Colonization of Bean Flowers by *Epicoccum purpurascens*

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ABSTRACT

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Emerging flowers, newly opened flowers, and senescent flowers of bean were sprayed with conidial suspensions of *Epicoccum purpurascens*, then sampled at 4, 8, 12, 24, and 96 h. The number of conidia per square millimeter of tissue surface was greater on newly opened or senescent flowers than on emerging flowers. Germination of conidia was greater on senescent petals than on younger tissue. However, conidia applied to emerging flowers germinated sufficiently such that resultant hyphae were able to completely colonize flowers as they senesced. The addition of malt extract to conidial suspensions improved germination on flowers and increased mycelial coverage on emerging flowers. Application of *E. purpurascens* did not accelerate senescence or affect chlorophyll content of bean leaves, nor did it affect yield or percentage of abscised flowers or pods. *E. purpurascens* did not penetrate leaves until they were in an advanced state of senescence, although the fungus readily colonized dead leaf tissue.

Additional keywords: biological control, *Phaseolus vulgaris, Sclerotinia sclerotiorum,* white mold.

Epicoccum purpurascens Ehrenb. & Schlecht. generally is considered a saprophytic fungus (6), although it is pathogenic on certain plants (12,16). In addition, it possesses antagonism to a number of plant pathogens, including *Sclerotinia sclerotiorum* (5,6,17). In nature, the fungus is found commonly on older or newly dead plant tissue but also can be recovered from the surface of young plants (10). Although *E. purpurascens* may colonize leaf surfaces early in the growing season, it does not penetrate leaf tissue until senescence has occurred (20). Leaf saprophytes may accelerate senescence and thereby reduce crop yield (11,22), however, *E. purpurascens* does not appear to affect leaf senescence (15).

Epidemics of white mold of snap bean (*Phaseolus vulgaris* L.) generally are initiated by ascospores of *S. sclerotiorum*. Hyphae arising from germinated ascospores colonize senescing bean flowers before invading adjacent plant tissues (1). Protection of flowers with fungicides (14) or antagonistic microorganisms, such as *E. purpurascens* (5,25), reduces flower colonization by *S. sclerotiorum,* thus reducing disease incidence. Application of conidia of *E. purpurascens* effectively suppresses white mold under both greenhouse and field conditions (5,25). Colonization of flowers and production of antifungal compounds by *E. purpurascens* are important elements in this control (5,17).

An antagonist must be selected not only for its activity against the pathogen but also for its capacities to survive adverse environmental conditions and maintain itself at effective population levels (4). Knowledge of the behavior of *E. purpurascens* on bean flowers and leaves is important, because it may lead to modification of application procedures and more efficient biological control of white mold. Also, it is essential to understand effects of biological agents on crops before antagonists can be used in practice. Thus, our objectives were to determine the effects of flower age and conidial suspension additives on the colonization of bean flowers by *E. purpurascens* and to evaluate the effects of *E. purpurascens* on flower abscission, pod abortion, pod yield, and senescence of leaves.

MATERIALS AND METHODS

Snap bean (cv. Strike) and strain R4000 of *E. purpurascens* (26) were used throughout this study. Beans were planted in 130-mm-diameter plastic pots containing Pro-Mix BX (Les Tourbières Premier Ltée, Rivière du Loup, Quebec) and fertilized every 2 wk with 0.1% N20/P20/K20 solution (Peters Fertilizer Products, W. R. Grace and Co., Fogelsville, PA). Conidia of *E. purpurascens* were produced on a wheat-seed medium prepared as described previously (25). Concentration of the suspension was adjusted to 10^6 conidia per milliliter in 0.01% Tween 80 (J. T. Baker Chemical Co., Phillipsburg, NJ). Flowers sampled in these studies were dissected immediately upon removal, and only banner (standard) petals were used for observations and measurements. Based on preliminary observations, these petals were assumed to represent the flower as a whole. Flowers were placed in one of three classes where emerging flowers were those having unopened petals, with the banner petal extending at least 5 mm past the tip of the sepal but still unopened and other petals not yet visible; newly opened flowers were those with the banner petal opened, all other petals visible and at least partly opened, with petals white and turgid; and senescent flowers were those where all petals were yellowish and flaccid but not dry, and all petals were still attached to the flower.

Behavior of *E. purpurascens* on bean flowers. Three plantings of bean, sown at 5-day intervals, were held on a growth bench (day/night temperatures of 22/19 C) with a 14-h photoperiod and a light intensity of 275 μmol photon m^{-2} s^{-1}. There were 10 plants per date. When 70% of the flowers on the oldest plants (45 days from planting) were open, all flowers, except those that were senescent, were removed. On the second group of plants (40 days), all but newly opened flowers were removed, and on the youngest plants (35 days), only emerging flowers were left. Conidial suspensions then were sprayed on the plants with a hand-held pump sprayer, with or without addition of 1% malt extract (ME; Difco Laboratories, Detroit, MI), until runoff (about 15 ml per plant). Suspensions were applied to the entire plant. Plants were air-dried for 1 h, then placed in a dew chamber (100% relative humidity [RH], 23 C) for 48 h. Two or more flowers per plant were sampled for various measurements at 4, 8, 12, 24, and 96

h after spraying. Banner petals were cleared with concentrated chloral hydrate solution (2 g/ml) for 24 h, then stained with lactophenol-cotton blue. Entire petals were used for open flowers, and only the exposed parts of the petal were used for emerging flowers. Each petal was treated as an experimental unit in a completely randomized design. Cleared petals were mounted on glass slides with clear lactophenol and observed under a microscope. The number of conidia on the petals at 4 h was estimated by determining the number of conidia in a microscope field (200×). These values were used to calculate the number of conidia per square millimeter. Five randomly selected fields were observed on each petal to obtain an average. Similarly, at least five fields were observed per petal to obtain values for percentage of conidial germination and length of germ tubes although, for these latter characters, only a subsample of six petals per treatment was examined. Where more than one germ tube was present, the longest germ tube was measured. To assess colonization by *E. purpurascens,* the whole petal surface first was observed, and two representative fields were recorded photographically (100×). Photographs (labeled on the back) were randomized, then rated as follows: 0, 0% of surface covered with mycelium; 1, less than 10%; 2, 10–30%; 3, 31–50%; 4, 51–75%; and 5, more than 75% of surface area covered.

A similar experiment was designed to test the effects of various additives (Fig. 1) on colonization of emerging flowers. Bean plants were produced as described previously. When one to two flowers per plant were open, open flowers and small (incompletely developed) inflorescences were removed, leaving only emerging flowers. Conidial suspensions of *E. purpurascens* plus the additives then were sprayed on plants, as described previously. Plants not receiving any application served as controls. Plants were placed in a greenhouse mist chamber for 4 days, with mist applied each night for 12 h and with the chamber open during the day (25). A completely randomized design with five plants per treatment was used. Newly opened flowers from treated plants were removed 4 days after application; senescent flowers were removed 6 days later. Four flowers per plant were sampled each time, with each flower treated as the experimental unit. Colonization of banner petals was recorded photographically through a dissecting microscope (38×). Photographs (labeled on the back) were randomized and rated for colonization where: 0, no conidia; 1, less than 25% area covered by conidia in sporodochia; 2, 25–50% area covered; 3, more than 50 but less than 75% area covered; and 4, 75% or more of the area covered.

Effects of *E. purpurascens* on bean. Plants were produced as described previously and arranged in a randomized complete block design with four blocks on a growth bench (day/night temperatures of 22/19 C, with a 14-h photoperiod). Treatments were conidia (10^6 conidia per milliliter) alone, conidia in 1% malt extract, malt extract alone, and an unsprayed control. Applications were made when more than 80% of the plants had at least one open flower and repeated 4 days later. The method of application was as described previously. Treated plants were placed in dew chambers (23 C, 100% RH without light) for 48 h before they were returned to the growth bench. After the first flower opened, plants were observed every third day. Number of inflorescences and number of flowers on each inflorescence were recorded when inflorescences were completely developed (no new flower buds present). Inflorescences and flowers (including emerging flowers with the banner petal extended at least 5 mm from the sepal) were counted and marked with an oil-base paint touching the peduncle of the inflorescence and sepals of the flower (24). The number of pods (greater than 1 cm long) also was determined, and each was marked with paint by touching the tip of the pod. The apex leaflet of the first trifoliolate leaf on each plant was similarly marked and observed for leaf senescence, expressed as the yellowish proportion of leaf. Pods were removed when at least 80% of them were judged ready to harvest commercially (70 days after planting and 20 days after the last application). Number of pods and fresh weight of pods per plant were recorded at harvest. Pods were dried in a 65 C oven for 96 h to obtain dry weight measurements. Percentage of abscised inflorescences was calculated with the formula: ([number of inflorescences developed − number of inflorescences at harvest]/number of inflorescences developed) × 100; percentage of abscised flowers was calculated with ([number of flowers − number of pods developed]/number of flowers) × 100, and percentage of abscised pods with ([number of pods formed − number of pods harvested]/number of pods formed) × 100 (24).

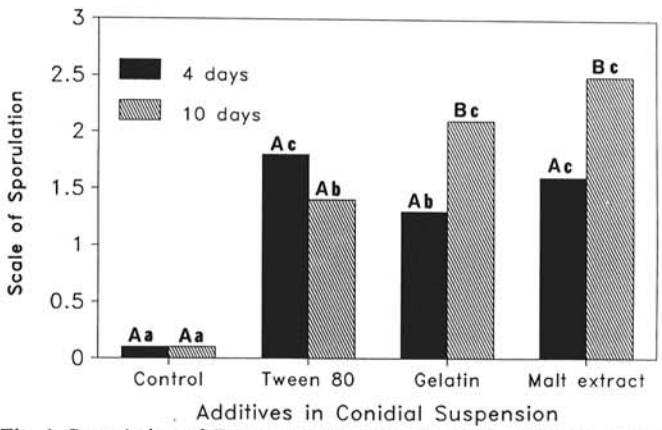

Fig. 1. Sporulation of *Epicoccum purpurascens* on bean flowers. Tween 80, gelatin, and malt extract were added at 0.01, 0.5, and 1%, respectively. Columns represent pooled means of 40 petals. Scale for assessing the amount of sporulation: 0, no conidia; 1, less than 25% area covered by conidia in sporodochia; 2, 25–50% area covered; 3, more than 50 but less than 75% area covered; and 4, more than 75% area covered. Means were separated by Dunn's multiple comparison procedure. Uppercase letters at the top of each column represent comparisons of sample times ($P = 0.05$) for each additive. Lowercase letters represent comparisons of additives ($P = 0.30$) for each sample time. For each type of comparison, means with same letter are not significantly different from one another.

Effects of *E. purpurascens* on chlorophyll content of leaves and behavior of *E. purpurascens* on leaves. Beans (produced as described previously) were planted in a greenhouse (16–26 C) and sprayed twice with suspensions of *E. purpurascens* or 0.01% Tween 80 (control). The time and methods for application and management after application were as described previously (25). Plants were arranged in a completely randomized design with three replicate plants per treatment. The first trifoliolate leaf of each plant was used for measurement of chlorophyll content, and samples were taken every 5 days following the second application. The method of Hiscox and Israelstam (13) was used to measure chlorophyll content. At each sampling date, three leaflets per treatment were cut into small pieces. A 100-mg subsample was placed in a test tube with 100 ml of dimethyl sulphoxide (Fisher Scientific Co., Fairlawn, NJ). Tubes then were placed in a 65 C water bath for 2 h. Light absorption of the resultant extract was measured immediately with a spectrophotometer (Spectronic 20, Bausch & Lomb, Rochester, NY) at 663 and 645 nm for chlorophyll a and b, respectively. Chlorophyll content was calculated with an equation used by Arnon (2), and total chlorophyll (a + b) was expressed as milligrams of chlorophyll per gram leaf fresh weight. Five 9-mm-diameter disks were cut from each sampled leaf and observed under the microscope after clearing with chloral hydrate. Leaf disks also were sampled from relatively young leaves (at top of the plant) and the first true leaf of the plant, which had begun to senesce at the time of sampling.

Data analyses. Data for number of conidia on flowers, area of flower surface colonized by *E. purpurascens,* and sporulation of *E. purpurascens* were analyzed by the Kruskal-Wallis test, and means were separated with Dunn's multiple comparison procedure using experimentwise error rates (9). Percent data and data on germ tube length, pod weight, and chlorophyll content were transformed to arcsines for analysis of variance. Differences among treatments were determined by Duncan's multiple range or LSD tests (21). All experiments were performed at least twice. Where variances were homogenous, data from trials of an experiment were pooled before analysis.

RESULTS

Behavior of *E. purpurascens* on bean flowers. After beans were inoculated with conidial suspensions of *E. purpurascens,* banner petals from emerging flowers had fewer conidia than those of newly opened or senescent flowers (Fig. 2). Addition of ME increased the number of conidia on emerging flowers, but not on newly opened or senescent flowers. Four hours after application, percent conidial germination varied from 6.1–42.9% (Table 1). Germination was greatest on flowers where ME had been added to the conidial suspension, with values for senescent flowers being greater than those for emerging flowers. Without added ME, percentages of germination among the flower groups were not significantly different. At 8 and 12 h, trends were similar. With ME, higher values were found on newly opened flowers than on emerging flowers at 8, but not at 4 or 12, h. Similar relationships were found for germ tube length (Table 2). However, at 4 h, no differences in germ tube length among the various flower groups were apparent, regardless of whether ME was added or not. Differences were apparent in the 8-h sample. With ME, the longest germ tubes were present on senescent flowers, followed by newly opened flowers, then emerging flowers. Without ME, germ tubes on emerging flowers were shorter than on newly opened or senescent flowers. With or without ME, a conidium initially produced one germ tube, but by 8 h two or more germ tubes generally were present on conidia found on open and senescent flowers. Such additional germ tubes usually were not formed on emerging flowers. Measurements could not be made at 12 or 24 h due to intertwining of elongated germ tubes. Conidiophores and immature conidia were present in clusters (sporodochia) at 24 h.

Colonization of banner petals by *E. purpurascens* varied with the flower stage (Table 2). A greater area of senescent flowers was covered by mycelium compared with newly opened flowers or emerging flowers. Mycelial coverage was greater with newly opened flowers than with emerging flowers. Addition of ME increased mycelial coverage on emerging flowers, but not on other stages. Within 96 h, emerging flowers became fully opened flowers, on which a few sporodochia of *E. purpurascens* could be seen. Flowers that were newly opened at the start of the experiment were senescent by this time, and sporodochia covered most of the surface. Flowers that were senescent at the start of the experiment were now dried up, and much of the tissue was filled with conidia of *E. purpurascens.*

Conidia of *E. purpurascens* applied to emerging flowers germinated and resultant germ tubes elongated as the flowers aged (Fig. 1). A few conidia were observed on five of the 40 flowers from unsprayed control plants at 4 and 10 days. Addition of ME and gelatin resulted in increased sporulation at 10 days, but not at 4 days.

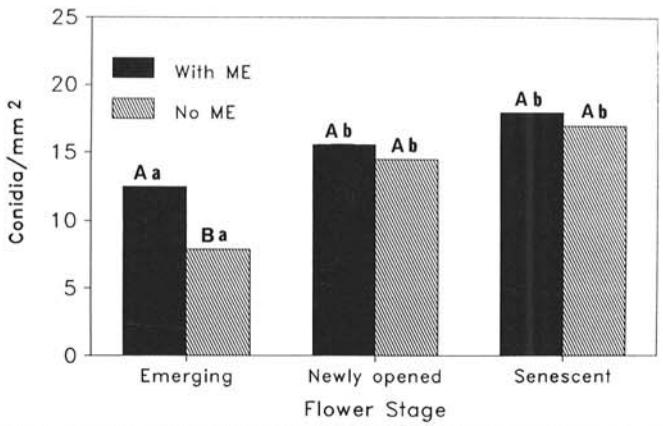

Fig. 2. Number of conidia of *Epicoccum purpurascens* present on bean flowers following spray application with and without malt extract (ME). Bean flowers were sampled 4 h after application of *E. purpurascens.* Columns represent means of 20 petals. Means were separated with Dunn's multiple comparison procedure. Uppercase letters at the top of each column represent comparisons of ME treatments with no ME treatments ($P = 0.05$), within each flower age. Lowercase letters represent comparisons among flower age groups ($P = 0.15$) for ME or no ME treatments. For each type of comparison, means with the same letter are not significantly different from one another.

Effects of *E. purpurascens* on bean. There were no significant differences among treatments with respect to fresh and dry pod weights or abscission of inflorescences, flowers, or pods.

Development of leaf senescence was followed until 20 days after harvest of pods (90 days after planting). Proportion of yellowed leaves (data not shown) was not affected by treatment with *E. purpurascens.* Percentage of yellowed area varied from 33.3 to 46.7% at harvest. The addition of ME appeared to maintain the green color of the leaves.

Effects of *E. purpurascens* on chlorophyll content of leaves. Chlorophyll contents of leaves sprayed with *E. purpurascens* were not significantly different from control leaves. However, means for leaves treated with *E. purpurascens* tended to be greater.

Observations of *E. purpurascens* on leaves. On disks from younger leaves, one-third to one-half of the conidia germinated with a single hypha. Hyphae usually did not elongate much on the surface of epidermal cells and were < 100 to a few hundred micrometers long (data not shown). Additional germ tubes were not produced. No penetration of leaf cells was observed. Similar phenomena were observed on disks from leaves used for

TABLE 1. Effects of bean flower age on percent germination of conidia of *Epicoccum purpurascens*

	Time after application (h)[x]					
	4		8		12	
Flower stage	ME[y]	No ME	ME	No ME	ME	No ME
Emerging	24.7 a*[z]	6.1 a	61.8 a*	40.8 a	86.4 a*	65.5 a
Newly opened	33.1 ab*	11.2 a	73.4 b	58.2 ab	88.8 ab*	76.7 a
Senescent	42.9 b*	15.9 a	84.9 c*	68.9 b	94.9 b*	80.2 a

[x]Bean flowers were sampled 4, 8, and 12 h after application of *E. purpurascens.*

[y]ME: 1% malt extract added to conidial suspension of *E. purpurascens*; no ME: no malt extract added.

[z]Values (means of six petals) in a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). For paired ME and no ME values in each row, the value indicated by an asterisk is significantly greater than the other value according to LSD tests ($P = 0.05$).

TABLE 2. Germ tube growth from conidia of *Epicoccum purpurascens* on bean flowers

	Length of germ tube (μm)[x]				Amount of flower flower surface covered by mycelium[y] (%)	
	4 h		8 h			
Flower stage	ME[z]	No ME	ME	No ME	ME	No ME
Emerging	27.3 a*	8.6 a	66.3 a*	48.9 a	2.5 a*	1.6 a
Newly opened	30.9 a*	14.7 a	106.1 b	105.7 b	2.9 b	2.7 b
Senescent	25.5 a*	11.9 a	130.5 c*	111.4 b	3.7 c	3.5 c

[x]Bean flowers were sampled 4 and 8 h after application of *E. purpurascens.* Means were separated by Duncan's multiple range test (columns) or the LSD test (rows; paired ME and no ME values), respectively. Values (means of six petals) in a column followed by the same letter are not significantly different ($P = 0.05$). For paired ME and no ME values in each row, the value indicated by an asterisk is significantly greater than the other value ($P = 0.05$).

[y]Bean flowers were sampled 24 h after application of *E. purpurascens* and rated as 0, 0%; 1, less than 10%; 2, 10–30%; 3, 31–50%; 4, 51–75%; and 5, more than 75% area covered by mycelium of *E. purpurascens.* Means were separated with Dunn's multiple range test. Values in a column followed by the same letter are not significantly different ($P = 0.15$). Values in rows followed by an asterisk are significantly greater than the other paired value ($P = 0.05$). Values are pooled means of 44 petals.

[z]The conidial suspension contained (ME) or did not contain (no ME) 1% malt extract.

chlorophyll measurements. On disks from older leaves (yellowed but not necrotic), hyphae were longer and more branched than those on young leaves. Hyphae, however, still were relatively short, and no conidiophores or conidia were formed. On necrotic spots on leaves, sporodochia of *E. purpurascens* containing mature and immature conidia were observed.

DISCUSSION

Colonization and growth of *E. purpurascens* were affected by the age of the tissue to which the conidia were applied. The number of conidia per square millimeter of flower was greater on newly opened or senescent flowers than on emerging flowers. In addition, conidial germination was greater on senescent flower petals than on younger tissue. These factors contributed to high rates of colonization of senescent flowers by *E. purpurascens.* It is likely that variations in conidial adherence, germination, and germ tube elongation mainly resulted from physical and chemical differences among the flower stages. Available nutrients leached or exuded from flowers (7,19), or received from other sources, may explain differences in growth, because quantities and compositions of leachates may vary with the age of the tissue being leached (3,23). As with tests in vitro (26), addition of ME greatly improved conidial germination of *E. purpurascens* on flowers. Also, ME stimulated an increase in mycelial coverage of emerging flowers. This, however, did not occur on newly opened or senescent flowers. Increases in germination and rates of growth following addition of nutrients may indicate that there is a limitation of nutrients available to *E. purpurascens* on emerging flowers.

Conidia of *E. purpurascens* were able to survive on emerging flowers and eventually colonize the flowers. Colonization, however, was not as extensive as when conidia were applied directly to older flowers. Nevertheless, it may be possible to apply conidia of *E. purpurascens* to earlier growth stages of beans to help protect flowers from infection by *S. sclerotiorum.* This may increase efficiency of white mold control by *E. purpurascens,* because ascospores of *S. sclerotiorum* infect young flowers of bean when artificially inoculated (1). However, additives to the spray suspension will have to include nutrients selected to assist *E. purpurascens* in the colonization of emerging flowers. Although ME is of use in this regard, additional nutrients may be required.

During these studies, plants used as controls were placed on a greenhouse bench together with plants treated with *E. purpurascens.* However, flowers on control plants were colonized only rarely by *E. purpurascens,* indicating that movement of conidia of *E. purpurascens* between plants was not great even though there was considerable air movement in the greenhouse during this period. In previous field experiments (25), we observed that plants not treated with *E. purpurascens* rarely had flowers colonized by this fungus, even when plants in adjacent plots had been inoculated with *E. purpurascens.* Although *E. purpurascens* violently releases conidia (18), it seems doubtful that conidia of *E. purpurascens* on leaves and stems could provide enough inoculum to efficiently colonize flowers on the same plant. Limited movement of conidia within and among plants may reduce the efficiency of biocontrol in the field, but it also may reduce chances for spread of *E. purpurascens* outside of the treated crop.

Application of *E. purpurascens* to plants under growth chamber conditions did not have deleterious effects. Yields and percentages of abscised flowers and pods were similar in all treatments. These results are similar to findings from earlier field and greenhouse studies (25). As with previous studies on wheat (15), application of *E. purpurascens* did not accelerate senescence of bean leaves or affect chlorophyll content. This may be because *E. purpurascens* did not penetrate cells of leaves until tissue was in an advanced state of senescence. The fungus also may produce auxin (8). However, it should be noted that, whereas snap beans are harvested when pods are fresh, dry beans are harvested when seed is mature. It is not known if *E. purpurascens* will accelerate senescence of bean leaves during maturing of bean seeds and affect seed quality, although this seems unlikely. Also, higher concentrations of *E. purpurascens* may result in harmful effects on plant growth, particularly if concentrations of these dark-colored conidia are great enough to interfere with light reception by leaves. Promotion of early colonization of young flowers by adding additional nutrients to spray suspensions may decrease yield if pod formation is affected.

Superior performance in bioassays (5,17), effective suppression of white mold in greenhouse and field trials (25), and the availability of improved strains (26), together with the results presented here, indicate that *E. purpurascens* has potential as a biological control agent. Knowledge of the mode of antagonism of *S. sclerotiorum* by *E. purpurascens* would provide useful additional information for development of formulation techniques and further improvements in efficiency of disease control.

LITERATURE CITED

1. Abawi, G. S., Polach, F. J., and Molin, W. T. 1975. Infection of bean by ascospores of *Whetzelinia sclerotiorum.* Phytopathology 65:673-678.
2. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris.* Plant Physiol. 24:1-15.
3. Blakeman, J. P. 1972. Effect of plant age on inhibition of *Botrytis cinerea* spores by bacteria on beetroot leaves. Physiol. Plant Pathol. 2:143-152.
4. Blakeman, J. P. 1988. Competitive antagonism of air-borne fungal pathogens. Pages 141-160 in: Fungi in Biological Control Systems. M. N. Burge, ed. Manchester University Press, Manchester, UK. 269 pp.
5. 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.
6. Booth, C. 1980. *Epicoccum purpurascens.* No. 680 in: Description of Pathogenic Fungi and Bacteria. Commonwealth Mycological Institute, Kew, Surrey, UK.
7. Brown, W. 1922. Studies in the physiology of parasitism. VIII. On the exosmosis of nutrient substances from the host tissue into the infection drop. Ann. Bot. 36:101-119.
8. Buckley, N. G., and Pugh, G. J. F. 1971. Auxin production by phylloplane fungi. Nature 231:332.
9. Daniel, W. W. 1978. Applied Nonparametric Statistics. Houghton Mifflin Co., Boston. 503 pp.
10. Dickinson, C. H. 1967. Fungal colonisation of *Pisum* leaves. Can. J. Bot. 45:915-927.
11. Fokkema, N. J. 1981. Fungal leaf saprophytes, beneficial or detrimental? Pages 433-454 in: Microbial Ecology of the Phylloplane. J. P. Blakeman, ed. Academic Press, London. 502 pp.
12. Goel, R. K., and Gupta, A. K. 1979. A new blotch disease of wheat in India. Plant Dis. Rep. 63:620.
13. Hiscox, J. D., and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332-1334.
14. Hunter, J. E., Abawi, G. S., and Crosier, D. C. 1978. Effect of timing, coverage, and spray oil on control of white mold of snap bean with benomyl. Plant Dis. Rep. 62:633-637.
15. Jachmann, H.-Th., and Fehrmann, H. 1989. Effects of phyllosphere micro-organisms on the senescence of wheat leaves. Z. Pflanzenkrankh. Pflanzenschutz 96:124-133.
16. Khatua, D. C., Maiti, S., and Chakravarty, D. K. 1977. Rice diseases newly recorded from India. Plant Dis. Rep. 61:511.
17. 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.
18. Meredith, D. S. 1966. Diurnal periodicity and violent liberation of conidia in *Epicoccum.* Phytopathology 56:988.
19. Mitchell, C. A. 1968. Detection of carbohydrates leached from above-ground plant parts. M.S. thesis. Cornell University, Ithaca, NY.
20. Pugh, G. J., and Buckley, N. G. 1971. The leaf surface as a substrate for colonization by fungi. Pages 432-445 in: Ecology of Leaf Surface Microorganism. T. F. Preece and C. H. Dickinson, eds. Academic Press, London. 640 pp.
21. SAS. 1987. Statistical Analysis System. SAS Institute, Cary, NC.
22. Smedegaard-Petersen, V., and Tolstrup, K. 1986. Saprophytic leaf fungi limit yield in cereals. Pages 160-171 in: Microbiology of the Phyllosphere. N. J. Fokkema and J. Van Den Heuvel, eds. Cambridge University Press, Cambridge, UK. 392 pp.
23. Stenlid, G. 1958. Salt losses and redistribution of salts in higher plants. Encycl. Plant Physiol. 4:615-637.
24. Wiebold, W. J., Ashley, D. A., and Boerma, H. R. 1981. Reproductive

abscission levels and patterns for eleven determinate soybean cultivars. Agron. J. 73:43-46.
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.