efficacy has been limited by the long dew period required for the germination and infection phases of this fungus.

In an attempt to overcome this limitation, a granular pre-emergence application of *P. convolvulus* has been evaluated (Vogelgsang et al., 1994). Inoculum produced on pot barley grains and applied onto the soil surface has effectively controlled seedlings and established stands of field bindweed under both controlled environment and field conditions (Vogelgsang et al., 1998). However, disease development and weed control efficacy in experiments performed under controlled environment conditions were generally lower than in corresponding experiments carried out under field conditions. This is unusual in bioherbicide research. Typically, the high levels of disease obtained under laboratory conditions are difficult to reproduce in the field. The narrow environmental range of conditions that is often required to attain high levels of infection has commonly been cited to explain the disparity in results between laboratory and field trials (Watson and Wymore, 1990).

The objective of this study is to gain additional knowledge about factors that might lead to different levels of disease by *P. convolvulus* under controlled environment conditions. Experiments presented here were designed to test the effect of moisture availability, inoculum production method, and planting substrate on disease development.

Materials and methods

Inoculum production of starter cultures

Single conidia isolates of *P. convolvulus* were maintained on potato dextrose agar (PDA; Difco, Detroit, MI, USA) at 4 °C. From these stock cultures, small pieces of mycelium were placed on 9cm diameter Petri dishes with PDA and incubated in the dark at 24 ± 1 °C. After four to five days, several mycelial plugs of 1cm diameter were transferred to PDA plates and incubated at room temperature (21 ± 2 °C) and 12 h day^{-1} near-ultraviolet light (F40 BLB Blacklight, General Electric Lighting, Cleveland, OH, USA). After three weeks, conidia were harvested by washing plates with 12 ml of sterile deionized water. Conidial density was then adjusted to 1×10^7 conidia ml^{-1} with the aid of a haemocytometer.

Preparation of granular barley inoculum

Twenty ml of deionized water were added to 20 g of pot barley grains (*Hordeum vulgare* L.) in 250 ml Erlenmeyer flasks and autoclaved (18 min, 100 kPa, 120 °C). Flasks were cooled to room temperature and inoculated with 1 ml of the previously prepared conidia suspension starter culture. Flasks were incubated at room temperature (21 ± 2 °C) and exposed to 12 h near-ultraviolet light day^{-1} and shaken every second day by hand in order to inhibit the substrate from clumping. Colonized barley grains were harvested after three weeks and milled using an electric coffee grinder (Braun® KSM 2, Lynnfield, MA, USA). The granules produced were air dried for two days and sieved, unless otherwise indicated, resulting in inoculum particles of < 710 μm diameter. In a previous study, inoculum of this size was found to have a high pre-emergence activity and a shelf-life of at least six months (Vogelgsang et al., 1994).

To determine conidia production and viability of inoculum produced on the barley grains, 1 g granule samples were routinely tested as described previously (Vogelgsang et al., 1998).

Plant production

Field bindweed seeds (Valley Seed Co., Fresno, CA, USA) were washed under warm running tapwater for 2 h and soaked overnight in deionized water. Imbibed seeds were then incubated on moist paper towels in a glass Petri dish in the dark at 24 ± 1 °C for 24 to 36 h. Four germinated seeds with emerged radicles were sown at a depth of 3 cm into 10 cm diameter plastic pots (500 ml volume) containing a commercial prepared potting medium (Pro-Mix™ BX, Les Tourbières Premier Ltée, Rivière-du-Loup, QC, Canada). Pots were placed in a growth chamber (2 m^3 volume) (Conviron, Model E-15, Controlled Environments, Winnipeg, MB, Canada) at 23/18 ± 1 °C day/night temperature with a 15 h photoperiod (350 μEm^{-2}s^{-1}).

General inoculation procedure and data collection

For each pot, 1 g of granules was manually spread onto the moistened soil surface (79 cm^2). Pots were immediately covered with large transparent plastic bags and the ends tucked under the base of the pot. Bags did not need support and were not misted with water. Bags were removed after six days. Unless otherwise specified, inoculum applications were carried out zero, one, and two days after sowing (0, 1, and 2 DAS). Foliar necrosis was evaluated at eight DAS using the following rating system: 0 = no visible symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, and 4 = 76-100% necrosis (Ormeno-Nuñez et al., 1988b). Disease rating was performed for each plant or shoot and results pooled and averaged for each pot. Mortality was assessed 13 DAS by counting the number of seedlings with completely necrotic hypocotyls. Above-ground and root dry biomass were determined 13 and 14 DAS, respectively. Plants were cut at the soil line, roots were carefully removed from the potting medium and living tissues dried in paper bags for four days at 60 °C, and weighed. Biomass data were recorded as total biomass per pot.

Effect of moisture availability on disease severity

Following application of *P. convolvulus* granules on the soil surface of pots containing four pre-germinated field bindweed seeds, two moisture regimes were compared. In one treatment, pots were covered with plastic bags and placed in a growth chamber under the same conditions used for seedling growth. In order to simulate field conditions, plants subjected to the second treatment were placed for 8 h day^{-1} in a dark dew chamber (100% RH, 21 $\pm$ 1 °C) during the normal night period, and subsequently returned to the same growth chamber containing the other treatment, pots covered with plastic bags. Plants were subjected to the two moisture regimes for six consecutive days and assessed for disease severity, mortality, above-ground biomass, and root biomass as described previously.

Effect of inoculum production method on disease severity

In previously performed field trials, larger-sized incubation containers (1 l) were used, and the sieving procedure after harvest of fungal conidia from the colonized barley was replaced by a second grinding step (Vogelgsang et al., 1998). In the present study *P. convolvulus* inoculum was produced in 1 l screw cap jars (100 g pot barley, 80 ml water) and also in 250 ml Erlenmeyer flasks (20 g pot barley, 20 ml water). Three weeks after inoculation, half of the colonized barley grains from the jars and flasks were ground in a coffee grinder and sieved resulting in particles of < 710 μm. The other half was not sieved after the initial grinding in a coffee grinder, but was ground a second time using an electric meat grinder (Quaker City Mill, Model 4-E, Westinghouse, PA, USA). This process resulted in a mixture of larger ($>$ 1 mm) and smaller-sized (150 μm) particles with over 70% of the particles being < 710 μm diameter. The four different inoculum treatments (250 ml flask, sieved; 250 ml flask, non-sieved; 1 l jar, sieved; 1 l jar, non-sieved) were applied 1 DAS. The experiment also included an uninoculated control treatment. Data collection was performed as described above.

Effect of two different planting substrates on disease severity

Two different planting substrates were evaluated; the commercial potting medium Pro-Mix and field soil, collected from a site adjacent (approximately 2 metres) to a research plot area where field trials with this host-pathogen system had been carried out four to five months previously. Pro-Mix is characterized by 80% peat moss, equal parts of perlite and vermiculite, and a limestone adjusted to a pH of 6.0 whereas the Chicot fine sandy loam field soil is 70% sand, 20% silt, 10% clay, has a pH of 5.3, and 3% organic matter. Four pre-germinated field bindweed seeds were sown in pots containing the respective soils, and treated with the granular inoculum as described in the general inoculation procedure.

Experimental design and data analysis

All three experiments were performed twice in a completely randomized design with four replicates per treatment. Mortality and biomass data were arcsin or $\log_{10}$ (x+1) transformed as appropriate prior to analysis of variance and differences between treatment means were determined using Tukey's W test (α = 0.05) (Steel and Torrie, 1980). Disease ratings were compared using the Kruskal-Wallis one-way analysis of variance by ranks, followed by a multiple-comparison procedure to evaluate differences between treatment means (Daniel, 1978). Due to significant interactions between main effects, data for each of the two moisture conditions and planting substrates were analyzed separately. In all experiments, results for the two trials were not pooled due to heterogeneity of variances as determined by Levene's test (Dufner et al., 1992).

Results

General

The amount of conidia produced as well as viability was similar for all experiments and methods of inoculum production. Consistently, 1 to 2 $\times$ 10^9 conidia g^{-1} were produced with 80-100% germination. Field bindweed seedlings typically emerged in all experiments two to three days after sowing.

Table 1. Effect of moisture availability on foliar necrosis and mortality of field bindweed caused by *Phomopsis convolvulus*[1]

Moisture condition	Treatment[2]	Disease Rating[3]		Mortality (%)	
		Trial 1	Trial 2	Trial 1	Trial 2
Plastic bags	control	0.1 (0.1) a[4]	0.1 (0.1) a	0 (0) a	0 (0) a
	0 DAS	1.1 (0.1) ab	1.7 (0.3) ab	0 (0) a	13 (7) a
	1 DAS	2.1 (0.2) b	2.4 (0.5) b	44 (12) b	44 (19) ab
	2 DAS	2.5 (0.3) b	3.2 (0.4) b	56 (12) b	88 (7) b
Dew (8 h day^{-1})	control	0.0 (0.0) a	0.1 (0.1) a	6 (6) ab	0 (0) a
	0 DAS	0.9 (0.1) ab	1.5 (0.2) b	0 (0) a	13 (7) a
	1 DAS	1.1 (0.1) ab	1.6 (0.4) b	6 (6) ab	25 (18) a
	2 DAS	1.7 (0.0) b	1.3 (0.2) ab	15 (6) b	6 (6) a

[1]Trials were not combined because variances were not homogeneous. Disease rating data recorded after eight days and mortality data after 13 days.
[2]0 DAS, 1 DAS, 2 DAS: Application of 1 g *P. convolvulus* granules pot^{-1} at zero, one, or two days after sowing, respectively. Plants were subjected to the moisture regimes for six days.
[3]Disease rating scale is 0 = no visible foliar symptoms, 1 = 1- 25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-100% necrosis.
[4]Data in parantheses are the SEM. For each moisture regime, means in each column with the same letter are not significantly different, according to the Kruskal-Wallis one-way analysis of variance test followed by a multiple comparison procedure (P = 0.15) (disease rating) or to Tukey's grouping (α = 0.05) (mortality).

Effect of moisture availability on disease severity

Disease symptoms were visible for all inoculum application dates under both moisture regimes. However, disease development and mortality was greatest for the delayed application treatments as well as pots covered with plastic bags (Table 1). Following inoculum application at 2 DAS, mortality of covered plants reached 56 and 88% in trial 1 and trial 2, respectively, whereas only 15 (trial 1) and 6% (trial 2) of seedlings were killed when pots were subjected to interrupted dew periods. Likewise, substantial shoot and root biomass reductions were achieved for all inoculation treatments, although the use of plastic bags to cover pots resulted in the greatest reductions (81 (trial 1) and 94% (trial 2) above-ground and 76 and 90% root biomass reduction, 2 DAS treatment) (Figure 1). In contrast, shoot and root biomass reductions of only 56 and 41% and 60 and 51%, respectively were obtained for the interrupted dew period treatment (2 DAS).

Effect of inoculum production method on disease severity

All granule applications resulted in similar disease development (Table 2). Differences in mortality and biomass reductions between varying inoculum production treatments were not significant. In trial 1, the number of seedlings killed following fungal application ranged between 50 and 81%, whereas in trial 2, efficacy was lower with mortality rates between 19 and 44% respectively (Table 2). Likewise, above-ground biomass in trial 1 was reduced between 76 and 94%, while in trial 2 reductions between 62 and 82% were obtained (Figure 2). Results for root biomass were similar.

Effect of planting substrate on disease severity

As in other experiments, seedling emergence occurred two to three DAS in both planting substrates; however, uninoculated plants emerging from field soil were shorter but more robust compared with plants growing in peat moss (Figure 3A). Similarly, root biomass from uninoculated plants in field soil consisted of a tap root that was considerably larger and thicker (Figure 3B) but had fewer secondary roots than seedlings in peat moss. When plastic bags were removed six DAS, soil particles covered by black pycnidia were commonly observed on the field soil surface. Foliar necrosis developed for all fungal treatments. Unexpectedly, plants in the uninoculated control treatment using field soil also showed some necrotic leaf spots (Table 3). Apparently, some *P. convolvulus* conidia moved from the field trial site to the adjacent area where the soil was collected. Effects of the inoculation treatments on peat moss-grown seedlings were similar to those obtained in previously performed experiments and led

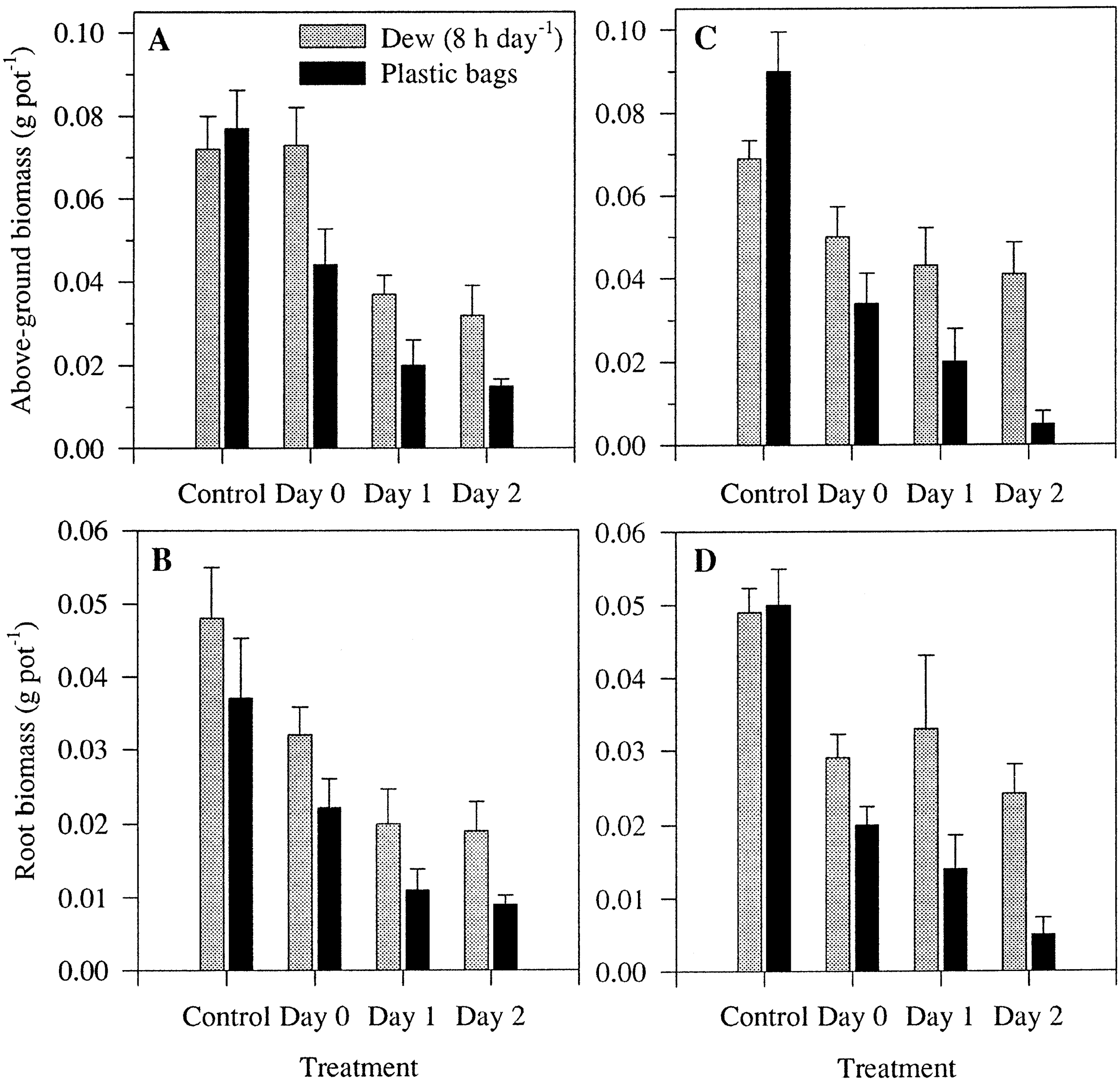

Figure 1. Effect of moisture regime on (A/C) above-ground, (B/D) root biomass of field bindweed after a pre-emergence application with *Phomopsis convolvulus*. (A), (B) and (C), (D) represent Trial 1 and Trial 2, respectively. Day 0, 1, 2 treatments refer to the application of 1 g of *P. convolvulus* granules pot^{-1} at zero, one, or two days after sowing to the soil surface, respectively. Plants were subjected to the two moisture regimes for six consecutive days. Shoots and roots were harvested 13 and 14 days after sowing, respectively. Each vertical bar is the standard error of the mean.

to significant differences in disease incidence and mortality between the various application dates. For example, fungal application on peat moss 0 DAS resulted in a relatively low mortality (50 and 25%) compared with the same application 2 DAS (94% for both trials) (Table 3). However, this pattern was not observed for plants grown in field soil with mortality being less dependent on date of application. Fungal applications carried out on the same day seeds were sown resulted in 81 and 56% seedling mortality. Biomass reductions followed the same trend as for disease incidence and mortality although no significant differences were obtained between application dates when compared by planting substrate. In trial 1, inoculum application at 0 DAS on peat moss resulted in an above-ground and root biomass reduction of 62 and 55% whereas in trial 2, 68 and 61% reductions were obtained, respectively. In contrast, inoculum application on the same day but on

Table 2. Effect of inoculum production method on foliar necrosis and mortality of field bindweed caused by *Phomopsis convolvulus*[1]

Treatment / Inoculum production[2] (Container size, processing)	Disease Rating[3] Trial 1	Disease Rating[3] Trial 2	Mortality (%) Trial 1	Mortality (%) Trial 2
control	0.1 a[4]	0.0 a	0 a	0 a
	(0.07)	(0.00)	(0.00)	(0.00)
250 ml, sieved	3.4 b	2.2 b	81 b	19 ab
	(0.42)	(0.36)	(11.97)	(11.97)
250 ml, non-sieved	2.5 ab	2.1 ab	50 ab	19 ab
	(0.54)	(0.16)	(17.68)	(11.97)
1 l, sieved	2.9 ab	2.2 b	75 b	44 b
	(0.28)	(0.17)	(10.21)	(6.25)
1 l, non-sieved	3.4 b	2.0 ab	75 b	44 b
	(0.39)	(0.23)	(17.68)	(6.25)

[1,3,4]As for Table 1.
[2]250 ml, 1 l: Production of *P. convolvulus* granules in 250 ml Erlenmeyer flasks or in 1 l jars, respectively. Sieved, non-sieved: After drying, inoculum was sieved (710 μm mesh size) or not sieved but ground a second time, respectively. One g *P. convolvulus* granules pot^{-1} was applied to the soil surface one day after sowing.

field soil, resulted in above-ground biomass reductions of 93% and 78% (Figure 3). With delayed application (3 DAS), efficacy of control was similar for both planting substrates, with 97 (or 99%) above-ground biomass reductions for plants grown in peat moss, and 100 (or 99%) reduction in biomass for plants grown in field soil. The relatively high biomass obtained following inoculation on field soil 1 DAS (Figure 3C) was probably due to two *C. arvensis* seedlings not coming into contact with fungal inoculum and thus escaping the treatment.

Discussion

This study examined the disease reaction of field bindweed to a pre-emergence application of the selective fungal pathogen, *P. convolvulus* under controlled environment conditions. Disease development was substantially lower with a moisture regime of interrupted dew periods compared with a continuous moisture period provided by covering pots with a plastic bag. This confirms results from earlier studies, where post-emergence treatments with *P. convolvulus* were more effective when accompanied by 18 h constant dew compared with three interrupted 6-h dew periods (Morin et al., 1989a). Similarly, in preliminary experiments with pre-emergence treatments, symptom development was greater in trials with continuous plastic bag coverage than in those with interrupted dew periods (Vogelgsang et al., 1994). However, these experiments were conducted independently. Although the granular inoculum is assumed to provide greater protection from desiccation (Boyette and Walker, 1985), reduced germination of conidia or penetration during interrupted dew periods may have led to lower disease development (Bashi and Rotem, 1974). For both moisture regimes, timing of fungal application was crucial for disease severity. The level of control declined with increasing time interval between fungal application and actual emergence. Despite the granular formulation, a considerable amount of inoculum might have lost viability by lying on the soil surface. However, reduced infection rates under changing moisture availability and at different application times were not observed in field trials (Vogelgsang et al., 1994; 1998). Consequently, moisture conditions are likely not to be a major factor contributing to the contradictory results obtained under the controlled environment and in the field.

The inoculum production methods used in our study did not significantly affect disease development. Although conidia quantities were similar for all fungal treatments, larger amounts of conidia matrix were observed within the larger-sized incubation jars, possibly suggesting a greater protection of conidia from desiccation (Sparace et al., 1991). However, application of inoculum produced in larger-sized jars did not lead to greater symptom development. Given that in this experiment, treatments were applied at a single date (1 DAS), the protective effect of a more abundant conidia matrix might have been masked. Further-

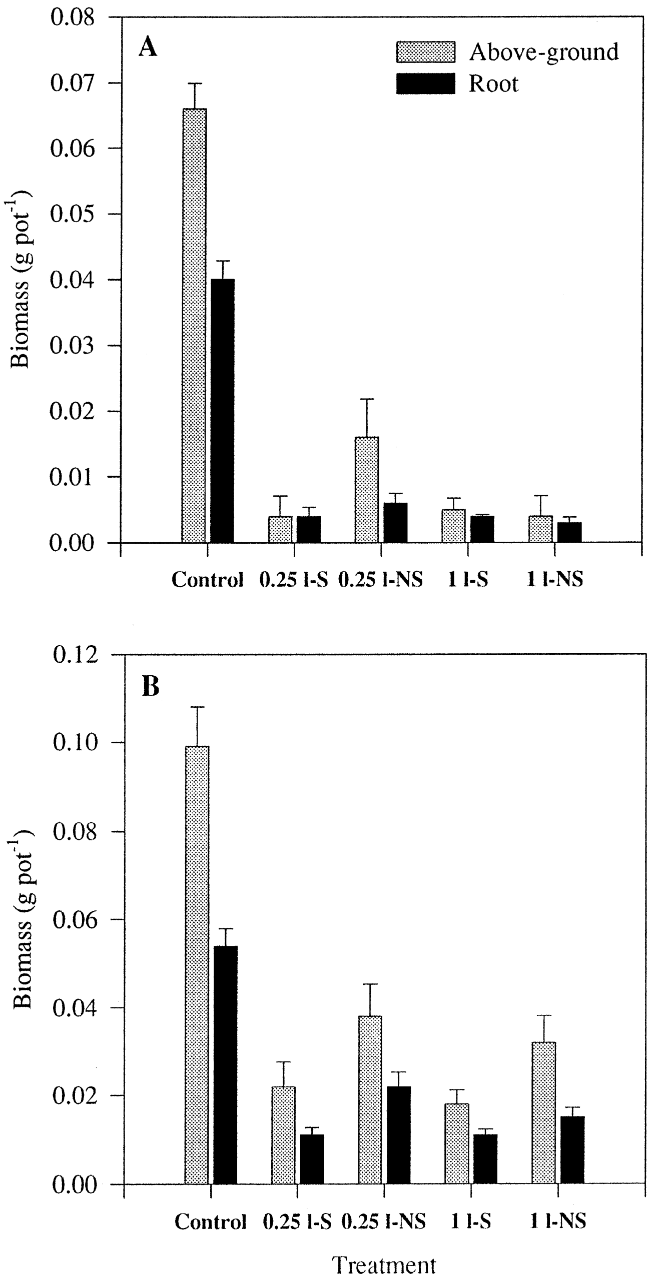

Figure 2. Effect of inoculum production method on field bindweed biomass in (A) Trial 1 and (B) Trial 2, after a pre-emergence application with *Phomopsis convolvulus*. Two hundred and fifty ml Erlenmeyer flasks and 1 l jars used as incubation containers are designated as 0.25 l and 1 l, respectively. S and NS refer to sieved (710 μm mesh size) and non-sieved inoculum, respectively. One g *P. convolvulus* granules pot^{-1} was applied one day after sowing to the soil surface. Shoots and roots were harvested 13, 14 days after sowing, respectively. Each vertical bar is the standard error of the mean.

more, *P. convolvulus* non-sieved inoculum, and thus less homogeneous granules, did not show a greater virulence compared with sieved inoculum. Therefore, the supposition that a mixture of smaller-sized particles serving as immediate infection sources, and larger-sized particles possibly being more persistent, could not be confirmed. A trial that would incorporate earlier

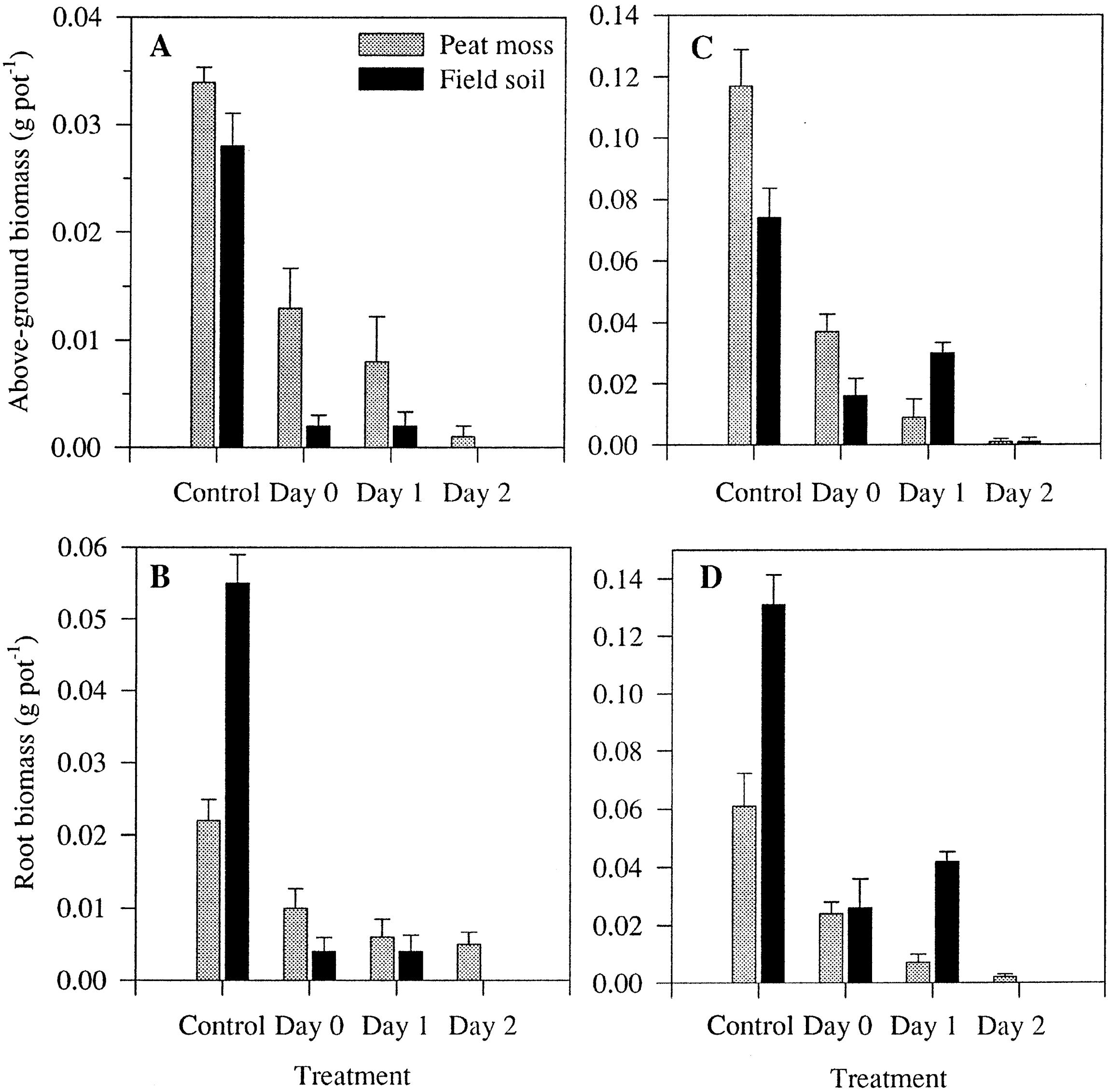

Figure 3. Effect of planting substrate on (A/C) above-ground, (B/D) root biomass of field bindweed after a pre-emergence application with *Phomopsis convolvulus*. (A), (B) and (C), (D) represent Trial 1 and Trial 2, respectively. Day 0, 1, 2 treatments refer to the application of 1 g of *P. convolvulus* granules pot^{-1} at zero, one, or two days after sowing, respectively. Shoots and roots were harvested 13, 14 days after sowing, respectively. Each vertical bar is the standard error of the mean.

application times (0 DAS) might, however, reveal such an effect.

The type of planting substrate used for growing field bindweed seedlings had a strong influence on the performance of *P. convolvulus*. Disease in plants grown in the sandy loam field soil was more pronounced than in plants grown on the prepared peat moss, with the greatest difference observed for the early pre-emergence inoculum application. Similar results were found with spore suspensions of *Ascochyta caulina* applied onto different soil types for the control of *Chenopodium album* (Kempenaar et al., 1996). Control of the target weed was substantially lower when inoculum was applied to peat compared with applications on a sandy soil, especially at the lower spore densities ($< 10^6$ spores/cm^2). Factors such as particle size, pH, and available nutrients have been shown to favour or inhibit the survival and activity of fungal pathogens

Table 3. Effect of planting substrate on foliar necrosis and mortality of field bindweed caused by *Phomopsis convolvulus*[1]

Planting substrate	Treatment[2]	Disease Rating[3]		Mortality (%)	
		Trial 1	Trial 2	Trial 1	Trial 2
Peat moss	control	0.0 (0.0) a[4]	0.0 (0.0) a	0 (0) a	0 (0) a
	0 DAS	2.0 (0.4) ab	1.8 (0.4) ab	50 (14) b	25 (10) a
	1 DAS	2.6 (0.6) b	3.5 (0.3) b	69 (16) bc	88 (7) b
	2 DAS	3.7 (0.2) b	3.7 (0.2) b	94 (6) c	94 (6) b
Field soil	control	0.4 (0.1) a	0.1 (0.1) a	6 (6) a	0 (0) a
	0 DAS	2.6 (0.2) ab	2.2 (0.4) ab	81 (6) b	56 (12) bc
	1 DAS	2.1 (0.4) ab	2.3 (0.3) ab	81 (12) b	25 (14) ab
	2 DAS	3.7 (0.1) b	3.7 (0.2) b	100 (0) b	94 (6) c

[1,2,3]As for Table 1. Analysis for each planting substrate carried out separately.

(Stotzky, 1974; Paulitz and Baker, 1987; Höpner and Alabouvette, 1996; Rosskopf et al., 1996). Moreover, the presence of numerous micro-organisms in our field soil could have led to intense competition for resources (Waksman, 1952), thus preventing *P. convolvulus* conidia from germinating rapidly and having an extensive period of saprophytic germ-tube growth prior to invasion of host tissue. Likewise, fungistasis particularly microbial in origin, may have initially inhibited fungal germination on the field soil (Lockwood, 1977). On the commercially prepared and sterilized peat moss medium, with presumably far fewer micro-organisms but higher moisture content, a substantial number of conidia might have germinated and perished before emergence of field bindweed. Furthermore, the physical structure of the sandy loam is more compact with characteristically smaller pore sizes compared with peat moss. The presence and distribution of different pore sizes have been shown to play a key role in determining the habitable space in some fungi which are often limited to colonizing pores of a given minimal diameter (Filip, 1979).

In field trials with *P. convolvulus*, applications of a granular formulation improved the capability of the fungus to withstand unfavourable weather conditions (Vogelgsang et al., 1994; 1998). However, in this study, only the direct comparison of disease severity, mortality, and biomass reduction in treatments using field soil versus peat moss potting mixture provided some answers that could possibly explain the differences observed between experiments conducted in the field with those under the controlled environment. Experiments incorporating a greater number of planting substrates and moisture levels may provide additional information on the impact of these two critical factors on the performance of *P. convolvulus* under both controlled and field conditions.

Further research on soil-incorporation of granules, fungal persistence in soil, dose response, and effect under competitive cropping situations are in progress and should provide additional information on the potential of *P. convolvulus* as an effective biological agent against field bindweed.

Acknowledgements

The senior author is grateful to the International Council of Canadian Studies for financial support through a postgraduate scholarship. Support from the Natural Sciences and Engineering Research Council of Canada (NSERC) through an operating grant to AKW is acknowledged. We also thank Dr. T. C. Paulitz for reviewing the manuscript.

References

Bashi E and Rotem J (1974) Adaptation of four pathogens to semi-arid habitats as conditioned by penetration rate and germination spore survival. Phytopathol 64: 1035–1039

Boyette CG and Walker HL (1985) Evaluation of *Fusarium lateritium* as a biological herbicide for controlling velvetleaf (*Abutilon theophrasti*) and prickly sida (*Sida spinosa*). Weed Science 34: 106–109

Daniel WW (1978) Applied Nonparametric Statistics. Houghton Mifflin Co., Boston, MASS, USA. 503 pp

DeGennaro FP and Weller SC (1984) Differential susceptibility of field bindweed (*Convolvulus arvensis*) biotypes to glyphosate. Weed Science 32: 472–476

Dufner J, Jensen U and Schumacher E (1992) Statistik mit SAS. Teubner, Stuttgart, Germany. 398 pp

Filip Z (1979) Wechselwirkungen von Mikroorgansimen und Tonmineralien - eine Übersicht. Z PflErn BodKunde 142: 555–561

Höpner H and Alabouvette C (1996) Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. Eur J Soil Biol 32: 41–58

Kempenaar C, Wanningen R and Scheepens PC (1996) Control of *Chenopodium album* by soil application of *Ascochyta caulina* under greenhouse conditions. Ann Appl Biol 129: 343–354

Kosinski W and Weller SC (1989) 5-Enolpyruvyl-shikimate-3-phosphate synthase activity in field bindweed, *Convolvulus arvensis* L., biotypes. Weed Science Society of America Abstracts 29: 76–77

Lockwood JL (1977) Fungistasis in soils. Biol Reviews 52: 1–43

Morin L, Watson AK and Reeleder RD (1989a) Effect of dew, inoculum density, and spray additives on infection of field bindweed by *Phomopsis convolvulus*. Can J Plant Pathol 12: 48–56

Morin L, Watson AK and Reeleder RD (1989b) Efficacy of *Phomopsis convolvulus* for control of field bindweed (*Convolvulus arvensis*). Weed Science 37: 830–835

Morin L, Watson AK and Reeleder RD (1990) Production of conidia by *Phomopsis convolvulus*. Can J Microbiol 36: 86–91

Ormeno-Nuñez J, Reeleder RD and Watson AK (1988a) A new species of *Phomopsis* recovered from field bindweed (*Convolvulus arvensis*). Can J Bot 66: 2228–2233

Ormeno-Nuñez J, Reeleder RD and Watson AK (1988b) A foliar disease of field bindweed (*Convolvulus arvensis* L.) caused by *Phomopsis convolvulus*. Plant Dis 72: 338–342

Paulitz TC and Baker R (1987) Biological control of Pythium damping-off with *Pythium nunn*: Influence of soil environment and organic amendments. Phytopathol 77: 341–346

Phillips RE, Blevins RL, Thomas GW, Frye WW and Phillips SH (1980) No-tillage agriculture. Science 208: 1108–1113

Rosskopf EN, Gaffney JF and Charudattan R (1996) Potential negative effects of CO_2 application and subsequent pH changes on biocontrol agent efficacy. Phytopathol 86 (11 Suppl.): S10

Sparace SA, Wymore LA, Menassa R and Watson AK (1991) Effects of the *Phomopsis convolvulus* conidia matrix on conidia germination and the leaf anthracnose disease of field bindweed (*Convolvulus arvensis*). Plant Dis 75: 1175–1179

Steel RGD and Torrie HT (1980) Principles and Procedures of Statistics. McGraw-Hill Book Co., NY, USA. 633 pp

Stotzky G (1974) Activity, ecology, and population dynamics of microorganisms in soil. In: Laskin AI and LeChevalier H (eds) Microbial Ecology. (pp. 57–135) C.R.C. Press, Palm Beach, FL, USA

Swan DG (1980) Field bindweed, *Convolvulus arvensis* L. College of Agriculture Research Center, Bulletin 0888, Washington State University, USA

Vogelgsang S, Watson AK and Hurle K (1994) The efficacy of *Phomopsis convolvulus* against field bindweed (*Convolvulus arvensis*) applied as a preemergence bioherbicide. J Plant Dis Prot, Spec. Iss XIV: 253–260

Vogelgsang S, Watson AK, DiTommaso A and Hurle K (1998) Effect of the pre-emergence bioherbicide *Phomopsis convolvulus* on seedling and established plant growth of *Convolvulus arvensis*. Weed Research (accepted January 9, 1998)

Waksman SA (1952) Soil Microbiology. John Wiley & Sons, Inc., New York, USA 356 pp

Watson AK and Wymore LA (1990) Identifying limiting factors in the biocontrol of weeds. In: Baker R and Dunn P (eds) New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases. UCLA Symposium of Molecular and Cellular Biology, New Series 112: 305–316

Whitworth JW and Muzik TJ (1967) Differential response of selected clones of bindweed to 2,4 D. Weeds 15: 275–280

Yerkes CD and Weller SC (1996) Diluent volume influences susceptibility of field bindweed (*Convolvulus arvensis*) biotypes to glyphosate. Weed Technol 10: 565–569