REPORT TO THE OREGON PROCESSED VEGETABLE COMMISSION

Title: Biological control and reduced-tillage for white mold management in Willamette Valley processed snap bean production.

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Introduction

White mold (WM, caused by *Sclerotinia sclerotiorum, Ss*) is a serious foliar and pod disease of snap beans grown for processing in western Oregon. Fields with > 6% infected bean pods are rejected by the processor, resulting in a complete crop failure. Ronilan (vinclozolin), a highly effective fungicide used through 2005 for the control of both white and gray mold (*Botrytis cinerea*), is no longer available to bean growers. The *Willamette Valley Bean Mold Task Force* (BMTF) is working on identifying short and long term solutions to this problem.

Biological control

Coniothyrium minitans (*Cm*) is a mycoparasite of *Ss* under natural conditions and was recently developed as a commercial product for WM suppression (Contans, www.prophyta.com). *Cm* can penetrate sclerotial tissue in less than 14 days. Infection of a sclerotium can be achieved by a single spore. *Cm* parasitizes sclerotia optimally over a temperature range of 50 - 68°F, with little activity occurring at > 80° or < 41°F. Contans has typically been applied at 2-6 pounds per acre several months before or at planting of susceptible crops, but this strategy is costly and not very effective.

This project investigated whether low rate (goal: one pound per acre) early fall Contans applications to flailed diseased residues left on the soil surface could effectively increase *Cm* inoculum and reduce *Ss* sclerotial viability. Colonized sclerotia produce pycnidia. If left on the surface, these pycnidia will produce conidial droplets under warm, wet conditions. During rain events, these droplets will splash and disperse, generating new sclerotial infections. Our hypothesis was that the mild, wet, winter conditions in western Oregon could generate a series of *Cm* colonization cycles - a "biological control epidemic" - thereby increasing *Cm* inoculum levels over time and increasing the efficacy of an initial low rate Contans application.

Summer burial and irrigation:

Some sclerotia will survive overwintering on the soil surface. What is the impact of subsequent management? Do irrigation and burial impact *Cm* colonization and survival of Ss sclerotia? This project investigated the impact of three irrigation application levels and burial at 1-2 inch depth.

Objectives: *to determine the impact of*

1. *fall Contans applications on Cm infection and survival of sclerotia left on the soil surface through the winter and summer*
2. *the impact of summer irrigation and burial on sclerotial survival and infection with Cm and other fungi*

Summary:

In two full year field experiments (one at OSU Vegetable Research Farm and the other at an on-farm site) we showed that fall Contans applications (late September and late November) to flailed diseased crops residues left on the soil surface over the winter did generate "biocontrol epidemics" of at least 12 and 6 months. In other words, *Cm* effectively colonized a proportion of the sclerotia in the field in the fall in both experiments, and those colonized sclerotia then provided a reservoir of *Cm* inoculum that generated *Cm* infections in additional sclerotia through the spring and summer. While biocontrol epidemics were successfully generated, we cannot know whether the biocontrol epidemics had an impact on sclerotial viability as there were no control plots. Control plots were established at both locations, but *Cm* splashed and moved to such a degree that those control plots were quickly colonized by *Cm*. This phenomenon is a benefit from an agricultural perspective, but a problem from a research perspective. In the future, we will create and manage distinct and widely separated *Cm*+ and *Cm*- fields.

In general, colonization of sclerotia by both *Cm* and other fungi and rate of sclerotial death were higher during the late spring and summer than during the winter, likely due to higher soil temperatures.

There were interesting differences between the two field experiments. By September 2008, many fewer sclerotia survived in the on-farm trial (approximately 1%) than in the OSU trial (approximately 17%). In addition, sclerotia in the OSU field trial were colonized by *Cm* more frequently than by other fungi, while the reverse was true for the on-farm trial. The reasons for the differences in the proportion of sclerotia colonized by *Cm* or other fungi between the two locations are not clear. Despite the differences, all sclerotia collected in September 2008 from both trials were colonized by fungi.

An irrigation and burial trial was established over the OSU field experiment in summer 2008. This trial demonstrated that both summer irrigation and shallow burial (1-2 inch depth) reduced sclerotial survival. While sclerotia left on the surface over the summer were much more likely to be colonized by *Cm* than buried sclerotia, the reverse was true for colonization by other fungi.

Full year field experiments 2007-08

Objective: to determine the impact of fall Contans applications on Cm infection and survival of sclerotia left on the soil surface through the winter and summer

Full Year Field Trial 1. Impact of fall Contans applications on *Cm* infection of sclerotia left on soil surface all year

Materials and methods:

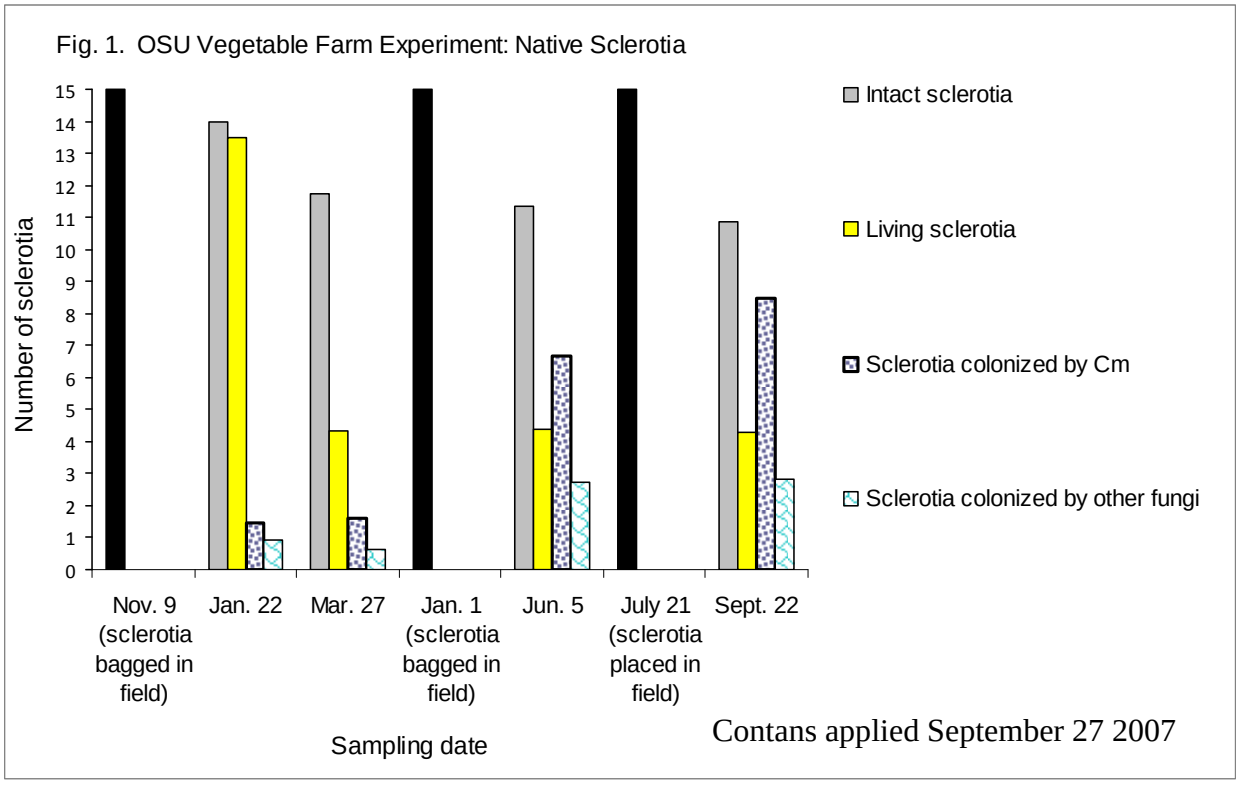
A 91G bean field was planted in July 2007 at the OSU Vegetable Research Farm and inoculated with *Sclerotinia sclerotiorum* mycelia at bloom. Crop development and disease progressed until September 27, when the field of mature diseased beans was flailed but not incorporated. A replicated complete block experiment consisting of 6 blocks of 3 treatments [0(water), 1, and 3 lbs per acre Contans] was applied to the field. Plots (20 ft^2) were separated in all directions with 20 ft buffer strips to minimize plot-plot contamination. Contans treatments were applied that afternoon directly to the surface of the plots with a backpack sprayer. It rained frequently and heavily over the next several weeks. Plating of sclerotia in November indicated that sclerotia in all plots were colonized by Cm, with no significant difference amongst treatments (data not

shown). For this reason, the plots were used subsequently only as sampling units, not as treatments.

Evaluation of native sclerotia:
Sclerotia were collected from the soil surface of each plot on November 8 (42 days after treatment). Sixty sclerotia were reapplied to the surface of the field in nylon mesh bags (4 bags of 15 sclerotia per bag per plot). The bags were affixed to the soil surface with metal-staked flags. Two bags per plot were collected on January 22 and the other two bags on March 27. On January 1, 30 native sclerotia were collected from the soil surface of each plot and 15 were placed into each of two mesh bags. The bags were sampled on June 5. Again, on June 5, 30 native sclerotia were collected from each plot and placed into 2 bags (15 sclerotia per bag), and those bags were collected on September 22. After bags were collected, intact sclerotia were counted, surface sterilized, and plated on PDA. Plates were evaluated for mycelial growth of *Sclerotinia sclerotiorum* at 1 week after plating and colonization by *Cm* and other fungi at 2 weeks after plating.

Evaluation of sterile sclerotia:
Sterile sclerotia were grown on PDA plates in the laboratory. Two mesh bags containing 15 sterile sclerotia were affixed to the soil surface in each plot on January 1 (collected February 15 and March 27), March 26 (collected June 5), and June 5 (collected September 22). After bags were collected, intact sclerotia were washed, counted, surface sterilized, and plated on PDA. Plates were evaluated for mycelial growth of *Sclerotinia sclerotiorum* at 1 week after plating and colonization by *Cm* and other fungi at 2 weeks after plating.

Results:



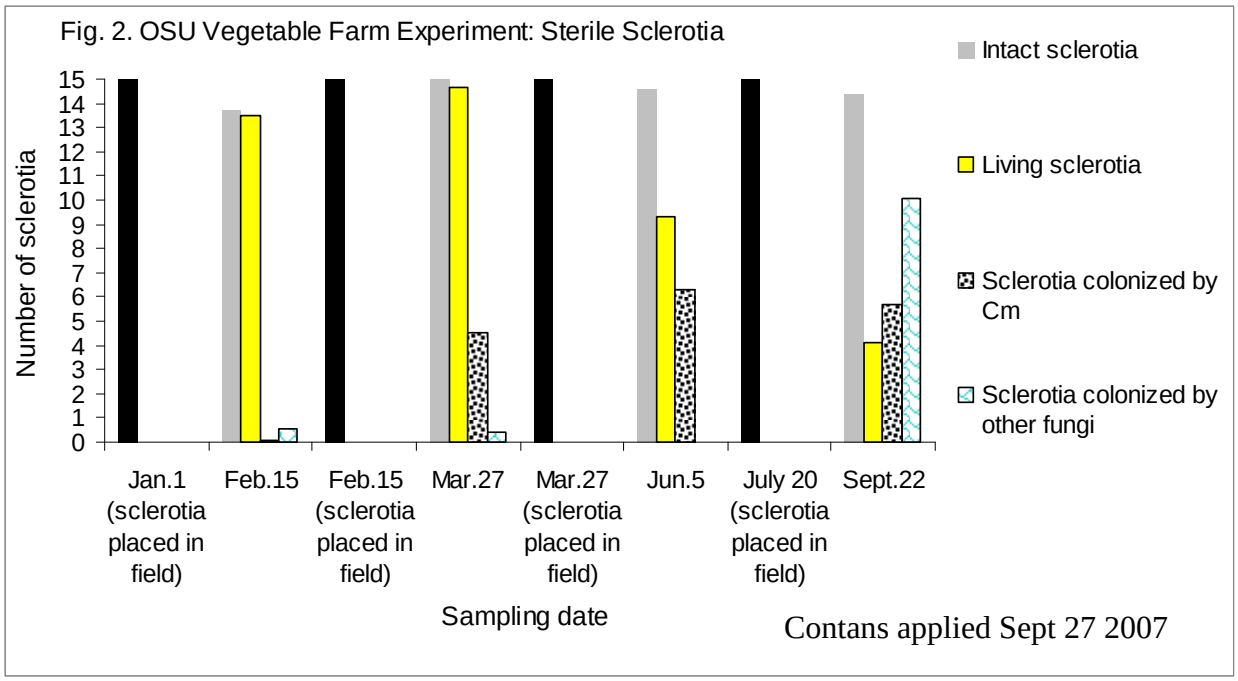


Fig. 1 Summary:

- There was approximately 10% *Cm* infection and 12% sclerotial death by Jan 22.
- Infection by *Cm* increased during the spring and summer; by the end of the summer most sclerotia remaining in the field were infected with *Cm*.
- Sclerotia were colonized by other fungi, and to a greater degree during the late spring and summer, but never to as great a degree as they were colonized by *Cm*.
- All sclerotia collected on Sept 22 were colonized by either *Cm* or other fungi (data not shown).

Fig. 2 Summary:

- *Cm* did not colonize sterile sclerotia during the period January 1- February 15. This would be expected as *Cm* cannot infect sclerotia when surface soil temperatures are less then 45 F.
- *Cm* infection of sterile sclerotia occurred from February 15 through September 22. Therefore, *Cm* applied in September 2007 created a "biocontrol epidemic" of at least 12 month duration.
- Sterile sclerotia were colonized by other fungi to a significant degree only during the summer months.

Full Year Field Trial 2. Impact of fall Contans applications on *Coniothyrium* infection of sclerotia left on soil surface (on-farm trial)

A fall cauliflower crop was grown at Pearmine Farm in Brooks Oregon and harvested in October. The 90 acre field has a recent history of snap bean production. Cauliflower yield was significantly reduced by a white mold epidemic initiated early in crop development. Crop residues were flailed in November and left on the soil surface for the winter. Contans was

applied across the field at 2 lbs/A on November 20. On 2 dates in 2008 (January 1 and May 14), native sclerotia were collected in the field. On January 1, the collected sclerotia were bagged in the field, and the bags were applied to the soil surface. The sclerotia collected May 14 were refrigerated for one month and applied to the field after corn planting on June 16. Of the bags applied on January 1, half (34 bags) were collected on March 22 and the other half (34 bags) on May 24. All bags (28) applied on June 16 were collected on September 11. On all collection dates, after bags were collected, intact sclerotia were washed, counted, surface sterilized, and plated on PDA. Plates were evaluated for mycelial growth of *Sclerotinia sclerotiorum* at 1 week after plating and colonization by *Cm* and other fungi at 2 weeks after plating.

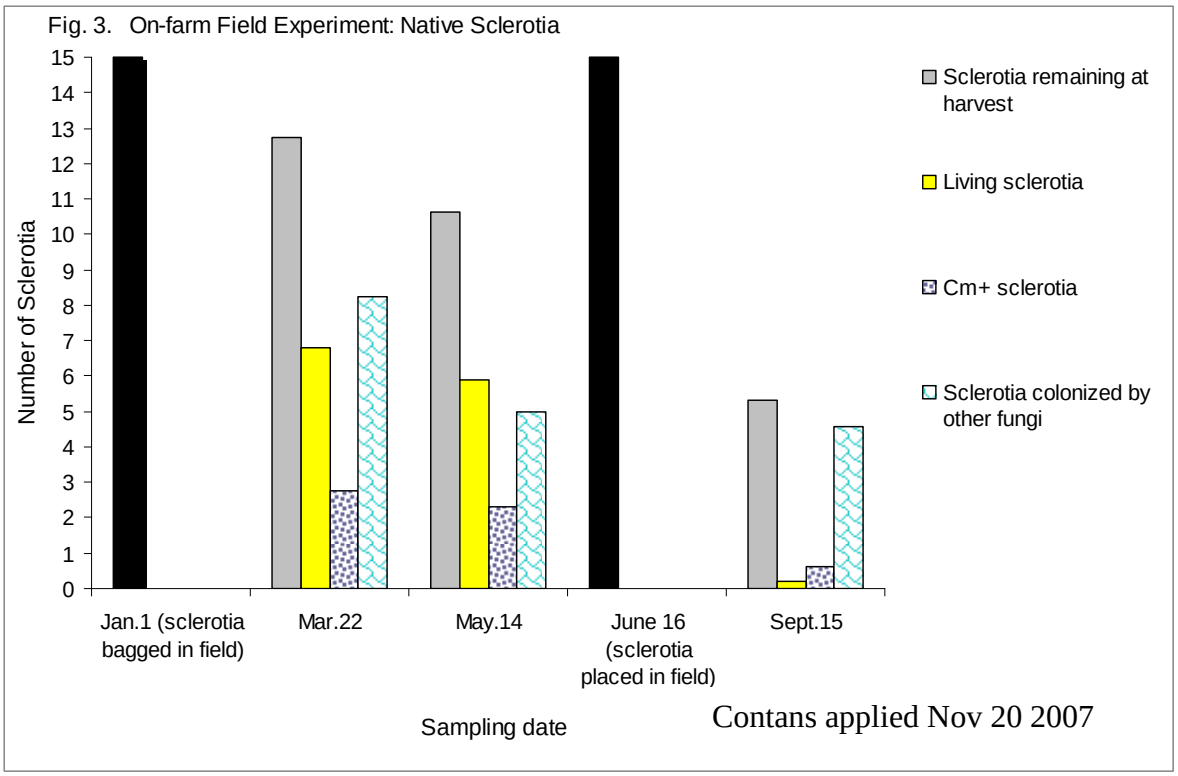


Fig. 3 Summary:

- In the on-farm trial, there was approximately 17% *Cm* infection, 55% infection by other fungi, and 65% sclerotial death by Mar 22. By May 14, there was approximately 14% Cm infection, 33% infection by other fungi, and 74% sclerotial death. By September 15, only about 4% of the recoverable sclerotia remained alive.
- Sclerotial colonization by other fungi was always higher than colonization by *Cm*.
- All sclerotia collected on Sept 15 were colonized by either *Cm* or other fungi (data not shown).

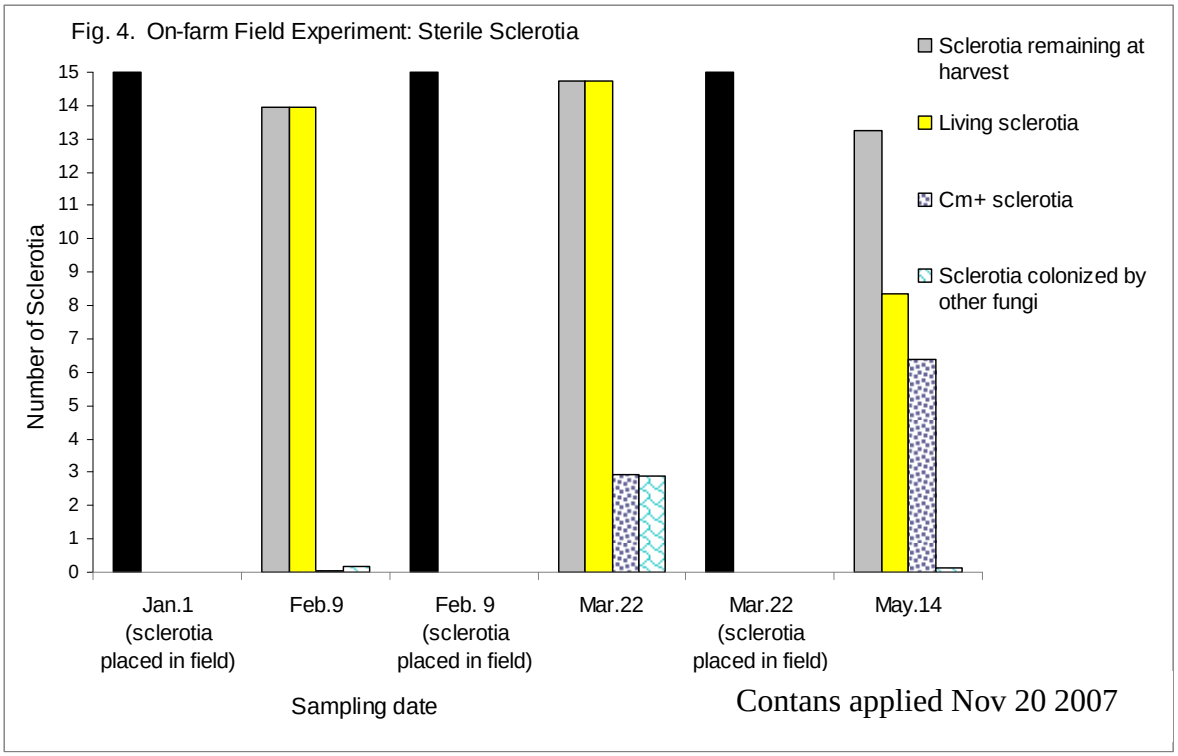


Fig. 4 Summary:

- *Cm* did not colonize sterile sclerotia during the period January 1- February 9. This would be expected as *Cm* cannot infect sclerotia when surface soil temperatures are less then 45 F.
- *Cm* infection of sclerotia was initiated by the fall 2007 Contans application. *Cm* infection of sterile sclerotia occurred from February 9 through May 14;; therefore, *Cm* applied in November 2007 created a "biocontrol epidemic" of at least 6 month duration.
- Sterile sclerotia were only colonized by other fungi to a significant degree during the period February 9 to March 22.

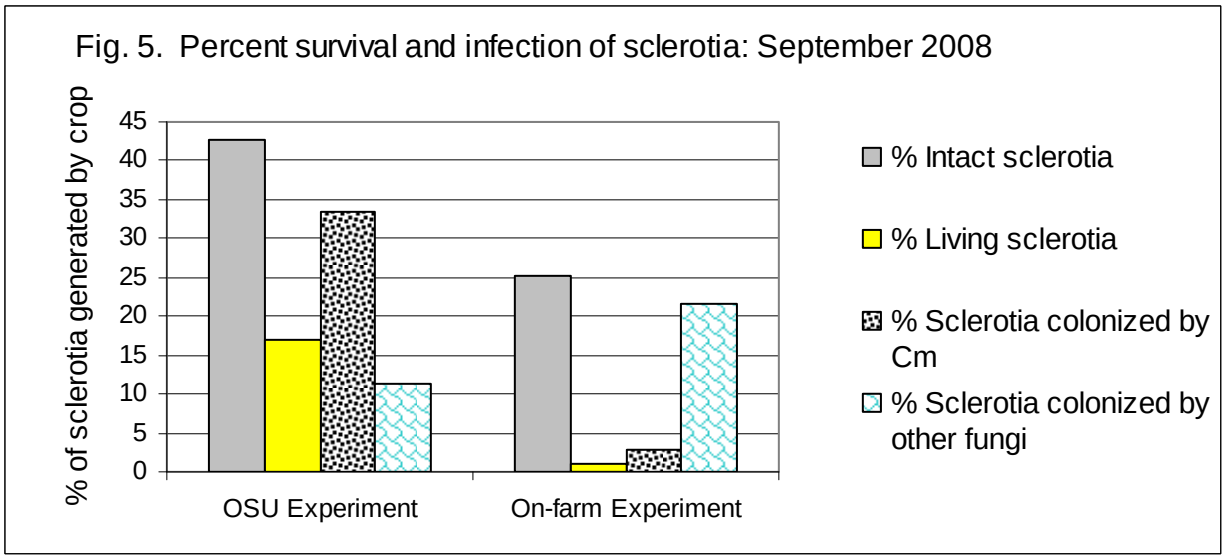


Fig. 5 Summary:

- In the OSU field trial, only 43% of the sclerotia generated by the bean crop were intact 12 months later, and only 17% of the original number of sclerotia was alive. Of the sclerotia collected from the field in September 2008:
    - 40% were alive (could produce mycelia)
    - 79% were colonized by *Cm*
    - 26% were colonized by other fungi
    - none were uncolonized (data not shown)

    .
- In the on-farm field trial, only 25% of the sclerotia generated by the cauliflower crop were intact 10 months later, and approximately 1% of the original number of sclerotia was alive. Of the sclerotia collected from the field in September 2008:
    - 4% were alive (could produce mycelia)
    - 10% were colonized by *Cm*
    - 88% were colonized by other fungi
    - none were uncolonized (data not shown)
- Fewer sclerotia survived in the on-farm trial than in the OSU trial.
- Sclerotia in the OSU field trial were colonized by *Cm* more frequently than by other fungi; the reverse was true for the on-farm trial.

Summer irrigation/burial field experiment 2008

Objective: to determine the impact of summer irrigation and burial on sclerotial survival and infection with Cm and other fungi

Materials and methods:

An irrigation/burial trial was conducted in the Full Year Field Trial 1 (OSU Vegetable Research Farm) described above in summer 2008. Native sclerotia were collected from the soil surface during the first two weeks of July and placed into mesh bags (15 sclerotia per bag). Irrigation was applied for 8 hours on July 20 to thoroughly wet the field; bags were placed in the field on July 21.

Bags were placed on the soil surface and also buried at 1-2 inches below the soil surface at 0, 40 and 60 feet from a single irrigation line run through the center of the field (10 reps per location and depth). The field was irrigated for 2.5 hours weekly for 8 weeks. No irrigation water was applied at 60 ft from the irrigation line. The volume of irrigation water applied at 40 ft from the irrigation line was on average 15% of the volume applied at 0 ft from the irrigation line. Gravimetric moisture contents of soil samples taken from the top 4 inches of soil were measured immediately before and several hours after irrigations for three irrigation cycles. Mean gravimetric soil moisture content at 60 ft from the irrigation line was 0.10. Mean gravimetric soil moisture contents at 40 ft from the irrigation line ranged from 0.16 to 0.20, and at 0 ft from the irrigation line from 0.19 to 0.24.

Bags were removed from the field on September 20. After bags were collected, intact sclerotia were washed, counted, surface sterilized, and plated on PDA. Plates were evaluated for mycelial growth of *Sclerotinia sclerotiorum* at 1 week after plating and colonization by *Cm* and other fungi at 2 weeks after plating.

Results:

Fig. 6. OSU Irrigation Trial: sclerotial survival

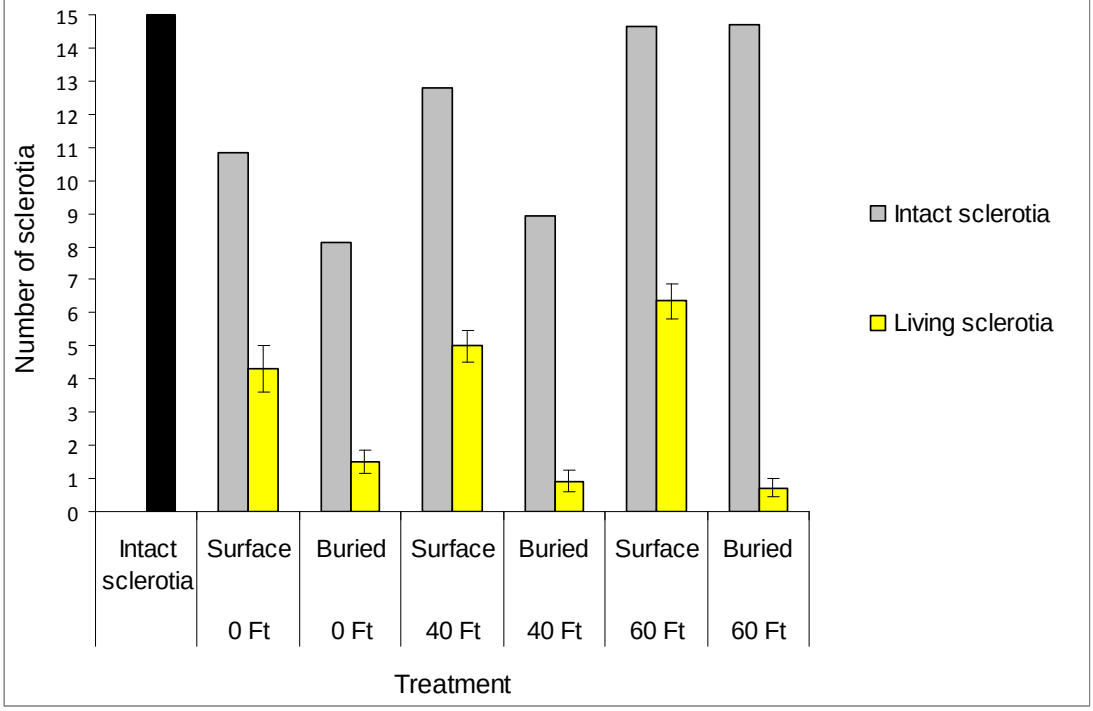


Fig. 7. OSU Irrigation Trial: *Cm* infection of native sclerotia

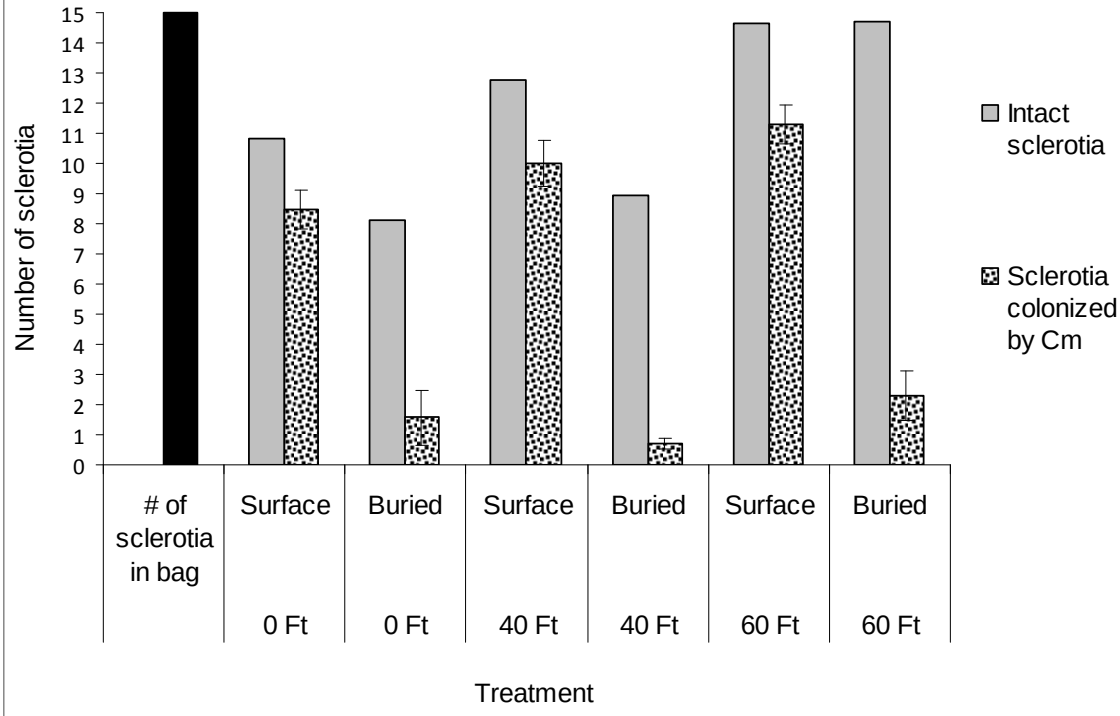


Fig. 6 Summary:

- In general, buried sclerotia were fewer in number and of lower viability than sclerotia left on the soil surface. The only exception to this trend was at 60 ft., where there was no difference in the number of buried and surface sclerotia.
- The number of intact sclerotia declined as the amount of irrigation water applied increased in both surface and buried sclerotia.
- There was a trend towards increased viability as the amount irrigation water applied decreased in surface sclerotia, while there was a slight trend in the opposite direction for buried sclerotia.

Fig. 7 Summary:

- Surface sclerotia were much more likely to be colonized by *Cm* than buried sclerotia.
- The proportion of sclerotia colonized by *Cm* was not affected by the amount of irrigation water applied.
- The number of intact sclerotia increased as the amount of irrigation water applied decreased in both buried and surface samples.
- There was a trend towards increased viability as the amount of irrigation water applied decreased in surface sclerotia, and a slight trend towards reduced viability as the amount of irrigation water applied decreased in buried sclerotia.

Fig. 8. OSU Irrigation Trial: sclerotia colonized by other fungi

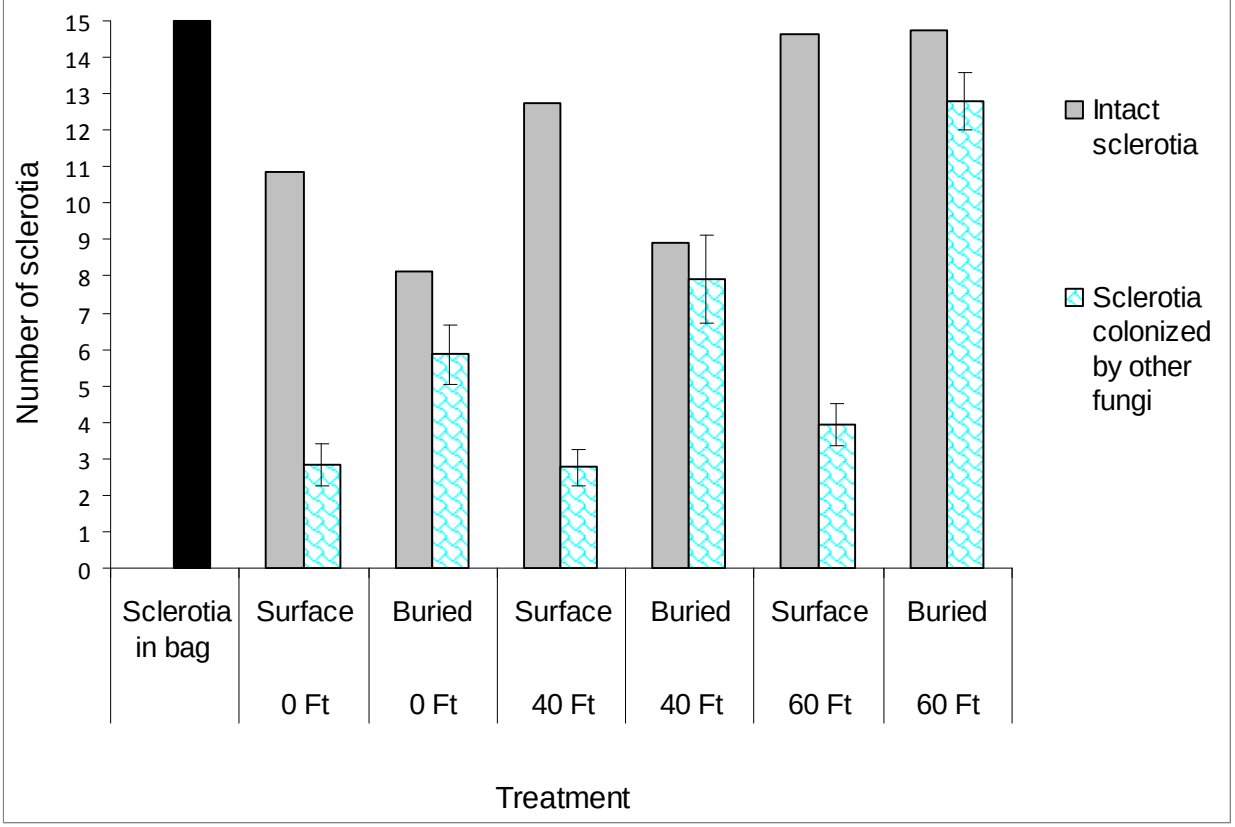


Fig. 8 Summary:

- Buried sclerotia were much more likely to be colonized by other fungi than surface sclerotia; this difference became more pronounced as the amount of irrigation water applied decreased.
- There was a trend towards increased colonization by other fungi as the amount of irrigation water applied decreased in buried sclerotia; the amount of irrigation water applied had little if any impact on colonization of surface sclerotia by other fungi.

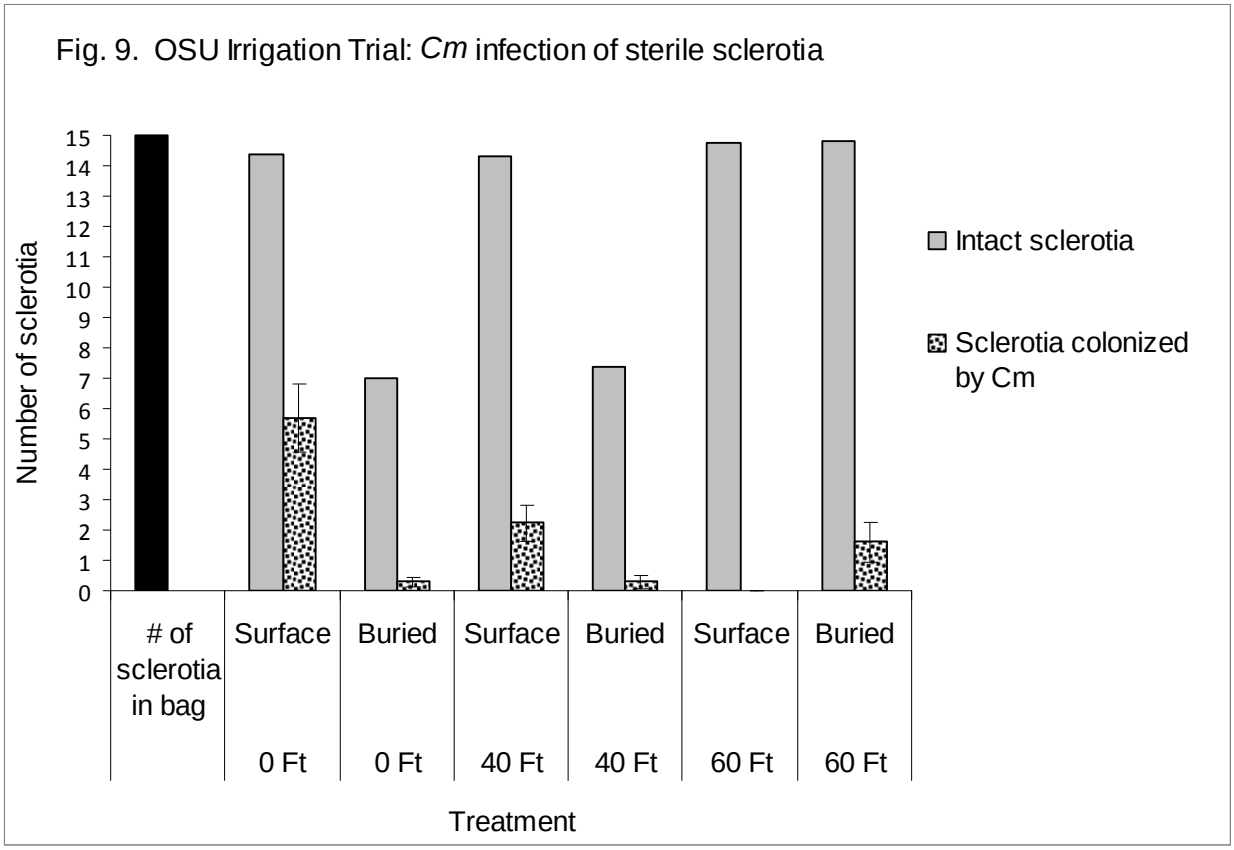


Fig. 9 Summary:

- Surface sclerotia were much more likely to be colonized by *Cm* than buried sclerotia at 0 and 40 ft from the irrigation line, while the reverse was true at 60 ft.
- Overall, colonization of sclerotia by *Cm* declined as the amount of irrigation water applied decreased.