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Control of White Mold of Bean by *Coniothyrium minitans*: Comparison of Soil and Foliar Treatments

Hung-Chang Huang[1,2] and R. Scott Erickson[1]

[1] Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, Alberta, T1J 4B1 Canada

[2] Corresponding author, E-mail: huangh@agr.gc.ca; Fax: +1-403-382-3156

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ABSTRACT

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A field study was conducted during 1998-2000 at Lethbridge, Alberta, Canada to determine the efficacy of different methods of applying the mycoparasite, *Coniothyrium minitans*, for control of white mold of common bean (*Phaseolus vulgaris*) caused by *Sclerotinia sclerotiorum*. Application of *C. minitans* grown on wheat bran to soil at the rate of 60 g/m^2 after seedling emergence resulted in a 97% reduction (P<0.05) in number of apothecia produced by sclerotia of *S. sclerotiorum*, whereas foliar application of a spore suspension of this mycoparasite during the early flowering stage had no effect on apothecial production. *C. minitans*, applied as a foliar spray, as a soil amendment, or as a combination of foliar spray plus soil amendment, as well as fungicide (benomyl) spray, all significantly reduced incidence and severity of white mold of bean, compared to the untreated control. The suppresion of white mold in the combined treatment of *C. minitans* foliar spray plus soil amendment was similar to that in the fungicide treatment, and these two treatments resulted in the highest seed yields. The study concludes that the combination of *C. minitans* foliar spray and soil amendment is more effective than either foliar spray or soil amendment alone for the control of white mold of bean.

Key words : *Sclerotinia sclerotiorum*, sclerotia, carpogenic germination, apothecia, soil amendment, foliar spray, biological control, mycoparasite, *Coniothyrium minitans*

INTRODUCTION

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a widespread and serious disease of dry bean (*Phaseolus vulgaris* L.) in western Canada [5, 10]. The primary source of inoculum for the disease on bean is airborne ascospores [16] that are released from apothecia arising from carpogenically germinated sclerotia [12]. Current methods used to control white mold of dry bean in commercial fields include crop rotation and application of fungicides.

Coniothyrium minitans Campbell [1] is a mycoparasite that attacks hyphae [11] and sclerotia [1, 9] of *S. sclerotiorum*. Previous reports [2, 3] indicate that *C. minitans* is effective in reducing the viability of sclerotia, the survival structures of *S. sclerotiorum* under field conditions. Field studies conducted in western Canada demonstrated that foliar application of *C. minitans* is effective in reducing incidence of white mold of bean, but is not as effective as fungicide (benomyl) spray application [4]. Other studies showed that soil treatment with *C. minitans* reduced the carpogenic germination of sclerotia of *S. sclerotiorum*, and thereby reduced production of apothecia in the host pulse crops of pea (*Pisum sativum* L.) and bean [6], as well as canola (*Brassica napus* L. and *B. rapa* L.) and safflower (*Carthamus tinctorius* L.) [8], and the non-host cereal crops of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) [13].

The purpose of this study was to determine the effectiveness of foliar spray, soil amendment, and combined (foliar spray plus soil amendment) methods of application of *C. minitans* for control of white mold of dry bean, and to compare the efficacy of these treatments with the standard agricultural practice of fungicide (benomyl) application.

MATERIALS AND METHODS

The pathogen used in this study was *Sclerotinia sclerotiorum* LRC 2148 (syn. sun-87), isolated from an infected sunflower (*Helianthus annuus* L.) near Lethbridge, Alberta, Canada. Sclerotia of *S. sclerotiorum* were produced by growing the pathogen on red kidney beans (Fraser Valley

Foods; Fraser Valley, British Columbia, Canada). Beans were transferred from cans into sterile 250-ml clear plastic containers (Twinpak, Regina, Saskatchewan, Canada), 25 ml each. Four potato dextrose agar (PDA) (Difco; Detroit, MI) plugs (5 mm diameter) containing 5-day-old mycelial mats of *S. sclerotiorum* were placed in each container, and the containers were incubated in the dark at 10℃ for 8 weeks. Sclerotia produced on the colony in each container were collected, washed in distilled water, and air-dried on paper towel. Sclerotia from 5 to 10 mm in diameter were selected, sorted into lots of 100, and stored at 10℃ for approximately two weeks prior to use for the field experiments.

The biocontrol agent used was the mycoparasite *Coniothyrium minitans* LRC 2137, isolated from an infected sclerotium of *S. sclerotiorum* near Morden, Manitoba, Canada. Solid inoculum of *C. minitans* for soil treatments was prepared according the method described by Huang and Erickson [6]. Wheat bran was moistened, autoclaved twice at 121℃ for 30 min with 48 h between autoclave cycles, and placed into sterile 250 ml clear plastic containers (Twinpak; Regina, Saskatchewan, Canada) with 25 g of bran in each container. Cultures of *C. minitans* grown at 20℃ on PDA in Petri dishes for 3 weeks were used to prepare a spore suspension (5.0 X 10^5 spores per ml), and 4 ml of the suspension was inoculated onto the bran substrate in each container. Wheat bran cultures were incubated at 20 ±2℃ under continuous fluorescent light for 28 days, air-dried, crushed into powder, and divided into lots of 15 g.

Liquid inoculum of *C. minitans* for spray treatments was prepared by the method of Huang *et al.* [4]. Cultures were grown on PDA at 20℃ under continuous fluorescent light for 3 weeks. Spore suspensions were prepared by washing the cultures with sterile distilled water while gently scraping them with a spatula and the resulting mixture was passed through four layers of cheesecloth. Spore concentrations in the suspension were determined using a hemacytometer.

Experiments were conducted in an irrigated field at the Lethbridge Research Centre during 1998 (2 locations), 1999 (1 location) and 2000 (1 location), using a viny type of dry bean, cultivar NW63. Each year, the experiment was conducted in an area of the field that was fallowed in the previous season. Prior to seeding, nitrogen fertilizer (34-0-0; N-P-K) was applied to the field at a rate of 200 kg per ha. Crops were seeded on 27 May 1998, 21 May 1999, and 25 May 2000, at the seeding rate of 229 kg per ha. Planting was done in a continuous strip, using a four-row seeder set at a depth of 2.5 cm and a row spacing of 22.5 cm. The experimental design consisted of six treatments arranged in a randomized complete block design with six replicates. The treatments were: (1) control without *S. sclerotiorum*; (2) control with *S. sclerotiorum*; (3) *S. sclerotiorum* + *C. minitans* spray; (4) *S. sclerotiorum* + *C. minitans* soil amendment; (5) *S. sclerotiorum* + *C. minitans* (spray plus soil amendment); and (6) *S. sclerotiorum* + fungicide spray (benomyl).

After seedling emergence, individual plots were established, with each plot consisting of twelve, 5 m - long rows. For all treatments except the control without *S. sclerotiorum*, five treated areas were set up in each plot by establishing trenches in the second, fourth, sixth, eighth and tenth inter-row spaces. Each trench was 2 m in length and 0.12 m in width. For the treatments of *S. sclerotiorum* + *C. minitans* soil amendment, and *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), 2 kg of soil was removed from each trench, mixed in a container with 30 g of inoculum of the biocontrol agent grown on bran, and half (1 kg) of the amended soil was put back in the trench. One hundred sclerotia were spread evenly in the trench and covered with the remaining amended soil (1 kg). A small amount of natural field soil was placed on top of the amended soil and trenches were tamped down to prevent the movement of inoculum or soil by the wind. For the treatments of control with *S. sclerotiorum*, *S. sclerotiorum* + *C. minitans* spray, and fungicide, 100 sclerotia per trench were buried in the soil without the *C. minitans* soil amendment. From the late vegetative growth stage (mid-June) to the late pod-filling stage (late August), irrigation was frequently provided to maintain high soil moisture for optimal apothecial development. Weeds were controlled during the growing season by hand weeding.

For the treatments of *S. sclerotiorum* + *C. minitans* spray, and *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), the biocontrol agent was sprayed onto plots twice during each year. The first application was done at 5% bloom (mid-July), and the second application was at 50% bloom, about 10 days after the first application. A spore suspension of 10^6 spores/ml was mixed with 5 g per liter PelGel (LiphaTech, Milwaukee, WI) and 0.17 ml per liter Tween 20 (polyoxyethylenesorbitan monolaurate; Fisher Scientific, Fair Lawn, NJ) and sprayed onto the crop using a garden hand-pump sprayer at a rate of 300 ml per m^2. Plots were not irrigated for 24 h after each spray. For the fungicide treatment, Benlate 50 WP containing 50% benomyl (methyl [1-[(butylamino)carbonyl]-1*H*-benzimidazol-2-yl]carbamate; DuPont, Mississauga, Ontario, Canada) was applied at the same time as *C. minitans*, at a rate of 1.0 kg per ha per application.

From late bloom (mid-August) to maturity (mid-September), data on the number of sclerotia that germinated carpogenically and the number of apothecia produced on sclerotia in each plot were collected weekly. Because apothecia might be produced from the same sclerotium at different times, color coded sticks representing the number of apothecia previously counted were used to allow cumulative counting of apothecia from each sclerotium. Plants were also rated for incidence of white mold by visual observation of symptoms [14] during the late pod-filling stage (early September). The ten middle rows of each plot were rated, ignoring the 0.5 m on each end of each row. Each plant was

rated on a scale of 1-4, where 1=healthy, 2=single stem infected, 3=multiple stems infected, and 4=dead. Disease incidence was defined as the percentage of plants infected by *S. sclerotiorum*. Disease severity was calculated as a weighted average of the plant ratings, DS = Σnr/t, where DS = disease severity (ranging from 1 - 4), n = number of plants, r = plant rating (1 - 4), and t = total number of plants rated. At maturity (mid-September), plants were harvested using a Nurserymaster Elite 2000 plot combine (Wintersteiger, Ried im Innkreis, Austria). Seed samples were dried at 20℃ for 4 weeks, cleaned, and weighed to determine seed yield for each plot. Seed yield was not determined for one of the experiments in 1998 due to frost damage.

Within each experiment, differences between treatments in number of apothecia produced, incidence of white mold, severity of white mold, and seed yield of bean were analyzed for statistical significance using analysis of variance (ANOVA). Means were separated using Duncan's multiple range test. All statistical analyses were conducted using the SAS/STAT® computer software [15].

RESULTS

Sclerotia of *S. sclerotiorum* germinated carpogenically during August and early September of each year to produce small, light brown colored apothecia. The number of apothecia produced per 100 sclerotia was significantly (P<0.05) lower for the treatments of *S. sclerotiorum* + *C. minitans* soil amendment and *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), than for the treatments of control with *S. sclerotiorum*, *S. sclerotiorum* + *C. minitans* spray, and *S. sclerotiorum* + fungicide spray (Table 1). For example, in the year 2000, the number of apothecia produced per 100 sclerotia for the treatment of control with *S. sclerotiorum* was 186, compared to 8 for the treatment of *S. sclerotiorum* + *C. minitans* soil amendment. The trend was consistent among all four location-years.

White mold symptoms appeared on bean plants during mid-August each year, and continued to develop and spread until the plants reached maturity. The incidence of white mold was highest in the treatment of control with *S. sclerotiorum*, ranging from 45% at site 1 in 1998, to 90% at site 2 in 1998 (Table 2). White mold incidences for all of the *C. minitans* treatments and the fungicide treatment were significantly (P<0.05) lower than for the control with *S. sclerotiorum*, in all four location-years. The lowest disease incidences were observed in the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), and *S. sclerotiorum* + fungicide,

Table 1. Effect of *Coniothyrium minitans* and fungicide treatments on production of apothecia from sclerotia of *Sclerotinia sclerotiorum*. (field experiments).

	Number of apothecia produced per 100 sclerotia			
	1998			
Treatments[1]	Site 1	Site 2	1999	2000
Control (with *Sclerotinia*)	168 a[2]	213 a	105 a	186 a
Coniothyrium minitans foliar spray	173 a	197 a	111 a	165 a
C. minitans soil amendment	5 b	9 b	6 b	8 b
C. minitans spray + soil amendment	4 b	3 b	6 b	6 b
Fungicide spray (Benlate)	191 a	211 a	119 a	180 a

[1] *Coniothyrium minitans* foliar spray: two applications of 10^6 spores/ml, ten days apart during early bloom stage. *C. minitans* soil amendment: wheat bran substrate application after seedling emergence at rate of 60 g/m^2. Fungicide spray: two applications at the rate of 1 kg/ha/application, at the same time as *C. minitans* spray.

[2] Means within a column followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.

Table 2. Effect of *Coniothyrium minitans* and fungicide treatments on incidence of white mold of common bean. (field experiments).

	Incidence of white mold (%)			
	1998			
Treatments[1]	Site 1	Site 2	1999	2000
Control (with *Sclerotinia*)	45 a[2]	90 a	61 a	84 a
Control (without *Sclerotinia*)	29 b	48 c	49 ab	60 b
Coniothyrium minitans foliar spray	34 b	68 b	32 b	29 c
C. minitans soil amendment	27 b	54 bc	32 b	23 c
C. minitans spray + soil amendment	23 b	44 c	15 c	6 d
Fungicide spray (Benlate)	29 b	58 bc	10 c	12 d

[1] *Coniothyrium minitans* foliar spray: two applications of 10^6 spores/ml, ten days apart during early bloom stage. *C. minitans* soil amendment: wheat bran substrate application after seedling emergence at rate of 60 g/m^2. Fungicide spray: two applications at the rate of 1 kg/ha/application, at the same time as *C. minitans* spray.

[2] Means within a column followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.

Table 3. Effect of *Coniothyrium minitans* and fungicide treatments on severity of white mold of dry bean. (field experiments).

	Severity of white mold[1]			
	1998			
Treatments[2]	Site 1	Site 2	1999	2000
Control (with *Sclerotinia*)	1.9 a[3]	2.7 a	2.1 a	3.0 a
Control (without *Sclerotinia*)	1.6 b	2.3 b	1.9 a	2.5 b
Coniothyrium minitans foliar spray	1.5 b	1.7 c	1.5 b	1.6 c
C. minitans soil amendment	1.5 b	1.5 cd	1.5 b	1.5 c
C. minitans spray + soil amendment	1.3 b	1.3 d	1.2 b	1.1 d
Fungicide spray (Benlate)	1.5 b	1.2 d	1.2 b	1.3 cd

[1] Severity index: 1=healthy, 2=single stem infected, 3=multiple stems infected, 4=dead.
[2] *Coniothyrium minitans* foliar spray: two applications of 10^6 spores/ml, ten days apart during early bloom stage. *C. minitans* soil amendment: wheat bran substrate application after seedling emergence at rate of 60 g/m^2. Fungicide spray: two applications at the rate of 1 kg/ha/application, at the same time as *C. minitans* spray.
[3] Means within a column followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.

Table 4. Effect of *Coniothyrium minitans* and fungicide treatments on seed yield of common bean. (field experiments).

	Bean seed yield (kg/ha)			
	1998			
Treatments[1]	Site 1	Site 2	1999	2000
Control (with *Sclerotinia*)	nd [2]	1770 a[3]	2154 a	2469 a
Control (without *Sclerotinia*)	nd	1855 a	2612 b	2615 a
Coniothyrium minitans foliar spray	nd	2431 b	2779 c	2911 b
C. minitans soil amendment	nd	2622 b	2862 c	2850 b
C. minitans spray + soil amendment	nd	2944 c	2879 c	3161 c
Fungicide spray (Benlate)	nd	2857 c	2921 c	3311 c

[1] *Coniothyrium minitans* foliar spray: two applications of 10^6 spores/ml, ten days apart during early bloom stage. *C. minitans* soil amendment: wheat bran substrate application after seedling emergence at rate of 60 g/m^2. Fungicide spray: two applications at the rate of 1 kg/ha/application, at the same time as *C. minitans* spray.
[2] nd = no data collected due to frost damage.
[3] Means within a column followed by the same letter are not significantly different (P>0.05) according to Duncan's multiple range test.

and these two treatments were not significantly different from each other. For example, in the year 2000, the disease incidence for the control with *S. sclerotiorum* was 84%, compared to 6% and 12% for the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), and *S. sclerotiorum* + fungicide, respectively (Table 2).

A similar trend was observed with respect to severity of white mold. The disease severity was highest in the treatment of control with *S. sclerotiorum*, ranging from 1.9 at site 1 in 1998, to 3.0 in 2000 (Table 3). Severity of white mold for all of the *C. minitans* treatments and the fungicide treatment was significantly (P<0.05) reduced in comparison to the control with S. sclerotiorum, in all four location-years. The lowest disease severities were observed in the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), and *S. sclerotiorum* + fungicide, and these two treatments were not significantly different from each other. For example, in the year 2000, the severity of white mold for the control with S. sclerotiorum was 3.0, compared to 1.1 and 1.3 for the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), and *S. sclerotiorum* + fungicide, respectively (Table 3).

Seed yield of bean was significantly (P<0.05) higher for all of the *C. minitans* treatments and the fungicide treatment, compared to the control with *S. sclerotiorum* (Table 4). Seed yields were highest in the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment), and *S. sclerotiorum* + fungicide, and these two treatments were not significantly different from each other in all three location-years. Seed yields for the treatments of *S. sclerotiorum* + *C. minitans* soil amendment and *S. sclerotiorum* + *C. minitans* spray were lower than for the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment) and *S. sclerotiorum* + fungicide in two out of three location-years. For example, in 2000, the bean seed yield for the control with *S. sclerotiorum* was 2469 kg/ha, whereas the yields for the treatments of *S. sclerotiorum* + *C. minitans* spray and *S. sclerotiorum* + *C. minitans* soil amendment were 2911 and 2850 kg/ha, respectively, and the seed yields for the treatments of *S. sclerotiorum* + *C. minitans* (spray plus soil amendment) and *S. sclerotiorum* + fungicide were 3161 and 3311 kg/ha, respectively (Table 4).

DISCUSSION

This study demonstrates that fungicide treatment and all three *C. minitans* biocontrol treatments (foliar spray, soil amendment and foliar spray + soil amendment) are effective means of reducing white mold of dry bean caused by *S. sclerotiorum* and thereby increasing bean seed yield. Among the biocontrol treatments, the most effective one was *C. minitans* foliar spray + soil amendment, which was similar to the fungicide treatment with respect to control of white mold and seed yield of bean. Huang *et al.* [4] reported that foliar application of *C. minitans* was effective in reducing incidence of white mold of bean, but not as effective as foliar application of fungicide. Another study demonstrated that soil treatment with *C. minitans* was effective in reducing apothecial production of *S. sclerotiorum* under the canopy of bean [7]. The current study indicates that the efficacy of biological control of white mold of bean can be enhanced by the combined strategy of *C. minitans* foliar spray and *C. minitans* soil amendment.

The effectiveness of the combined treatment of spray + soil amendment with *C. minitans* can be attributed to two mechanisms: (1) soil treatment of *C. minitans* resulting in reduction of sclerotial population in the soil and thereby reducing ascospore inoculum of *S. sclerotiorum*; and (2) foliar spray of *C. minitans* resulting in protection of vulnerable plant parts such as senescent flowers and leaves from colonization by ascospores of *S. sclerotiorum*. This two-pronged approach of soil treatment and air spray uses complementary modes of action of *C. minitans* that control both sclerotial inoculum in the soil and ascospore inoculum on above ground parts of plant tissues. Therefore, the combined treatment of *C. minitans* can achieve a higher level of control of white mold of bean than the treatments of either soil application or air spray of *C. minitans*.

The fact that *C. minitans* is a hyperparasite of *S. sclerotiorum* [1], combined with the finding that *C. minitans* can survive Canadian prairie winters in the soil [7], suggests that biological control using *C. minitans* may have the additional benefit of persistent reduction of sclerotia, the primary source of inoculum of *S. sclerotiorum* in the soil. This prolonged effect of *C. minitans* on survival of sclerotia of *S. sclerotiorum* suggests that the use of this mycoparasite may have a distinct advantage over the use of chemical fungicides for control of white mold of bean. Although an examination of the durability of protection against white mold of bean by *C. minitans* was beyond the scope of the current study, future investigations in this area are warranted. These and other efforts to further improve the efficacy and practicality of *C. minitans* for the control of white mold of bean will likely result in a reduction of producers' reliance on chemical pesticides, thus enhancing the sustainability of dry bean production.

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摘 要

黃鴻章[1,2]、Erickson, R. S.[1] 2004. 比較土壤處理與植株噴佈超寄生菌 (*Coniothyrium minitans*) 防治菜豆菌核病的效果. 植病會刊 13:171-176. ([1.] 加拿大農業及農業食品部 Lethbridge研究中心；[2.] 聯絡作者，電子郵件：huangh@agr.gc.ca；傳眞：+1-403-382-3156)

在加拿大亞伯達省Lethbridge 研究中心的試驗田進行試驗，比較超寄生眞菌 *Coniothyrium minitans* 的使用方法 (土壤處理或植株噴佈) 對菜豆菌核病 (*Sclerotinia sclerotiorum*) 的防治效果。由西元1998 到2000 年的三年試驗結果顯示，將培養在麥麩上、經風乾的 *C. minitans* 以60g/m^2 的用量，在播種期處理土壤，可以顯著減少土壤中病原菌菌核的發芽率與子囊盤的形成率達97%。但是於菜豆開花期間噴佈 *C. minitans* 的孢子懸浮液於植株上，卻無法顯著減少田間菌核產生子囊盤的數量。以土壤處理或植株噴佈 *C. minitans*，或是兩者併用均可顯著地降低菜豆菌核病的發病率與發病嚴重度，並增加菜豆產量；尤其是以土壤處理合併植株噴佈時效果最佳，此一併用方式處理 *C. minitans* 與噴佈億力 (Benlate) 殺菌劑的防病效果無異。本項試驗證明 *C. minitans* 以土壤處理與植株噴佈兩種方法合併使用，是防治菜豆菌核病的最有效方法。

關鍵詞：菌核病菌、菌核、子囊盤、子囊孢子、生物防治、超寄生眞菌