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Soil Treatment with Fungal Agents for Control of Apothecia of *Sclerotinia sclerotiorum* in Bean and Pea Crops

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ABSTRACT

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A two-year field study was conducted to determine the effects of soil treatment with four mycoparasites and one antagonist on production of apothecia of *Sclerotinia sclerotiorum* under the canopy of dry bean and dry pea. Among the five species of fungi assessed, *Coniothyrium minitans* and *Talaromyces flavus* were the most effective agents for reducing carpogenic germination of sclerotia of *S. sclerotiorum* in bean and pea crops, whereas *Tricothecium roseum* and *Trichoderma virens* were effective only in bean, and *Epicoccum purpurascens* was ineffective for both crops. Treatment with *C. minitans* significantly reduced formation of apothecia of *S. sclerotiorum* in bean and pea. This study suggests that, under Canadian Prairie conditions, soil treatment with mycoparasites such as *C. minitans* can be an effective measure for reducing the production of ascospores, which are the primary source of inoculum for white mold of bean and sclerotinia blight of pea.

Key words : Dry bean, *Phaseolus vulgaris*, dry pea, *Pisum sativum*, white mold, *Sclerotinia sclerotiorum*, sclerotia, carpogenic germination, apothecia, ascospores, biological control, mycoparasites

INTRODUCTION

Sclerotinia rot or white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is an important disease of pulse crops such as dry bean (*Phaseolus vulgaris* L.) [17] and dry pea (*Pisum sativum* L.) [20] in western Canada. White mold of dry bean is particularly serious in southern Alberta because the crop is grown under sprinkler irrigation, which creates a conducive environment for development of the disease. A survey conducted in 1984-1987 showed that white mold of dry bean occurred in all the fields surveyed in southern Alberta [17].

Sclerotia of *S. sclerotiorum* germinate carpogenically to produce apothecia, which release ascospores that serve as the primary source of inoculum for white mold of bean [1] and pod rot of pea [14]. Blad *et al*. [3] reported that irrigation and canopy structure of bean were important factors influencing the development of white mold in the field. Crop rotation and the use of chemical fungicides [19] are the methods currently available for control of white mold of bean.

Fungi such as *Coniothyrium minitans* Campbell [7, 9, 26], *Talaromyces flavus* Klöcker A.C.Stock & R.A. Sansom [22], *Trichoderma viride* Pers. ex Fr. [11], *Trichoderma virens* (Miller, Giddens and Foster) Arx, *Trichothecium roseum* Pers. ex Fr. [15], Gliocladium virens Miller & Foster [29], and *Gliocladium catenulatum* Gilman & Abbott [11] are destructive mycoparasites of sclerotia of *S. sclerotiorum*. Some species including *C. minitans* [13], *T. flavus* [21] and *G. catenulatum* [10] are also parasitic on growing hyphae of *S. sclerotiorum*.

Previous reports indicate that spraying spores of mycoparasites such as *C. minitans* [18] or antagonists such as *Epicoccum purpurascens* Ehrenb. ex Schlecht. [30] on bean plants is effective in reducing the incidence of white mold. The objective of this study was to determine the effect of soil treatment with mycoparasites or antagonists, on production of apothecia by *S. sclerotiorum* under the canopy of dry bean and dry pea.

MATERIALS AND METHODS

The biocontrol agents used in this study included four mycoparasites: *C. minitans* isolate LRS 2137, *T. flavus* isolate LRS 2152, *T. virens* isolate LRS 2425, *T. roseum* isolate LRS 2424, and one antagonist *E. purpurascens* isolate LRS 2114. The three fungi that originated from Manitoba were: *C. minitans* from an infected sclerotium of *S. sclerotiorum*, *T. flavus* from rhizosphere of a sunflower (*Helianthus annuus*

L.) plant, and *T. virens* from a sclerotium of *Botrytis cinerea* Pers. : Fr. that formed on a diseased sunflower head. The other two fungi that originated from Alberta were: *T. roseum* from a sclerotium of *S. sclerotiorum*, and *E. purpurascens* from a safflower (*Carthamus tinctorius* L.) seed.

Inoculum of each biocontrol agent was prepared by inoculation of sterilized wheat bran with a spore suspension. Water was added to wheat bran to adjust its moisture level to 85% (w/v). The moistened wheat bran was autoclaved twice at 121℃ for 30 min with 48 h between autoclaving, and transferred to individual sterile 250 ml clear plastic containers (Twinpak; Regina, Saskatchewan, Canada) using 25 g per container. Spore suspensions were prepared, using 14- to 28-day-old cultures grown on potato dextrose agar (PDA) (Difco; Detroit, MI) at 20℃. The concentration of the suspensions was adjusted to 5.0 X 10^5 spores per mL, and the bran substrate in each container was inoculated with 4 mL of the spore suspension. After incubation under continuous fluorescent light at 20±2℃ for 28 days, the cultures were air-dried, crushed manually, and divided into either 15 or 30 g lots.

Sclerotia of *S. sclerotiorum* isolate LRS 2148 were produced by growing the pathogen on a cooked bean substrate. Red kidney beans (Fraser Valley Foods; Fraser Valley, British Columbia, Canada) were discanted from cans, pureed in a food processor and poured into sterile 250 mL clear plastic containers (Twinpak, Regina, Saskatchewan, Canada), 25 mL each. Each container was inoculated with four PDA plugs (5 mm diameter) containing 5-day-old mycelial mats of *S. sclerotiorum*. After incubation in the dark at 10℃ for 8 weeks, sclerotia produced on the colony in each container were harvested by washing them in distilled water. The sclerotia were air-dried on paper towel, divided into lots of 100, and stored at 10℃ for approximately two weeks. The size of sclerotia used in this study ranged from 5 to 10 mm in diameter.

Experiments were conducted in an irrigated field at the Lethbridge Research Centre during 1993 and 1994, using a viny type of dry bean, cultivar NW 63, and a long-vined dry pea, cultivar Trapper. Prior to seeding, the herbicide trifluralin (2,6-dinitro-N,N-dipropyl-4- (trifluoromethyl)benzenamine) (DowElanco, Calgary, Alberta, Canada) was incorporated into soil at a rate of 960 g a.i. per ha. No fertilizer was applied in 1993, but in 1994, nitrogen fertilizer (34-0-0; N-P-K) was broadcast prior to seeding at a rate of 200 kg per ha for both crops. Crops were seeded on 25 May 1993 and 17 May 1994, with a seeding rate of 229 kg per ha for both crops. 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 experiment was repeated in 1994 by setting up the treatments in a fallowed area of the field.

After seedling emergence, individual plots were established, with each plot consisting of five, 2.5 m - long rows. Two treated areas were set up in each plot by establishing trenches in the second and third inter-row spaces. Each trench was 1 m in length and 0.12 m in width. One kg of soil was removed from each trench, mixed with inoculum of each biocontrol agent grown on bran, 15 g per trench for low rate and 30 g per trench for high rate, in a container, and half (500 g) 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 (500 g). 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 controls, 100 sclerotia per trench were buried in the soil without biocontrol agent. Treatments were set up each year approximately 3 weeks after seeding, and were arranged in a randomized complete block design with four replications. 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.

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 (Fig. 1a, b). Analyses of variance were carried out for each crop and year separately, for the percent of sclerotia germinated carpogenically, number of apothecia produced per plot, and number of apothecia produced per germinated sclerotium. Since similar results were obtained for both years, an analysis over years was carried out and the combined data were presented. For percent germination of sclerotia, a log transformation was performed to stabilize the variances. All statistical analyses were conducted using SAS/STAT® software [25].

RESULTS

Carpogenic germination of sclerotia of *S. sclerotiorum* occurred when the inter-row spaces were completely covered by the crop canopy. Small, light brown colored apothecia first appeared on the ground in late July in 1993 and early August in 1994, and new apothecia were produced throughout the rest of the season until late August in 1993 and early September in 1994, when the crop was near maturity (Fig. 1a, b). Apothecia produced from individual sclerotia were in clusters, and the number of apothecia often varied between clusters (Fig. 1a). In plots where large numbers of apothecia were present, spore clouds resulting from the release of ascospores from apothecia were frequently visible when the canopy was gently opened. By late August, the bean canopy collapsed in all the control plots due to infection of plants by ascospores of *S. sclerotiorum*.

Fig. 1. Bean plots, showing numerous apothecia (arrows) of *S. sclerotiorum* in untreated plot (a) and few apothecia in *C. minitans* -treated plot (b).

Under the dry bean canopy, amendment of soil with *C. minitans* or *T. flavus* significantly reduced carpogenic germination of sclerotia of *S. sclerotiorum* (Fig. 2). The frequency of sclerotia germinated carpogenically was 11% in 1993 and 2% in 1994 for the treatment of *C. minitans*, whereas the frequency for untreated controls was 48% in 1993 and 42% in 1994. Treatment with *C. minitans* (Fig. 1b) or *T. flavus* also resulted in a significant reduction in apothecial production of *S. sclerotiorum*. The number of apothecia produced per plot for the *C. minitans* treatment was 53 in 1993 and 10 in 1994, compared to 503 in 1993 and 291 in 1994 in the untreated controls. Treatment with *T. flavus* resulted in carpogenic germination of 27% and 14% in 1993 and 1994, respectively, and apothecial production of 221 and 94 apothecia per plot in 1993 and 1994, respectively. *T. roseum* and *T. virens* reduced the number of apothecia produced per plot in dry bean ($P<0.05$), but did not reduce the frequency of carpogenic germination of sclerotia ($P\geq0.05$). Soil treatment with *E. purpurascens* did not result in a significant reduction in carpogenic germination of sclerotia or

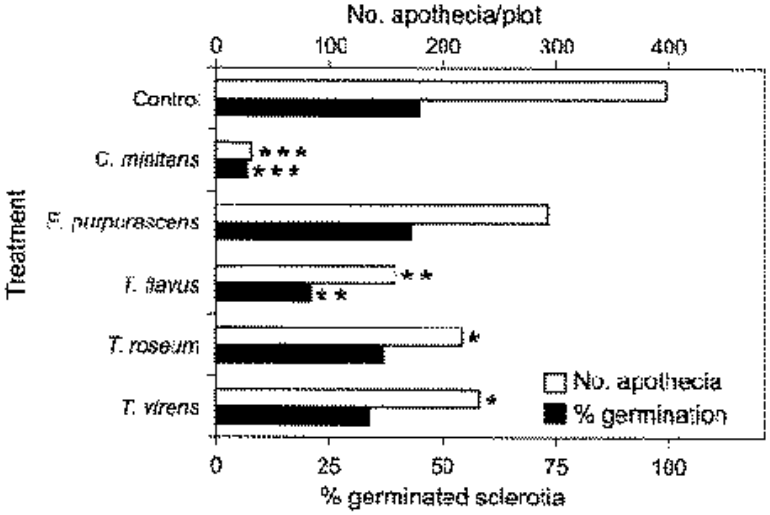

Fig. 2. Control of carpogenic germination and apothecial production of *S. sclerotiorum* by biocontrol agents under the canopy of dry bean. Data presented are the combined analysis of the results of the field trials in 1993 and 1994. Biocontrol agents were *Coniothyrium minitans*, *Epicoccum purpurascens*, *Talaromyces flavus*, *Trichothecium roseum*, and *Trichoderma virens*. ***,**,* = Significantly different from the control; $P<0.001$, $P<0.01$, and $P<0.05$, respectively.

the production of apothecia (Fig. 2). No difference was observed between the high (30 g per trench) and low (15 g per trench) rates of amendment for any of the biocontrol agents (data not presented).

Under the canopy of dry pea, treatment of soil with *C. minitans* or *T. flavus* significantly reduced carpogenic germination of sclerotia of *S. sclerotiorum* (Fig. 3). The frequency of carpogenic germination for the treatment of *C. minitans* was 2% in 1993 and 2% in 1994, compared to 7% in 1993 and 33% in 1994 for the untreated controls. Amendment of soil with *C. minitans* significantly reduced production of apothecia. The number of apothecia produced per plot for the *C. minitans* treatment was 5 in 1993 and 6 in 1994, compared to 28 in 1993 and 173 in 1994 for the untreated controls. There was no significant difference between high (30 g per trench) and low (15 g per trench) application rates of the biocontrol agents (data not presented).

Amendment of soil with *C. minitans* also significantly ($P<0.01$) reduced the number of apothecia produced per germinated sclerotium in dry bean, from 4.4 in the untreated control, to 2.8 in the *C. minitans* treatment (data not presented). In dry pea, the number of apothecia per germinated sclerotium was 2.3 in the untreated control, compared to 1.8 in the *C. minitans*- treated plots. Among the other biocontrol agents tested, none significantly reduced the number of apothecia produced per sclerotium under the canopy of either dry bean or dry pea.

DISCUSSION

This study reveals that soil treatment with fungal biocontrol agents can be effective in reducing carpogenic germination of sclerotia of *S. sclerotiorum*, and can thereby reduce the production of apothecia. Mycoparasitism appears to be the mechanism for the effective control of apothecial production as the treatment of soil with the non-mycoparasitic species *E. purpurascens* did not result in a significant reduction of carpogenic germination of sclerotia under the canopy of dry bean or dry pea. Among the four mycoparasites tested, *C. minitans* was the most effective agent for control of sclerotial germination and apothecial production of *S. sclerotiorum*. This suggests that *C. minitans* is well adapted to the conditions of the southern Canadian prairies, because the organism is widespread in fields [12] and is active against *S. sclerotiorum* under a wide range of field conditions [10, 11, 16, 23]. Another mycoparasite, *T. flavus*, also effectively reduced sclerotial germination and apothecial production under the bean canopy, but was less effective than *C. minitans* in the prairie soil. The less effective control of carpogenic germination of sclerotia by *T. virens* and *T. roseum* observed in the present study suggests that survival of these two indigenous species of mycoparasites in the prairie soil may have been poor compared to that of *C. minitans* and *T. flavus*.

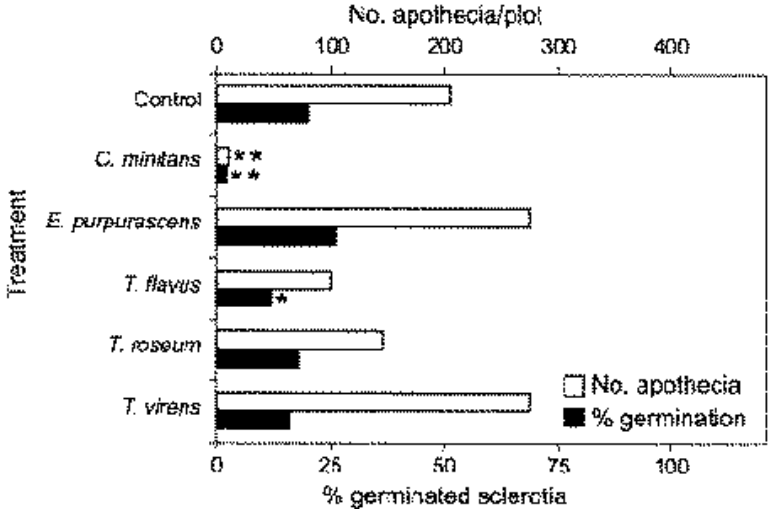

Fig. 3. Control of carpogenic germination and apothecial production of *S. sclerotiorum* by biocontrol agents under the canopy of dry pea. Data presented are the combined analysis of the results of the field trials in 1993 and 1994. Biocontrol agents were *Coniothyrium minitans*, *Epicoccum purpurascens*, *Talaromyces flavus*, *Trichothecium roseum*, and *Trichoderma virens*. **,* = Significantly different from the control; $P<0.01$, and $P<0.05$, respectively.

Talaromyces flavus, *Trichoderma virens*, and *Trichothecium roseum* were all less effective in suppressing the production of apothecia of *S. sclerotiorum* under the canopy of dry pea than under dry bean. The survival and activity of these biocontrol agents in the field depends on micro-environmental conditions that are influenced by crop canopy structure. It is likely that the low, dense canopy of dry bean resulted in more favorable conditions for the interaction between these biocontrol agents and *S. sclerotiorum* than the taller, more open canopy of dry pea. Further studies of the effect of environmental factors on the control of carpogenic germination of *S. sclerotiorum* by mycoparasites are warranted.

Coniothyrium minitans is a versatile mycoparasite, which attacks sclerotia of *S. sclerotiorum* buried in the soil [6, 10, 26, 28], or formed on the above-ground tissues of diseased plants [8, 18, 24]. Several attempts have been made to control white mold of bean using aerial application of *C. minitans*, but these often resulted in inconsistent control of the disease [4, 18, 27]. The present study indicates that soil treatment with mycoparasites, *C. minitans* in particular, is effective in reducing the production of apothecia from sclerotia of *S. sclerotiorum*. Since ascospores released from apothecia are the primary source of inoculum for white mold of bean [2] and sclerotinia blight of pea [14], it may be possible to enhance the control of sclerotinia diseases of dry bean and dry pea by *C. minitans*, using both soil treatment and aerial application methods.

Although spraying spores of *E. purpurascens* was effective in reducing incidence of white mold of bean [18, 30], the control is apparently achieved by antagonism as culture filtrates of *E. purpurascens* are inhibitory to hyphal growth and/or spore germination of plant pathogenic fungi including *Pythium* [5] and *S. sclerotiorum* [31]. Despite the rare occurrence of coiling of hyphae of *S. sclerotiorum* by *E. purpurascens* observed in dual culture [31], the inability to control carpogenic germination and apothecial production of sclerotia of *S. sclerotiorum* in the present study indicates that *E. purpurascens* is lacking in mycoparasitic activities against *S. sclerotiorum*. Therefore, the mycoparasite *C. minitans* would offer more potential than the antagonist *E. purpurascens* for control of *S. sclerotiorum* in dry bean and dry pea fields on the Canadian prairies, because soil treatment with the former can reduce the source of ascospore inoculum. Further studies to assess the control of sclerotinia rot of pulse crops by combination of soil treatment and aerial application of mycoparasites are in progress.

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摘　要

黃鴻章[1]、Erickson, R.S. 2000. 利用拮抗真菌抑制菜豆及豌豆田中菌核病菌 (*Sclerotinia sclerotiorum*) 的菌核發芽與子囊盤產生. 植病會刊 9:53-58. (Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, Alberta, T1J 4B1 Canada, [1.] 聯絡作者：電子郵件信箱：huangh@em.agr.ca；傳真機：403-3823156)

在菜豆田及豌豆田進行兩年試驗，比較五種真菌包括四種真菌寄生菌 (Mycoparasites) *Coniothyrium minitans*, *Talaromyces flavus*, *Trichothecium roseum* 及 *Trichoderma virens* 和一種拮抗菌 (antagonist) *Epicoccum purpurascens* 對抑制防治菌核病菌 (*Sclerotinia sclerotiorum*) 之菌核發芽與子囊盤產生效果。將固體培養的真菌與菌核於豆苗出土後 (約播種後二週) 施於土中，然後於豆子開花至成熟期定期檢查菌核發芽及子囊盤產生數目。兩年試驗結果顯示：*C. minitans* 與 *T. flavus* 的抑制效果最好；*T. roseum* 及 *T. virens* 僅在菜豆田有效果；而 *E. purpurascens* 在兩種豆田均無防治效果。*C. minitans* 施用於菜豆田或豌豆田均能顯著降低菌核的發芽數及子囊盤形成的數目。因此，在加拿大西部草原環境下，如果利用 *C. minitans* 來控制菜豆及豌豆田裡的菌核發芽形成子囊盤，繼而抑制產生子囊孢子，將有助於此一病害的防治。

關鍵詞：菜豆、豌豆、菌核病菌 (*Sclerotinia sclerotiorum*)、菌核、子囊盤、子囊孢子，生物防治、真菌寄生菌、*Coniothyrium minitans*