

# Application of *Epicoccum purpurascens* Spores to Control White Mold of Snap Bean

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## ABSTRACT

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The efficacy of *Epicoccum purpurascens* in controlling white mold of snap bean, caused by *Sclerotinia sclerotiorum*, was assessed in greenhouse and field trials in 1987 and 1988. Treatments were applied to plants during flowering, and plants were subsequently sprayed with ascospores of *S. sclerotiorum*. In two field trials in 1987, applications of an iprodione-tolerant strain of *E. purpurascens* (in 1% malt extract) and/or iprodione (0.5 kg a.i./ha) significantly reduced disease incidence and disease index relative to control plots. In two field trials in 1988, three applications of *E. purpurascens* in 1% malt extract and two applications of iprodione significantly reduced disease incidence, disease index, and percentage of diseased pods. Similar results were obtained in greenhouse studies.

Additional keywords: biocontrol, *Phaseolus vulgaris*

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most common and destructive diseases of bean (*Phaseolus vulgaris* L.) in temperate regions (16). Epidemics usually are initiated by ascospores (16) that land on senescing or dead flower petals. After colonizing the flower, mycelium can infect adjacent pods, leaves, or stems. Thus, white mold epidemics generally occur during or after flowering of the crop (16).

Hunter et al (8) reported that spraying blossoms with fungicide effectively controlled the disease, but no control was obtained when all aboveground plant parts except blossoms were covered with fungicide. This suggests that the key to

controlling white mold is to protect blossoms from colonization by ascospores of *S. sclerotiorum*. Colonization of blossoms by organisms antagonistic to *S. sclerotiorum* might suppress epidemics of white mold (10). If so, such biocontrol agents could augment or replace fungicides used in the management of this disease.

*Epicoccum purpurascens* Ehrenb. ex Schlecht. (syn. *E. nigrum* Link) is a saprophytic fungus commonly found on plant surfaces (11). It inhibited the elongation of germ tubes of ascospores in vitro and reduced infection of lettuce by *S. sclerotiorum* in growth chamber tests (10). Our objective in this study was to determine the efficacy of *E. purpurascens* in controlling white mold of snap bean under greenhouse and field conditions. A portion of these results has been reported previously (17).

## MATERIALS AND METHODS

**Spore production.** Spores of an isolate of *E. purpurascens*, recovered from the surface of a lettuce leaf (11), were treated with short-wave ultraviolet irradiation.

Irradiated spores were placed on an iprodione-amended medium, and strains that grew were assayed for tolerance to iprodione. One of the resulting strains (R4000), tolerant to 2,000  $\mu\text{g/ml}$  iprodione (compared to 10  $\mu\text{g/ml}$  for the original isolate), was used in all experiments and was maintained under sterile mineral oil on potato-dextrose agar (PDA) slants at 8 C.

For all experiments, spores of *E. purpurascens* were obtained from 14- to 20-day-old cultures grown on a wheat kernel medium held at  $22 \pm 2$  C. To prepare the medium, cleaned wheat kernels were soaked in water for 12 hr at 40 C (or boiled for 20 min), then 30-40 ml of the seeds was placed in a 125-ml Erlenmeyer flask and autoclaved at 121 C, 104 kPa, for 1 hr. After flasks had cooled, spores from PDA slant cultures or wheat kernel cultures were added to the flasks.

Ascospores of *S. sclerotiorum* (isolate MACF-152) were produced and maintained as described previously (10).

**Preparation of spore suspensions.** Spores of *E. purpurascens* were washed from wheat kernel cultures with distilled water containing 0.01% Tween 80. Mycelium and wheat kernels were removed by filtering through three layers of cheesecloth. For field tests, the filtrate was adjusted to  $1 \times 10^6$  spores per milliliter with distilled water containing 0.01% Tween 80. For greenhouse tests, spores were washed twice by centrifugation before the concentration was adjusted to  $1 \times 10^6$  spores per milliliter. For some treatments, suspensions of *E. purpurascens* were amended with 1% malt extract, 1% potato-dextrose broth, and/or iprodione (Rovral 50WP) before application.

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Ascospores of *S. sclerotiorum* ( $1 \times 10^6$  spores per milliliter) were suspended in phosphate buffer (0.01 M, pH 6.0) containing 0.01% Tween 80. The concentrations of *E. purpurascens* spores and *S. sclerotiorum* ascospores were determined with a hemacytometer.

**Greenhouse tests.** Snap beans (cv. Strike) were seeded in Pro-Mix BX (Les Tourbières Premier Ltée., Rivière du Loup, Quebec) in pots 130 mm in diameter to provide 10 replications of one plant per pot for each treatment. Plants were fertilized with a 0.1% NPK (20-20-20) solution every second week after emergence. Greenhouse temperatures were maintained at  $24 \pm 2$  C, with a 14-hr photoperiod. Spore suspensions of *E. purpurascens* and/or nutrient solutions (see Table 1 for treatment combinations) were applied when all plants had at least one open flower and again 4 days later. Tween 80 (0.01%) was added to all treatments as a wetting agent.

Plants were inoculated with ascospores of *S. sclerotiorum* 24 hr after the second treatment. For each inoculation, plants were sprayed with a hand-held pump sprayer until runoff (about 15 ml per plant, applied over the entire surface), permitted to air-dry for 1 hr, and then placed in a plastic mist chamber in a greenhouse. Plants were misted each night from 8 p.m. to 8 a.m. with an electric atomizer. Plastic sheets were removed from the sides of the chamber to permit air circulation during the day. This regime was continued until disease symptoms appeared. No mist was provided after that time, and plastic sheets were removed from the sides of the chamber. The number of lesions and the percentage of diseased leaf and stem area on each plant were determined 6 and 14 days, respectively, after application of ascospores. The experiment was performed twice.

**Field trials.** A randomized complete block design with four replications of each treatment was used for field trials in 1987 and 1988. Snap beans (cv. Strike) were seeded at a rate of 20 seeds per meter of row, with 0.5 m between rows. Each plot consisted of four rows 3.2 m long. Treatments were applied during flowering (which began 36–41 days after planting) and, except where indicated, all plants were inoculated with ascospores of *S. sclerotiorum* 1 day after the last treatment. All inoculations were performed with a hand-held compressed-air sprayer in the evening (7–10 p.m.), 2–3 hr after 1 hr of sprinkler irrigation. About 1.5 L of suspension was used for each plot, and care was taken to ensure that flowers and buds were covered, although stem and leaf tissue was also sprayed.

During the first week after inoculation, plots were sprinkler-irrigated for 30 min every second day to ensure high levels of disease. The number of lesions on 20 plants in each plot was determined 6 days after inoculation. Pod weight, disease incidence, disease severity (percentage of leaf and stem area diseased), and the percentage of infected pods were determined at harvest from two 2-m sections of each of the two middle rows of each plot.

All trials were located at the Macdonald College Research Station, Ste. Anne de Bellevue, Quebec, on land that was cropped to lettuce in 1985 and 1986. The lettuce plants had been artificially inoculated with *S. sclerotiorum* in both years.

In 1987, two trials with similar treatments were seeded on 1 June and 2 July and harvested on 6 August and 3 September, respectively. The treatments used in one trial are listed in Table 2. The first treatments were applied when 70% of plants had at least one open flower. In 1988, two trials with similar

treatments were seeded on 7 and 28 June and harvested on 10 and 31 August, respectively. The treatments used in one trial are listed in Table 2. The first treatments were applied when all plants had at least one open flower. In each year, one trial contained a treatment where *E. purpurascens* was applied three times without subsequent inoculation with ascospores of *S. sclerotiorum*.

In 1988, 30–40 senescing flowers were collected from each plot, and at least 10 of these were examined under a stereomicroscope ( $\times 30$ ) for the presence of sporulating colonies of *E. purpurascens*. Petals were then cleared with chloral hydrate (3) and observed under a compound microscope ( $\times 200$ ). Samples of leaves also were collected and examined similarly.

**Data analyses.** Data from the greenhouse trials for the number of lesions were analyzed using the Kruskal-Wallis test (4). Treatment means were separated using Dunn's multiple comparison procedure (4). Similar data from the field trials were analyzed with the Friedman analysis of variance (ANOVA) and the associated multiple comparison procedure (4).

Percentage data (disease incidence, disease index, and percentage of pods with white mold) were arcsine-transformed before ANOVA when the range of percentages among treatments was greater than 40 (9). Yield data were not transformed before ANOVA. Treatments were compared using Duncan's multiple range test ( $\alpha = 0.05$ ) (15).

## RESULTS

**Greenhouse tests.** Typical symptoms of white mold appeared 4 days after inoculation with ascospores of *S. sclerotiorum*. All treatments containing *E. purpurascens* significantly reduced the number of white mold lesions per plant compared to the water control (Table 1). Addition of nutrients to spore suspensions of *E. purpurascens* and treatment with nutrients alone had no significant effect on the number of lesions. When applied alone, malt extract (1%) and potato-dextrose broth (1%) appeared to reduce the percentage of leaves and stems with disease symptoms, but these effects were not significant. Treatment with *E. purpurascens*, however, resulted in significantly less diseased tissue than treatment with water or nutrients. *E. purpurascens* was observed to colonize senescent petals. Both trials provided similar results.

**Field experiments.** In 1987, plants that received four applications of *E. purpurascens* in 1% malt extract and those that received two applications of *E. purpurascens* combined with iprodione had significantly fewer white mold lesions than plants in control plots (Table 2). Treatment with *E. purpurascens* plus 1% malt extract (two or

**Table 1.** Effect of application of *Epicoccum purpurascens* and nutrient solutions on the development of white mold on greenhouse-grown snap beans

| Treatment <sup>a</sup>                            | Number of plants with symptoms <sup>w</sup> | Number of lesions <sup>x</sup> | Diseased tissue <sup>y</sup> (%) | Pod rot <sup>z</sup> (%) |
|---|---|--------------------------------|----------------------------------|--------------------------|
| 1% Malt extract                                   | 9   | 6.5 a                          | 59.0 b                           | 15.9                     |
| <i>E. purpurascens</i> + 1% malt extract          | 7   | 1.3 b                          | 10.0 c                           | 10.9                     |
| 1% Potato-dextrose broth                          | 10  | 5.4 a                          | 41.5 b                           | 17.6                     |
| <i>E. purpurascens</i> + 1% potato-dextrose broth | 5   | 0.9 b                          | 12.0 c                           | 9.0                      |
| <i>E. purpurascens</i>                            | 8   | 1.2 b                          | 17.5 c                           | 7.8                      |
| Water   | 10  | 6.5 a                          | 81.0 a                           | 26.6                     |

<sup>a</sup> Treatments were applied when all plants had at least one open flower and again 4 days later. Tween 80 (0.01%) was added to all applications. Ascospores of *Sclerotinia sclerotiorum* were applied to all plants 1 day after the second application of each treatment.

<sup>w</sup> Ten plants were observed.

<sup>x</sup> Data were collected 6 days after inoculation with ascospores of *S. sclerotiorum*. Values followed by the same letter are not significantly different by the Kruskal-Wallis test ( $p > 0.0001$ ) and Dunn's multiple comparison procedure (experimentwise error rate = 0.75).

<sup>y</sup> Percentage of leaf and stem area diseased, assessed 14 days after inoculation. Values in a column followed by the same letter are not significantly different by Duncan's multiple range test ( $\alpha = 0.05$ ). Percentage data were arcsine-transformed before analysis.

<sup>z</sup> Percentage of pods with white mold among all 10 plants in each treatment.

four times), *E. purpurascens* plus iprodione (two times), and iprodione (0.5 kg a.i./ha) alone (one time) significantly reduced disease incidence, disease index, and the percentage of pods with white mold. However, two applications of *E. purpurascens* in 1% malt extract and one application of iprodione alone at 0.25 kg a.i./ha had less effect on the percentage of pods with mold. Yield was not significantly affected in one trial (Table 2), although in the other trial (*data not shown*) applications of *E. purpurascens* in 1% malt extract (three or five times), iprodione (0.5 kg a.i./ha) alone (two times), and iprodione combined with *E. purpurascens* (three times) did significantly increase yields.

Typical white mold symptoms also appeared in plots where plants were treated with *E. purpurascens* but not inoculated with *S. sclerotiorum* ascospores (*data not shown*). Disease in these plots was assumed to be the result of natural infection. Although these plots had disease ratings similar to those of plots that received *E. purpurascens* and/or iprodione followed by *S. sclerotiorum*, yield did not differ significantly from the control.

In 1988, plants in plots that received *E. purpurascens* in 1% malt extract (three times), iprodione (0.5 kg a.i./ha) (two times), and iprodione (0.25 kg a.i./ha) plus *E. purpurascens* (two times) had significantly lower values for the number of lesions, disease incidence, disease index, and percentage of pods with white mold than those in plots that received only water (Table 2). *E. purpurascens* alone (three times) had a similar efficacy except in the number of lesions. Two applications of *E. purpurascens* in 1% malt extract resulted in significantly lower values than the control only with respect to disease incidence and the percentage of pods with white mold. Treatment with 1% malt extract alone provided some reduction in the percentage of pods with white mold; however, the effect was significantly less than that achieved with three applications of *E. purpurascens* in 1% malt extract. Yields in plots receiving *E. purpurascens* in 1% malt extract (three times), iprodione (two times), and iprodione plus *E. purpurascens* (two times) were significantly higher than those of the control plots. Results of both trials were similar. When *E. purpurascens* was applied three times but not followed by inoculation with *S. sclerotiorum*, disease ratings were similar to those obtained for the same treatment in 1987 except that yield was significantly higher than in the control plot (*data not shown*).

Colonies of *E. purpurascens* with spores and sporodochia were readily detected with the stereomicroscope on all samples of senescing flowers from plants treated with *E. purpurascens*. In most cases, colonies completely covered the

petal surface. Colonization by *E. purpurascens* did not appear to be inhibited on flowers that received mixtures of *E. purpurascens* and iprodione, although quantitative measurements were not made. No colonies of *E. purpurascens* were observed on healthy leaves, although many spores could be seen. Flowers from plots where *E. purpurascens* was not applied rarely possessed colonies of *E. purpurascens*.

## DISCUSSION

Results from both greenhouse and field experiments indicate that *E. purpurascens* can reduce disease incidence and disease index of white mold of bean. At least one of the treatments containing *E. purpurascens* resulted in yields significantly higher than those in control treatments in three out of four field trials.

Spores of *E. purpurascens* can be found on the surface of plants in early stages of growth but usually are more frequent on older or newly dead plant tissue (5). The fungus appears to establish itself and sporulate quickly on senescent or dead plant tissue. This ability, plus the production of antifungal compounds (2), makes *E. purpurascens* an attractive

choice for controlling *S. sclerotiorum* on beans and other crops (10).

No previous studies have shown that *E. purpurascens* or other antagonists control white mold of bean under field conditions. *E. purpurascens* also may be useful for controlling gray mold of beans caused by *Botrytis cinerea*, which also infects bean blossoms before invading other parts of the plant (13).

The effects of malt extract in promoting disease control did not appear to be very great in this study; however, other nutrients may prove to be useful additives to antagonist suspensions (12).

The isolate of *E. purpurascens* used in these studies was tolerant to iprodione, and observations of petals collected from the field suggest that it colonized petals well in the presence of iprodione. More critical studies are needed, but tolerance to fungicides appears to be a useful characteristic for biocontrol agents. Tolerant strains may be combined with a fungicide to provide increased control of the pathogen (either by eliminating saprophytic microflora competing with the antagonist or through the chemical's direct effects on the pathogen) and may allow a biocontrol agent for one pathogen to be packaged with a chemical

**Table 2.** Effect of application of *Epicoccum purpurascens* and iprodione on the development of white mold of snap bean in the field

| Treatment <sup>w</sup>                               | Dates of treatment | Number of lesions <sup>x</sup> | Disease incidence <sup>y</sup> (%) | Disease index <sup>z</sup> (%) | Pod rot <sup>y</sup> (%) | Yield <sup>y</sup> (tons/ha) |
|--|--------------------|--------------------------------|------------------------------------|--------------------------------|--------------------------|------------------------------|
| 1987 (2 July)  |                    |                                |                                    |                                |                          |                              |
| Control  |                    | 26 a                           | 78.2 a                             | 27.0 a                         | 12.2 a                   | 16.65 a                      |
| <i>E. purpurascens</i> + 1% malt extract             | 10,16 Aug.         | 13 a                           | 60.9 b                             | 17.0 b                         | 5.2 bc                   | 17.23 a                      |
| <i>E. purpurascens</i> + 1% malt extract             | 6,10,14, 17 Aug.   | 8 b                            | 51.7 b                             | 12.1 b                         | 4.0 c                    | 18.35 a                      |
| Iprodione (0.5 kg a.i./ha)                           | 10 Aug.            | 15 a                           | 43.5 b                             | 14.3 b                         | 4.4 c                    | 16.85 a                      |
| <i>E. purpurascens</i> + iprodione (0.25 kg a.i./ha) | 10,16 Aug.         | 7 b                            | 40.8 b                             | 10.1 b                         | 3.5 c                    | 17.30 a                      |
| Iprodione (0.25 kg a.i./ha)                          | 10 Aug.            | 17 a                           | 61.2 b                             | 15.3 b                         | 6.7 b                    | 16.35 a                      |
| 1988 (28 June)                                       |                    |                                |                                    |                                |                          |                              |
| Water  | 6,9,12 Aug.        | 24 a                           | 82.1 a                             | 27.7 a                         | 24.0 a                   | 13.97 b                      |
| 1% Malt extract                                      | 6,9,12 Aug.        | 16 a                           | 73.8 ab                            | 21.3 ab                        | 17.3 b                   | 14.02 b                      |
| <i>E. purpurascens</i>                               | 6,9,12 Aug.        | 9 ab                           | 61.8 bc                            | 16.7 bc                        | 12.1 bc                  | 16.15 ab                     |
| <i>E. purpurascens</i> + 1% malt extract             | 6,9,12 Aug.        | 4 b                            | 52.1 c                             | 13.0 bc                        | 7.5 cd                   | 17.45 a                      |
| Iprodione (0.5 kg a.i./ha)                           | 8,12 Aug.          | 6 b                            | 48.5 c                             | 11.2 c                         | 5.4 d                    | 16.86 a                      |
| <i>E. purpurascens</i> + iprodione (0.25 kg a.i./ha) | 8,12 Aug.          | 5 b                            | 57.7 bc                            | 15.3 bc                        | 9.2 cd                   | 17.95 a                      |
| <i>E. purpurascens</i> + 1% malt extract             | 8,12 Aug.          | 12 ab                          | 62.3 bc                            | 20.9 ab                        | 11.2 cd                  | 14.05 b                      |

<sup>w</sup>Control plants (1987) were inoculated only with *Sclerotinia sclerotiorum*. Water treatment (1988 control treatment) was distilled water containing 0.01% Tween 80. Tween 80 (0.01%) was added to all suspensions of *E. purpurascens*. Ascospores of *S. sclerotiorum* were applied to all plots on 18 August in 1987 and on 13 August in 1988.

<sup>x</sup>Number of lesions was recorded from 20 plants in each plot on 24 August in 1987 and on 19 August in 1988. The data were analyzed using Friedman analysis of variance. Values followed by the same letter are not significantly different (multiple comparison test, experimentwise error rate = 1.05).

<sup>y</sup>Values for each year in a column followed by the same letter are not significantly different by Duncan's multiple range test ( $\alpha = 0.05$ ). Percentage data of disease incidence were arc-sine-transformed before analysis.

<sup>z</sup>Disease severity classes: 0 = 0%, 1 = 1–10%, 2 = 11–30%, 3 = 31–50%, 4 = 51–75%, 5 = 76–100% of stem and leaf area diseased. Disease index =  $(\sum S_i n_i / 5N) \times 100$  ( $i = 1, 2, \dots, 5$ ), where  $S$  is the appropriate disease class,  $n$  = number of diseased plants in the same class, and  $N$  = number of plants rated.

targeted for a second pathogen. In our studies, combining *E. purpurascens* with iprodione did not appear to provide levels of control greater than those provided by either agent alone, indicating that there were no additive or synergistic effects.

Although *E. purpurascens* was effective in the field in this study, much work needs to be done before it can be offered as an alternative or a supplement to chemical control. In these studies, two to four applications of *E. purpurascens* were required to provide control similar to that provided by fewer applications of iprodione. *E. purpurascens* was applied before *S. sclerotiorum* to give *E. purpurascens* some opportunity to colonize flowers before the arrival of ascospores, but this may not always be the case under conditions of natural infection. Although *E. purpurascens* generally is considered to be a nonpathogen, some reports have shown that it can act as a weak pathogen on certain plants (6). Moreover, *E. purpurascens* has been reported as an allergen, and culture extracts have been shown to be toxic to mice (7,14). Nevertheless, *E. purpurascens* appears to have good potential as a biocontrol agent, and strain selection to overcome these problems should be a major goal.

The usefulness of additives such as nutrients and other compounds to promote the growth and survival of *E. purpurascens* needs to be investigated.

The ability of bean stems, leaves, and flower buds to act as sources of spore populations for colonization of senescing flowers should be assessed. Other microorganisms may also be potential antagonists (10), and they merit more extensive testing, either alone or in combination with *E. purpurascens*. When coupled with the development of techniques to increase the rate of destruction of soilborne sclerotia (1), the approach outlined here may result in integrated control of white mold of bean and similar diseases.

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