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Biological Control of Botrytis Stem and Blossom Blight of Lentil

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ABSTRACT

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Greenhouse and field studies were conducted to assess the effectiveness of 32 antagonistic isolates of bacteria and fungi for control of stem and blossom blight of lentil caused by *Botrytis cinerea*. All of the isolates were effective in reducing the incidence and severity of the disease in the greenhouse when applied as foliar sprays at the early bloom stage. Four of the isolates including *Pantoea agglomerans, Pseudomonas fluorescens, Penicillium griseofulvum* and *Trichoderma hamatum* tested in the greenhouse, increased the seed yield and reduced the rate of seed infection by *B. cinerea*. In field experiments of 1998-2000, the same four isolates significantly reduced incidence and severity of botrytis stem and blossom blight of lentil, and increased seed yield compared to the *Botrytis*-inoculated control. *Trichoderma hamatum* was the most consistent among these four agents. This study suggests that foliar application of microbial antagonists may be an effective measure for the control of botrytis stem and blossom blight of lentil.

Key words : Lentil, *Lens culinaris*, botrytis stem and blossom blight, *Botrytis cinerea*, biological control, *Trichoderma hamatum*, *Penicillium griseofulvum, Pantoea agglomerans*, *Pseudomonas fluorescens*

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important pulse crop on the western Canadian prairies. Canadian lentil production has increased steadily in recent years, reaching 698,900 hectares in 2000, with more than 95 percent of production occurring in the province of Saskatchewan [(4)]. Canada has recently achieved the status of the world's largest producer and exporter of lentil [(1)].

Botrytis stem and blossom blight, caused by *Botrytis cinerea* Pers.:Fr., is a disease which affects lentil plants during the flowering stage, and can reduce the yield and seed quality of the crop. The disease is serious and widespread in Saskatchewan [(15)] and southern Alberta [(6,7,8)]. At present, control of botrytis stem and blossom blight is largely dependent on cultural practices such as crop rotation and use of healthy seed, and no resistant lentil varieties are available.

Studies conducted by Huang *et al.* [(13)] on the use of foliar-applied biocontrol agents have shown that some fungal agents such as *Coniothyrium minitans* Campbell and *Trichoderma virens* (Miller, Giddens and Foster) Arx. are effective in reducing the incidence of white mold of dry bean caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. The aim of the current study was to evaluate bacteria and fungi collected in the Canadian prairies, as microbial agents for control of botrytis stem and blossom blight of lentil, using a foliar spray technique.

MATERIALS AND METHODS

Thirty plant samples including alfalfa (*Medicago sativa* L.), alsike clover (*Trifolium hybridum* L.), aspen (*Populus tremuloides* Michx.), barley (*Hordeum vulgare* L.), canola (*Brassica napus* L. and *B. rapa* L.), common labrador tea (*Ledum groenlandicum* Oeder), common wild rose (*Rosa woodsii* Lindl.), cow parsnip (*Heracleum lanatum* Michx.) crested wheat grass (*Agropyron cristatum* (L.) Gaertn.), fireweed (*Epilobium angustifolium* L.), pea (*Pisum sativum* L.), sagebrush (*Artemisia* spp.), saskatoon-berry (*Amelanchier alnifolia* Nutt.), tamarack (*Larix laricina* (Du Roi) K. Koch), wheat (*Triticum aestivum* L.), white clover (*Trifolium repens*

L.) and yarrow (*Achillea millefolium* L.) were collected from central and southern Alberta during July 1996, air-dried at room temperature (20±2℃) and stored in paper bags until used. Using the isolation and dual culture methods described by Liang *et al.* [14], 281 bacterial and 89 fungal isolates were obtained from the rhizospheres of the plant samples, and were tested together with 24 additional cultures (15 bacteria and 9 fungi) from the culture collection at the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta, Canada, for antagonism to *B. cinerea* isolate LRC 2421.

Twelve fungal isolates and 20 bacterial isolates showing antagonistic effects to *B. cinerea* in dual culture were selected for further testing of control of botrytis stem and blossom blight of lentil by foliar spray in the greenhouse. The bacteria were 2 isolates of *Bacillus cereus* Frankland & Frankland (LRC 805, LRC 1195), 1 isolate of *Bacillus circulans* Jordan (LRC 1675), 3 isolates of *Bacillus subtilis* (Ehrenberg) Cohn (LRC 886, LRC 1105, LRC 1786), 1 isolate of *Erwinia rhapontici* (Millard) Burkholder (LRC 946), 3 isolates of *Paenibacillus polymyxa* (Prazmowski) Ash *et al.* (LRC 806, LRC 959, LRC 1784), 2 isolates of *Pantoea agglomerans* (Beijerinck) Gavini *et al.* (LRC 954, LRC 1787), 6 isolates of *Pseudomonas fluorescens* (Trevisan) Migula (LRC 939, LRC 941, LRC 1192, LRC 1654, LRC 1673, LRC 1788), and 2 isolates of *Pseudomonas putida* (Trevisan) Migula (LRC 945, LRC 1785). The fungi were *Coniothyrium minitans* LRC 2137, *Epicoccum purpurascens* Ehrenb.:Schlect. LRC 2250, *Gliocladium catenulatum* Gilman & Abbott LRC 2087, *Gliocladium roseum* (Link.) Bainier LRC 2090, *Penicilllium aurantiogriseum* Dierckx LRC 2450, 2 isolates of *Penicillium griseofulvum* Dierckx (LRC 2461, LRC 2464), *Talaromyces flavus* (Klöcker) A.C. Stock & R.A. Sampson LRC 2152, *Trichoderma hamatum* (Bonord.) Bainier LRC 2475, *Trichoderma harzianum* Rifai LRC 2428, *Trichoderma virens* LRC 2425 and *Trichoderma viride* Pers.:Fr. LRC 2424.

Seeds of lentil, cultivar Laird, were sown into Cornell Peat-Lite Mix™ [3] in plastic pots (15 cm diameter x 12 cm deep), 5 seeds per pot. Plants were thinned to 3 per pot after emergence of seedlings, and were grown in the greenhouse until they reached the early flowering stage. Each experiment consisted of six treatments including four microbial treatments (isolates), a *Botrytis*-inoculated control, and an uninoculated control. Treatments were arranged in a completely randomized design, with three pots (replicates) for each treatment. Each experiment was performed twice.

Biocontrol agents were grown on potato dextrose agar (PDA) at 20℃ under continuous fluorescent light for 3 days for bacterial agents or 21 days for fungal agents. The cultures were flooded with sterile distilled water, colonies were gently scraped, and the resulting suspensions of bacteria or fungal spores were passed through four layers of cheesecloth. The concentrations of the suspensions were adjusted to 10^8 colony forming units (cfu)/ml for bacteria, and 10^7 spores/ml for fungi, except for *E. purpurascens*, which was adjusted to 10^6 spores/ml. Suspensions were mixed with PelGel™ (Lipha-Tech, Milwaukee, WI) at 5 g/liter and Tween 20 (polyoxyethylenesorbitan monolaurate; Fisher Scientific, Fair Lawn, NJ) at 0.17 ml/liter and sprayed onto the plants at the early flowering stage at a rate of 150 ml/pot. The control pots received water containing the same rate of PelGel™ and Tween 20. Plants were covered with plastic bags (45 cm wide x 80 cm long) for 48 h, and were then inoculated with *B. cinerea*.

Botrytis cinerea isolate LRC 2421 was grown under continuous fluorescent light for 21 days on PDA at 20℃, and spores were harvested by the method previously described for the biocontrol agents, to make suspensions containing 10^7 spores/ml. Plants were uncovered, sprayed with the suspension of *B. cinerea* at a rate of 150 ml/pot, covered again with the same plastic bags, and returned to the greenhouse.

Ten days after inoculation with *B. cinerea*, plastic bags were removed from the plants, and the number of infection loci on each plant was counted. In addition, each plant was rated for overall severity of stem and blossom blight, based on a rating scale of 1 to 4, where 1=healthy plant, 2=one stem infected, 3=more than one stem infected, and 4=dead plant. Infected pods and stems were collected and plated on PDA to verify infection by *B. cinerea* and development of the disease. Seeds were harvested from each plant at maturity and weighed, and the number of healthy and infected seeds was determined. To verify infection by *B. cinerea*, seeds were surface sterilized for 90 sec in 70% ethanol, dried on paper towel, plated on PDA in petri plates, incubated at 20℃ for 10 days, and examined for the presence of the pathogen.

Field experiments were conducted at the Fairfield Farm of the Lethbridge Research Centre during 1998, 1999 and 2000, using the lentil cultivar Laird. The field was treated with ammonium nitrate fertilizer (34-0-0; N-P-K) prior to seeding, at a rate of 200 kg/ha. Lentil crops were seeded on 22 May 1998, 21 May 1999 and 15 May 2000, at a seeding rate of 100 kg/ha. Planting was done in a continuous strip, using a four row plot seeder set at a depth of 4 cm and a row spacing of 22.5 cm. In each year, the experiment was conducted in a fallowed area of the field.

After seedling emergence, individual plots were established, with each plot consisting of eight, 3.5 m- long rows. Treatments were set up each year, arranged in a randomized block design with four replications. Uninoculated and *Botrytis*-inoculated controls were included in the experiments.

Four biocontrol agents were selected for use in the field experiments, based on the results of the greenhouse experiments. The two bacterial isolates were *Pantoea*

agglomerans LRC 954 and *Pseudomonas fluorescens* LRC 1788, and the two fungal isolates were *Penicillium griseofulvum* LRC 2461 and *Trichoderma hamatum* LRC 2475. The inoculum of each biocontrol agent was prepared by the method described in the greenhouse experiments. The biocontrol agents were sprayed onto the plots during the early flowering stage (early July) using a garden hand-pump sprayer at a rate of 300 ml/m^2. Ten days after the initial spray, the biocontrol agents were sprayed for a second time, using the same amount of inoculum as for the first spray. Forty-eight hours after each application of biocontrol agents, plots were sprayed with a spore suspension of *B. cinerea*, also prepared as for the greenhouse experiments. Plots were irrigated with a sprinkler system, from the late vegetative growth stage (late June) to the late pod-filling stage (late August). Weeds were controlled during the growing season by hand weeding.

Lentil plants were rated for botrytis stem and blossom blight during the late pod-filling stage (late August) by scoring each plant for infection based on the scale of 1 to 4 described above. The middle 4 rows of each plot were rated, excluding the end plants on each row. Disease incidence was calculated for each plot as the percentage of plants infected by *B. cinerea*. Disease severity was defined as a weighted average of the plant ratings using the formula DI= Σ(NS)/T, where N=number of plants for each severity rating, S=severity rating (1-4), and T=total number of plants. At maturity (early September), plots were harvested using a Nurserymaster Elite 2000 plot combine (Wintersteiger, Ried im Innkreis, Austria). Seed samples were dried at 32°C for 1 week, cleaned using a Clipper™ seed cleaner (Ferrel-Ross, Bluffton, IN), and weighed.

Data of disease incidence, disease severity, seed yield, and seed infection from greenhouse and field experiments were analysed using analysis of variance, and separation of means was accomplished using Duncan's multiple range test. All statistical analyses were conducted using SAS/STAT® software [18].

RESULTS

Dual culture experiments showed that, of the 296 isolates of rhizobacteria and 98 isolates of fungi tested for antagonistic effects on *Botrytis cinerea*, 147 isolates showed some degree of antagonism. Zones of inhibition among these isolates ranged from 0-16 mm in width, corresponding to a range of 0-65% inhibition of growth of *B. cinerea*. The antagonistic isolates included both bacteria and fungi, representing a wide range of species. A total of 32 isolates (20 bacteria and 12 fungi) was selected from laboratory experiments and used for the greenhouse study.

In a series of eight greenhouse experiments, lentil plants which were sprayed only with a suspension of *B. cinerea* developed lesions on stems, flowers and pods. Spraying lentil plants with any of the 32 microbial agents resulted in a significant (P<0.001) reduction in the number of infection loci per plant, and in disease severity (Table 1). The number of infection loci for plants sprayed with microbial agents ranged from 1 to 7 per plant, whereas the number for the *Botrytis*-inoculated control ranged from 18 to 25 per plant, and the untreated control had none. The disease severity index for plants sprayed with biocontrol agents ranged from 1.3 to 2.3, compared to 2.5 to 3.2 for the *Botrytis*-inoculated control, and 1.0 for the untreated control (Table 1).

Lentil plants which were sprayed with the biocontrol agents *P. agglomerans* LRC 954, *P. griseofulvum* LRC 2461, *P. fluorescens* LRC 1788, or *T. hamatum* LRC 2475, had higher seed yield than plants from the *Botrytis*-inoculated control (P<0.001), but were not significantly different from the uninoculated control (P>0.05) (Table 2). Seed yield ranged from 4.8 to 5.0 g per plant for the biocontrol agents, compared to 3.0 g per plant for the *Botrytis*-inoculated control, and 4.8 g per plant for the untreated control.

Spraying of plants with *P. agglomerans*, *P. griseofulvum*, *P. fluorescens*, or *T. hamatum*, also resulted in a lower frequency of seed infection by *B. cinerea*, compared to the *Botrytis*-inoculated control (P<0.001) (Table 2). The rates of seed infection by *B. cinerea* for plants treated with the biocontrol agents and the untreated control were not significantly different (P>0.05). The frequency of seed infection ranged from 1 to 2% for the treatments of biocontrol agents, compared to 41% for the *Botrytis*-inoculated control, and 0% for the untreated control.

In the three field experiments, lentil plants in the *Botrytis*-inoculated control plots developed lesions on stems, flowers and pods by early August, with conidiophores of *B. cinerea* readily visible to the naked eye. Lesions on stems usually developed in association with plant injuries or senescent plant tissues such as petals or leaves. Infected plants ripened prematurely, turning brown before pods were fully formed or filled (Figure 1, left). The incidence of botrytis stem and blossom blight for the *Botrytis*-inoculated control plots ranged from 57% in 1999 to 86% in 1998 (Table 3). In comparison, the disease incidence in the untreated control plots ranged from 10% in 2000 to 22% in 1998. Spraying lentil plants with *P. agglomerans* LRC 954, *P. fluorescens* LRC 1788, *P. griseofulvum* LRC 2461, or *T. hamatum* LRC 2475 resulted in a significant (P<0.001) reduction in the incidence of botrytis stem and blossom blight in all three years, with the disease incidence in the treatments of biocontrol agents ranging from 10% for *P. griseofulvum* in 2000, to 49% for *P. agglomerans* in 1998 (Table 3). *Trichoderma hamatum* was the most consistent agent among the four biocontrol agents tested (Table 3, Figure 1).

Fig. 1. Lentil plants treated with *B. cinerea* alone (left) or with *B. cinerea* and *T. hamatum* (right) (Field Experiment, 1998). Note that the disease was severe in the plot without biocontrol agents (left), but light when *T. hamatum* was applied (right).

Treatment of lentil plots with any of the four biocontrol agents significantly (P<0.001) reduced the severity of botrytis stem and blossom blight in all three years of field experiments (Table 3). The disease severity index for the treatments of biocontrol agents ranged from 1.1 for *P. griseofulvum* in 2000, to 2.0 for *P. agglomerans* in 1998, compared to a range of 1.1 to 1.4 for the untreated control, and 2.1 to 3.0 for the *Botrytis*-inoculated control.

Lentils which were sprayed with the biocontrol agents *P. agglomerans*, *P. griseofulvum*, *P. fluorescens*, or *T. hamatum*, produced higher seed yield than plants sprayed with *B. cinerea* alone (P<0.001) in 1999 and 2000, but not in 1998 (P>0.05) (Table 3). The seed yield for the treatments of biocontrol agents ranged from 1113 kg/ha for *P. agglomerans* in 1999, to 2312 kg/ha for *T. hamatum* in 2000, compared to a range of 810 kg/ha to 1722 kg/ha for the *Botrytis*-inoculated control, and 1641 kg/ha to 2230 kg/ha for the untreated control. The *Botrytis*-inoculated control had lower seed yield than the untreated control in 1999 and 2000 (P<0.001) (Table 3).

DISCUSSION

Our studies in the greenhouse and in the field reveal that the bacteria *P. agglomerans* LRC 954 and *P. fluorescens* LRC 954, and the fungi *P. griseofulvum* LRC 2461 and *T. hamatum* LRC 2475, have potential as biocontrol agents for botrytis stem and blossom blight of lentil. The reduction in disease and increase in seed yield observed in this study may be due to the effective protection of potential infection sites by these biocontrol agents. Previous reports indicate that pollen and senescent flower petals are important sources of nutrients for plant pathogenic fungi such as *B. cinerea* [11,12], and mycoparasitic fungi such as *Gliocladium catenulatum* [10] and *Coniothyrium minitans* (H.C. Huang, unpublished), or antagonistic bacteria such as *Bacillus cereus* [9]. Since both pathogenic fungi and non-pathogenic fungi or bacteria must compete for the exogenous nutrients released by senescent tissues such as pollen and petals, the outcome of this competition for essential nutrients becomes an important factor in determining the success or failure of each biological control strategy.

In addition to the occupation of niche by biocontrol agents, and competitive exclusion of *B. cinerea*, other mechanisms may have played a role in suppressing the development of infection loci on lentil plants. Bacteria and fungi are both capable of producing antibiotics, such as griseofulvin in the case of *P. griseofulvum* [16], and polymyxin in the case of *P. polymyxa* [2]. *Trichoderma* species produce a wide range of toxic metabolites [17], and *T. hamatum* can also grow mycoparasitically on pathogenic fungi such as *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc. [5]. It is possible that, once biocontrol agents became established on

Table 1. Control of botrytis stem and blossom blight of lentil by spray of bacterial or fungal agents (Eight greenhouse experiments).

Isolate	No. Loci per plant	Disease Severity	Isolate	No. Loci per plant	Disease Severity
Experiment 1			**Experiment 5**		
Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]	Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]
Pseudomonas fluorescens 1673	2 ab	1.3 ab	*Bacillus subtilis* 1786	4 b	1.9 b
Pseudomonas fluorescens 1654	3 b	1.3 ab	*Bacillus subtilis* 1105	5 bc	2.0 bc
Pseudomonas fluorescens 1192	3 b	1.4 b	*Pseudomonas fluorescens* 939	6 bc	2.1 bc
Pseudomonas fluorescens 1788	4 b	1.5 b	*Pantoea agglomerans* 1787	7 c	2.2 c
Control (with *Botrytis*)	22 c	3.2 c	Control (with *Botrytis*)	25 d	3.0 d
Sig (F)	0.001	0.001	Sig (F)	0.001	0.001
Experiment 2			**Experiment 6**		
Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]	Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]
Bacillus cereus 805	3 b	1.9 b	*Penicillium griseofulvum* 2461	1 ab	1.5 b
Bacillus cereus 1195	4 b	1.9 b	*Trichoderma harzianum* 2428	1 ab	1.6 bc
Bacillus subtilis 886	4 b	1.9 b	*P. aurantiogriseum* 2450	2 b	1.8 c
Paenibacillus polymyxa 1784	4 b	2.0 b	*Gliocladium catenulatum* 2087	2 b	1.8 c
Control (with *Botrytis*)	20 c	2.8 c	Control (with *Botrytis*)	19 c	2.6 d
Sig (F)	0.001	0.001	Sig(F)	0.001	0.001
Experiment 3			**Experiment 7**		
Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]	Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]
Pseudomonas putida 1785	4 b	1.9 b	*Pantoea agglomerans* 954	1 a	1.5 b
Paenibacillus polymyxa 959	6 b	2.1 bc	*Erwinia rhapontici* 946	4 b	1.9 c
Paenibacillus polymyxa 806	7 b	2.0 bc	*Pseudomonas fluorescens* 941	4 b	1.9 c
Bacillus circulans 1675	7 b	2.3 c	*Pseudomonas putida* 945	5 b	2.1 c
Control (with *Botrytis*)	23 c	2.9 d	Control (with *Botrytis*)	20 c	2.5 d
Sig (F)	0.001	0.001	Sig(F)	0.001	0.001
Experiment 4			**Experiment 8**		
Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]	Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]
Coniothyrium minitans 2137	2 b	1.7 b	*Trichoderma hamatum* 2475	1 ab	1.4 b
Talaromyces flavus 2152	3 b	1.8 b	*Epicoccum purpurascens* 2250	2 abc	1.7 c
Gliocladium roseum 2090	4 b	1.9 b	*Penicillium griseofulvum* 2464	3 bc	1.9 c
Trichoderma virens 2425	5 b	1.9 b	*Trichoderma viride* 2424	4 c	1.9 c
Control (with *Botrytis*)	18 c	2.9 c	Control (with *Botrytis*)	19 d	2.7 d
Sig (F)	0.001	0.001	Sig (F)	0.001	0.001

[1] Means within a column followed by the same letter are not significantly different at $P<0.001$ level (Duncan's multiple range test). Figures presented represent the combined results of two runs of the experiment.

[2] Disease severity index: 1=healthy; 2=one stem infected; 3=two or more stems infected; 4=dead.

senescent tissues, they may have further supressed *B. cinerea* through antibiosis and/or mycoparasitism. Further studies to elaborate the mechanisms involved in biological control of botrytis stem and blossom blight may provide important information leading to improvement of the biocontrol technique.

The timing of application of biocontrol agents relative to the introduction of the pathogen may be another important factor for consideration in biocontrol. While pre-inoculation of plants with the biocontrol agents led to effective control of botrytis stem and blossom blight in this study, further investigations on timing of application of *B. cinerea* and biocontrol agents would generate useful information on the optimum strategy for control of this disease. Also, large-scale trials in commercial fields would be necessary to determine if this biocontrol technique is robust enough for practical application on a farm scale.

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Table 2. Control of botrytis stem and blossom blight of lentil by spray of bacterial or fungal agents (Greenhouse experiment).

Isolate	No. Loci per Plant	Disease Severity	Seed Yield per Plant (g)	Seed Infection (%)
Control (without *Botrytis*)	0 a[1]	1.0 a[1,2]	4.8 a[1]	0 a[1]
Trichoderma hamatum 2475	1 a	1.4 b	5.0 a	1 a
Pseudomonas fluorescens 1788	1 a	1.6 b	4.8 a	1 a
Penicillium griseofulvum 2461	1 a	1.6 b	5.0 a	1 a
Pantoea agglomerans 954	1 a	1.7 b	4.8 a	2 a
Control (with *Botrytis*)	20 b	2.6 c	3.0 b	41 b
Sig (F)	0.001	0.001	0.001	0.001

[1] Means within a column followed by the same letter are not significantly different at $P<0.001$ level (Duncan's multiple range test). Figures presented represent the combined results of two runs of the experiment.
[2] Disease severity index: 1=healthy; 2=one stem infected; 3=two or more stems infected; 4=dead.

Table 3. Effect of spraying biocontrol agents on incidence and severity of botrytis stem and blossom blight, and seed yield of lentil (Field experiments, 1998-2000).

	Disease Incidence (%)			Disease Severity (1-4)			Seed Yield (kg/ha)		
Isolate	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control (without *Botrytis*)	22 a[1]	15 a	10 a	1.4 a[2]	1.2 a	1.1 a	1903 a	1641 b	2230 a
Trichoderma hamatum 2475	29 b	27 b	16 b	1.6 a	1.4 b	1.2 a	2009 a	1945 a	2312 a
Pseudomonas fluorescens 1788	39 c	29 b	36 c	1.8 b	1.5 b	1.6 b	1707 a	1537 b	2160 a
Penicillium griseofulvum 2461	38 c	48 d	10 a	1.8 b	1.8 c	1.1 a	1537 a	1238 c	2123 a
Pantoea agglomerans 954	49 d	39 c	38 c	2.0 c	1.7 c	1.7 b	1338 a	1113 c	2189 a
Control (with *Botrytis*)	86 e	57 e	73 d	3.0 d	2.1 d	2.5 c	1295 a	810 d	1722 b
Sig (F)	0.001	0.001	0.001	0.001	0.001	0.001	3.660	0.001	0.001

[1] Means within a column followed by the same letter are not significantly different at $P<0.001$ level (Duncan's multiple range test).
[2] Disease severity index: 1=healthy; 2=one stem infected; 3=two or more stems infected; 4=dead.

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摘 要

黃鴻章[1,2]、Erickson, R. S [1]. 2002. 扁豆莖與花灰黴病的生物防治. 植病會刊 11:7-14. ([1] 加拿大農部 Lethbridge 研究中心；[2] 聯絡作者，電子郵件：huangh@em.agr.ca；傳眞：403-382-3156)

進行溫室與田間試驗，以測定 32 種拮抗眞菌與細菌對防治扁豆 (*Lens culinaris*) 莖與花灰黴病 (*Botrytis cinerea*) 的防治效果。結果顯示，所有菌株在開花初期噴布扁豆全株均有降低灰黴病罹病率與罹病度的效果。其中 4 個菌株，包括 *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Penicillium griseofulvum* 及 *Trichoderma hamatum*，在溫室實驗時，可以增加扁豆的產量與降低灰黴病感染率。在 1998-2000 年田間試驗時，與對照接種處理比較，此四個菌株可以顯著的降低莖與花灰黴病的罹病度，而且其處理的產量顯著增加。其中以 *Trichoderma hamatum* 的效果最佳且最穩定。本試驗認爲“葉面噴布拮抗微生物”爲防治扁豆莖與花灰黴病的一種有效方法。

關鍵詞：扁豆 (*Lens culinaris*)、灰黴病、*Botrytis cinerea*、生物防治、拮抗微生物、*Pantoea agglomerans*、*Pseudomonas fluorescens*、*Penicillium griseofulvum*、*Trichoderma hamatum*