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Effect of Soil Treatment of Fungal Agents on Control of Apothecia of *Sclerotinia sclerotiorum* in Canola and Safflower Fields

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

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ABSTRACT

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A two-year field study was conducted to determine the effects of fungal agents on control of apothecia of *Sclerotinia sclerotiorum* when applied as soil treatment in canola and safflower fields. Among the five fungal species tested, *Coniothyrium minitans* was the most effective biocontrol agent for reducing carpogenic germination of sclerotia and production of apothecia of *S. sclerotiorum* under the canopies of canola and safflower, whereas *Talaromyces flavus* was only effective under the canopy of safflower. Soil treatment with *Trichothecium roseum*, *Trichoderma virens* or *Epicoccum purpurascens* did not cause a significant reduction on apothecial production under the canopy of either canola or safflower. The study suggests that soil treatment with fungal biocontrol agents such as *C. minitans* is an effective method for reducing the production of ascospores of *S. sclerotiorum* in canola and safflower fields, and thereby reducing incidence of sclerotinia stem rot of canola and sclerotinia head rot of safflower on the western Canadian prairies.

Key words: Canola, *Brassica napus*, *Brassica rapa*, safflower, *Carthamus tinctorius*, stem rot, head rot, *Sclerotinia sclerotiorum*, sclerotia, carpogenic germination, apothecia, ascospores, biological control, mycoparasites

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is an important fungal pathogen with cosmopolitan distribution that has a wide host range [22] including oilseed crops such as canola (*Brassica napus* L. and *Brassica rapa* L.) [24], safflower (*Carthamus tinctorius* L.) [21] and sunflower (*Helianthus annuus* L.) [6]. Oilseed crop losses due to sclerotinia diseases can often reach economic proportions. For example, an outbreak of sclerotinia blight in western Canada in 1982 resulted in an estimated loss of 15 million Canadian dollars to the canola crop [17].

Sclerotia of *S. sclerotiorum* in the soil can germinate carpogenically to produce airborne ascospores which are the primary source of inoculum for causing sclerotinia stem rot and pod rot of canola [7] and head rot of safflower [21]. High soil moisture conditions created by frequent rains or irrigation, and thick crop canopy are conducive to the carpogenic germination of sclerotia and consequently, the development of sclerotinia diseases in the field [2,15]. Producers in Canada currently use crop rotation and chemical fungicides for control of sclerotinia stem blight of canola and crop rotation for control of sclerotinia head rot of safflower. Recent studies on biocontrol of sclerotinia diseases have identified several mycoparasitic or antagonistic fungi with potential for use as biocontrol agents, including *Coniothyrium minitans* Campbell [5,8,25], *Epicoccum purpurascens* Ehrenb. ex Schlecht. [27], *Talaromyces flavus* Klöcker A.C.Stock & R.A. Sansom [18], *Trichoderma viride* Pers. ex Fr. [9], *Trichoderma virens* (Miller, Giddens and Foster) Arx., and *Trichothecium roseum* Pers. ex Fr. [12]. Soil treatment with *C. minitans* was effective in reducing incidence of diseases caused by *S. sclerotiorum* including sclerotinia wilt of sunflower [3,9,19], wilt or drop of lettuce (*Lactuca sativa* L.) [4] and white mold of dry bean (*Phaseolus vulgaris* L.) [10,14]. Several reports [9,13] indicated that destruction of sclerotial inoculum in the soil by the mycoparasites is one of the mechanisms contributing to the reduction of sclerotinia diseases. The objective of this study was to determine the effect of five fungal agents on production of apothecia by *S.*

sclerotiorum when applied as soil treatment under the canopy of canola and safflower.

MATERIALS AND METHODS

The five biocontrol agents used in this study were one antagonist, *E. purpurascens* isolate LRC 2114, and four mycoparasites, *C. minitans* isolate LRC 2137, *T. flavus* isolate LRC 2152, *T. virens* isolate LRC 2425, and *T. roseum* isolate LRC 2424. These fungi were originated from the southern Canadian prairies [10]. The inoculum of each biocontrol agent was prepared by growing the organism on wheat bran that was moistened with distilled water, and sterilised by autoclaving it twice at 121℃ for 30 min with a 48-h interval. The sterilised bran was measured into sterile 250 mL clear plastic containers (Twinpak; Regina, Saskatchewan, Canada), at a rate of 25 g per container. An aqueous spore suspension of each biocontrol agent was prepared from 14- to 28-day-old cultures grown on potato dextrose agar (PDA) (Difco; Detroit, MI) at 20℃. The spore suspensions were diluted to a concentration of 5.0×10^5 spores per mL, and 4 mL of the suspension was added to the wheat bran in each container. The plastic containers were sealed and incubated for 28 days under continuous fluorescent light at 20℃, and the resulting cultures were air-dried, crushed manually, and weighed into lots of either 15 or 30 g.

Sclerotinia sclerotiorum isolate LRC 2148 was grown on a cooked bean substrate to produce sclerotia for use in this study by the following process. Canned red kidney beans (Fraser Valley Foods; Fraser Valley, British Columbia, Canada) were transferred into a sterilised food processor, pureed, and dispensed into sterile 250 mL clear plastic containers (Twinpak, Regina, Saskatchewan, Canada), 25 mL per container. Four PDA plugs (5 mm diameter) containing 5-day-old mycelial mats of *S. sclerotiorum* were added to each container and the containers were incubated at 10℃ in the dark for 8 weeks. Sclerotia produced on the colony in each container were harvested by washing them in distilled water and air-drying on paper towel. Sclerotia in the size range of 5 to 10 mm diameter were selected for the study, counted into lots of 100, and stored at 10℃ for approximately two weeks prior to use in the field.

The experiments were conducted in an irrigated field at the Lethbridge Research Centre during 1993 and 1994, using canola cv. Westar and safflower cv. Saffire. Prior to planting, 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. For both crops, fertilizer was not applied in 1993, but nitrogen fertilizer (34-0-0; N-P-K) was applied to the fields prior to seeding in 1994 at a rate of 200 kg/ha. Crops were seeded at the rate of 17 kg/ha for canola and 35 kg/ha for safflower, with seeding for both crops taking place on 25 May 1993 and 17 May 1994. Plots of both canola and safflower were seeded in continuous blocks with a four row seeder, to a depth of 2.5 cm and a row spacing of 22.5 cm. The experiment in both years was established on a section of the field that had been fallowed in the previous year.

After seedling emergence, individual plots were established within the seeded block of each crop. Each plot consisted of five rows of crop with 2.5-m row length and 23-cm row spacing. Two sclerotia burial areas were established in each plot by scraping 2-cm deep trenches, 1 m long by 0.12 m wide each, in the second and third inter-row spaces. For each trench, 1 kg of soil was removed and mixed with inoculum of biocontrol agent grown on bran, at the rate of 15 g per trench for the low rate and 30 g per trench for the high rate. Half of the biocontrol agent - amended soil was put back in the trench, 100 sclerotia were spread evenly over the surface, and the sclerotia were covered with the remaining amended soil. A thin layer of natural field soil was spread over the amended soil and the trenches were tamped down to prevent movement of the soil by wind. Controls were established by burying 100 sclerotia per trench without biocontrol agent. Treatments were established each year after seedling emergence (approximately 3 weeks after seeding), and were arranged in a randomized block design with four replicates. Irrigation was provided frequently to create conducive conditions for carpogenic germination of sclerotia and development of apothecia, from the late vegetative growth stage (mid-June) to the late pod-filling stage (late August).

The number of carpogenically germinated sclerotia and number of apothecia produced was counted weekly for each plot, from late bloom (mid-August) to maturity (mid-September). Color-coded sticks representing the number of apothecia previously counted were used to allow cumulative counting of apothecia from each sclerotium. The percent of sclerotia germinated carpogenically and number of apothecia produced per plot were statistically analysed for each crop and year separately, using analysis of variance. A combined analysis over years was also performed for each crop. For percent germination of sclerotia, the data were converted by a log transformation for statistical analyses. All statistical analyses were conducted using SAS/STAT™ software [23].

RESULTS

As the canopy of canola and safflower developed and covered the inter-row spaces in late July, sclerotia of *S. sclerotiorum* buried in each plot began to germinate carpogenically, resulting in the production of clusters of small, tan-colored apothecia on the ground surface. The production of apothecia continued until the end of the growing season (early September). The release of airborne ascospores from clusters of apothecia in the form of spore-cloud was often visible when the canopy was gently parted in plots.

Among the five fungal agents tested in canola fields, only the soil treatment with *C. minitans* significantly ($P<0.05$) reduced carpogenic germination of sclerotia and production of apothecia compared to the untreated control (Table 1). The efficacy of *C. minitans* was consistent for both years. The frequency of carpogenic germination under the canopy of canola was 7.4% in 1993 and 1.1% in 1994 for the treatment of *C. minitans* compared to 27.4% in 1993 and 16.0% in 1994 for the untreated control. The number of apothecia produced per plot for the treatment of *C. minitans* was 28 in 1993 and 3 in 1994, compared to 183 in 1993 and 86 in 1994 for the untreated control (Table 1).

Under the canopy of safflower, soil treatment with *C. minitans* resulted in significant ($P<0.05$) reductions in carpogenic germination of sclerotia and production of apothecia in both 1993 and 1994 compared to the untreated control (Table 2). For the treatment of *C. minitans*, the frequency of carpogenic germination under the canopy of safflower was 10.2% in 1993 and 2.7% in 1994, compared to 39.2% in 1993 and 51.8% in 1994 for the untreated control. The number of apothecia produced per plot for the treatment of *C. minitans* under the canopy of safflower was 36 in 1993 and 13 in 1994, compared to 268 in 1993 and 443 in 1994 for the untreated control. Soil treatment with *T. flavus* significantly ($P<0.05$) reduced carpogenic germination of sclerotia and production of apothecia under the canopy of safflower in 1994, but not in 1993 (Table 2). The frequency of carpogenic germination under the canopy of safflower for treatment of *T. flavus* was 40.9% in 1993 and 15.5% in 1994, compared to 39.2% in 1993 and 51.8% in 1994 for the untreated control. The number of apothecia produced per plot under the canopy of safflower for the treatment of *T. flavus* was 219 in 1993 and 86 in 1994, compared to 268 in 1993 and 443 in 1994 for the untreated control.

Results of the two-year tests showed a significant difference ($P<0.05$) in carpogenic germination and apothecial production of *S. sclerotiorum* between the canopies of canola and safflower. The frequency of carpogenic germination of sclerotia and number of apothecia produced in safflower plots were higher than in canola plots. Results also showed that no effect of rate of amendment (high versus low rate) was observed on carpogenic germination of sclerotia or apothecial production, for any of the biocontrol agents in any year or under either crop canopy.

DISCUSSION

This study demonstrates that of the five microbial agents

Table 1. Effect of soil treatment with fungal biocontrol agents on carpogenic germination of sclerotia of *S. sclerotiorum* under the canopy of canola (Field experiments, 1993 and 1994)

	Carpogenic germination (%)		No. apothecia per plot	
Treatment[1]	1993	1994	1993	1994
Control	27.4 b[2]	16.0 b	183 b	86 b
Coniothyrium minitans	7.4 a	1.1 a	28 a	3 a
Epicoccum purpurascens	30.6 b	21.8 b	181 b	115 b
Talaromyces flavus	19.5 b	21.2 b	145 b	108 b
Trichothecium roseum	33.9 b	21.2 b	138 b	107 b
Trichoderma virens	17.5 b	18.2 b	92 b	94 b

[1] Soil in inter-row spaces was amended with powdered biocontrol agents (on wheat bran substrate), at the rate of 15 or 30 g/kg soil, after seedling emergence. Soil in the control was not amended.
[2] Means within each column followed by the same letter are not different significantly ($P>0.05$) (LSD test).

Table 2. Effect of soil treatment with fungal biocontrol agents on carpogenic germination of sclerotia of *S. sclerotiorum* under the canopy of safflower (Field experiments, 1993 and1994)

	Carpogenic germination (%)		No. apothecia per plot	
Treatment[1]	1993	1994	1993	1994
Control	39.2 b[2]	51.8 b	268 b	443 b
Coniothyrium minitans	10.2 a	2.7 a	36 a	13 a
Epicoccum purpurascens	34.1 b	51.7 b	230 b	382 b
Talaromyces flavus	40.9 b	15.5 a	219 b	86 a
Trichothecium roseum	33.6 b	41.6 b	183 b	276 b
Trichoderma virens	33.3 b	47.6 b	184 b	388 b

[1] Soil in inter-row spaces was amended with powdered biocontrol agents (on wheat bran substrate), at the rate of 15 or 30 g/kg soil, after seedling emergence. Soil in the control was not amended.
[2] Means within each column followed by the same letter are not different significantly ($P>0.05$) (LSD test).

applied as soil treatment, *C. minitans* is the most effective agent for control of carpogenic germination of sclerotia of *S. sclerotiorum* under the canopies of canola and safflower. The consistency of *C. minitans* in this study is similar to previous findings on sunflower [8,19] and in soil [11,26]. The high level of effectiveness of *C. minitans* is likely due to its destructive mycoparasitic activity against sclerotia of *S. sclerotiorum* [8], combined with its adaptation to soil conditions in temperate climates. The ineffective control of carpogenic germination of sclerotia of *S. sclerotiorum* by *E. purpurascens* may be because it is an antagonistic fungus and not a mycoparasite [27], and therefore is incapable of destroying sclerotia of *S. sclerotiorum*. Although *T. flavus* [18], *T. roseum* [12] and *T. virens* are all mycoparasites capable of infecting sclerotia of *S. sclerotiorum*, they are less effective than *C. minitans* because they are not well adapted to prairie conditions (H. C. Huang and R. S. Erickson, unpublished) [20]. A comprehensive assessment of the interactions between various biocontrol agents and *S. sclerotiorum* under a range of environmental conditions would provide valuable information to guide future efforts in biological control of *S. sclerotiorum.*

This study also shows that oilseed crop canopy affects carpogenic germination of sclerotia of *S. sclerotiorum* and subsequent production of apothecia. The higher carpogenic germination and apothecial production observed under the canopy of safflower were likely due to the dense foliage, which provided a moist, cool microenvironment suitable for proliferation of ascospores of *S. sclerotiorum.* In contrast, the canola canopy was sparser and more open, resulting in a drier microenvironment that was less favorable for carpogenic germination of sclerotia. This finding is similar to that of Huang and Erickson [10], which demonstrated higher carpogenic germination and apothecial production under the dense canopy of dry bean, compared to the sparser canopy of dry pea.

Coniothyrium minitans is an effective biocontrol agent for control of diseases caused by *S. sclerotiorum* in a wide range of crops including canola [16] and vegetable crops [1,4]. Results of two years of field testing in this study suggest that *C. minitans* is the most promising biocontrol agent for control of sclerotinia diseases of canola and safflower in western Canada. This is not only because of its effectiveness in suppressing apothecial production of *S. sclerotiorum*, but also because of its ability to survive Canadian prairie winters [11] and to induce soil suppression of *S. sclerotiorum* [13].

Control of apothecial production by mycoparasites is only one of the strategies currently being investigated for suppression of diseases caused by *S. sclerotiorum* in pulse and oilseed crops. *In vitro* studies by Huang et al. [14] demonstrated that soil amendment with crop residues can also suppress production of apothecia of *S. sclerotiorum*, and further proved that the technique is compatible with soil application of mycoparasites such as *C. minitans*. Soil treatment is an important method for reducing sclerotial populations in soil, and thereby reducing the primary inoculum for development of sclerotinia diseases. For diseases such as blossom blight, pod rot and head rot caused by airborne ascospores of *S. sclerotiorum*, an approach combining soil treatment and foliar spray of *C. minitans* may offer improved protection of plants. Further investigation in this area of study is warranted.

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摘 要

黃鴻章[1,2]、R. S. Erickson[1]. 2004. 土壤處理眞菌防治油菜與紅花田之菌核病菌產生子囊盤的效果. 植病會刊 13: 1-6.([1.]加拿大農部及農業食品部 Lethbridge 研究中心; [2.]聯絡作者，電子郵件：huangh@agr.gc.ca，傳眞：+1-403-382-3156)

在油菜及紅花田裡採用土壤處理的方式，比較五種眞菌 (包括 *Coniothyrium minitans, Talaromyces flavus, Trichothecium roseum, Trichoderma virens* 及 *Epicoccum purpurascens*) 防治菌核病菌 (*Sclerotinia sclerotiorum*) 之菌核發芽與產生子囊盤 (apothecia) 的效果。從西元 1993 及 1994 兩年的試驗結果顯示，在油菜及紅花田理處理超寄生菌 *C. minitans*，均可顯著降低菌核的發芽率與減少子囊盤的形成數目。惟 *Talaromyces flavus* 僅於 1994 年紅花田造成菌核發芽率及子囊盤形成數目顯著地下降；至於其他三種眞菌卻沒有防治的效果。這項試驗証明，在油菜或紅花作物覆蓋情況下，用 *C. minitans* 處理田間土壤，可以有效地減少菌核病菌產生子囊盤及子囊孢子 (ascospores)。所以在加拿大西部的環境下，用土壤處理 *C. minitans* 的方式可以有效防治油菜菌核病及紅花爛頭病。

關鍵詞：油菜、*Brassica napus*、*Brassica rapa*、紅花、*Carthamus tinctorius*、莖枯、爛頭、*Sclerotinia sclerotiorum*、菌核、apothecia、生物防治、超寄生菌、mycoparasites