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Biological control of botrytis leaf blight of onion by *Gliocladium roseum* applied as sprays and with fabric applicators

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Abstract

Gliocladium roseum (5×10^6 conidia ml^{-1}) and chlorothalonil (Bravo 500) were compared in two field tests for effectiveness in suppressing leaf blight caused by *Botrytis squamosa* in cooking onions. The biological control agent and fungicide were applied with sprayers and with specially designed fabric applicators that were mounted interchangeably on an aluminium carriage with bicycle wheels. In the applicators, inoculum and fungicide gravitated from a reservoir down curtains of denim strips and onto portions of onion leaves contacted by the strips. Initial applications were timed using a leaf blight forecasting system (BOTCAST) and three or four subsequent applications were made at weekly intervals. When compared to water checks, *G. roseum* applied as sprays or by applicators reduced density of leaf spots by about 50-58% during middle and late stages of epidemics. The antagonist was about half as effective as chlorothalonil in suppressing density of spots, regardless of application method. The applicators delivered inoculum in similar densities to proximal and distal halves of leaves, and used at least 35% less volume of inoculum compared to the sprayers. Density of inoculum on leaves treated by applicators was about the same as in spray-treated leaves when canopies were sparse, but less when canopies were dense. It is concluded that *G. roseum* has good potential for controlling leaf blight sufficiently to avoid economic yield losses.

Introduction

Leaf blight of onion (*Allium cepa* L.), caused by *Botrytis squamosa*, is usually managed by means of fungicide sprays applied at regular intervals or timed according to leaf blight forecasting systems [Sutton *et al.*, 1986; Vincelli and Lorbeer, 1989]. BOTCAST, the chief leaf blight forecasting system used in cooking onion crops in Canada, optimizes the timing of the first spray and reduces fungicide use by 20–60% in most crop seasons [Sutton *et al.*, 1986]. To meet public demands for further reduction in fungicide use, alternative methods such as biological control will probably be needed. Biological control has yet to be exploited for onion leaf blight. However, biological control, as or more successful than fungicides, was developed for use against several diseases caused by *Botrytis cinerea* Pers.:Fr., including grey mold of strawberry and black spruce [Sutton and Peng, 1991, 1993a; Zhang *et al.*, 1994]. Effective agents and efficient application methods will be needed for biological control to be acceptable for leaf blight control in commercially-grown onions.

In preliminary studies at Guelph, the biological control agent *Gliocladium roseum* Bainier showed considerable potential for suppressing leaf blight in onion crops [J.C. Sutton, K.E. Nelson, and T.D.W. James, 1991, unpublished observations]. The antagonist performed best among 22 fungi evaluated in field plots. It substantially suppressed leaf blight, but less effectively than did chlorothalonil. The biocontrol fungi (10^6 conidia ml^{-1} water plus surfactant) and fungicide were applied weekly after leaf blight lesions first appeared. In the Netherlands, however, weekly applications of a different isolate of *G. roseum* (10^6 conidia ml^{-1}) in onion field plots failed to suppress leaf blight

[Köhl *et al.*, 1992] but the timing of treatments in relation to leaf blight development was not specified.

Further evaluation of *G. roseum* for biological control of leaf blight appeared justified in view of the effectiveness of the antagonist against *B. squamosa* in onions at Guelph, and against *B. cinerea* in strawberry, black spruce and other hosts [Peng and Sutton, 1991; Sutton, 1994; Sutton and Peng, 1993a, 1993b; Zhang *et al.*, 1994]. Investigations were needed also of methods to improve application efficiency of biocontrol agents to onions in which inoculum applied as sprays is usually wasted. Studies were therefore conducted to compare the effectiveness of *G. roseum* and of chlorothalonil (a standard fungicide used in Canada on onions) for suppressing leaf blight when applied as sprays and by specially-constructed fabric applicators at times determined by the BOTCAST forecaster. Efficiency of inoculum application by sprayers and by applicators was also compared.

Materials and methods

Field plots

Plots of onion cv. Bingo were established on mineral soil at the Arkell Research Station near Guelph, Ontario in 1992 and 1993, and on organic soil at the Muck Research Station, Bradford, Ontario, in 1993. Preceding crops at the respective sites were strawberries and onions. The onions were sown in early May in rows 41 cm apart (35–40 seeds m^{-1} row) using precision seeders. Seeds sown at Bradford were treated with carbathiin (carboxin) plus thiram (Pro-Gro, Uniroyal Chemical, Elmira, Ontario N3B 3A3, Canada) to control smut caused by *Urocystis cepulae* Frost. Weeds were controlled at Arkell with chlorthaldimethyl (Dacthal 75 WP), fluazifop-butyl (Fusilade 250 EC) and by hand, and at Bradford with sethoxydim (Poast 20 EC), oxyfluorfen (Goal 2 EC), and pendimethalin (Prowl 4 EC). At Bradford, onion maggots (*Hylemya antigua* (Meig.)) were controlled with chlorpyrifos (Lorsban 15 G) and thrips (*Thrips tabaci* Lindeman) with cypermethrin (Ripcord 400 EC). Metalaxyl (Ridomil 2.4 EC) was applied to onion foliage at Arkell to control downy mildew caused by *Peronospora destructor* (Berk.) Casp. All of the pesticides were applied at recommended doses [Ontario Ministry of Agriculture and Food, 1992–1993; 1993]. Trickle and sprinkler irrigation was applied as needed at Arkell and Bradford, respectively. Other production practices followed local recommendations [Ontario Ministry of Agriculture and Food, 1992–1993].

Inoculum production

Isolate 710 of *G. roseum* obtained from strawberry [Peng and Sutton, 1991] was used in all studies. In 1992, inoculum was produced by growing the fungus on potato dextrose agar (PDA) in petri dishes beneath cool-white fluorescent lamps (14-h photoperiod) at 20–23 °C for 15–20 days. Conidia were recovered in sterile distilled water plus surfactant (50 μl Triton X-100 100 ml^{-1} water) and the spore suspension was filtered through four layers of cheesecloth. In 1993, inoculum was produced on wheat grain which was moistened with water (1:1, w/v), steamed for 2–3 h, drained of any excess water, placed in Mason jars (200 g grain 1 l^{-1} jar), and autoclaved. The sterilized wheat in each jar was inoculated with 2–3 ml of a spore suspension (5×10^7 conidia ml^{-1} water) of *G. roseum*, and incubated under conditions described for the PDA cultures. The jars were shaken manually at 1–2 day intervals. After 18 days, when the grains were heavily colonized, metal inserts of the jar caps were substituted with sterilized photocopy paper, which allowed the grains to dry slowly and the fungus to sporulate profusely by 25–30 days. Conidial suspensions were produced by stirring the infested grain in deionized water plus the surfactant (2 g grain l^{-1}) for 10 min. Grain fragments were removed by decanting the suspensions through a fine sieve. Suspensions of conidia recovered from PDA and wheat were adjusted to $5.0–6.5 \times 10^6$ spores ml^{-1} with the aid of a hemacytometer for use in the biocontrol studies.

Application of inoculum and fungicide

Conidial suspensions of *G. roseum* [$5.0–6.5 \times 10^6$ spores ml^{-1}] and the fungicide chlorothalonil [Bravo 500 (500 g a.i. l^{-1}), ISK Biotech Limited, London, Ontario N5Z 3H9, Canada], at a rate of approximately 5 L product in 800 l ha^{-1}, were applied to onion foliage by means of sprayers and fabric applicators. Hand-held sprayers of 5 l capacity were used at Arkell and backpack sprayers of 10 l capacity were used at Bradford. All sprayers operated by means of compressed air and each was equipped with one hollow-cone nozzle.

In the fabric applicators, inoculum or fungicide gravitated from a reservoir down curtains of denim strips and onto those portions of the onion leaves contacted by the strips in a manner similar to that of wiper applicators used for herbicides [Derting, 1987; Welker

and Peterson, 1987, 1989] (Fig 1A-D). The reservoir was constructed using polyvinyl chloride (PVC) pipe 115 cm long and 22 cm in circumference (1992) and 120 cm long and 31 cm circumference (1993) with the ends sealed by plastic caps. A slot about 10 cm wide was cut lengthwise to form an upper opening in the reservoir, and two parallel slots 5 cm apart and each 2 mm wide were cut in the lower part of the reservoir. A piece of pre-washed denim (Len's Mill Store, Guelph, Ontario N1E 2Z5, Canada) was passed upwards through one of the parallel slots, across the base of the reservoir and downwards through the second slot to form two curtains that extended downwards from the reservoir. The denim was sealed to the base of the reservoir with silicone cement. Each curtain was 54 cm long and cut into strips 2.5 cm or 1.5 cm wide. Fraying of the fabric was minimized by use of pinking shears to cut the narrow strips and by coating the edges of the wider strips with latex rubber. For use in the field, the applicator was mounted on an aluminium carriage supported on bicycle wheels and pushed by hand. The carriage was equipped with an adjustable holder for the reservoir to allow height alteration of the denim strips above the soil. In 1992, the reservoir of each applicator was 6 cm deep, and each curtain (heavy grade denim, 330 g m^{-2}, thick weave) was 90 cm wide and cut into 36 strips each 2.5 cm wide. In 1993, the reservoir of each applicator was 9 cm deep, and each curtain (medium grade denim, 317 g m^{-2}, thin weave) was divided into three units 32 cm wide and approximately 5 cm apart. The curtains were comprised of 39 strips each 2.5 cm wide (wide-strip applicators) or 63 strips each 1.5 cm wide (narrow-strip applicators). In both years, separate applicators were used for the biocontrol agent, fungicide treatments, and the water checks.

Immediately before the applicators were used to apply treatments, the denim curtains were wetted with water, squeezed, and subsequently immersed and agitated in the treatment material (spore suspension, fungicide, or water plus surfactant) for about 1 min. Treatment material was poured into the reservoir and allowed to gravitate until it dripped steadily from the base of the denim strips. The apparatus (applicator plus carriage) was then pushed along the rows of onions at 2 km $hour^{-1}$, with the base of the denim curtains about 5 cm above the soil, and in both directions such that all onions were treated twice to ensure adequate coverage of foliage (roughly double the spray application of *G. roseum* and the fungicide).

Inoculum gravitation in applicators

Concentration of *G. roseum* conidia in effluent drops from narrow and wide denim strips of stationary applicators was estimated over time. The strips were wetted and immersed in inoculum as described above, and the reservoir was filled with conidial suspensions (5.0–5.5 $\times 10^6$ conidia ml^{-1}). Inoculum dripping from the free ends of the strips was sampled at 2-min intervals for a total of 16 min, which approximated the time needed to apply a biocontrol treatment in the field plots. Conidia in four subsamples of each inoculum sample were counted with the aid of a hemacytometer. The experiment was repeated once.

Estimation of leaf blight

At each time of estimation, leaf blight lesions were counted on the lowest, partially green leaf, on each of ten arbitrarily sampled onion plants in each plot. The data were expressed as numbers of lesions per 10 cm length of green tissue.

Biological control tests

Studies were conducted in onion plots at Arkell in 1992 and at Bradford in 1993. At Arkell, onion residues of the 1991 crop season containing sclerotia of *B. squamosa* were scattered in all plots after the onions emerged to provide a source of initial inoculum of the pathogen. The residues had been kept over winter on the soil surface near the plot area. Initial inoculum at Bradford was from natural sources.

In the Arkell studies, *G. roseum*, chlorothalonil, and water plus surfactant were each applied with fabric applicators with wide denim strips, and by means of hand sprayers. The date of initial application was determined using BOTCAST [Sutton *et al.*, 1986]. In BOTCAST, microclimatic measurements are used to predict daily inoculum incidence and the likelihood of infection by *B. squamosa*. A disease severity index for the day is computed using these data. The daily indices are added as a cumulative disease severity index (CDSI) from onion emergence until values of fungicide spray thresholds have been reached.

Leaf blight severity was estimated one day after the first treatment (day 56), and on days 75, 82 and 90 after the onions emerged. There were four replicate plots per treatment, and the treatments were arranged in a randomized complete block design. Plots within blocks were contiguous and each consisted of 1.5 m $\times$ 1.5 m of onions. Blocks were separated by a 2 m strip of bare soil.

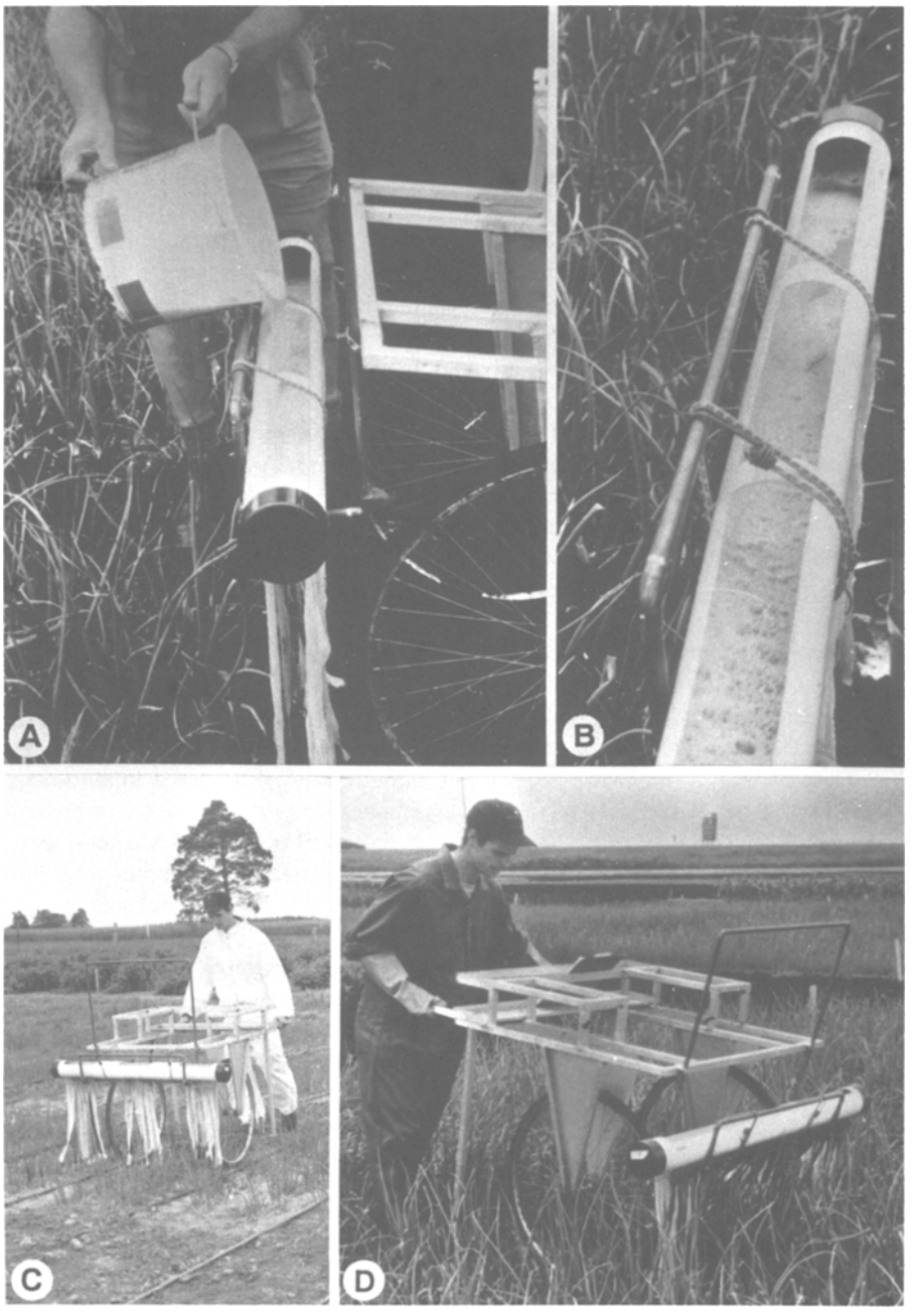

Fig. 1. Fabric applicators used to apply water, fungicide and inoculum of *Gliocladium roseum* to onion foliage. (A) Reservoir of applicator being filled with inoculum of *Gliocladium roseum*; (B) full reservoir prior to application of inoculum. Treatment material being applied by fabric applicator to onion foliage: (C) early in the season at Arkell; (D) late in the season at Bradford.

Treatments at Bradford were similar to those at Arkell except that fabric applicators with narrow denim strips and with wide denim strips were used, and sprays were applied with backpack sprayers. The first application was timed using BOTCAST. Leaf blight severity was estimated on days 65, 71, 77 and 90 after the onions emerged. The plots were contiguous within blocks and each consisted of 1.5 × 5.3 m of onions.

Blocks were separated by bare soil or 2 m-wide buffer strips of non-treated onions.

Inoculum application rates

Application rates (1 ha^{-1}) of *G. roseum* inoculum and of water plus surfactant to onion foliage by applicators with narrow and wide denim strips, and by the backpack sprayer, were estimated in the biocontrol and water check plots each time treatments were applied at Bradford in 1993. Application rates by the applicator and sprayer were estimated approximately from the decrease in volume of conidial suspension or water in the reservoir and tank, respectively, when three onion plots were treated, and from the area of the treated plots.

Inoculum density on onion leaves

Density of *G. roseum* propagules on untreated onion leaves and on leaves treated with the antagonist (ca. 5×10^6 conidia ml^{-1}) by means of fabric applicators with wide and narrow denim strips, and with a hand sprayer was estimated in the Arkell plots in 1993. Treatments were applied on 13 July (trial 1) and 21 July (trial 2) when the onions were at the eighth and tenth leaf stages, respectively. In both trials, five leaves were taken abitrarily from each plot 1 h after the treatments were applied; in the second trial five leaves were also taken 10 min before the treatments were applied. Sampled leaves were immediately placed on grids above moist paper towels in sealed plastic containers and delivered to the laboratory within 1 h. Each leaf was cut transversely in half and the proximal and distal halves of the five leaves from each plot were grouped. The groups of half leaves were placed in known volumes of deionized water plus surfactant (50 μl Triton X-100 100 ml^{-1}) in erlenmeyer flasks and agitated on a rotary shaker at 110 rpm for 30 min. Undiluted aliquots (0.5 ml) of the aqueous suspensions in each flask were transferred as five 0.1 ml bands onto PDA amended with Triton X-100 and streptomycin sulphate (final concentrations of 0.2% and 100 ppm, respectively, [PDTSA; Peng *et al.*, 1992] in petri dishes. The dishes were kept on the laboratory bench at 20–23 °C for 5–7 days, after which colonies of *G. roseum* were counted and numbers of colony forming units (cfu) of the antagonist in the suspensions were estimated. To measure the surface area of the leaf halves in each flask, each leaf piece was cut longitudinally and flattened beneath a thin sheet of glass on a photocopier. Areas of leaf photocopies were measured using a digitizer (model MOP-3, Carl Zeiss Canada Ltd., Don Mills, Ontario M3B 2S6, Canada). The cfu values and leaf area measurements were used to estimate the density of *G. roseum* propagules on the leaves. In trial 2, density values of *G. roseum* in pre-treatment samples were subtracted from post-treatment samples to account for possible inoculum carryover from trial 1. Treatment plots were replicated four times and arranged as an randomized complete block design. The plots were contiguous and each consisted of 2 m $\times$ 2 m of onion rows within blocks. Blocks were separated by 2 m-wide strips of bare soil.

Mycoflora of dead leaf tips

Dead leaf tips of onions in plots treated with *G. roseum* using sprayers or fabric applicators with wide denim strips were examined for *G. roseum*, *B. squamosa*, and other microfungi at Arkell and Bradford in 1993. Leaf samples were taken at 5 and 7 days after the last application of *G. roseum* at Arkell and Bradford respectively. The tips (3–10 cm long) of each of ten leaves sampled arbitrarily in each plot were cut, and separated into two subsamples of five tips. One subsample from each plot was placed in a humidity chamber, and the second subsample was cut into segments 1–2 cm long and placed on PDTSA or water agar (WA) in petri dishes. All leaf pieces were incubated at 20–23 °C for 1 wk and then examined microscopically for microfungi.

Microclimatic measurements

Air temperature, relative humidity, duration of leaf wetness, and rainfall were monitored continuously from when the onions emerged until they lodged. The microclimatic variables were monitored in the onion canopies with electronic sensors [Sutton *et al.*, 1984, 1988], connected to microprocessor-based dataloggers (model 21X, Campbell Scientific Canada Corp., Edmonton, Alberta T5E 2P4, Canada). The microclimatic data were used to calculate disease severity indices of BOTCAST [Sutton *et al.*, 1986; Sutton *et al.*, 1989], and total monthly rainfall.

Data analyses

Observations of inoculum gravitation in applicators were subjected to regression analysis and the regressions were tested for equality of slope and elevation using analysis of covariance [Snedecor and Cochran, 1980]. Numbers of lesions 10 cm^{-1} leaf were transformed to values of $\log_{10} + 1$ or to square root + 0.5

before the data were subjected to analyses of variance (ANOVA), but are reported as untransformed means. Treatment means were separated using the Waller-Duncan Bayesian K-ratio t test [Waller and Duncan, 1969; Duncan, 1975]. Untransformed observations of inoculum application rates and of inoculum density of *G. roseum* on onion leaves were subjected to ANOVA and means were separated using the Waller-Duncan Bayesian K-ratio t test. The Statistical Analysis System (SAS) [SAS Institute Inc., Cary, North Carolina 27512–8000, USA] was used for all ANOVAs. Analyses of covariance and the regression analyses used a statistics package compiled by James [1985].

Results

Biological control tests

Treatments at Arkell in 1992 were initiated at 55 days after the onions emerged (15 July), when the cumulative disease severity index (CDSI) of BOTCAST had reached threshold 2 (31–40 units), the spray threshold previously established for fungicide treatments (Fig. 2A). Four treatment applications subsequently were made at intervals of 7–9 days. In the period of 0–48 days after the onions emerged, the CDSI was characterized by several, and often long, periods of zero increase interspersed by brief periods of moderate or rapid increase (Fig. 2A). From day 49 to day 89, however, the CDSI increased almost continuously and usually rapidly. Rainfall during July (days 41 to 71) totalled 151 mm, which was 69 mm above the long-term average; rainfall in August (days 72 to 103) was also high (130 mm, or 48 mm above average). Wet weather after day 42 contributed to the rapid increase in CDSI.

A few lesions were found in the plot area on day 42 (2 July) which coincided with the beginning of threshold 1. Number of leaf blight lesions in check onions that were sprayed with water plus surfactant was still low (0.3 lesions 10 cm^{-1} green leaf) on day 56, which was near the end of threshold 2 and one day after the treatments were first applied, but increased progressively thereafter (Table 1). The progressive disease increase coincided with the steep increase in CDSI (Fig. 2A). The increase in lesions was especially rapid between days 82 and 90 (11 and 19 August). Number of lesions in onions treated with water plus surfactant by means of the fabric applicator did not differ significantly from those treated with the hand sprayer except on day 75 (4 August) (Table 1).

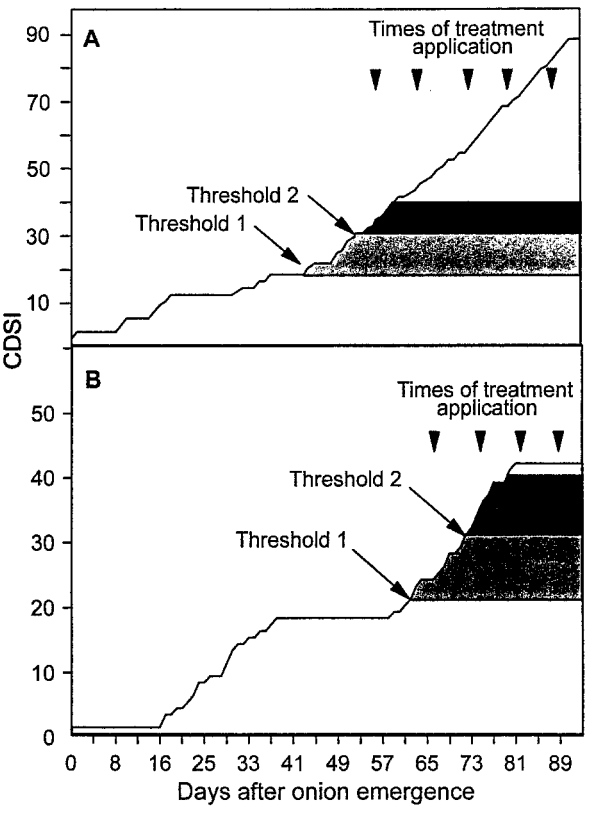

Fig. 2. Cumulative disease severity index (CDSI) and fungicide treatment thresholds of the BOTCAST leaf blight forecaster at: (A) the Arkell Research Station in 1992; and (B) the Muck Research Station near Bradford in 1993.

Disease severity was suppressed moderately by *G. roseum* and markedly by chlorothalonil (Table 1). Estimated numbers of lesions in the various treatments did not differ significantly on day 56, but were significantly suppressed by *G. roseum* and chlorothalonil, whether applied as sprays or by fabric applicators, at all other times of disease assessment (days 75, 82, and 90). *Gliocladium roseum* in most instances suppressed the number of lesions as effectively, and by approximately 50–57%, when applied using an applicator and when applied as a spray. Chlorothalonil suppressed numbers of lesions almost completely when applied as a spray or by the applicator, except late in the epidemic (day 90) when numbers of lesions had increased in fabric-treated leaves but not in spray-treated leaves. The onions began to lodge on day 91.

Treatments at Bradford in 1993 were initiated at 63 days after the onions emerged (26 July) shortly after the onset of threshold 1 of BOTCAST (CDSI = 21–30

Table 1. Effects of *Gliocladium roseum* and of chlorothalonil, applied as sprays and by fabric applicators, on estimated number of lesions of botrytis leaf blight on leaves of onions in field plots at Arkell in 1992 and at Bradford in 1993

	Mean number of lesions 10 cm^{-1} green leaf[2,3]							
	Arkell 1992				Bradford 1993			
Treatment[1]	Day 56 (Leaf 3)	Day 75 (Leaf 3)	Day 82 (Leaf 3)	Day 90 (Leaf 4)	Day 65 (Leaf 3)	Day 71 (Leaf 3)	Day 77 (Leaf 3)	Day 90 (Leaf 4)
Sprayer								
Water	0.3 a	3.2 a	3.3 a	10.3 a	0.5 ab	0.4 bc	2.3 a	12.9 b
G. roseum	0.2 a	1.9 b	1.4 c	5.6 b	0.6 a	0.7 a	2.2 a	8.6 cd
Chlorothalonil	0.2 a	0.4 c	0.4 d	0.3 d	0.4 ab	0.2 c	0.9 b	1.4 e
Applicator								
Water	0.3 a	1.3 b	4.2 a	9.6 a	0.4 ab	0.7 a	1.1 b	20.4 a
G. roseum	0.3 a	1.4 b	2.5 b	5.7 b	0.3 b	0.5 ab	1.6 ab	10.0 bc
Chlorothalonil	0.2 a	0.6 b	0.7 d	1.9 c	0.4 ab	0.5 ab	1.1 b	6.8 d

[1]Treatments were water plus surfactant (50 μl Triton X-100 100 ml^{-1}), conidial suspensions of *G. roseum* (5 – -6×10^6 conidia ml^{-1}), and chlorothalonil (5 l Bravo 500 in 800 l water ha^{-1}), applied five times (Arkell) or four times (Bradford).

[2]For analysis, data for Arkell were transformed to $\log_{10} + 1$ and those for Bradford were transformed to square root + 0.5. All data are reported as untransformed means.

[3]Means in a column followed by the same letter are not different (Waller- Duncan Bayesian K-ratio t test).

units), the spray warning threshold for fungicide treatments (Fig. 2B). The onions were subsequently treated three times at 6–7 day intervals. The CDSI did not increase during three long periods of days 1–16 (25 May-9 June), 37–56 (30 June-19 July) and 82–92 (13–24 August) after emergence. The first and second of these periods markedly delayed the BOTCAST thresholds. Increase in CDSI was prevented in the first of the periods by short durations of leaf wetness associated with lack of rain, and in the second and third periods by frequent incidence of high daytime temperatures (mean hourly temperatures $\geq$30 °C for $\geq$4 h [Sutton *et al.*, 1986]). The CDSI increased rapidly between days 16 and 36, and 58 to 81 when rain was more frequent and daily leaf wetness periods were long (12–14 h).

Leaf blight lesions were first found in the Bradford plots on 14 July. Number of lesions in check onions that were sprayed with water plus surfactant was low on days 65, 71 and 77 (0.4, 0.5, and 2.3 lesions 10 cm^{-1} green leaf, respectively) and moderate (12.9 lesions) on day 90 (Table 1). A similar pattern of lesion numbers was observed in checks that were treated with water plus surfactant by the fabric applicators, except that more lesions were found than in spray checks on day 90. The sharp increase in number of lesions after day 77 occurred a few days after threshold 2 of BOTCAST (Fig. 2B).

In the Bradford plots, *G. roseum* suppressed leaf blight moderately when applied as a spray and by fabric applicators, and chlorothalonil suppressed the disease moderately and markedly when applied by the applicators and as a spray, respectively (Table 1). On days 65, 71 and 77, number of lesions on leaves of the various treatments in some instances differed significantly. However, marked differences between treatments were found only on day 90. Numbers of lesions in onions treated using applicators with narrow and wide denim strips did not differ significantly (Waller-Duncan test) thus these data were combined for analysis and presentation. The onions began to lodge on day 91.

Inoculum gravitation in applicators

Inoculum was observed to gravitate as films on the surfaces of the denim strips. Estimated concentration of conidia of *G. roseum* in effluent drops from free ends of the strips was less than in the reservoirs (Fig. 3). Conidial concentrations in drops collected from applicators with narrow and wide strips at 2 min after the reservoirs were filled were 23% and 45% lower, respectively, than in the reservoir in trial 1, and 26% and 14% lower, respectively, in trial 2. Conidial concentration in effluent drops from wide and narrow strips generally declined slowly from 2 to 16 min after the reservoirs were filled. In trial 1, regressions of conidial concen-

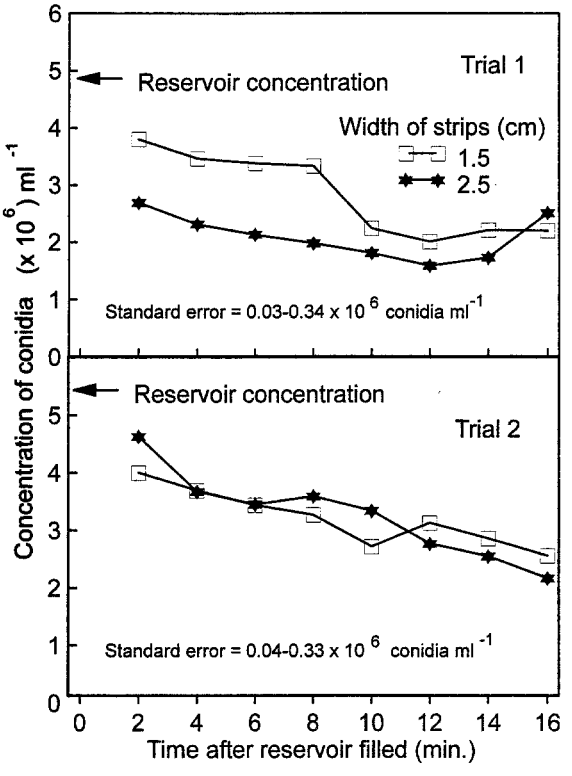

Fig. 3. Concentration of conidia of *Gliocladium roseum* in effluent from free ends of narrow and wide denim strips of fabric applicators in relation to time after the applicator reservoirs were filled with inoculum of *G. roseum* in each of two trials.

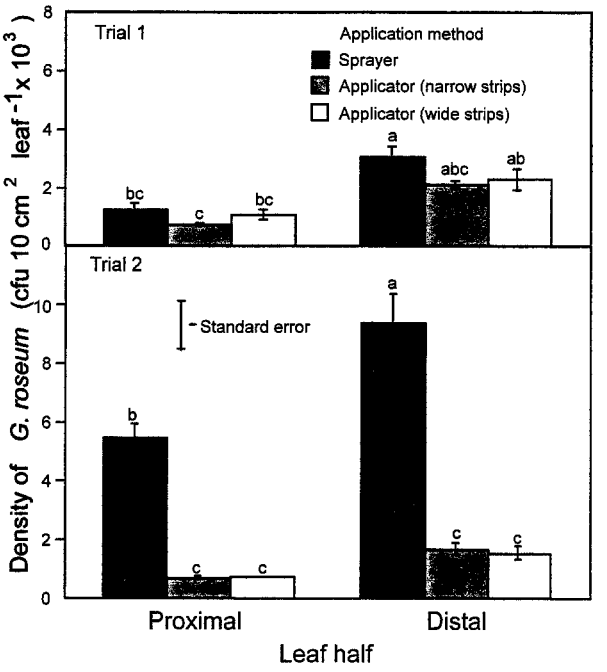

Fig. 4. Effects of method of inoculum application on estimated colony forming units of *Gliocladium roseum* on proximal and distal portions of onion leaves in two trials in field plots at the Arkell Research Station in 1993. Bars with the same letter above them are not different (Waller-Duncan Bayesian K-ratio t test).

tration with time (2–16 min) differed significantly in elevation but not slope ($P > F_{0.05}$, analysis of covariance). In trial 2, neither the elevation nor the slopes of the regression lines differed significantly ($P > F_{0.001}$). Combined observations of the two applicators in the two trials indicated that the inoculum concentration in the effluent averaged over the 16 min of sampling was 1.7×10^6 conidia ml^{-1} less than the reservoir concentration of 5.2×10^6 conidia ml^{-1}.

Inoculum application rate

In the onions at Bradford, the sprayers applied 1690 l water ha^{-1} and 1927 l inoculum of *G. roseum* ha^{-1}. The applicators with narrow (1.5 cm) and wide (2.5 cm) denim strips significantly applied approximately 35% and 21% less water, and 40% and 31% less inoculum (Waller-Duncan K-ratio t test), respectively, than did the backpack sprayers. Delivery rates of water and *G. roseum* inoculum by applicators with narrow strips and those with wide strips were not significantly different (Waller-Duncan t test). The standard error range for this experiment was 2.7–140.0 l ha^{-1}.

Inoculum density on onion leaves

In the first trial, estimated density of *G. roseum* propagules on proximal halves and on distal halves of the onion leaves was numerically but not statistically higher when inoculum of the antagonist was applied by hand sprayers than when applicators with narrow or wide denim strips were used (Fig. 4). Width of the strips did not significantly affect estimated density of inoculum on either proximal or distal leaf halves. Density of *G. roseum* on distal halves of leaves, averaged across treatments, was 41% higher than on the proximal halves.

In the second trial, estimated density of *G. roseum* propagules on proximal and distal halves of the leaves, respectively, was about six and seven times higher when the inoculum was applied by hand sprayers as opposed to applicators (Fig. 4). These treatment differences were highly significant ($P > F_{0.001}$). Propagule density when inoculum was applied by hand sprayers was lower on proximal halves than on distal halves

of leaves. In leaves treated by applicators, propagule density was numerically, but not significantly higher on the distal halves. No propagules of *G. roseum* were recovered from untreated leaves.

Mycoflora of leaf tips

The chief fungi found in dead tips of leaves from Arkell were *G. roseum* and species of *Alternaria*, *Cladosporium*, *Epicoccum*, and *Stemphylium*. *Alternaria* spp., chiefly *Alternaria alternata* Fr. Keissler, and *Stemphylium botryosum* Wallr. were abundant on dead leaf tips of all treatments that were incubated in humidity chambers or on WA. *Gliocladium roseum* sporulated frequently on leaf tips from plots that had been sprayed with the antagonist, infrequently on leaf tips from applicator-treated plots, and not at all on leaf tips from untreated check onions. Incubation of leaves in humidity chambers or on PDTSA medium allowed frequent detection of *G. roseum*, but the antagonist was not found on leaves plated on WA. The antagonist frequently grew over mycelium and conidiophores of *Alternaria* and *Stemphylium*, but possible mycoparasitism was not investigated. *Gliocladium roseum* produced chiefly penicillate conidiophores on dead leaves and verticillate conidiophores on mycelium growing over WA and over mycelium and spores of *Alternaria* and *Stemphylium*. No spore production of *B. squamosa* was observed in any samples.

Tip portions of all leaves collected at Bradford were covered with heavily sporulating *S. botryosum*, regardless of incubation method. No other fungi were found on these leaf tips.

Discussion

Gliocladium roseum was moderately effective in suppressing leaf blight in onion field plots in each of the two years. The antagonist substantially reduced the number of lesions produced by *B. squamosa* when applied as a spray and by fabric applicators, but in all instances was less effective than chlorothalonil. The observed effectiveness of *G. roseum* agreed with the preliminary observations made at Guelph [J.C. Sutton, K.E. Nelson, and T.D.W. James, 1991, unpublished], but contrasted with the lack of disease suppression obtained with an isolate of *G. roseum* in The Netherlands [Köhl *et al.*, 1992]. Although less effective than the fungicide, *G. roseum* may suppress leaf blight sufficiently to avoid yield losses. Mild leaf blight late in the crop season is desirable in Canada because it facilitates drying of the onion tops and constriction of the onion necks. Data on relationships of *G. roseum* treatments to leaf dieback, and of blight severity to yield loss, are needed to determine whether *G. roseum* is sufficiently effective that economic loss in onion bulbs are avoided. Rapid drying and effective neck constriction are important for control of neck rot caused by *Botrytis alli* Munn. and other diseases that develop when the onions are stored [Sherf and MacNab, 1986].

The mechanism by which *B. squamosa* was suppressed by *G. roseum* is not clear, but the available evidence is consistent with competitive colonization of the leaf tissues. From the frequent sporulation of *G. roseum* on dead tips of onion leaves collected in the Arkell study, the antagonist substantially colonized the leaves in competition with other microflora which included species of *Alternaria*, *Cladosporium*, *Epicoccum*, and *Stemphylium*. Whether the leaves were green, senescent, or dead at the time of colonization is unknown. *G. roseum* may have penetrated the green leaves and rapidly colonized the tissues when they senesced and died, as was observed in biocontrol studies of *B. cinerea* in strawberry leaves [Sutton and Peng, 1993b]. Rapid colonization and possession of the tissues by *G. roseum* could have suppressed colonization and conidia production by *B. squamosa* in the leaves and resulted in the reduced frequencies of lesions observed in plots treated with the antagonist. Numbers of viable propagules of *G. roseum* recoverable from the phylloplane of onions in field plots can decline by 94 to 97% in 24–48 h after application [T.D.W. James, 1994, unpublished data], so it is unlikely that weekly applications of the antagonist as used in the present study would effectively suppress infection of the leaves by *B. squamosa*.

The detection of *G. roseum* on the leaf tips in the Arkell study contrasted with the lack of recovery of the fungus from tips of treated leaves in a study in The Netherlands [Köhl *et al.*, 1992]. Methodology, however, can markedly influence the success of detection. In our study, *G. roseum* sporulated on leaves kept in humidity chambers and on leaves incubated on PDTSA, an agar medium that is selective for the antagonist [Peng *et al.*, 1992], but not on leaves placed on WA. In the study of Köhl *et al.* [1992], necrotic leaves were homogenized and plated onto malt agar containing tetracycline, but information on detection efficiency of this procedure was not given. It is possible also that conditions in the study of Köhl *et al.* [1992]

did not favour progressive colonization of onion leaves by *B. squamosa*.

The use of BOTCAST to time applications of *G. roseum* did not necessarily optimize biological control by the antagonist. As in earlier studies [Sutton *et al.*, 1983, 1986], the timing of the initial treatments by BOTCAST coincided with a shift from linear to exponential increase in number of leaf blight lesions. Initiation of fungicide programs according to BOTCAST efficiently controlled leaf blight in both of the field experiments, and consistently did so in earlier work [Sutton *et al.*, 1986]. However, there is not yet any evidence that the time of transition from linear to exponential disease increase is optimal for the initial biocontrol treatment, or that subsequent treatments are best applied at weekly intervals. Should *G. roseum* suppress *B. squamosa* chiefly by substrate competition and suppression of spore production on foliage of the current onion crop, it may be advantageous to start treatments well before the pathogen begins to sporulate on the dead leaf tips and initiates the exponential phase of leaf blight. Such adaptation of BOTCAST or other forecasting systems [Vincelli and Lorbeer, 1989] could increase effectiveness of *G. roseum* in leaf blight control.

The fabric applicators efficiently treated onion foliage with *G. roseum* and with fungicide. The estimated average reduction (35%) in volume of inoculum of *G. roseum* used by the applicators compared to the sprayers was highly conservative because the applicators lacked a means to stop inoculum flow and continued to use inoculum when moved between plots and blocks. Estimated density of inoculum on leaves treated by applicators and on those treated with sprayers were similar when the onion canopy was sparse (eighth leaf stage), but lower in applicator-treated leaves when the canopies were dense (tenth leaf stage), probably because of a reduction in the proportion of leaf surface contacted by the fabric strips. Nonetheless treatment programs delivered by applicators suppressed leaf blight as effectively as those applied by sprayers. The similar densities of inoculum applied by applicators to proximal and distal halves of the leaves may have contributed to the effectiveness of the delivered inoculum. The applicator technology probably has the additional advantages of reduced potential for drift of, and operator exposure to, the treatment material, especially under windy conditions.

Possible disadvantages of the fabric applicators are potential abrasive removal of epicuticular wax from leaves and tissue by the fabric, which could predispose the foliage to infection by *B. squamosa* and other pathogens [Sutton *et al.*, 1984]. Such factors possibly contributed to the higher densities of lesions when water or chlorothanonil were applied with applicators as opposed to sprayers at Bradford in 1993 (day 90 only).

The fabric applicators were similar in principle to gravity-fed contact applicators developed to apply herbicides in various crops [Derting, 1987; Boerboom and Wyse, 1988; Welker and Peterson, 1987, 1989]. To our knowledge, no similar technologies have been developed for applying biological control agents or fungicides to manage diseases in crops. Future development of the experimental prototypes used in the present study should include evaluation of fabrics that have greater resistance to abrasion and biodegradation than does denim (cotton), which is not sufficiently durable for commercial use [Derting, 1987]. The ideal wick fibre for fabric applicators should combine high 'wicking' properties, that is, a high moisture load capacity and recharge rate, with good abrasion resistance [Derting, 1987]. Neither denim nor nylon, which is used in herbicide applicators, meet both criteria. Fabric applicators of various levels of sophistication could be designed for efficient application of biocontrol agents and fungicides, and to meet the needs of subsistence agriculture as well as advanced agricultural systems.

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