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Interactions of air temperature, relative humidity and biological control agents on grey mold of bean

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Abstract

The interactions of *Botrytis cinerea* and seven biological control agents (BCAs) were examined in controlled environments to determine the influence of selected relative humidities (RH) (90, 95, and 100%) and air temperatures (20, 24 and 28 °C) on grey mold of bean. All main effects and interactions were significant ($P \leq 0.05$) among the 72 treatments. In the control, lesions of grey mold developed under all environmental conditions but were largest at 24 °C × 95 and 100% RH, and 28 °C × 95% RH. Interactions of environment, BCAs and grey mold were complex. *Alternaria alternata, Drechslera* sp., *Myrothecium verrucaria, Trichoderma viride, Gliocladium roseum* and an unidentified pink yeast were all highly dependent on environment for biological control efficacy, and changes of 4 °C or 5% RH were associated with variability in disease suppression that ranged from ≤ 15 to 100%. Efficacy of *Epicoccum purpurascens* appeared independent of environment and this BCA suppressed disease by 100% in all of the environmental treatments. Suppression of grey mold by many of the BCAs was most effective under environmental conditions least conducive to disease. Therefore, evaluations of potential BCAs in environmental conditions that are marginal for disease can overestimate their efficacy in field environments. Assessments of biological control efficacy in various environments can be used to more accurately assess the potential of BCAs.

Introduction

Botrytis cinerea Pers. is a ubiquitous and destructive pathogen, inciting disease on many crops. Diseases induced by this pathogen, often collectively referred to as grey mold, cause losses among many vegetable, fruit, ornamental and field crops [Agrios, 1978]. Among protected glasshouse crops, grey mold inflicts more losses than any other pathogen [Nelson, 1978]. The pathogen causes blights of most plant parts. *Botrytis cinerea* initially invades senescing or dead plant tissue before it spreads to healthy plant tissue [Jarvis and Nuttall, 1981]. Infected flowers of many crops provide a nutrient-rich, saprotrophic base for infection of adjoining fruit or leaves by the pathogen [Jarvis, 1962]. After colonization of such nutrient sources, the pathogen invades adjoining living tissue and initiates disease.

Several factors are known to influence the growth and survival of *B. cinerea*. Air temperature, relative humidity (RH), and duration of surface wetness affect the germination of spores and growth of mycelium, and influence the ability of *B. cinerea* to colonize susceptible plant organs [Jarvis, 1977]. The pathogen must also compete with other filamentous fungi, bacteria and yeasts that are known to inhabit senescing petals and the crop phyllosphere [Jarvis, 1977]. Several of these microorganisms are antagonistic to *B. cinerea* and have been used in biological control experiments. Isolates of *Alternaria alternata* (Fr.) Keissler, *Drechslera* sp., *Epiccocum purpurascens* Ehrenb. *ex* Schlecht, *Fusarium graminearum* Schwabe, *Gliocladium roseum* Link: Bainier, *Myrothecium verrucaria* (Alb. & Schw.) Ditm. *ex* Steudel and *Trichoderma viride* Pers., among other microorganisms, have been shown to reduce the incidence and severity of disease caused by *B. cinerea* among a variety of crops

[Peng and Sutton, 1990, 1991; McLean, 1988; Nelson and Powelson, 1988; Redmond *et al.*, 1987; Dubos *et al.*, 1983; Tronsmo and Ystaas, 1980; Tronsmo and Dennis, 1977; Bhatt and Vaughan, 1963]. While many of these naturally-occurring microorganisms have effectively suppressed grey mold, the conditions under which they were tested were often artificial. Many of these experiments have been conducted only at high relative humidities ($\geq$ 90%) [Redmond *et al.*, 1987; Nelson and Powelson, 1988]. Application of BCAs in field trials has sometimes resulted in poor or variable disease control [Bisiach *et al.*, 1985; McLean, 1988; Bhatt and Vaughan, 1963], perhaps due to microclimatic factors [Bisiach *et al.*, 1985; McLean, 1988].

Discrepancies in biological control that occur between controlled and field environments suggest that various environmental factors influence the efficacy of BCAs [Baker, 1946; Burpee, 1989; Harman *et al.*, 1981]. Evaluation of BCAs in relation to environmental factors may therefore be a useful method to evaluate such discrepencies and to enhance the selection of microbial biological agents for disease control. The objective of this study was to examine the influence and interactions of seven BCAs, relative humidity and temperature on the development of grey mold in white bean and the ability of the BCAs to colonize bean petals.

Materials and methods

Bean plant cultivation. Bean plants (*Phaseolus vulgaris* cv. Strike) were cultivated in a growth room at 22 to 26 °C with a 14-h photoperiod at 150 μE m^{-2} sec^{-1}. Seeds were planted in either a 1:1 mixture of Pro-Mix (Les Tourbieres Premier Ltee, Riviere du Loup, PQ, Canada) and Turface (Applied Industrial Material Corp, Deerfield, IL, USA), or Metro-Mix 245 (W.R. Grace Co. of Canada Ltd., Ajax, ON, Canada), in 180 cm^3 pots, placed in plastic flats (Kord Products, Burlington, ON, Canada), and initially watered with a solution of 20:20:20 N:P:K fertilizer (1 g/l). The flats were covered with transparent plastic for 4 to 5 d, until the seedlings emerged. Plants were grown until the primary leaves had expanded (approximately 10 to 14 d).

Collection and sterilization of bean petals. After plants had started flowering (3–4 wk), bean petals at stages A (attached, recently opened flowers) and B (attached flowers that had started to wilt or senesce) [Boland and Inglis, 1989] were collected daily until flowering ceased. The petals were sterilized using propylene oxide according to Inglis [1989]. Aliquots of 2 ml propylene oxide at 4 ° were pipetted into desiccation chambers where the petals were arranged in single layers on a perforated screen. Petals were fumigated in the chambers at room temperature for 12 to 14 h in a fume hood and then aerated in a laminar-flow transfer chamber for one hour. Sterile petals were aseptically removed from the chambers using forceps and placed into sterile petri dishes. Petals were stored at 4 °C for up to 4 months before use.

Collection and preparation of spore suspensions. Isolates of *A. alternata* (B639), and *E. purpurascens* (D224) were cultured from rapeseed petals [Inglis, 1989]. *Drechslera* sp. (B10), *Myrothecium verrucaria* (Alb. & Schw.) Ditm. *ex* Steudel (B24), and *Trichoderma viride* Pers. *ex* (BC34) were isolated from rapeseed or bean petals [Boland and Inglis, 1989]. Isolates of *Gliocladium roseum* Bain. (1710), and an unidentified pink yeast (Y3) were received from G. Peng, University of Guelph, Guelph, ON N1G 2W1, Canada. An isolate of *B. cinerea* was cultured from strawberry fruit (G. Peng, University of Guelph).

Botrytis cinerea was cultured on thinly-poured strawberry agar (200 ml steamed, fresh strawberries, 800 ml deionized water, 20 g agar) in petri dishes. The cultures were incubated for 15 to 21 d under fluorescent lights with a 16 h photoperiod at room temperature. Spores were vacuum-harvested from the cultures using a device which trapped the dislodged spores in a side-arm flask containing 2 ml of sterile water amended with 0.01% Tween 80. The flask was flushed using small amounts of Tween 80-amended water. The resultant spore suspension was filtered through cheesecloth and the filtrate was collected in a sterile 15 ml screw-top test tube. The spore concentration was estimated using a haemocytometer and adjusted to 2×10^5 spores per ml.

Most BCAs were cultured on potato dextrose agar (PDA) under ultraviolet lights (12 h photoperiod; 310 to 420 nm) for 7 to 21 d, except *E. purpurascens* which was cultured on V-8 agar (200 ml Campbell's V-8 juice, 3 g $CaCO_3$, 20 g agar, 800 ml deionized water) for the same length of time, all at 20–22 °C. *Trichoderma viride* and the unidentified pink yeast (Y3) were grown under fluorescent lights at room temperature and in an incubator at 21 °C, respectively. Spore suspensions were prepared by flooding the individual cultures with 3 to 8 ml of sterile deionized water amended with

0.01% Tween 80 and agitating with a sterile glass rod. Spore concentrations were adjusted to 2×10^5 spores per ml. The viability of BCAs and *B. cinerea* were determined by plating three replicate samples of 0.5 ml of spore suspension on PDA for 12 h at 20 °C. The percentage of germinated spores was determined by counting at least 100 spores per replication [Boland and Hunter 1988].

Petal inoculations. Seven co-inoculum spore suspensions were prepared by combining 0.5 ml of individual BCA spore suspensions with 0.5 ml of *B. cinerea* spore suspension in sterile test tubes. An eighth spore suspension (control treatment) was prepared by combining 0.5 ml of *B. cinerea* spore suspension with an equal volume of Tween 80-amended sterile water. All suspension were agitated using a vortex stirrer to ensure adequate mixing of the co-inoculum treatments. Each co-inoculum treatment was added to 20 sterile bean petals in a sterile 15 ml test tube and gently agitated for 10 sec until the co-inoculum was evenly dispersed on the petals. Petals were removed from each tube, placed into 90-mm diameter petri dishes and air-dried in a laminar-flow transfer chamber for one hour, and then applied to the primary leaves of the bean seedlings.

Environmental chambers. Environmental chambers were developed that maintained selective relative humidities within plexiglass enclosures measuring 50 × 50 × 150 cm [Hannusch *et al.*, 1994]. Relative humidity (RH) was maintained at 90% ± 0.4% and 95% ± 0.5% through the use of micrologger-controlled (Model 21×, Campbell Scientific, Edmonton, Alberta, Canada) ultrasonic mister that cycled between narrow set points. To maintain consistent RH, misters were activated at approximately 5 and 3 min intervals at 90% and 95% RH, respectively. Periods of unbroken leaf wetness were obtained through continuous operation of the misters. Air temperatures of 20–28°C were maintained at ± 0.5 °C by regulation of growth room controls.

Bean seedling inoculation and incubation. The environmental chambers were adjusted to 90, 95, or 100% RH and growth room air temperature was set at 20, 24 or 28 °C. The experiment was conducted as a factorial design with three air temperatures × three relative humidities × eight biological control treatments (including the control). The treatments each consisted of a different combination of temperature, RH and four bean seedlings, with one infested petal inoculated onto each of two primary leaves per treatment (eight observations). All treatments were repeated once. A tray of 32 seedlings were inoculated per trial. Individual treatments, trials, and chambers were administered in a random sequence.

Disease evaluations. In each trial, inoculated plants were incubated for 96 h, the optimum time required for disease development according to preliminary experiments [Inglis, 1989], and disease was then assessed. The diameter of lesions that formed beneath the infested petals on bean leaves were measured using callipers. Petals were examined with a hand-held magnifying glass to estimate the incidence of petal colonization by the BCAs. A modified Horsfall-Barratt scale [Horsfall and Cowling, 1978] of 0–11, where 0 = 0% of the surface area of each petal covered by mycelium or spores of each BCA and 11 = 100% of the surface area covered, was used to assess colonization by the BCAs. The colonization of petals by *B. cinerea* was also evaluated by rating the amount of mycelial growth and sporulation on each petal and the diseased area (if present), also using the modified Horsfall-Barratt scale.

Statistical analysis. Factorial analyses of variance (ANOVA) of the lesion diameter data were performed using PROC ANOVA of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). Repeated experiments were examined for homogeneity of variance prior to pooling of data [Snedecor and Cochran, 1980]. Horsfall-Barratt values of surface colonization for each treatment were transformed to percentage values, averaged, and statistically compared. For each BCA/pathogen treatment (including the pathogen control) the mean of each environmental treatment was compared using the protected Least Squares Difference (LSD) test ($P = 0.05$) (Statistix 4.0, Analytical Software, St. Paul, Mn, USA).

Results

All main effects and interactions were significant among the 72 treatments (3 temperatures × 3 relative humidities × 8 organisms examined for influence on mean diameter of grey mold lesions on bean (Table 1). Main effects and interactions for percent colonization of petals by BCAs (SCP), and percent surface colonization of petals and lesions by *B. cinerea* (SCPL) were also significant among all treatments (ANOVA not

Table 1. Analysis of variance of the influence of temperature and relative humidity on the diameter of lesions formed by *Botrytis cinerea* on leaves of bean seedlings[a] treated with bean petals co-inoculated with the pathogen and biological control agents

Source	df	SS	F	Pr>F
Temperature (A)	2	21440.8	1530.2	0.0001
Relative humidity (B)	2	9959.8	710.8	0.0001
Biological control agents (C)	7	9942.4	202.7	0.0001
A * B	4	6362.5	227.0	0.0001
A * C	14	4319.9	44.0	0.0001
B * C	14	3960.7	40.4	0.0001
A * B * C	28	6138.7	31.3	0.0001
Error	504	3530.9		
Total	575	65655.7		

[a] Lesion diameters (mm) on bean seedlings were recorded 96 h post inoculation.

presented). According to ANOVAs for individual BCAs, F values were significant for all temperature × relative humidity interactions. Therefore, the results are presented as main effects for individual fungi.

Influence of environmental variables on grey mold

Lesions of grey mold were formed in the control treatment (inoculum of *B. cinerea* applied alone) in all environmental treatments, with lesion diameters ranging from 2.4 to 37.7 mm (Table 2, Fig. 1A). Environmental treatments which produced the largest grey mold lesions were 24 ° × 95% and 100% RH, and 28 °C × 95% RH. The remaining combinations of relative humidity and temperature resulted in lesions less than 19.0 mm in diameter. Sizes of grey mold lesions in treatments with BCAs applied ranged from 0 mm (several) to 32.9 mm (Y3 at 24 °C × 100% RH) (Tables 3 to 9, Fig. 1B to 1H). The percent suppression of grey mold by BCAs co-inoculated with *B. cinerea*, relative to the controls inoculated with *B. cinerea* alone, ranged from 0% to 100% (cf. Table 2 with Tables 3 to 9, Fig. 1A with Fig. 1B to 1H). The colonization of petals and lesions by *B. cinerea*, and of petals by BCAs, varied considerably among the treatments (Tables 2 to 9).

Influence of environmental variables on biological control of grey mold

Alternaria alternata. Grey mold lesions formed in five of nine environmental treatments in which petals were co-inoculated with *A. alternata* and *B. cinerea*. Lesions were generally large in environments conducive to grey mold development, with diameters of 27.6 mm and 12.9 mm at 24 °C × 100% RH, and 20 °C × 95% RH, respectively (Table 3, Fig. 1B). *Alternaria alternata* suppressed development of grey mold lesions most

Table 2. The influence of relative humidity and temperature on the development of grey mold lesions, and the proportion of surface colonization of petals and lesions on bean leaves by *Botrytis cinerea*[a]

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	2.5 e[b]	17.0 b
20	95	18.4 bc	35.8 b
20	100	17.0 cd	97.9 a
24	90	8.8 de	10.2 b
24	95	27.2 b	75.0 a
24	100	37.7 a	78.2 a
28	90	2.4 e	15.5 b
28	95	23.8 bc	86.9 a
28	100	4.7 e	27.9 b

[a] Pathogen treatment applied as spore suspensions at 1.0 × 10^5 spores/ml.

[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Lest Squares Difference Test. Data are the pooled results of two trials.

Table 3. The influence of relative humidity, temperature and *Alternaria alternata* on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by *A. alternata*[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *A. alternata* on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 c[b]	20.2 b	1.8 b
20	95	12.9 b	2.8 b	32.6 a
20	100	5.5 c	82.6 a	12.0 b
24	90	3.7 c	1.3 b	2.5 b
24	95	7.0 bc	67.3 a	5.3 b
24	100	27.7 a	3.1 b	41.6 a
28	90	0.2 c	16.0 b	1.4 b
28	95	0.5 c	78.1 a	1.2 b
28	100	0.0 c	65.0 a	1.2 b

[a] Biological control agent and pathogen treatments applied as a 50:50 cell and spore suspension at 2.0 × 10^5 spores/ml.

[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

effectively (>94.0%) at 28 °C at all relative humidities, and at 20 °C × 90% RH. The fungus was least suppressive (29.9% and 26.5%) at 20 °C × 95% RH, and 24 °C × 100% RH, respectively. In treatments where the BCA suppressed grey mold lesions by nearly 100%, and at 20 °C × 100% RH or 24 °C × 95% RH, *A. alternata* was more prevalent on the bean tissues than *B. cinerea*. Surface colonization of petals by *A. alternata* was most extensive at 28 °C.

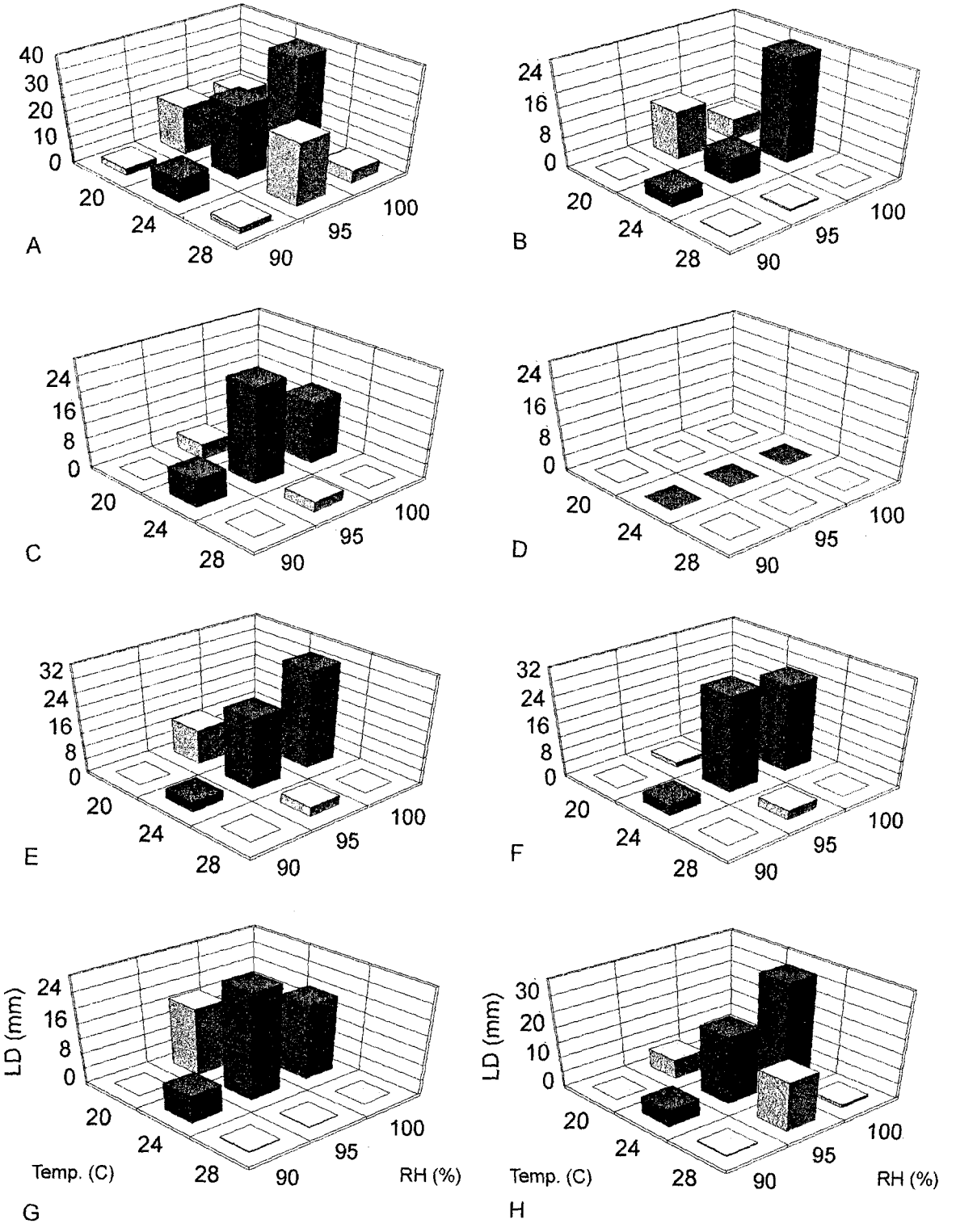

Fig. 1. The influence of relative humidity and temperature on the development of grey mold lesions caused by *Botrytis cinerea* on bean leaves alone, and in combination with seven biological control agents. (A) *Botrytis cinerea* inoculated alone; (B) *B. cinerea* coinoculated with *Alternaria alternata*; (C) *B. cinerea* coinoculated with *Drechslera* sp.; (D) *B. cinerea* coinoculated with *Epicoccum purpurascens*; (E) *B. cinerea* coinoculated with *Gliocladium roseum*; (F) *B. cinerea* coinoculated with *Myrothecium verrucaria*; (G) *B. cinerea* coinoculated with *Trichoderma viride*; and (H) *B. cinerea* coinoculated with an unidentified yeast (Y3).

Table 4. The influence of relative humidity, temperature and *Drechslera* sp. on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by *Drechslera* sp.[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *Drechslera* sp. on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 d[b]	17.6 cd	3.4 b
20	95	4.1 cd	23.2 cd	2.8 b
20	100	0.0 d	80.2 a	4.3 b
24	90	7.4 c	4.7 d	12.7 b
24	95	25.1 a	10.0 d	63.6 a
24	100	16.3 b	54.1 b	17.1 b
28	90	0.0 d	6.7 d	1.2 b
28	95	2.7 c	40.7 bc	3.1 b
28	100	0.0 d	83.8 a	1.2 b

[a] Biological control agent and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores/ml.
[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

Table 5. The influence of relative humidity, temperature and *Epicoccum purpurascens* on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by *E. purpurascens*[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *E. purpurascens* on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 a[b]	20.0 c	1.2 b
20	95	0.0 a	15.8 c	1.2 b
20	100	0.0 a	98.8 a	1.2 b
24	90	0.0 a	14.9 c	1.9 a
24	95	0.0 a	76.8 ab	1.2 b
24	100	0.0 a	82.2 ab	1.2 b
28	90	0.0 a	30.5 c	1.2 b
28	95	0.0 a	73.1 ab	1.2 b
28	100	0.0 a	70.3 b	1.2 b

[a] Biological control agent and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores/ml.
[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

Drechslera sp. Grey mold lesions formed on bean leaves in five of nine environmental treatments in which petals were co-inoculated with *Drechslera* sp. and *B. cinerea*, mainly in environments conducive to disease development. Lesion diameters ranged from 2.7 to 25.1 mm and disease suppression ranged from 7.8% to 100% (Table 4, Fig. 1C). Grey mold lesions were, largest (25.1 mm and 16.3 mm) at 24 °C × 95% and 100% RH, respectively, and these treatments suppressed disease the least (7.8% and 56.8%). Grey mold was highly suppressed (77.7% and 88.7%) at 20 °C and 28 °C × 95% RH. At 20 °C and 28 °C × 90% and 100% RH, *Drechslera* sp. completely suppressed growth of the pathogen. At all air temperatures, SCP by *Drechslera* sp. was most apparent at 100% RH, while SCPL by *B. cinerea* was most evident in all relative humidities at 24 °C.

Epiccocum purpurascens. In all of the environmental conditions tested, *E. purpurascens* prevented lesion formation on leaves by *B. cinerea* (Table 5, Fig. 1D). *Epicoccum purpurascens* aggressively colonized petals of all the treatments, but was less abundant (<31% of petal colonized) at 20 °C × 90% and 95% RH, and at 24 °C × 90% RH. At 20 °C × 100% RH, over 98% of the petal area was colonized by *E. purpurascens*. *Botrytis cinerea* occurred at very low levels (<2% of petals and lesions colonized) in all of the treatments co-inoculated with *E. purpurascens*.

Gliocladium roseum. When bean petals were co-inoculated with *G. roseum* and *B. cinerea*, grey mold lesions developed in five of nine environmental treatments. Lesion diameters on leaves ranged form 2.8 to 29.3 mm (Table 6, Fig. 1E). At 24 °C × 95% and 100% RH, lesion diameters of 21.3 mm and 29.3 mm were observed, respectively. The smallest lesions (3.0 mm and 2.8 mm) developed at 24 °C × 90% RH and at 28 °C × 95% RH. Grey mold suppression ranged from 22.3% to 100%. *Gliocladium roseum* suppressed grey mold entirely at 20 °C and 28 °C × 90% and 100% RH, and by 88.2% at 28 °C × 95% RH. In all environmental conditions examined, the SCP by *G. roseum* was never greater than 49%. Colonization of petals and lesions by *B. cinerea* when co-inoculated with *G. roseum* was greatest at RH and temperature treatments similar to those which produced the largest lesions in the control (cf. Table 2 and 6, Fig. 1A and 1E).

Myrothecium verrucaria. Grey mold lesions were formed in five of nine environmental conditions tested when petals were co-inoculated with *M. verrucaria* and *B. cinerea* (Table 7, Fig. 1F). The largest lesions (28.5 mm and 26.0 mm) developed under the same environmental conditions as those which produced the largest grey mold lesions in the control (24 °C × 95% and 100% RH) (cf. Tables 2 and 7, Fig. 1A and 1F). The lesions ranged from 1.4 to 28.5 mm in diameter, while disease suppression ranged from 0% to 100%.

Table 6. The influence of relative humidity, temperature and *Gliocladium roseum* on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by *G. roseum*[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *G. roseum* on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 d[b]	15.4 b	1.2 b
20	95	10.6 c	1.5 b	31.5 b
20	100	0.0 d	48.9 a	28.1 bc
24	90	3.0 cd	2.4 b	4.8 cd
24	95	21.3 b	5.7 d	75.2 a
24	100	29.3 a	2.7 b	63.1 a
28	90	0.0 d	4.9 b	1.2 d
28	95	2.8 cd	15.8 b	7.8 bcd
28	100	0.0 d	12.6 b	1.2 d

[a] Biological control agent and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores/ml.
[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

Table 7. The influence of relative humidity, temperature and *Myrothecium verrucaria* on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by *M. verrucaria*[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *M. verrucaria* on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 b[b]	2.9 c	1.6 b
20	95	1.4 b	11.1 c	2.5 b
20	100	0.0 b	90.8 a	1.2 b
24	90	3.2 b	4.0 c	2.8 b
24	95	28.5 a	3.2 c	94.1 a
24	100	26.0 a	46.1 b	6.0 b
28	90	0.0 b	7.9 c	2.3 b
28	95	2.8 b	39.2 b	1.4 b
28	100	0.0 b	46.8 b	1.2 b

[a] Biological control agent and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores/ml.
[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

Where grey mold lesions formed, control of 92% or more by the BCA was achieved at 20 °C and 28 °C × 95% RH but control was less than 64% at 24 °C. At all other environmental regimes, *M. verrucaria* completely suppressed grey mold. Colonization of petals by *M. verrucaria* was most extensive at all temperatures where the RH was 100%, as well as at 28 °C × 95% RH. Surface colonization of petals and lesions by *B. cinerea* was slight, except at 24 °C × 95% RH under which it exceeded 94%.

Trichoderma viride. Grey mold lesions were formed in only four of nine environmental treatments in which petals were co-inoculated with *T. viride* and *B. cinerea*. Lesions ≥7 mm diameter were found at 20 °C × 95% RH, and 24 °C × 90%, 95% and 100% RH, respectively (Table 8, Fig. 1G). Lesion size was ≤0.3 mm in all the other environmental regimes. Where grey mold lesions developed, *T. viride* suppressed grey mold by 87.4% and 99.1% at 28 °C × 90% and 95% RH, respectively, but by less than 48% in the other treatments. In all the other environmental regimes, the BCA completely suppressed grey mold. Surface colonization of petals by *T. viride* was most extensive at 100% RH at all temperatures, and at 28 °C × 95% RH. Significant colonization of petals and lesions by *B. cinerea* only occurred at 20 °C × 95% RH, and 24 °C × 95% and 100% RH.

Table 8. The influence of relative humidity, temperature and *Trichoderma viride* on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by *T. viride*[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: *T. viride* on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 c[b]	3.2 d	6.6 c
20	95	17.9 b	1.2 d	41.8 b
20	100	0.0 c	50.2 a	1.2 c
24	90	7.2 c	1.4 d	5.9 c
24	95	27.7 a	2.2 d	86.5 a
24	100	19.9 ab	18.5 cd	29.2 b
28	90	0.3 c	2.7 d	3.9 c
28	95	0.2 c	35.6 bc	1.2 c
28	100	0.0 c	40.3 b	1.2 c

[a] Biological control agent and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores/ml.
[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

Unidentified pink yeast. Lesions of grey mold developed in eight of the nine environmental treatments when petals were co-inoculated with the yeast and *B. cinerea* (Table 9, Fig. 1H). Lesion diameters varied from 0.4 to 32.9 mm and disease suppression ranged from 13% to 100%. The greatest control of grey mold by the BCA (100%, 81.7% and 80.9%) was achieved at 20 °C × 90% RH, and at 28 °C × 90% and 100% RH, respectively (cf. Tables 2 and 9, Fig. 1A and 1H). Grey mold was not controlled (12.5% and 18.4% suppression of lesion development) at 24 °C × 95% and

Table 9. The influence of relative humidity, temperature and yeast (Y3) on the development of grey mold lesions on bean leaves and the proportion of surface colonization of petals and/or lesions by Y3[a] and *Botrytis cinerea*

Temp (°C)	RH (%)	Lesion Diameter (mm)	Surface colonization: Yeast on petals (%)	Surface colonization: *B. cinerea* on petals plus lesions (%)
20	90	0.0 d[b]	57.6 a	6.8 c
20	95	6.4 cd	56.6 a	9.0 c
20	100	9.2 c	34.6 b	54.2 ab
24	90	5.6 cd	10.6 c	5.0 c
24	95	22.2 b	3.2 c	48.0 ab
24	100	32.9 a	1.2 c	69.4 a
28	90	0.4 cd	2.4 c	3.1 c
28	95	16.0 b	12.9 c	41.6 b
28	100	0.9 cd	1.2 c	14.9 c

[a] Biological control agent and pathogen treatments applied as a 50:50 cell and spore suspension at 2.0×10^5 spores/ml.
[b] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to the protected Least Squares Difference Test. Data are the pooled results of two trials.

100% RH, respectively. When co-inoculated with the yeast, *B. cinerea* colonized the petals and lesions extensively under conditions of high humidity (≥95% RH) at all temperatures. Petal colonization by the yeast, in contrast, was greatest at all relative humidities in the coolest temperature of 20 °C.

Discussion

The results of this study provide new information on the influence of environment on disease caused by *B. cinerea*, and on the interactions of environment, disease, and biological control agents. We believe this study is the first to quantitatively demonstrate that *B. cinerea* is able to form lesions on bean seedlings when relative humidity is less than 95%. Lesions of grey mold developed at all of the combinations of relative humidity and temperature examined. In addition, *B. cinerea* was able to grow saprotrophically in these environmental conditions as indicated by the surface colonization of petals and lesions (SCPL) by the fungus. According to Morgan [1984] and Tezuka *et al.* [1983], infection of glasshouse tomatoes by *B. cinerea* was favoured by temperatures greater than 20 °C, humidities greater than 95%, and the presence of liquid water. In the present study, *B. cinerea* produced lesions greater than 22 mm in diameter at 95% relative humidity at 24 °C and 28 °C, and less than 19 mm in diameter at 20 °C. Lesions produced at 100% relative humidity were greater than 17 mm at 20 °C and 24 °C, and less than 5 mm at 28 °C. The ability of *B. cinerea* to initiate disease on bean at relative humidities of 90 and 95% should be accounted for when assessing the influence of environment on disease and in the development of disease prediction models.

The influence of air temperature on grey mold of bean coincided with earlier disease reports on other hosts [Baker, 1984; Bulger *et al.*, 1987]. Temperatures of 18–25 °C were optimal for lesion development, and lesion formation was greatly reduced above 25 °C. In our study, the influence of temperature on disease was similar except for the interaction of temperature with relative humidity where small grey mold lesions developed at 28 °C in 100% relative humidity but larger lesions developed at 28 °C in 95% relative humidity. A small droplet of water that tended to collect between the petal and leaf surface in the 100% RH treatments may have interrupted the development of disease in the 28 °C × 100% treatment, although it did not appear to affect the 20 and 24 °C × 100% RH treatments (Table 2). Observations during the experiment did not indicate that potential bacterial contamination around the inoculated petal was a significant factor in disease development, although such observations would only be expected to identify heavily contaminated petals. The temperature and relative humidity treatments chosen for this study represented ranges which commonly occur in field environments in southern Ontario and included known temperature and moisture requirements for grey mold.

Two main patterns of disease suppression by the BCAs were evident. In most cases, including *A. alternata, Drechslera* sp., *M. verrucaria, T. viride, G. roseum* and the unidentified pink yeast, the efficacy of biological control was dependent on the prevailing environmental conditions. For many of these BCAs, disease suppression ranged from ≤15 to 100%. Relatively small changes in air temperature or relative humidity (e.g. 4 °C or 5% relative humidity) often resulted in large changes in disease suppression. These results suggest that the effects of the BCAs were highly responsive to environmental factors and would produce variable results in field environments. Variation in disease suppression was also caused by sub-optimal conditions for *B. cinerea.*

A second pattern of disease suppression displayed by one of the BCAs, *E. purpurascens*, was that efficacy of biological control was independent of the environment. *Epicoccum purpurascens* completely suppressed grey mold on bean seedlings in all of the

environmental treatments tested. This BCA has also proved moderately to highly successful in controlling white mold (*Sclerotinia sclerotiorum* [Lib.] de Bary) on bean [Inglis and Boland, 1992], and grey mold on strawberry [Peng and Sutton, 1991], in the field. The behaviour of *E. purpurascens* may be attributable to its ecological competence or to a different mechanism of biocontrol action, such as antibiosis, which might be independent of the atmospheric environment. Studies of the mechanism(s) of action of this BCA have reported that both competition and antibiosis may be important in disease suppression [Boland and Hunter, 1988; Boland and Inglis, 1989; Mercier and Reeleder, 1987; Zhou and Reeleder, 1990; Zhou and Reeleder, 1991; Zhou *et al.*, 1991].

An important trend was observed in the efficacy of biological control for all of the BCAs, with the exception of *E. purpurascens*. Suppression of disease was most effective under environmental conditions least conducive to disease. Nearly 100% control of grey mold by the BCAs was observed at 28 °C and 100% relative humidity; however, the lesions formed under these conditions in the control were invariably small ($\leq$5 mm diameter). Many of the BCAs suppressed grey mold in environmental conditions marginal for growth of the pathogen. Control treatments for the BCAs were not included in the experiments, therefore, the influence of temperature and relative humidity on the BCAs alone are not available.

The BCAs tested in this study were originally selected and shown to suppress grey mold or white mold [Inglis, 1989; Boland and Inglis, 1989] under conditions of 24 °C to 28 °C and 100% relative humidity. When evaluated under the more variable environmental conditions presented in this study, most of the BCAs (except *E. purpurascens*) were primarily disease-suppressive under environmental conditions which were non-optimal for disease, as evidenced by smaller grey mold lesions in the control. We believe this phenomenon may explain the frequently-reported discrepencies between efficacy of BCAs in suppressing disease in controlled versus field environments. Evaluations of potential BCAs in environmental conditions that are marginal for disease can overestimate their efficacy in field environments. Controlled environment assessments of biological control efficacy should be made under conditions optimal for disease.

The surface colonization of petals (SCP) and the SCPL by *B. cinerea* were measures of the pathogen's response to temperature, relative humidity, and the co-inoculated BCA treatments (e.g. competition). The SCP for *A. alternata* increased with increasing temperature, whereas the SCP for *T. viride* and *Dreschlera* sp. increased with increasing relative humidity. The SCPL by *B. cinerea*, in general, was closely related to the size of the lesions formed. The SCP by many of the BCAs was closely related to the degree of disease suppression. Environmental treatments which resulted in high SCPL values (24 °C × 95% RH; 28 °C × 95% RH; 20 °C × 100% RH and 24 °C × 100% RH) often coincided with conditions under which the largest grey mold lesions developed. Conversely, treatments in which high SCP values developed often coincided with small lesion diameters. These results suggest that competition for senescing bean petals as infection sites may be the mechanism of action for many BCAs [Boland and Hunter, 1988].

Our study has established that *B. cinerea* can cause disease on bean seedlings under conditions of high relative humidity, and that extended periods of plant surface wetness are not required. Interactions among environmental conditions, BCAs, and grey mold on bean are highly complex. Most of the BCAs studied were highly dependent on the environment for efficacy, but *E. purpurascens* was comparatively independent of the environment. The efficacy of BCAs in environmental conditions that are non-optimal for disease may be overestimated and assessments of biological control efficacy in various environments can be used to more accurately assess the potential of individual agents in controlling disease.

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References

Agrios GN (1978) Plant Pathology. Academic Press, New York, 703 pp.

Baker KF (1946) Observations on some *Botrytis* diseases in California. Plant Disease Reporter 30: 145–155

Bhatt DD and Vaughan EK (1963) Inter-relationships among fungi associated with strawberries in Oregon. Phytopathology 53: 217–220

Bisiach M, Minervini G, Vercesi A and Zerbetto F (1985) Research on protection against *Botrytis* in viticulture using microbial competitors. Difesa delle Piante 8(4): 429–439

Boland GJ and Hunter JE (1988) Influence of *Alternaria alternata* and *Cladosporium cladosporiodes* on white mold of bean caused by *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 10: 172–177

Boland GJ and Inglis GD (1989) Antagonism of white mold (*Sclerotinia sclerotiorum*) of bean by fungi from bean and rapeseed flowers. Canadian Journal of Botany 67: 1775–1781

Bulger MA, Ellis MA and Madden LV (1987) Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. Phytopathology 77: 1225–1230

Burpee LL (1989) The influence of abiotic factors on biological control of soilborne plant pathogenic fungi. Canadian Journal of Plant Pathology 12: 306–317

Dubos B, Roudet J, Bulit J and Bugaret Y (1983) L'utilisation du *Trichoderma harzianum* Rifai dans la pratique vitiole pur lutter contre la pourriture grise (*Botrytis cinerea* Pers.). Les Colloques de l'INRA 18: 289–296

Hannusch DJ, James TDW, Gillespie TJ and Boland GJ (1994) Simple and inexpensive control of relative humidity in a flow-through environmental chamber. Environmental and Experimental Botany. In press.

Harman GE, Chet I and Baker R (1981) Factors affecting *Trichoderma hamatum* applied to seeds as biocontrol agents. Phytopathology 71: 569–572

Horsfall JG and Cowling EB (1978) Phytopathometry: the measurement of plant disease. In: Horsfall JG and Cowling EB (eds) Plant Disease: An Advanced Treatise Vol. II (pp. 120–135) Academic Press, New York, 436 pp

Inglis GD (1989) Biological control of white mold (*Sclerotinia sclerotiorum*) on aerial surfaces of bean with fungi isolated from flowers. M.Sc. Thesis, University of Guelph, Guelph, ON, Canada, 135 pp

Inglis GD and Boland GJ (1992) Evaluation of filamentous fungi isolated from petals of bean and rapeseed for suppression of white mold. Canadian Journal of Microbiology 38: 124–129

Jarvis WR (1962) The infection of strawberry and raspberry fruits by *Botrytis cinerea* Fr. Annals of Applied Biology 50: 569–575

Jarvis WR (1977) *Botryotinia* and *Botrytis* species: Taxonomy, Physiology, and Pathogenicity. Monograph No. 15. Research Branch, Canada Department of Agriculture Research Station, Harrow, ON, Canada, 195 pp

Jarvis WR and Nuttall VW (1981) Cucumber Diseases. Agriculture Canada Publication Publ. No. 1684

Mercier J and Reeleder RD (1987) Interactions between *Sclerotinia sclerotiorum* and other fungi on the phylloplane of lettuce. Canadian Journal of Plant Pathology 65: 1633–1637

McLean MM (1988) The microflora of strawberry in relation to biological control of grey mold fruit not caused by *Botrytis cinerea* Pers. *ex* Fr. M.Sc. Thesis. University of Guelph, Guelph, ON, Canada, 106 pp

Morgan WM (1984) The effect of night temperature and glasshouse ventilation on the incidence of *Botrytis cinerea* in a late-planted tomato crop. Crop Protection 3: 243–251

Nelson PV (1978) Greenhouse Operation and Management. Reston Publishing & Company Incorporated. Reston, Virginia, USA

Nelson ME and Powelson ML (1988) Biological control of grey mold of snap beans by *Trichoderma hamatum*. Plant Disease 72: 727–729

Peng G and Sutton JC (1990) Biological methods to control grey mold of strawberry. Proceedings of the Brighton Crop Protection Conference, Pests Diseases, Volume 1, pp 233–240

Peng G and Sutton JC (1991) Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. Canadian Journal of Plant Pathology 13: 247–257

Redmond JC, Marois JJ and MacDonald JD (1987) Biological control of *Botrytis cinerea* on roses with epiphytic microorganisms. Plant Disease 71: 799–802

Snedecor GW and Cochran WG (1980) Statistical methods. Iowa State University Press, Ames, Iowa, USA

Tezuka N, Ishii M and Watanabe Y (1983) Effect of relative humidity on the development of grey mold of tomato in greenhouse cultivation. Bulletin of Vegetable and Ornamental Crops Research Station A (Ishinden-Ogoso) 11: 105–112

Tronsmo A and Dennis C (1977) The use of *Trichoderma* species to control strawberry fruit rots. Netherlands Journal of Plant pathology 83(Suppl. 1): 449–455

Tronsmo A and Ystaas J (1980) Biological control of *Botrytis cinerea* on apple. Plant Disease 64: 1009

Zhou T and Reeleder RD (1990) Selection of strains of *Epicoccum purpurascens* for tolerance to fungicides and improved biocontrol of *Sclerotinia sclerotiorum*. Canadian Journal of Microbiology 36: 754–759

Zhou T and Reeleder RD (1991) Colonization of bean flowers by *Epicoccum purpurascens*. Phytopathology 81: 774–778

Zhou T, Reeleder RD and Sparace SA (1991) Interactions between *Sclerotinia sclerotiorum* and *Epicoccum purpurascens*. Canadian Journal of Botany 69: 2503–2510