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Biological Control of Sclerotinia Stem Rot of Canola Using *Ulocladium atrum*

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ABSTRACT

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Field studies were conducted in 2004 and 2005 in Lethbridge, Alberta, Canada to determine the efficacy of control of Sclerotinia stem rot of canola caused by *Sclerotinia sclerotiorum* by the antagonistic fungus *Ulocladium atrum*. Compared to the untreated control, foliar application of a 10^6 spore/mL suspension of *U. atrum* at the rate of 300 mL/m^2 onto canola plants at the early flowering stage resulted in reductions in incidence and severity of Sclerotinia stem rot, a reduction in contamination of harvested canola seeds by sclerotia of *S. sclerotiorum*, and an increase in canola seed yield. The effectiveness of *U. atrum* was similar to the treatment of the mycoparasite *Coniothyrium minitans*, but not as good as foliar application of 750 g/ha of the fungicide Lance (boscalid). Suggestions for further research on enhancing the effectiveness of *U. atrum* as a biological control agent for Sclerotinia diseases caused by *S. sclerotiorum*, and assessing its eco-friendliness, are discussed.

Key words: antagonism, biological control, boscalid, *Brassica* spp., canola, *Coniothyrium minitans*, foliar application, Sclerotinia stem rot, *Sclerotinia sclerotiorum*, *Ulocladium atrum*.

INTRODUCTION

Sclerotinia stem rot is the most prevalent disease of canola (*Brassica napus* L. and *B. rapa* L.) in western Canada [8,22,23]. It is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, a fungal pathogen with worldwide distribution [6] and a host range that includes numerous plant species [5]. Canola crop losses due to Sclerotinia stem rot can have serious economic impact. For example, an outbreak in 1982 in western Canada resulted in an estimated loss of 15 million Canadian dollars [3]. Although fungicides are available and effective for control of the disease, their use may be limited because of problems associated with cost as well as environmental and health concerns [25].

Senescent canola tissues such as flower petals play an important role in infection of plants by *S. sclerotiorum*. When airborne ascospores of *S. sclerotiorum* land on senescent petals, they germinate, and produce mycelia that invade the plant tissues [9]. Thus, protection of senescent petals from colonization by *S. sclerotiorum* by biocontrol agents may be an effective strategy for control of sclerotinia stem rot of canola. For example, foliar application of the mycoparasite *Coniothyrium minitans* Campbell was effective in reducing incidence and severity of white mold of bean (*Phaseolus vulgaris* L.) [13] and sclerotinia stem rot of oilrape (*Brassica* spp.) [21]. Another example showed that foliar application of the antagonistic fungus *Epicoccum purpurascens* Ehrenb.: Schlecht. also resulted in reduced incidence of white mold of bean [26].

Ulocladium atrum Preuss is an antagonistic fungus

that is effective in the control of gray mold of crops caused by *Botrytis cinerea* Pers.: Fr.[15,16,18]. In a 3-year field study, Huang and Erickson[10] reported that foliar application of *U. atrum* reduced incidence and severity of white mold of bean caused by *S. sclerotiorum*. In an indoor study, Li *et al.*[20] reported that *U. atrum* reduced the colonization and infection of petals of canola (*Brassica napus* L.) and alfalfa (*Medicago sativa* L.) by ascospores of *S. sclerotiorum*. The objective of this study was to determine the efficacy of foliar application of *U. atrum* as a biological control agent for control of sclerotinia stem rot of canola under field conditions.

MATERIALS AND METHODS

Sclerotinia sclerotiorum LRC 2148 (syn. sun-87), used for this study, was isolated from an infected sunflower plant near Lethbridge, Alberta, Canada[12]. Sclerotia were produced by growing fungal cultures on canned kidney beans (Fraser Valley Foods; Fraser Valley, British Columbia, Canada). Kidney beans were placed into sterile 250-mL clear plastic containers (Twinpak; Regina, Saskatchewan, Canada) with 25 mL in each container, and each container was inoculated with five 5-mm diameter agar plugs from a 5-day-old mycelial culture of *S. sclerotiorum* grown on potato dextrose agar (PDA) (Difco; Detroit, Michigan, USA). The kidney bean cultures were covered with plastic bags and incubated for 10 weeks at 10 ℃ in the dark. Sclerotia were harvested from the cultures by washing in distilled water, separating out the sclerotia, and air-drying them overnight on paper towel. The sclerotia were stored at 10℃ in a paper bag for 2-3 weeks, until used as disease inoculum for the field experiments.

The biological control agents used for the study were: *Ulocladium atrum* LRC 2535, isolated from an apothecium of *S. sclerotiorum* near Lethbridge, Alberta, Canada; and *Coniothyrium minitans* LRC 2137, isolated from an infected sclerotium of *S. sclerotiorum* on a wilted sunflower plant near Morden, Manitoba, Canada. Inoculum of both biocontrol agents was prepared according to the method of Huang *et al.*[13]. The fungi were grown on PDA at 20℃ under fluorescent lights for 21 days. Spore suspensions were prepared by rinsing each culture with sterile distilled water while gently scraping with a spatula, and filtering the suspension through four layers of sterile cheesecloth. The spore concentration of the suspensions was checked using a hemacytometer, and adjusted by dilution to 10^6 spores/mL. Methyl cellulose (Sigma-Aldrich; Milwaukee, Wisconsin, USA) at the rate of 5 g/L and Tween 20 (polyoxyethylenesorbitan monolaurate; Fisher Scientific, Fair Lawn, New Jersey, USA) at the rate of 0.2 ml/L were added to the spore suspension of each fungus prior to application in the field.

The experiments were performed during 2004 and 2005 in an irrigated field at the Agriculture and Agri-Food Canada Research Centre near Lethbridge, Alberta, Canada. The experiments were conducted each year in a portion of field that was fallowed during the previous year. The experimental area was fertilized prior to seeding with nitrogen fertilizer (34-0-0; N-P-K) at the rate of 200 kg/ha, and was infested with *S. sclerotiorum* by spreading sclerotia onto the soil surface at the rate of 30 sclerotia/m^2. The sclerotia were buried to a depth of 3 cm by shallow cultivation with a Triple K cultivator (Kongskilde; Exeter, Ontario, Canada). Canola cv. Westar was sown on 28 May 2004 and 31 May 2005 at the rate of 17 kg/ha, with a seeding depth of 1.5 cm and row spacing of 22.5 cm. The dimensions of each plot were 5 m long by 3 m wide. Experiments were conducted using a randomized block design with three replicates and four treatments: (1) untreated control; (2) *U. atrum* spray; (3) *C. minitans* spray; and (4) fungicide spray. Treatments were applied simultaneously to the plots at the rate of 300 mL/m^2 using a garden hand-pump sprayer. There were two applications during each year, with the first application in mid-July (approximately 5% bloom) and the second application 10 days later (approximately 50% bloom). The plots were not irrigated for 24 hours following each application. The fungicide used was Lance (boscalid; BASF, Toronto, Ontario, Canada) at the rate of 750 g/ha/application. From mid-June (late vegetative growth stage) to late August (late pod-filling stage), irrigation was provided frequently to maintain moist soil conditions conducive for carpogenic germination and production of ascospores from the sclerotia in the soil, and thereby promote development of sclerotinia stem rot. Weeds were manually removed from the plots during the growing season.

Canola plants were assessed for incidence and severity of sclerotinia stem rot in mid- September (maturity stage) of each year. Each plant in the ten middle rows of each plot was rated on a scale of 1 to 4, where 1=healthy, 2=single stem infected, 3=multiple stems infected, and 4=dead plant. The last 0.5 m on both ends of each row was disregarded. The incidence of sclerotinia stem rot of canola was defined as the percentage of plants infected by *S. sclerotiorum*, and the disease severity was calculated as a weighted average of the plant ratings, using the formula $DS=\Sigma nr/t$, where DS=disease severity (ranging from 1 to 4), n=number of plants, r=plant rating (scale 1 to 4), and t=total number of plants rated. Plots were harvested in mid-September using a Nurserymaster Elite 2000 plot combine (Wintersteiger; Ried im Innkreis, Austria). Samples were air-dried for 2 weeks in paper bags on wire racks in a room at 20℃, and were cleaned and

weighed to determine the canola seed yield for each plot. The number of sclerotia in each sample was counted during seed cleaning.

Analysis of variance (ANOVA) at the P=0.05 level was used to determine significant differences between treatments for the data of incidence of sclerotinia stem rot, severity of sclerotinia stem rot, canola seed yield, and number of sclerotia in harvested seeds. Treatment means were separated using Duncan's multiple range tests. The data for each year was analyzed separately. Statistical analyses were conducted using SAS/STAT® computer software, version 8.2 [24].

RESULTS

During late July (mid-bloom stage) to late August (early maturity stage) of each year, small tan-colored apothecia were produced by carpogenic germination of the sclerotia of *S. sclerotiorum* in the soil. The apothecia released airborne ascospores for infection of canola plants. Plants with symptoms of stem rot began to appear in early August and the number of infected plants increased until the crop canopy deteriorated due to maturity. The incidence of sclerotinia stem rot was highest in the untreated control, reaching 42% in 2004 and 39% in 2005 (Table 1). Significant (P<0.05) reductions in disease incidence were observed for the treatments of *U. atrum*, *C. minitans*, and fungicide, compared to the untreated control. The incidence of sclerotinia stem rot for the fungicide treatment was significantly lower than for all the other treatments (Table 1). For example, the incidences of sclerotinia stem rot for the treatments of *U. atrum*, *C. minitans* and fungicide in 2004 were 17, 19 and 7%, respectively, compared to 42% for the untreated control. The difference in disease incidence of the treatments of *U. atrum* and *C. minitans* treatments was not significant in 2004, but was significant in 2005 (Table 1).

The trends observed for incidence of sclerotinia stem rot of canola were also apparent in the data for disease severity. The highest disease severity ratings were observed in the untreated control, at 1.92 in 2004 and 1.81 in 2005 (Table 1). Foliar spray with any of the treatments of *U. atrum*, *C. minitans*, or fungicide resulted in significant (P<0.05) reductions in severity of sclerotinia stem rot, compared to the untreated control. The lowest disease severity ratings were observed for the fungicide treatment, and these were significantly lower than any of the other treatments (Table 1). For example, the severity index of sclerotinia stem rot in 2005 was 1.13 for the fungicide treatment, compared to 1.24 for the treatment of

Table 1. Effect of foliar application of *Ulocladium atrum* on incidence and severity of sclerotinia stem rot of canola (field experiments in 2004 and 2005)

	Stem rot incidence (%)		Stem rot severity (1-4) [1]	
Treatment	2004	2005	2004	2005
Control (untreated)	42 a [2]	39 a	1.92 a	1.81 a
Coniothyrium minitans [3]	19 b	20 b	1.46 b	1.45 b
Ulocladium atrum [3]	17 b	14 c	1.41 b	1.24 c
Lance (boscalid) [4]	7 c	6 d	1.16 c	1.13 d

[1] Severity index: 1=healthy, 2=single stem infected, 3=multiple stems infected, 4=plant dead.
[2] Means within a column followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test).
[3] Biocontrol agents applied twice at the rate of 300 mL/m^2. Data collected 5 weeks after the second application.
[4] Fungicide applied twice at the rate of 750 g/ha/application. Data collected 5 weeks after the second application.

Table 2. Effect of foliar application of *Ulocladium atrum* on seed yield of canola and contamination of sclerotia of *Sclerotinia sclerotiorum* in harvested seeds (field experiments in 2004 and 2005)

	Canola seed yield (kg/ha)		Sclerotia in harvested canola seeds (no. sclerotia/kg seed)	
Treatment	2004	2005	2004	2005
Control (untreated)	895 a [1]	1109 a	26 a	13 a
Coniothyrium minitans [2]	1311 b	1291 b	6 b	2 b
Ulocladium atrum [2]	1364 b	1349 b	4 bc	2 b
Lance (boscalid) [3]	1712 c	1557 c	1 c	2 b

[1] Means within a column followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test).
[2] Biocontrol agents applied twice at the rate of 300 mL/m^2. Data collected 5 weeks after the second application.
[3] Fungicide applied twice at the rate of 750 g/ha/application. Data collected 5 weeks after the second application.

U. atrum, 1.45 for the treatment of *C. minitans*, and 1.81 for the untreated control. The disease severity index for the treatment of *U. atrum* was significantly lower than for the treatment of *C. minitans* in 2005, but not in 2004 (Table 1).

The canola seed yields for the treatments of *U. atrum*, *C. minitans*, or fungicide were all significantly ($P<0.05$) higher than the untreated control in both years (Table 2). Also, seed yield for the fungicide treatment was significantly ($P<0.05$) higher than for all the other treatments. For example, in 2004, the canola seed yield for the fungicide treatment was 1712 kg/ha, compared to 1364 kg/ha for *U. atrum*, 1311 kg/ha for *C. minitans* and 895 kg/ha for the untreated control (Table 2). The seed yields for the treatments of *U. atrum* and *C. minitans* were not significantly different ($P>0.05$) from each other. The number of sclerotia in harvested canola seeds was also significantly ($P<0.05$) reduced in both years for the treatments of *U. atrum*, *C. minitans* or fungicide, compared to the untreated control (Table 2). In the 2004 experiment, for example, there were 4, 6 and 1 sclerotia/kg canola seed for the treatments of *U. atrum*, *C. minitans* and fungicide, respectively, compared to 26 sclerotia/kg canola seed for the untreated control. There was no significant difference ($P>0.05$) in sclerotial contamination of canola seeds between the treatments of *U. atrum* and *C. minitans*.

DISCUSSION

This study demonstrates that foliar application of *U. atrum* to canola at the early flowering stage results in reduced incidence and severity of sclerotinia stem rot, increased canola seed yield, and reduced contamination of harvested canola seeds by sclerotia of *S. sclerotiorum*. Although *U. atrum* is an antagonist [14] and *C. minitans* is a mycoparasite [11], the effects of foliar application of these two biocontrol agents were similar with respect to the parameters measured in this study. The results of this study are also similar to previous reports of *C. minitans* [13] and *U. atrum* [10] on biological control of white mold of bean caused by *S. sclerotiorum*. Neither of the biocontrol agents were as effective as the fungicide Lance (boscalid), suggesting that there is ample room for further improvement in the efficacy of these agents. Strategies to improve the effectiveness of the biocontrol agents might include determination of optimal formulations, application rates and times, and combination of different treatments, both biological and fungicidal.

Numerous studies have shown that *U. atrum* has potential as a biocontrol agent for plant diseases caused by *Botrytis* spp. [14,15,16,17,18]. However, the assessment of *U. atrum* as a biocontrol agent for diseases caused by *S. sclerotiorum* in the field has so far been limited to the current study on stem blight of canola, and a previous study on white mold of common bean [10], despite the fact that *S. sclerotiorum* has a very broad range of hosts, including numerous economically important crops [5]. Therefore, further studies are warranted to determine whether *U. atrum* can control diseases of *S. sclerotiorum* on other host crops. These studies could also include Sclerotinia diseases that occur in other climates such as the tropical and subtropical regions, since the studies to date have only addressed Sclerotinia diseases in the temperate zone of Canada.

More information is needed on the basic biology and ecology of *U. atrum*, in order to determine its eco-friendliness as a biological control agent, and to reveal possible ways to improve its effectiveness. *U. atrum* has been characterized as a saprophyte or a weak pathogen of crops [7], and was reported to play a positive role in soil humification and development of soil structure [1, 2]. Variation in the effectiveness of different strains of *U. atrum* has been observed [4], suggesting that selection and use of certain strains may enhance the effectiveness of *U. atrum* as a biocontrol agent. Li *et al.* [19] showed that a strain of *U. atrum* was tolerant to the fungicides benomyl (DuPont, Wilmington, DE, USA) and vinclozolin (BASF, Parsippany, NJ, USA), but these fungicides are no longer registered for control of Sclerotinia diseases in Canada. Although the existing information is all useful, more knowledge is needed on the effects of environmental factors (temperature, humidity, light, dessication) and nutritional factors on growth, sporulation, and production of enzymes and antifungal substances by *U. atrum*. This new information would provide a basis to guide future efforts in the development of *U. atrum* for commercial use for control of diseases caused by *S. sclerotiorum*, thereby enhancing the sustainability of agricultural crop production in the future.

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