Developing New Integrated Strategies for Controlling White Rot in Garlic

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Introduction

White rot caused by *Sclerotium cepivorum* Berk is a significant threat to the garlic and onion industry in the United States. The pathogen produces a great number of poppy-seed-sized sclerotia, which can survive in soil for many years. Populations of just a few sclerotia per liter of soil can potentially cause severe disease and result in crop failure. Once the land has been infested, it is generally considered not suitable for garlic or onion production for up to 40 years or more. One of the most effective control methods was fumigation with methyl bromide, but it is not cost effective and has been phased out due to its harmful environmental effects.

Sclerotia of *S. cepivorum* are dormant in the absence of an Allium crop, but one natural compound from Alliums, diallyl disulfide (DADS), which is also recoverable from petroleum, stimulates them to germinate. The germinated sclerotia will exhaust nutrient reserves and die without an Allium crop. Efforts have been made to apply DADS in the absence of Alliums to reduce soilborne sclerotia (Crowe et al. 2007, Davis et al. 2007). When DADS was applied in commercial fields, it killed over 90 percent of the sclerotia within 3 months of treatment (Davis et al. 2007). However, the remaining sclerotia were still sufficient to cause considerable root rot and yield losses in the subsequent Allium crops (Davis et al. 2007). Multiple DADS treatments are considered impractical due to high cost, little gain in disease control, and the time it takes for treatment because the optimal treatment period usually occurs only in spring or fall of a year. Therefore, a new method that can be either used alone or integrated with DADS to reduce soilborne sclerotia is needed.

In a study in Tulelake, California, flooding fields significantly reduced viable soilborne sclerotia to no more than 8 percent, but not low enough to achieve disease control (Crowe et al. 2005). This indicated flooding may be potentially employed as a measure to reduce the sclerotium density in the soil. The inadequate disease control might have been partially due to relatively low soil temperature (59.0-73.4^{o}F in the summer). Wu et al. (2008) found high temperatures were critical for the accelerated decay of sclerotia where high moisture or low oxygen combined with high temperatures accelerated decay of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*, two pathogens that commonly survive as sclerotia in the soil for multiple years. Similar results were also reported in a laboratory study where sclerotia of *Sclerotium cepivorum* decayed completely within 21 days in moist soil at 80.6 and 86^{o}F. McLean et al. (2001) found that sclerotial viability of *S. cepivorum* could be reduced to 10.7 percent in 28 days at 68^{o}F and to 0 percent in 16 days at 86^{o}F in laboratory, and when mean soil temperature in commercial fields was increased to 76.3 ~83.8^{o}F by using a cover of 50-µm-thick polythene film, sclerotial viability reduced 46.7 ~ 91.3 percent compared with the uncovered control. Solarization was consistently found to reduce the viable inoculum density in the soil and provided good control of white rot of garlic in Spain and Mexico. Given the sunny weather during the summer in the main onion and garlic production areas in the U.S., solarization may increase the soil temperature above 86^{o}F even at 8-inch depth, which is likely to dramatically reduce the inoculum density in the soil, and therefore provide good control of white rot in garlic and onion in addition to control of other soilborne diseases and weeds.

Biological soil disinfestation (BSD), achieved by incorporating easily decomposable organic materials into irrigated soil that is covered with plastic film, has been used as an alternative to methyl bromide fumigation for controlling plant diseases caused by a wide range of soilborne fungal pathogens, including *Fusarium, Verticillium, Rhizoctonia*, and the nematodes *Meloidogyne,* and *Pratylenchus* (Melero-Vara et al. 2000, Mattner et al. 2008, Momma 2008). The mechanisms of BSD include a reduction of soil pH, deficiency of oxygen, and the accumulation of toxic levels of organic acids produced by anaerobic bacteria. It has been considered a promising environmentally friendly method for reducing inoculum levels of various soilborne plant pathogenic fungi.

The objectives of the study are to quantify the temporal changes in viability of *S. cepivorum* sclerotia under different soil treatments (incorporation of fresh-cut oat, solarization, application of DADS), and to compare the efficacy of the different treatments for controlling white rot in commercial onion and garlic fields.

Materials and Methods

A field trial was conducted in a commercial field infested with sclerotia of *S. cepivorum* at the Central Oregon Agricultural Research Center in Madras, Oregon. Six treatments were arranged in a randomized complete block design with four replications. The treatments were: 1) untreated control: the field was left fallow during the spring and summer; 2) DADS: DADS was applied at 0.535 gal/acre on May 19, irrigated, and left fallow during the summer; 3) solarization: untreated in the spring, tilled, irrigated, and then covered with a 2-mil clear polyethylene film since July 30; 4) incorporation of fresh-cut oat: untreated in the spring, fresh-cut oat was incorporated at 250 lb fresh weight per plot (5,978 lbs dry weight /acre) on July 30 and then left fallow; 5) BSD: untreated in the spring, fresh-cut oat was incorporated at 250 lb fresh weight/plot on July 30, and plots then irrigated and covered with a 2-mil clear polyethylene film; and 6) DADS followed by BSD: DADS was applied at 0.535 gal/acre on May 19, fresh-cut oat incorporated at 250 lb/acre on July 30, then plots irrigated and covered with a 2-mil clear polyethylene film. The plot sizes were 20 ft by 20 ft.

A 1,000-ml soil sample was collected from the top 6 inches of soil in each plot monthly, starting immediately before the DADS treatment in the spring, until incorporation of fresh-cut oat, and then samples were taken at 2, 4, and 8 weeks after the treatments began. A 250-ml subsample was drawn from each sample for assay (if the number of sclerotia was <10, then remaining soil would also be assayed). Soil was blended briefly and sclerotia were concentrated from soil by size (sieving through screens) and by density (flotation on a sucrose solution). Remaining soil residue with sclerotia was collected and observed under a binocular microscope. The number of sclerotial bodies remaining intact was counted. If more than 50 intact sclerotia were counted, then 50 sclerotia were randomly selected and tested for viability as per Crowe and Hall (1980) on water agar (Bactoagar, Difco). If 50 or fewer sclerotia were counted, then all intact sclerotia were tested for viability. Sclerotia were washed, surface disinfected for 2.5 min in 0.5 percent sodium hypochlorite, rinsed with sterilized water, cracked using forceps, and placed on water agar plates to induce growth. Sclerotia that developed characteristic mycelial growth and clumps of microconidia in the agar were identified as viable sclerotia of *S. cepivorum*. Sclerotia ungerminated in 3 weeks were considered to be dead.

Garlic (cultivar ‘California Early’) was planted in 2 rows per 36-inch bed at spacing of 9 plants/ft row on October 5, 2010 and irrigated as was needed. Incidence of white rot will be monitored

monthly in the spring, and the marketable yield will be determined for each plot at harvest. After harvest and tillage, a 1000-ml soil sample will be collected from the top 6 inches of soil in each plot and assayed for the viable inoculum level in the soil as described above.

Results and Discussion

The results revealed that sclerotium density of *S. cepivorum* were not reduced by DADS as expected (Figure 1). The number of viable sclerotium remained high more than 2 month after DADS application, when fresh-cut oat was incorporated and solarization started on July 30. By then, around 95 percent of sclerotia recovered from soil samples were still viable when tested on water agar. The total number of viable sclerotia ranged from 185 to 435/l soil (Figure 1). The possible explanations for no efficacy of DADS treatment might include temperature less than optimal in early spring, poor penetration of DADS, and poor quality of product used.

Soil temperature at 2-inch depth was consistently higher (an average of 11.4^{o}F) in the plots covered with a polyethylene film than in the plots without coverage (Figure 2). Just over 2 weeks after covering with polyethylene film, the number of viable sclerotia as determined by germination on water agar dramatically declined on August 17 in all solarization plots (Figure 1), although no significant decline was detected in total sclerotial density in solarization plots (data not shown). Surprisingly, the total viable sclerotia increased on the next two sampling dates of September 5 and 30 in solarization plots compared with the numbers on August 17 (Figure 1). By then, no significant difference was detected among the different treatments (Figure 1, Table 1). The daily maximum soil temperature declined dramatically starting from late August in solarization plots (Figure 2). It remains unclear whether the low number of viable slcerotia on August 17 was due to a false negative result in the germination test. It might also be possible that many sclerotia of *S. cepivorum,* rather than being killed, had gone dormant in response to high soil temperature. Subsequently, they become active again after the temperature dropped back into the optimal range for the fungus. Further studies are required to confirm this hypothesis and determine whether sclerotia of *S. cepivorum* can recover from a long period of high soil temperature treatment.

Table 1. Analysis of variance on total number of viable sclerotia of *Sclerotium cepivorum* per liter in the top 6 inches of soil in plots subjected different treatments[1], Central Oregon Agricultural Research Center, Madras, OR, 2010.

Date	Source	DF	Type III SS	Mean Square	*F* Value	Pr. > *F*
May 13	Treatment	5	9104.61	1820.92	0.71	0.6262
	Block	3	6030.61	2010.20	0.78	0.5222
Jun 16	Treatment	5	2005.47	401.09	0.25	0.9347
	Block	3	3643.11	1214.37	0.75	0.5399
Jul 30	Treatment	5	9318.43	1863.69	0.82	0.5561
	Block	3	1935.33	645.11	0.28	0.837
Aug 17	Treatment	5	50676.30	10135.26	4.44	0.0111
	Block	3	2090.94	696.98	0.31	0.8211
Sep 5	Treatment	5	11757.04	2351.41	1.11	0.3963
	Block	3	1482.34	494.11	0.23	0.8719
Sep 30	Treatment	5	15619.17	3123.83	1.28	0.3252
	Block	3	267.76	89.25	0.04	0.9903

Note: [1] Treatments include: 1) untreated control; 2) DADS; 3) solarization; 4) incorporation of fresh-cut oat; 5) BSD; and 6) DADS followed by BSD.

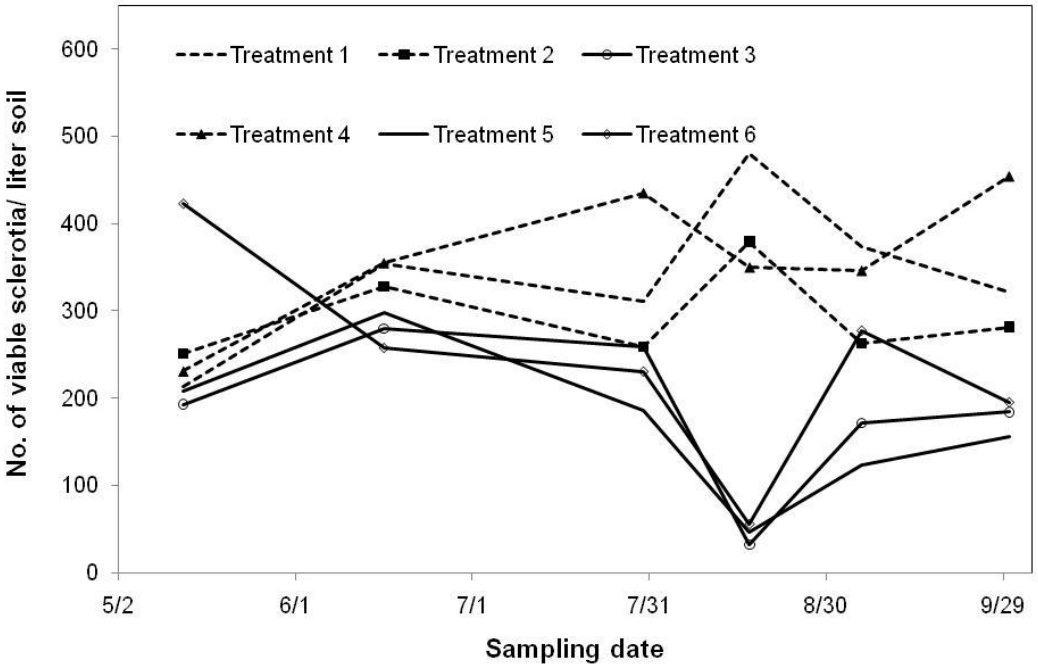

Figure 1. Number of viable sclerotia of *Sclerotium cepivorum* per liter of the top 6 inches of soil in plots subjected to different treatments: 1) untreated control; 2) DADS; 3) solarization; 4) incorporation of fresh-cut oat; 5) BSD; and 6) DADS followed by BSD. Central Oregon Agricultural Research Center, Madras, OR, 2010.

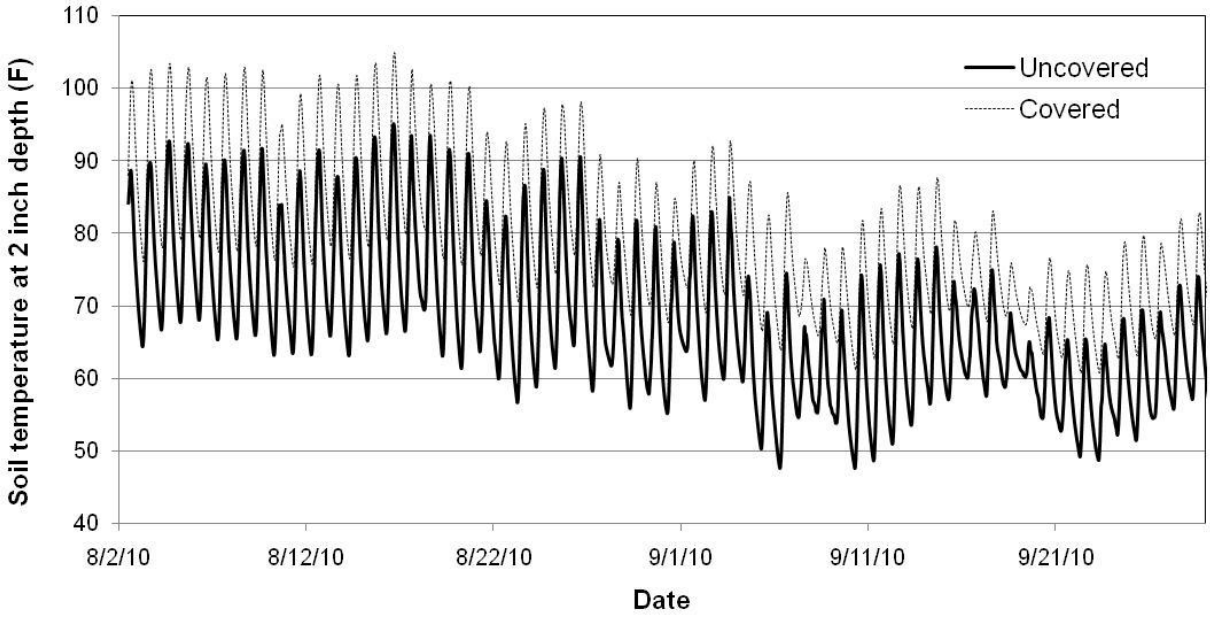

Figure 2. Half-hourly averages of soil temperature at 2-inch depth in plots covered with a 2-mil clear polyethylene film and in uncovered plots, Central Oregon Agricultural Research Center, Madras, OR, 2010.

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