Influence of Air Temperature and Relative Humidity on Biological Control of White Mold of Bean (*Sclerotinia sclerotiorum*)

D. J. Hannusch and G. J. Boland

Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.
The financial assistance of the Ontario Ministry of Environment and the Natural Sciences and Engineering Research Council of Canada are gratefully acknowledged.
We thank E. A. Smith for technical assistance.
Accepted for publication 22 October 1995.

ABSTRACT

Hannusch, D. J., and Boland, G. J. 1996. Influence of air temperature and relative humidity on biological control of white mold of bean (*Sclerotinia sclerotiorum*). Phytopathology 86:156-162.

The interactions of *Sclerotinia sclerotiorum* 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 biological control of white mold of bean. All main effects and interactions were significant ($P \leq 0.0001$) among the 72 treatments. In the control treatment, lesions of white mold developed in all environmental treatments but were largest at 20°C × 100% RH, 24°C × 95 and 100% RH, and 28°C × 95% RH. Interactions of environment, BCAs, and white mold were highly complex. *Alternaria alternata*, *Drechslera* sp., *Myrothecium verrucaria*, *Trichoderma viride*, *Gliocladium roseum*, and an unidentified pink yeast were all highly dependent on environment for efficacy. Changes of 4°C or 5% RH were associated with variability in disease suppression that ranged from ≤25 to 100%. *Epicoccum nigrum* was comparatively independent of environment and suppressed disease by 100% in all environments. Suppression of disease by many of the BCAs was most effective under environmental conditions that were least conducive for disease. Assessments of biological control efficacy in various environments can be used to more accurately assess the potential of individual BCAs.

Additional keywords: environmental interactions, *Phaseolus vulgaris*.

The ascomycete *Sclerotinia sclerotiorum* (Lib.) de Bary is a ubiquitous and destructive pathogen, inciting disease on up to 408 plant species (5). Diseases induced by this pathogen, often collectively referred to as white mold, cause yield losses in numerous cultivated crops, such as sunflower, canola, carrots, and beans (12,24). Before infecting healthy plant tissues, ascospores of *S. sclerotiorum* require an exogenous source of energy. In white mold of bean (*Phaseolus vulgaris* L.), the most common energy source is senescing flower parts (1,19). After colonization of such nutrient sources, the pathogen invades adjoining living tissue (2) and initiates disease.

Several factors are known to influence the colonization of senescing flower petals by *S. sclerotiorum*. Air temperature, relative humidity (RH), water potential (9,11,23), and duration of plant surface wetness (4) affect the germination of ascospores and growth of mycelium and influence the ability of *S. sclerotiorum* to colonize senescing petals. The pathogen must compete with other filamentous fungi, bacteria, and yeasts known to inhabit bean and rapeseed petals. Several of these microorganisms are antagonistic to *S. sclerotiorum* (6,7,17,25). In laboratory, growth room, and greenhouse trials, isolates of *Cladosporium cladosporioides* (Fresen) G.A. De Vries, *Alternaria alternata* (Fr.) Keissl., *Drechslera* sp., *Epicoccum purpurascens* Ehrenb.:Schlechtend., *Fusarium graminearum* Schwabe, and *F. heterosporum* Nees:Fr. reduced the incidence and severity of disease caused by *S. sclerotiorum*. However, when these biological control agents (BCAs) were applied to bean crops in naturally infested field conditions, disease reduction varied (18,25).

Discrepancies in the efficacy of biological control between controlled and field environments suggest that environmental factors influence the efficacy of BCAs (3,8,10,14). Evaluation of BCAs in various environments may be a useful method to explain such discrepancies and to enhance the selection of BCAs for foliar diseases. The objective of this study was to examine the influence and interactions of seven BCAs, RH, and temperature on the development of white mold and the ability of the BCAs to colonize petals.

MATERIALS AND METHODS

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

Collection and sterilization of bean petals. Flower petals at stages B and C (7) were collected daily until flowering ceased. The petals were sterilized with propylene oxide gas (16). Aliquots (2 ml) of propylene oxide at 4°C 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 1 h. 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.

Publication no. P-1995-1227-01R

Collection and preparation of spore suspensions. Isolates of *A. alternata* (B639) and *E. nigrum* Link (D224) were cultured from rapeseed petals (16). *Drechslera* sp. (B10), *Myrothecium verrucaria* (Albertini & Schwein.) Ditmar.:Steudel (B24), and *Trichoderma viride* Pers.:Fr. (BC34) were cultured from rapeseed or bean petals (7). Isolates of *Gliocladium roseum* Bainier (I710) and an unidentified pink yeast (Y_3) were received from G. Peng, University of Guelph, Guelph, Ontario, Canada. Preconditioned (e.g., vernalized) sclerotia of *S. sclerotiorum* (21) were collected from overwintered bean stubble at the Arkell Research Station, Arkell, Ontario, Canada.

Sclerotia of *S. sclerotiorum* were surface-disinfested in 0.6% sodium hypochlorite for 2 min, rinsed twice in sterile distilled water, and placed into moist, sterile silica sand in 9-cm-diameter petri dishes. Sclerotia were incubated for 3 to 4 weeks at 18°C with a 14-h photoperiod until carpogenic germination commenced. Mature apothecia were positioned with their stipes in a thin film of lanolin jelly in the lid of a 30-mm-diameter petri dish that was inverted over the bottom of the dish and incubated at room temperature and high RH. Ascospores deposited on the bottom of the plastic dish were used immediately or allowed to air-dry for 10 to 15 min and stored over calcium chloride at 4°C for up to 10 days (16). To prepare spore suspensions, sterile water amended with 0.01% Tween 80 (Fisher Scientific Co., Toronto) was added to the petri dish, and spores were dislodged with a clean artists' brush. Ascospore suspensions were poured into sterile 15-ml test tubes. Spore concentration was estimated using a haemocytometer and adjusted to 2×10^5 spores per ml.

Most BCAs were cultured on potato dextrose agar under UV lights (12-h photoperiod; 310 to 420 nm) for 7 to 21 days, except *E. nigrum*, which was cultured on V8-juice agar (200 ml of V8 juice, 3 g of $CaCO_3$, 20 g of agar, 800 ml of deionized water) for the same length of time. *T. viride* and the unidentified pink yeast (Y_3) 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. Individual suspensions were poured into sterile test tubes through a single layer of cheesecloth. The concentration was estimated using a haemocytometer and adjusted to 2×10^5 spores per ml. The viability of BCAs and *S. sclerotiorum* was determined by the method of Boland and Hunter (6).

Petal inoculations. Seven coinoculum spore suspensions were prepared by combining 0.5 ml of individual BCA spore suspensions with 0.5 ml of *S. sclerotiorum* spore suspension in sterile test tubes. An eighth spore suspension (control treatment) was prepared by combining 0.5 ml of *S. sclerotiorum* spore suspension with an equal volume of Tween-amended sterile water. All suspensions were agitated using a vortex stirrer to ensure adequate mixing of the coinoculum treatments. Each coinoculum treatment was added to 20 sterile bean petals in a sterile 15-ml test tube and gently agitated for 10 s until the coinoculum was dispersed on the petals. Petals were removed from each tube, placed into partially covered 90-mm-diameter petri dishes and air-dried in a laminar-flow transfer chamber for 1 h.

Environmental chambers. Environmental chambers were developed that maintained selected RHs within Plexiglas chambers measuring 50 × 50 × 150 cm (13). RH was maintained at 90 ± 0.4% and 95 ± 0.5% through the use of a micrologger-controlled 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. Continuous leaf wetness was obtained through continuous operation of the ultrasonic misters. Air temperatures of 20 to 28 ± 0.5°C were maintained by regulation of growth room controls.

Bean seedling inoculation and incubation. The environmental chambers were adjusted to either 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 (20, 24, and 28°C) × three RH (90, 95, and 100%) × eight BCAs (including the pathogen control). Because there were only two environmental chambers, nine individual trials were conducted to complete the nine environmental settings examined. In preliminary experiments, no significant differences ($P = 0.05$) were detected between simultaneous or sequential experiments conducted in the two chambers (data not shown). Therefore, results from individual trials were combined for statistical analysis (22). The entire experiment was repeated once.

Each trial (e.g., environmental chamber) consisted of one combination of temperature and RH and included a tray of 32 seedlings inoculated with the pathogen and all of the BCAs. The pathogen and BCAs were inoculated by placing one infested petal onto each of two primary leaves of four bean seedlings per trial (eight observations). Bean seedlings were randomly placed within the environmental chambers, and individual trials 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 (16), and disease was then assessed. Lesions that formed beneath the infested petals on the bean leaves were measured using calipers (Manostat, Fisher Scientific). Petals were examined with a hand-held magnifying glass to estimate the extent of petal colonization by the BCAs. A modified Horsfall-Barratt scale (15) of 0 to 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 *S. sclerotiorum* also was evaluated by rating the amount of mycelial growth on each petal and the diseased area (if present) using the modified Horsfall-Barratt scale.

Data analysis. Factorial analyses of variance (ANOVA) of the lesion diameter data were performed using Statistical Analysis System software (SAS Institute, Inc., Cary, NC). The repeated experiment was examined for homogeneity of variance prior to pooling of data (22). Horsfall-Barratt values of surface colonization for each treatment were transformed to percent values, averaged, and compared statistically. For each BCA/pathogen treatment (including the pathogen control), the mean of each environmental treatment was compared using the protected least significant difference (PLSD) test ($P = 0.05$) (Statistix 4.0, Analytical Software, St. Paul, MN).

RESULTS

All main effects and interactions were significant ($P = 0.0001$) among the 72 treatments (three temperatures × three RH × eight organisms) examined for influence on mean lesion diameter of white mold on bean. Main effects and interactions for percent surface colonization of petals by BCAs (SCP) and percent surface colonization of petals and lesions by *S. sclerotiorum* (SCPL) also were significant ($P = 0.0001$) among all treatments. According to the ANOVAs for individual BCAs, F values were significant for all temperature × RH interactions. Therefore, the results are presented as simple effects for individual fungi.

Influence of environmental variables on white mold. White mold lesions were formed in the control treatment (inoculum of *S. sclerotiorum* applied alone) in all environmental treatments, with lesion diameters ranging from 1.7 to 42.9 mm (Fig. 1A; Table 1). Environmental treatments that produced the largest white mold lesions were 20°C × 100% RH (41.9 mm), 24°C × 95 and 100% RH (41.2 and 42.9 mm, respectively), and 28°C × 95% RH (40.0 mm). The remaining environments produced lesions less than 15.5 mm in diameter.

Influence of environmental variables on biological control of white mold. For all of the BCAs, except *E. nigrum*, RH and air temperature affected the efficacy of biological control. White mold

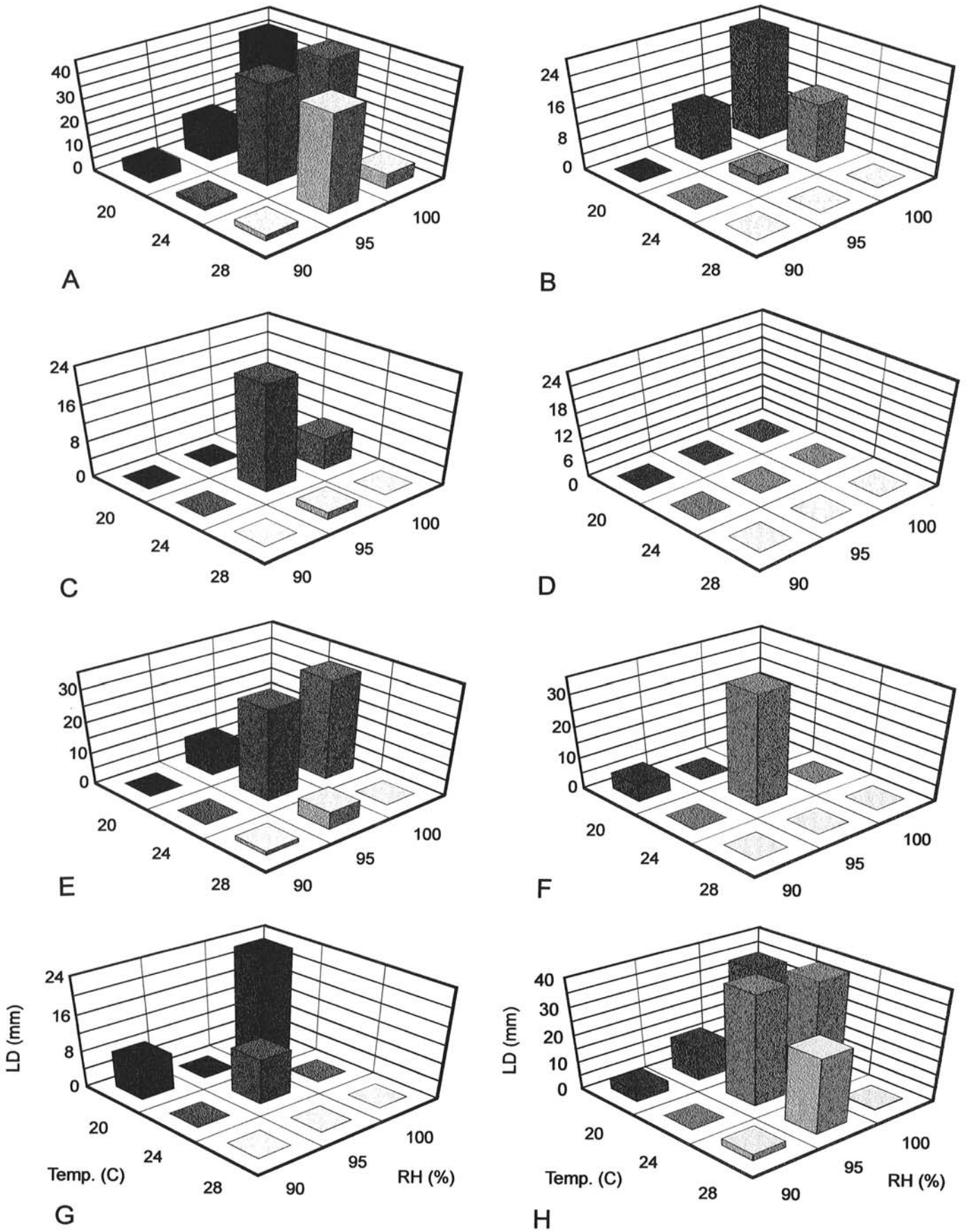

Fig. 1. The influence of relative humidity (RH) and temperature (Temp.) on the development of white mold lesions (LD = lesion diameter) caused by *Sclerotinia sclerotiorum* on bean leaves, alone and in combination with seven biological control agents. **A,** *S. sclerotiorum* inoculated alone. **B,** *S. sclerotiorum* coinoculated with *Alternaria alternata*. **C,** *S. sclerotiorum* coinoculated with *Drechslera* sp. **D,** *S. sclerotiorum* coinoculated with *Epicoccum nigrum*. **E,** *S. sclerotiorum* coinoculated with *Gliocladium roseum*. **F,** *S. sclerotiorum* coinoculated with *Myrothecium verrucaria*. **G,** *S. sclerotiorum* coinoculated with *Trichoderma viride*. **H,** *S. sclerotiorum* coinoculated with an unidentified yeast (Y_3).

lesions for treatments containing BCAs ranged from 0 mm (several BCAs) to 39.9 mm (unidentified pink yeast at 24°C × 95% RH) in diameter (Fig. 1B to H; Tables 2 to 8). The percent suppression of white mold by BCAs fluctuated from 0 to 100%, compared to the control treatment inoculated with *S. sclerotiorum* alone. The SCPL by *S. sclerotiorum* and the SCP by BCAs varied considerably among treatments (Tables 1 to 8).

A. alternata. No white mold lesions were found in five of nine environmental treatments in which petals were coinoculated with *A. alternata* and *S. sclerotiorum* (Fig. 1B; Table 3). Lesion diameters ranged from 2.4 to 27.1 mm, and disease suppression varied between 22.1 and 100% compared to the control treatment. *A. alternata* completely suppressed the development of white mold lesions at all levels of RH at 28°C and at 90% RH at 20 and 24°C. The fungus was least suppressive (22.1 and 35.3%, respectively) at 95 and 100% RH at 20°C. *A. alternata* had a greater SCP than the SCPL for *S. sclerotiorum* in all treatments in which control was near 100%. Petal colonization by *A. alternata* was

TABLE 1. The influence of relative humidity (RH) and temperature (Temp.) on the development of white mold lesions and the proportion of surface colonization of petals and lesions on bean leaves by *Sclerotinia sclerotiorum*[v]

Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	Surface colonization on petal + lesion[y,z] (%)
20	90	5.4 b	16.3 b
20	95	15.5 b	32.4 b
20	100	41.9 a	98.8 a
24	90	1.7 b	2.6 b
24	95	41.2 a	98.8 a
24	100	42.9 a	90.9 a
28	90	2.4 b	8.5 b
28	95	40.0 a	93.2 a
28	100	6.2 b	13.2 b

[v] Pathogen treatment applied as spore suspensions at 1.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals was determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal and lesion colonized by *S. sclerotiorum*. Values were transformed to percent values for statistical analysis.

TABLE 2. The influence of relative humidity (RH), temperature (Temp.), and *Alternaria alternata* on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with *A. alternata* and *S. sclerotiorum*[v]

			Surface colonization[y,z]	
Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	*A. alternata* on petal (%)	*S. sclerotiorum* on petal + lesion (%)
20	90	0.0 c	14.3 c,d	1.2 b
20	95	12.1 b	2.7 d	14.8 b
20	100	27.1 a	4.8 d	68.1 a
24	90	0.0 c	18.8 c,d	1.3 b
24	95	2.4 c	48.8 b	7.2 b
24	100	15.2 b	11.5 c,d	14.6 b
28	90	0.0 c	31.6 b,c	1.2 b
28	95	0.0 c	51.2 b	1.2 b
28	100	0.0 c	86.0 a	1.2 b

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals was determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by *S. sclerotiorum*. Values were transformed to percent values for statistical analysis.

TABLE 3. The influence of relative humidity (RH), temperature (Temp.), and *Drechslera* sp. on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with *Drechslera* sp. and *S. sclerotiorum*[v]

			Surface Colonization[y,z]	
Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	*Drechslera* sp. on petal (%)	*S. sclerotiorum* on petal + lesion (%)
20	90	0.0 c	6.5 c	1.4 c
20	95	0.0 c	9.5 c	1.2 c
20	100	15.1 a,b	51.1 b	47.7 a
24	90	0.0 c	3.2 c	1.2 c
24	95	23.2 a	17.2 c	28.6 b
24	100	7.0 b,c	77.2 a,b	7.2 c
28	90	0.0 c	19.5 c	1.2 c
28	95	1.6 c	61.7 a,b	1.3 c
28	100	0.0 c	78.3 c	1.2 c

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals was determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by *S. sclerotiorum*. Values were transformed to percent values for statistical analysis.

TABLE 4. The influence of relative humidity (RH), temperature (Temp.), and *Epicoccum nigrum* on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with *E. nigrum* and *S. sclerotiorum*[v]

			Surface colonization[y,z]	
Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	*E. nigrum* on petal (%)	*S. sclerotiorum* on petal + lesion (%)
20	90	0.0 a	44.7 c,d	1.2 a
20	95	0.0 a	22.3 d,e	1.2 a
20	100	0.0 a	97.5 a	1.2 a
24	90	0.0 a	10.3 e	1.2 a
24	95	0.0 a	57.2 c	1.2 a
24	100	0.0 a	90.7 a,b	1.2 a
28	90	0.0 a	16.3 e	1.2 a
28	95	0.0 a	60.5 c	1.2 a
28	100	0.0 a	64.8 b,c	1.2 a

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals was determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by *S. sclerotiorum*. Values were transformed to percent values for statistical analysis.

highest at 28°C × 100% RH, while the greatest SCPL by *S. sclerotiorum* was at 20°C × 100% RH.

***Drechslera* sp.** White mold lesions developed in four of nine environmental treatments in which petals were coinoculated with *Drechslera* sp. and *S. sclerotiorum* (Fig. 1C; Table 3). Lesion diameters ranged from 1.6 to 23.2 mm, and disease suppression ranged from 43.6 to 100%. White mold lesions were largest (15.1 and 23.2 mm) at 20°C × 100% RH and 24°C × 95% RH, respectively, and consequently, these treatments were associated with the least suppression of disease (64.0 and 43.6%). White mold was

TABLE 5. The influence of relative humidity (RH), temperature (Temp.), and *Gliocladium roseum* on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with *G. roseum* and *S. sclerotiorum*[v]

Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	Surface colonization[y,z]: *G. roseum* on petal (%)	Surface colonization[y,z]: *S. sclerotiorum* on petal + lesion (%)
20	90	0.0 c	3.6 b	1.6 b
20	95	8.9 b,c	1.2 b	17.0 b
20	100	15.3 b	11.3 a	41.6 a
24	90	0.0 c	3.3 b	1.3 b
24	95	28.9 a	3.1 b	49.8 a
24	100	31.6 a	7.5 a,b	48.4 a
28	90	1.3 c	3.1 b	12.2 b
28	95	5.5 b,c	6.3 a,b	13.3 b
28	100	0.0 c	6.3 a,b	1.2 b

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by the BCA and *S. sclerotiorum*, respectively. Values were transformed to percent values for statistical analysis.

TABLE 6. The influence of relative humidity (RH), temperature (Temp.), and *Myrothecium verrucaria* on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with *M. verrucaria* and *S. sclerotiorum*[v]

Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	Surface colonization[y,z]: *M. verrucaria* on petal (%)	Surface colonization[y,z]: *S. sclerotiorum* on petal + lesion (%)
20	90	5.4 b	2.5 c	7.5 b
20	95	0.0 b	1.7 c	1.6 b
20	100	6.0 b	53.9 a,b	16.7 b
24	90	0.0 b	1.7 c	1.2 b
24	95	34.9 a	1.2 c	70.5 a
24	100	0.0 b	51.2 a,b	1.2 b
28	90	0.0 b	2.9 c	1.2 b
28	95	0.0 b	36.4 b	1.2 b
28	100	0.0 b	64.0 a	1.2 b

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by the BCA and *S. sclerotiorum*, respectively. Values were transformed to percent values for statistical analysis.

TABLE 7. The influence of relative humidity (RH), temperature (Temp.), and *Trichoderma viride* on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with *T. viride* and *S. sclerotiorum*[v]

Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	Surface colonization[y,z]: *T. viride* on petal (%)	Surface colonization[y,z]: *S. sclerotiorum* on petal + lesion (%)
20	90	8.2 b	2.9 c	13.6 b
20	95	0.0 c	2.5 c	1.2 b
20	100	23.1 a	4.5 c	43.4 a
24	90	0.0 c	1.5 c	3.4 b
24	95	9.9 b	5.2 c	14.0 b
24	100	0.0 c	33.4 b	1.2 b
28	90	0.0 c	1.5 c	1.5 b
28	95	0.0 c	49.1 a,b	1.2 b
28	100	0.0 c	56.5 a	1.2 b

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by the BCA and *S. sclerotiorum*, respectively. Values were transformed to percent values for statistical analysis.

TABLE 8. The influence of relative humidity (RH), temperature (Temp.), and an unidentified pink yeast (Y_3) on the development of white mold lesions caused by *Sclerotinia sclerotiorum* on bean leaves and the proportion of surface colonization of petals and lesions coinoculated with Y_3 and *S. sclerotiorum*[v]

Temp.[w] (C)	RH[w] (%)	Lesion diameter[x,y] (mm)	Surface colonization[y,z]: Yeast on petal (%)	Surface colonization[y,z]: *S. sclerotiorum* on petal + lesion (%)
20	90	3.9 c	4.9 c	13.4 b
20	95	12.2 c	40.9 a	9.9 b
20	100	34.1 a,b	1.2 c	85.5 a
24	90	0.0 c	25.1 b	1.3 b
24	95	39.9 a	4.0 c	70.3 a
24	100	37.7 a,b	1.5 c	78.8 a
28	90	2.4 c	10.2 c	3.2 b
28	95	27.3 b	6.1 c	66.5 a
28	100	0.3 c	1.2 c	2.0 b

[v] Biological control agent (BCA) and pathogen treatments applied as a 50:50 spore suspension at 2.0×10^5 spores per ml.
[w] Temp. and RH were controlled and monitored using environmental chambers. Measured Temp. and RH values varied by ±0.5°C and ±0.5% RH.
[x] Mean lesion diameters on bean seedlings were recorded 96-h postinoculation. Significant main effects and interactions were detected in all treatments ($P = 0.05$).
[y] Values not followed by a common letter are significantly different at $P \leq 0.05$ according to least squares difference test. Data are the pooled results of two trials.
[z] Surface colonization of petals determined 96-h postinoculation using a modified Horsfall-Barratt scale of 0 to 11, where 0 = 0% and 11 = 100% of surface of petal or surface of petal and lesion colonized by the BCA and *S. sclerotiorum*, respectively. Values were transformed to percent values for statistical analysis.

suppressed most effectively (83.7 and 96.1%) at 24°C × 100% RH and 28°C × 95% RH. In all other environmental treatments, *Drechslera* sp. completely suppressed white mold. At all temperatures, SCP by *Drechslera* sp. was most apparent at 95 and 100% RH, whereas SCPL by *S. sclerotiorum* was most evident at 20°C × 100% RH and 24°C × 95% RH.

E. nigrum. In all environmental conditions tested, *E. nigrum* prevented lesion formation by *S. sclerotiorum* (Fig. 1D; Table 4). *E. nigrum* aggressively colonized the petals of all treatments but was least prevalent (<45% of petals colonized) at 20°C × 95% RH and 24 and 28°C × 90% RH. At 20°C × 100% RH, an average of 97.5% of petal surfaces was colonized by *E. nigrum*. *S. sclerotiorum* was not visible on petals or lesions of any treatments when coinoculated with *E. nigrum*.

G. roseum. When bean petals were coinoculated with *G. roseum* and *S. sclerotiorum*, white mold lesions formed in six of nine environmental treatments (Fig. 1E; Table 5). Lesion diameters ranged from 1.3 to 31.6 mm. At 20°C × 100% RH and 24°C × 95 and 100% RH, lesion diameters of 15.3, 28.9, and 31.6 mm were observed, respectively. Generally, the smallest lesions were formed in all RH at 28°C and in all temperatures at 90% RH. White mold suppression ranged from 26.4 to 100%. *G. roseum* suppressed white mold entirely at 90% RH × 20 and 24°C and 100% RH × 28°C; and by 86.2% RH at 95% × 28°C. In all environmental conditions examined, the SCP by *G. roseum* was never greater than 12%. Colonization of petals and lesions by *S. sclerotiorum* when coinoculated with *G. roseum* was greatest at environmental treatments similar to those that produced the largest lesions in the control treatment (Tables 1 and 5).

M. verrucaria. White mold lesions formed in only three of nine environmental conditions tested when petals were coinoculated with *M. verrucaria* and *S. sclerotiorum* (Fig. 1F; Table 6). Lesions developed under environmental conditions similar to those that produced the largest white mold lesions in the control treatment (20°C × 100% RH, 24°C × 95% RH, and 20°C × 90% RH). Lesion diameters ranged from 5.4 to 34.9 mm. In the three environmental treatments in which white mold did develop, disease was suppressed by 85.8% at 20°C × 100% RH, but the two remaining environmental treatments had less than 16% suppression of white mold. In all other environmental regimes, *M. verrucaria* completely suppressed white mold. Colonization of petals by *M. verrucaria* was most extensive at all temperatures when the RH was 100%, as well as 28°C × 95% RH. Surface colonization of petals and lesions by *S. sclerotiorum* only occurred at 20°C × 100% RH and 24°C × 95% RH.

T. viride. White mold lesions were formed in only three of nine environmental treatments when petals were coinoculated with *T. viride* and *S. sclerotiorum* (Fig. 1G; Table 7). Lesions formed in the environmental treatments of 20°C × 100% RH, 24°C × 95% RH, and at 20°C × 90% RH. Where white mold lesions developed, *T. viride* suppressed white mold by 76.0% at 24°C × 95% RH but by less than 45% for the other treatments. In all other environmental regimes, the BCA completely suppressed white mold. Surface colonization of petals by *T. viride* was most prevalent at 24°C × 100% RH and 28°C × 95 and 100% RH. Colonization of petals and lesions by *S. sclerotiorum* only occurred at 20°C × 90 and 100% RH and 24°C × 95% RH.

Unidentified pink yeast. Lesions of white mold developed in eight of the nine environmental treatments when petals were coinoculated with the yeast and *S. sclerotiorum* (Fig. 1H; Table 8). Lesion diameters ranged from 0.3 to 39.9 mm, and disease suppression ranged from 0 to 100%. The greatest white mold control (100 and 94.6%) was achieved at 24°C × 90% RH and 28°C × 100% RH, respectively. White mold was not controlled (0 to 3% disease suppression) at 28°C × 90% RH and 24°C × 95% RH, respectively. When coinoculated with the yeast, *S. sclerotiorum* colonized the petals and lesions most extensively during conditions of high humidity (≥95% RH). Petal colonization by the BCA was most prevalent at lower humidities.

DISCUSSION

These studies provide important new information on the influence of environment on disease caused by *S. sclerotiorum* and on the interaction of these variables with BCAs. To our knowledge, this is the first report that *S. sclerotiorum* was able to form lesions on bean seedlings when RH was less than 100%. Although bean petals were consistently air-dried prior to being placed on bean seedlings and the chambers quickly reached equilibrium (13), white mold lesions developed at all of the RH and air temperature settings examined. In addition, *S. sclerotiorum* was able to grow saprotrophically at these environmental settings, as indicated by the SCPL assessments. Observations during the experiments did not indicate any visible moisture around the inoculated petals at 90 or 95% RH, although such observations would be expected to identify only condensation or small droplets of water. RH sensors were located within the canopy of the seedlings, where they accurately measured the RH of air within this region. Small discrepancies may exist between measurements within this region and the boundary layer surrounding the inoculated petals on the foliar surface, depending on the net radiative flux of the foliar environment.

More than 14 h of continuous plant surface wetness previously was thought to be required for infection by *S. sclerotiorum* in field conditions (1,4). In the present study, *S. sclerotiorum* produced lesions greater than 40 mm in diameter at 95% RH × 24 and 28°C but less than 16 mm at 95% RH × 20°C. This pattern was opposite to that at 100% RH, which produced lesions greater than 40 mm at 20 and 24°C and less than 10 mm at 28°C. These discrepancies in the environmental conditions required for white mold of bean likely are due to differences in experimental methods (e.g., field versus controlled environments) and a more accurate control of RH in the present experiments. The ability of *S. sclerotiorum* to initiate disease at 90 and 95% RH is an important consideration in the development of disease prediction models and should be taken into account when assessing the influence of environment on disease.

The influence of air temperature on white mold was similar to previous reports. Temperatures of 15 to 25°C were optimal for lesion development, and lesions did not form at 30°C (1,4). In our study, the influence of temperature on disease was similar except for the interaction of temperature with RH where small lesions developed at 28°C in 100% RH but larger lesions developed at 28°C in 95% RH. The temperature and RH treatments chosen for this study represent ranges that commonly occur in field environments in southern Ontario and include known temperature and moisture requirements for white mold.

Two patterns of disease suppression by the BCAs were evident in this study. 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 environmental conditions. For many of these BCAs, disease suppression ranged from ≤25 to 100% within the nine environmental treatments examined. Relatively small changes in air temperature or RH (e.g., 5°C or 4% RH) often produced dramatic changes in disease suppression. These results establish that most of the BCAs examined in this study were highly responsive to environmental factors and also would be expected to produce variable results in field environments.

A second pattern of disease suppression displayed by one of the BCAs, *E. nigrum*, was that efficacy of biological control was independent of the environmental conditions examined. In this treatment, disease was completely suppressed at all environmental settings. It is unclear why the efficacy of *E. nigrum* was unaffected by these environments, but different mechanisms of action, such as antibiosis, may be responsible. Studies on the mechanisms of action of *E. nigrum* have reported that both competition and antibiosis may be important in disease suppression (6,7,20,26,27,28).

One important trend was observed in the efficacy of biological control for all of the BCAs, except *E. nigrum*. Suppression of disease was most effective under environmental conditions that were least conducive for disease. For example, nearly 100% control of white mold was observed at 28°C × 100% RH. However, the lesions that formed in this environment in the control treatment were small. Many of the BCAs were most effective under environmental conditions considered marginal for the pathogen (e.g., small lesion diameters in control treatment). However, because control treatments for the BCAs were not included in the experimental design, the relative influence of temperature and RH on the BCAs alone is not available for comparison. Almost all of the BCAs were least effective under the same environmental conditions that resulted in the largest lesion diameters in the control treatment.

E. nigrum and *A. alternata*, two BCAs that have significantly suppressed white mold in field conditions (18), also suppressed white mold by 100% in the most optimal conditions for disease in the present study, 24°C × 95% RH. All the BCAs evaluated in this study previously have been shown to suppress disease under conditions of 24 to 28°C × 100% RH and, therefore, were selected for continued evaluation. However, according to the results of this study, only *E. nigrum* and *A. alternata* provided adequate control of white mold under conditions favorable for disease. Thus, environmental assessments of potential BCAs can provide an additional method for selection of effective BCAs.

The surface colonization of petals by the BCAs and the surface colonization of petals and lesions by *S. sclerotiorum* provided a measurement of response of the organisms to temperature, RH, and coinoculation treatments (e.g., competition). The SCP for some BCAs, such as *A. alternata*, *T. viride*, and *Drechslera* sp., increased with increasing temperature, whereas the SCP for *Drechslera* sp. and *A. alternata* increased with increasing RH. The SCPL by *S. sclerotiorum* was closely related to the size of lesions formed, and the SCP by many of the BCAs was closely related to the degree of disease suppression. For example, treatments that had high SCPL values (24°C × 95% RH, 28°C × 95% RH, 20°C × 100% RH, and 24°C × 100% RH) often coincided with treatments in which the largest white mold lesions occurred. Conversely, treatments that had high SCP values often coincided with small lesion diameters. As mentioned previously, there was little relationship between SCP by *E. nigrum* and disease control. Statistical correlations were not conducted because of the significant interactions among all of the variables. These results support the hypothesis that competition for senescing bean petals as infection sites may be the mechanism of action for many, but not all, of the BCAs used in this strategy of biological control (6).

The results of this study establish that *S. sclerotiorum* can incite disease under conditions of high RH and that extended periods of plant surface wetness are not required to incite white mold of bean. Interactions of environment, BCAs, and white mold are highly complex. Most of the BCAs examined were highly dependent on environment for efficacy, but the effectiveness of *E. nigrum* was comparatively independent of environment. Evaluations of potential BCAs in environmental conditions that are marginal for disease can overestimate their potential efficacy. Finally, assessments of biological control efficacy in various environments can be used to more accurately assess the potential of individual agents.

LITERATURE CITED

1. Abawi, G. S., and Grogan, R. G. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. Phytopathology 65:300-309.
2. Abawi, G. S., Polach, F. J., and Molin, W. T. 1975. Infection of bean by ascospores of *Whetzelinia sclerotiorum*. Phytopathology 65:673-678.
3. Baker, R. 1968. Mechanisms of biological control of soilborne pathogens. Annu. Rev. Phytopathol. 6:263-294.
4. Boland, G. J., and Hall, R. 1987. Epidemiology of white mold of white bean in Ontario. Can. J. Plant Pathol. 9:218-224.
5. Boland, G. J., and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 16:93-108.
6. Boland, G. J., and Hunter, J. E. 1988. Influence of *Alternaria alternata* and *Cladosporium cladosporioides* on white mold of bean caused by *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 10:172-177.
7. Boland, G. J., and Inglis, G. D. 1989. Antagonism of white mold (*Sclerotinia sclerotiorum*) of bean by fungi from bean and rapeseed flowers. Can. J. Bot. 67:1775-1781.
8. Burpee, L. L. 1989. The influence of abiotic factors on biological control of soilborne plant pathogenic fungi. Can. J. Plant Pathol. 12:306-317.
9. Caesar, A. J., and Pearson, R. C. 1983. Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. Phytopathology 73: 1024-1030.
10. Diem, H. G. 1971. Effect of low humidity on the survival of germinated spores commonly found in the phylloplane. Pages 211-219: Ecology of Leaf Surface Microorganisms. T. F. Preece and C. H. Dickinson, eds. Academic Press, London.
11. Grogan, R. G., and Abawi, G. S. 1975. Influence of water potential on growth and survival of *Whetzelinia sclerotiorum*. Phytopathology 65: 122-138.
12. Haas, J. H., and Bolwyn, B. 1972. Ecology and epidemiology of Sclerotinia wilt of white beans in Ontario. Can. J. Plant Sci. 52:525-533.
13. Hannusch, D. J., James, T. D. W., Gillespie, T. J., and Boland, G. J. 1995. Simple and inexpensive control of relative humidity in a flow-through environmental chamber. Environmental and Experimental Botany. 35(3):411-415.
14. Harman, G. E., Chet, I., and Baker, R. 1981. Factors affecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. Phytopathology 71:569-572.
15. Horsfall, J. G., and Cowling, E. B. 1978. Phytopathometry: The measurement of plant disease. Pages 120-135: Plant Disease: An Advanced Treatise. Vol. 2. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
16. Inglis, G. D. 1989. Biological control of white mold (*Sclerotinia sclerotiorum*) on aerial surfaces of bean with fungi isolated from flowers. M.S. thesis. University of Guelph, Guelph, Ontario, Canada.
17. Inglis, G. D., and Boland, G. J. 1990. The microflora of bean and rapeseed petals and the influence of the microflora of bean petals on white mold. Can. J. Plant Pathol. 12:129-134.
18. Inglis, G. D., and Boland, G. J. 1992. Evaluation of filamentous fungi isolated from petals of bean and rapeseed for suppression of white mold. Can. J. Microbiol. 38:124-129.
19. McLean, D. M. 1958. Role of dead flower parts in infection of certain crucifers by *Sclerotinia sclerotiorum*. Plant Dis. Rep. 42:663-666.
20. Mercier, J., and Reeleder, R. D. 1987. Interactions between *Sclerotinia sclerotiorum* and other fungi on the phylloplane of lettuce. Can. J. Bot. 65:1633-1637.
21. Smith, E. A., and Boland, G. J. 1989. A reliable method for the production and maintenance of germinated sclerotia of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 11:45-48.
22. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. Iowa State University Press, Ames.
23. van den Berg, L., and Lentz, C. P. 1968. The effect of relative humidity and temperature on survival and growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Can. J. Bot. 46:1477-1481.
24. Wallen, V. R., and Sutton, M. D. 1967. Observations on Sclerotinia rot of field beans in southwestern Ontario and its effect on yield. Can. Plant Dis. Surv. 47:116.
25. Zhou, T., and Reeleder, R. D. 1989. Application of *Epicoccum purpurascens* spores to control white mold of snap bean. Plant Dis. 73:639-642.
26. Zhou, T., and Reeleder, R. D. 1990. Selection of strains of *Epicoccum purpurascens* for tolerance to fungicides and improved biocontrol of *Sclerotinia sclerotiorum*. Can. J. Microbiol. 36:754-759.
27. Zhou, T., and Reeleder, R. D. 1991. Colonization of bean flowers by *Epicoccum purpurascens*. Phytopathology 81:774-778.
28. Zhou, T., Reeleder, R. D., and Sparace, S. A. 1991. Interactions between *Sclerotinia sclerotiorum* and *Epicoccum purpurascens*. Can. J. Bot. 69: 2503-2510.