

EVALUATING BIOLOGICAL CONTROL PRODUCTS TO MANAGE SCLEROTINA STEM ROT IN CANOLA

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ABSTRACT

Sclerotinia stem rot (SSR), also known as white mold, is caused by the soilborne fungal pathogen *Sclerotinia sclerotiorum*. This disease can cause yield losses in canola exceeding 50%. Currently, there is little information available on the effectiveness of biofungicide products for the control of *S. sclerotiorum* in canola alone or in complement with chemical control. It is pertinent to refine application timings and frequency of current biofungicides and to compare their efficacy to conventional fungicides. Additionally, there are limited biofungicide products commercially available, and novel products may provide growers with additional tools to manage SSR. The goal of this study was to evaluate the effectiveness of biological control products for the management of SSR under field conditions in Minnesota and to investigate new biological management tools. Results indicated that the current, commercially available biofungicide products evaluated (Contans, Serenade OPTI, and Double Nickel LC) did not reduce disease, enhance sclerotia degradation, or improve yields compared to conventional fungicides or untreated controls. However, in Roseau, MN, Endura, applied at early bloom, provided better SSR control than untreated plots. Proline applied at early bloom followed by Endura a week later, provided the most effective disease reduction. Yields were also improved with this “premium” application compared to two biological control applications. However, since yields were not greater than untreated controls, the treatments evaluated would not be economical under the disease pressures evaluated. So far, seven bacteria and over 10 fungal isolates isolated from sclerotia have exhibited antagonism to *S. sclerotiorum*. Work to identify antagonists of *S. sclerotiorum* is an initial step towards the discovery of novel biofungicides and further studies on the suppression mechanisms of these microbes. Future work may isolate metabolites, antibiotics, or other compounds that may have applications for control of *S. sclerotiorum* in canola.

INTRODUCTION

Sclerotinia stem rot (SSR), also known as white mold, is caused by the soilborne fungal pathogen *Sclerotinia sclerotiorum*. This disease can cause yield losses in canola exceeding 50% per infected plant (canolacouncil.org). *S. sclerotiorum* also has a broad host range that includes many broadleaf crops that may be grown in rotation with canola such as soybean, sunflower, potato, and dry bean.

Managing SSR is complicated by a variety of factors. Crop rotation does not eradicate the pathogen since it can infect a wide range of crop plants. The hardened survival structures of the fungus, called sclerotia, can survive for many years in the soil and will initiate new infections when conditions are favorable. Chemical control can be effective, but there is a narrow range during which to apply fungicides (20-50% flowering), and chemical control may not be economical if more tolerant varieties are planted (Dr. Damon Smith, badgercropdoc.com) or if infection levels are below an incidence of ~17% (depending on current crop and chemical prices, del Rio et al., 2007). Complementary management strategies are needed for durable, economical, and effective management of the disease.

Currently, there is little information available on the effectiveness of biofungicide products for the control of *S. sclerotiorum* in canola. Biofungicides can work in multiple ways. They can control pathogens through 1) the production of toxins, 2) parasitism that attacks the pathogen, 3) competition for resources, or 4) induced resistance of the crop plant. Current products labeled for SSR control on canola include Contans (Bayer), a product derived from a fungus which directly attacks the pathogen's resting structures called sclerotia. Double Nickel LC (Certis Biologicals) and Serenade OPTI (Bayer) are protectants, derived from bacteria that impede infection. It is pertinent to refine application timings and frequency of these biofungicides and to compare their efficacy to conventional fungicides. Additionally, there are limited biofungicide products commercially available, and novel products would provide growers with additional tools to manage SSR.

PROJECT OBJECTIVES AND CORRESPONDING RESULTS

The goal of this study was to evaluate the effectiveness of biological control products for the management of SSR under field conditions in Minnesota and to investigate new biological management tools.

Objectives of this work:

- 1) To evaluate the efficacy of current, diverse biological control products at reducing disease and inoculum
- 2) To evaluate the economic cost-benefit of using biocontrol products in sclerotinia infested fields
- 3) To evaluate the potential of fungi and bacteria isolated from sclerotia for biological disease control

Corresponding results of this work:

- 1) Results indicated that the current, commercially available biofungicide products evaluated (Contans, Serenade OPTI, and Double Nickel LC) did not reduce disease, enhance sclerotia degradation, or improve yields compared to conventional fungicides or untreated controls. However, in Roseau, MN, Endura, applied at early bloom, provided better SSR control than untreated plots. Proline applied at early bloom followed by Endura a week later, provided the most effective disease reduction.
- 2) Since yields were not greater than untreated controls, the treatments evaluated would not be economical under the disease pressures evaluated.

- 3) A total of 69 bacterial and 29 fungal isolates were recovered from sclerotia and tested for antagonistic effects against *S. sclerotiorum*. So far, seven bacteria and ten fungal isolates have exhibited antagonism and are undergoing genetic identification, as described above. Work to identify antagonists of *S. sclerotiorum* is an initial step towards the discovery of novel biofungicides and further studies on the suppression mechanisms of these microbes.

MATERIALS AND METHODS

Field trial establishment

Field trials were conducted at two University of Minnesota research farm locations in St. Paul and Roseau, Minnesota. The canola variety LL 345PC, treated with Helix Vibrance + Lumiderm seed treatment, was seeded on 4/23/24 in St. Paul and on 5/29/24 in Roseau at a seeding rate of 12 PLS, with individual plots measuring 6x30 feet and trimmed to 20 feet. Fields were infested right after planting with sclerotia sourced from a sunflower confectionary at a rate of ~60k sclerotia/acre. In Roseau, the field was prepared with a high-speed disk in early May, followed by fertilizer application (140-30-20-20S) incorporated before planting. Canola emergence was uniform, with average early-season vigor. A hailstorm on 6/13 caused significant leaf damage. Canola was direct harvested on 9/14. In St. Paul, canola emerged uniformly, showing good early-season. Plots in St. Paul were misted to encourage disease development through early flowering (June 7th).



Image 1. Misting to encourage apothecia production and the development of SSR

Objective 1) In order to evaluate current, commercially available biofungicides for control of *Sclerotinia sclerotiorum*, the timing of three current products and one combined treatment were evaluated and compared to an untreated control and early flowering chemical application of Endura, a boscalid fungicide that is effective at controlling SSR when applied at flowering (Bradley et al., 2006) and a dual application of Proline at early flowering and Endura a week later (Table 1). Each treatment was repeated four times and randomized within each block of

treatments (randomized complete block design). Biofungicide applications were applied with a backpack sprayer at the timings indicated in table 1. In St. Paul, the first application was on 6/13, and the second application was 6/19. In Roseau, the first application was on 7/15 and the second application on 7/22. The Contans treatment at St. Paul was applied 4/22 and 5/15 in Roseau and lightly harrowed after application.

Table 1. Treatments for 2024 biofungicide trial

Treatment	Product	Active ingredient	Application timing	Application rate
1	Untreated control	NA	NA	NA
2	Contans	<i>Coniothyrium minitans</i>	May, pre-plant soil application	4 lbs/ac
3	Double Nickel LC	<i>Bacillus amyloliquefaciens</i>	20-30% bloom	5 quarts/ac
4	Double Nickel LC	<i>Bacillus amyloliquefaciens</i>	50-60% bloom	5 quarts/ac
5	Double Nickel LC	<i>Bacillus amyloliquefaciens</i>	20-30% bloom + 7 days later	5 quarts/ac
6	Serenade OPTI	<i>Bacillus subtilis</i>	20-30% bloom	20 oz/ac
7	Serenade OPTI	<i>Bacillus subtilis</i>	50-60% bloom	20 oz/ac
8	Serenade OPTI	<i>Bacillus subtilis</i>	20-30% bloom + 7 days later	20 oz/ac
9	Contans + Serenade OPTI	<i>Coniothyrium minitans</i> + <i>Bacillus subtilis</i>	May, pre-plant + 20-30% bloom	4 lbs/ac + 20 oz/ac
10	Endura	Boscalid	20-30% bloom	5.5 oz/ac
11	Proline + Endura	Prothioconazole, boscalid	20-30% bloom + 7 days later	5.5 + 5.7 oz/ac

Plots were evaluated for disease at full pod from 15' of the center row of each plot (July 3rd, St. Paul and August 6th, Roseau). Data were collected on both the incidence and severity of disease. The disease severity index was calculated per plot (Canola Council of Canada) by rating each plant as follows:

0 = No symptoms

1 = Superficial stem lesions not causing wilt

2 = Lesions on stems causing premature wilt up to 25% of the plant

3 = Lesions on stems causing premature wilt up to 50% of the plant

4 = Lesions on stems causing premature wilt up to 75% of the plant

5 = Lesions on stems causing premature wilt on more than 75% of the plant

Disease severity index (DSI) was then calculated as follows:

$$100 * [(\#plants * 1) + (\#plants * 2) + (\#plants * 3) + (\#plants * 4) + (\#plants * 5)] / (\text{Total number of plants in the sample} * 5)$$

At planting, bags of sclerotia (15 sclerotia per bag) were buried 2.5" in the untreated control and Contans- only containing plots to assess viability after Contans treatments. Prior to harvest, buried sclerotia bags were excavated from plots (Treatment 2, Table 1) from Roseau to assess the impact of Contans on sclerotia viability. Sclerotia were counted, weighed, and surface sterilized by rinsing in sterile DI water (SDW), submergence in 20% bleach for 5 minutes, two additional rinses in SDW, and submergence in 70% ethanol for 5 minutes. After sterilization, sclerotia were plated onto potato dextrose agar from each bag to assess viability (three sclerotia per plate and two plates per bag). If there was fungal growth besides for *Sclerotinia sclerotiorum*, the colony was subcultured to better understand internal colonizers of the sclerotia and its effect on sclerotia viability.

Objective 2) Yield was collected from each treatment. Plots were harvested on August 14th in St. Paul and September 15th in Roseau. Yield values were converted to 8.5% moisture. Grain oil and protein were also assessed using a NIR grain analyzer.

Objective 3)

Eighteen sclerotia per bag underwent serial dilutions to isolate fungi and bacteria from the sclerotia surface. Sclerotia were removed from bags, weighed, and underwent serial dilutions in 0.9% NaCl, followed by plating on selective media (½ LBA + Cycloheximide for bacteria and ½ PDA + antibiotics for fungi) to isolate microbial communities. Colony-forming units (CFUs) were counted at multiple time points to quantify microbial abundance estimated based on dilution factors and plating volumes, and distinct morphologies were described and tracked over the course of 5 days. Both the sclerotia surface inhabiting isolates and the non-*S. sclerotiorum* fungal isolates collected from viability assays were tested for inhibition against *Sclerotinia sclerotiorum* in dual culture inhibition assays. Areas of growth inhibition were assessed as indicators of inhibition (Image 2).

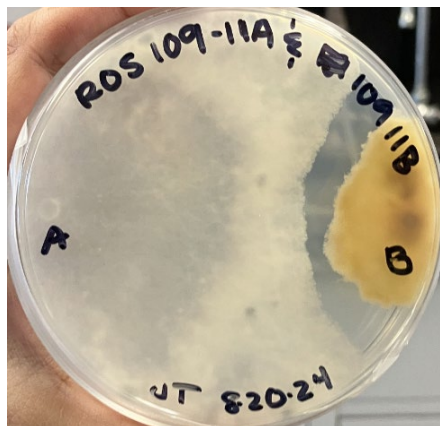


Image 2: Zone of inhibition observed around antagonistic fungus "B", preventing further growth of *S. sclerotiorum*, "A."

Statistical analysis

All analyses were conducted in R Studio software. Disease metrics were conducted using a Beta regression model, then Tukey HSD for multiple comparisons. Yield and quality data were assessed using Analysis of Variance (ANOVA) followed by Tukey HSD post hoc test for multiple comparisons.

RESULTS AND DISCUSSION

Objective 1) The disease incidence and disease severity index did not differ between biological and chemical treatments in St. Paul and infection did not exceed 5% of plants of any plots. In Roseau, significant differences were detected for DI (Figure 1, $p < 0.001$) and DSI (Figure 2, $p < 0.001$) between treatments. Only the Endura at early bloom (Trt 10) and the combined Proline and Endura treatment (Trt 11) resulted in a lower mean incidence of SSR (2.74% and 1.37%) compared to the untreated control (10.5%, $p = 0.01$ and $p < 0.001$, respectively). The mean disease severity index was lower in the plots treated with Endura (Trt 10, 1.89%) compared to all biofungicide treatments (Tukey's HSD, $p < 0.05$) and the untreated control (Trt 1, 5.7%), and the lowest mean disease severity index was achieved with an application of Proline at early flowering, followed by Endura a week later (Trt 11, 0.91%). It would be beneficial to evaluate treatments in an additional field season under higher disease pressure. Due to the very wet weather, plants did exhibit lodging in St. Paul, and this may have influenced conditions related to disease development.



Image 3. Stem rot caused by *S. sclerotiorum* in St. Paul, 2024.

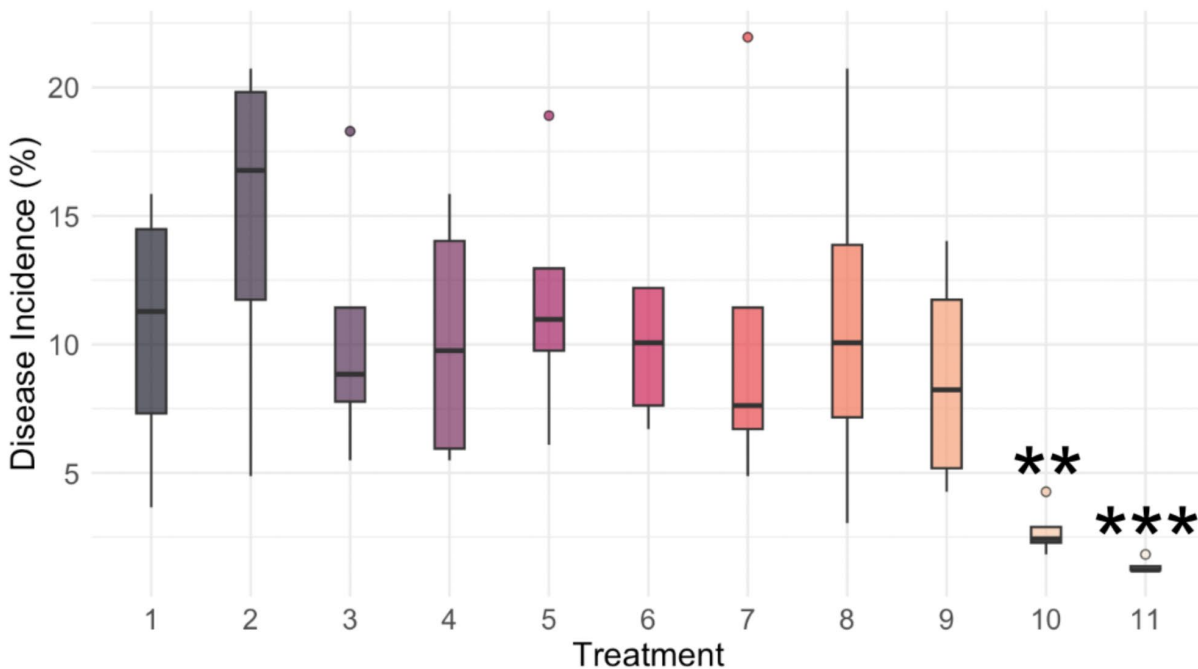


Figure 1. Disease incidence of biofungicide treatments from Roseau MN in 2024. Treatments with ** indicates a significant difference of $p < 0.01$ and *** of $p < 0.001$ compared to the untreated control, Trt1.

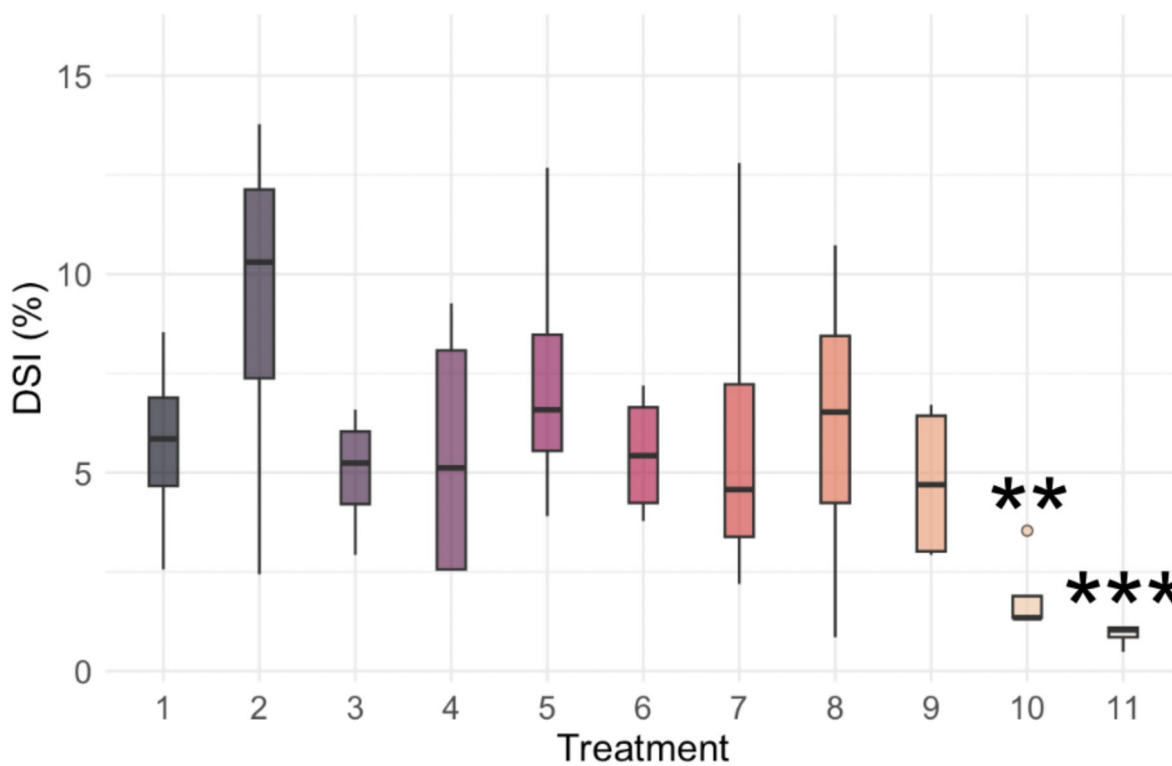


Figure 2 Disease severity index of biofungicide treatments from Roseau MN in 2024. Treatments with ** indicates a significant difference of $p < 0.01$ and *** of $p < 0.001$ compared to the untreated control, Trt1.

Approximately 65% of sclerotia buried in each treatment degraded after burial for one field season. Remaining sclerotia (22 from untreated and 21 from Contans-treated plots) were screened for viability *in vitro*. *S. sclerotiorum* germination was similar in both treatments (45% for control and 46% for Contans-treated). This indicated that pre-season applications of Contans was not effective at reducing the germination of sclerotia.

Objective 2) Yields did not differ significantly between treatments in the St. Paul trial. Differential yields were observed in Roseau (Figure 3, $p = 0.045$). Proline at 20% bloom followed by Endura one week later (Trt 11) resulted in higher yields (2358 lbs/ac) compared to Serenade OPTI applied during late bloom (Trt 7, 1900 lb/ac, Tukey's HSD, $p < 0.05$) and plots treated with Contans before planting and Serenade OPTI during early bloom (Trt 9, 1730 lb/ac, Tukey's HSD, $p < 0.05$). However, since yields were not greater than untreated controls, the conventional and biofungicides evaluated would not be economical to apply under the disease pressures evaluated. It would be beneficial to evaluate treatments in an additional field season under higher disease pressure. Grain quality (oil and protein) did not differ between treatments.

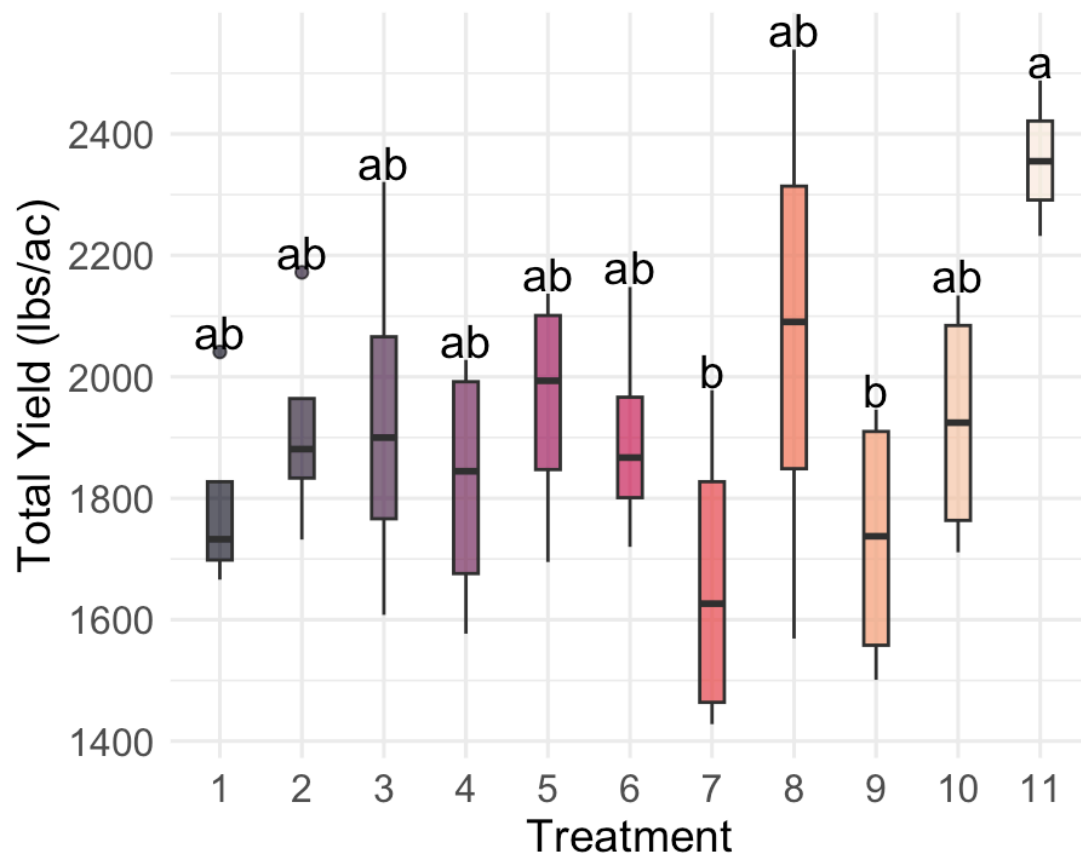


Figure 3. Yields of biofungicide treatments from Roseau MN in 2024. Treatments with the same letter are not significantly different, Tukey's HSD, $p < 0.05$.

Objective 3) A total of 69 bacterial and 29 fungal isolates were recovered from sclerotia and tested for antagonistic effects against *S. sclerotiorum*. So far, seven bacteria and 10 fungal isolates exhibited antagonism and are undergoing genetic identification, as described above. Work to identify antagonists of *S. sclerotiorum* is an initial step towards the discovery of novel biofungicides and further studies on the suppression mechanisms of these microbes. Future work may isolate metabolites, antibiotics, or other compounds that may have applications for control of *S. sclerotiorum* in canola.

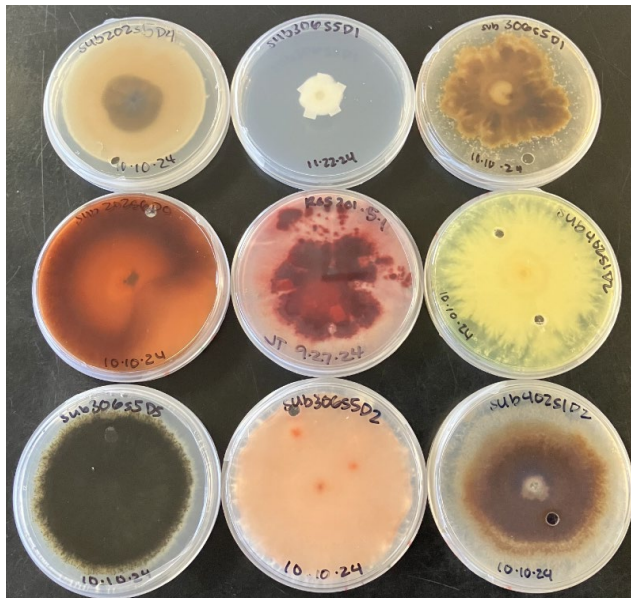


Image 4. Examples of inhibitory isolates that are undergoing molecular identification this winter

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KEYWORDS

White mold, *Sclerotinia sclerotiorum*, *Sclerotinia* stem rot, biological control, biofungicides