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 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 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 or more years. One of the most effective 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 a compound from Allium root exudates, 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% of the sclerotia within three 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. 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 in a year. Therefore, a new method that can be either used alone or integrated with DADS to reduce soilborne sclerotia is needed.

In a previous study conducted in Tulelake, California, flooding significantly reduced viable soilborne sclerotia to no more than 8%, 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 (59-73.4^{o}F in the summer) since sclerotia of *S. cepivorum* decayed completely within 21 days in moist soil at 80.6 and 86^{o}F (Crowe and Hall, 1980). McLean et al. (2001) also found that sclerotial viability of *S. cepivorum* could be reduced to 10.7% in 28 days at 68^{o}F and to 0% 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 using 50µm thick polythene film, sclerotial viability reduced 46.7% ~ 91.3% 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 to provide good control of white rot in garlic and onion in addition to control of other soil borne 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 of methyl bromide fumigation for controlling plant diseases caused by a wide range of soil borne fungal pathogens, including *Fusarium, Verticillium, Rhizoctonia*, and the nematodes *Meloidogyne,* and *Pratylenchus* (Melero-Vara et al., 2000; Mattners 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 soil borne 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 combinations of them); 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, OR. Six treatments were arranged in a randomized complete block design with 4 replications. The 6 treatments were: 1) Untreated control -the field was left fallow during the spring and summer; 2) DADS- DADS was applied at 0.535 gal/A 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 lbs fresh weight per plot (5978 lbs dry weight per acre) on July 30 and then left fallow; 5) BSD: untreated in the spring, fresh cut oat was incorporated at 250 lb fresh weight per 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 lbs per acre on July 30, then plots irrigated and covered with a 2-mil clear polyethylene film. The plot sizes were 20 feet × 20 feet.

An 1000-ml soil sample was collected from the top 6 inch soil in each plot monthly starting immediately before the DADS treatment in the spring, until incorporation of fresh cut oat, starting from then, samples were taken at 2, 4, 8 weeks after treatments start. A 250-ml subsample was drawn from each sample for assay (if the number of sclerotia was lower than 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 et al. (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 minutes in 0.5% 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 two rows per 36 inch bed at spacing of 9 plants per foot row on October 5, 2010 and irrigated as it 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 inch 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 two month after DADS application when fresh cut oat was incorporated on July 30. By then, around 95% 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 per liter soil (Figure 1). The possible explanations for the inefficacy of DADS treatment might include less than optimal temperature in early spring, poor penetration of DADS without incorporation, and poor quality of product used.

During solarization period, soil temperature at 2 inch depth was consistently higher in the plots covered with a polyethylene film than that in the plots without coverage, and the difference between them was 11.4^{o}F on the average (Figure 2). Just over two weeks after covering with a 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 number of viable sclerotia came back up on the next two sampling dates, September 5 and September 30 in solarization plots compared with the numbers on August 17 (Figure 1). By then, no significant difference was detected among different treatments (Figure 1, Table 1). It was also observed that 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 sclerotia on August 17 was due to false negative result in the germination test. It might also be possible that many sclerotia of *S. cepivorum*, rather than being killed, had turned into dormancy in response to high soil temperature. Subsequently, they became active again after the temperature dropped back into 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.

References

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Table 1. Analysis of variance on total number of viable sclerotia of *Sclerotium cepivorum* per liter top 6 inch soil in plots subjected different treatments[1].

Date	Source	DF	Type III SS	Mean Square	*F* Value	Pr. > *F*
5/13/2010	Treatment	5	9104.61	1820.92	0.71	0.6262
	Block	3	6030.61	2010.20	0.78	0.5222
6/16/2010	Treatment	5	2005.47	401.09	0.25	0.9347
	Block	3	3643.11	1214.37	0.75	0.5399
7/30/2010	Treatment	5	9318.43	1863.69	0.82	0.5561
	Block	3	1935.33	645.11	0.28	0.837
8/17/2010	Treatment	5	50676.30	10135.26	4.44	0.0111
	Block	3	2090.94	696.98	0.31	0.8211
9/5/2010	Treatment	5	11757.04	2351.41	1.11	0.3963
	Block	3	1482.34	494.11	0.23	0.8719
9/30/2010	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.

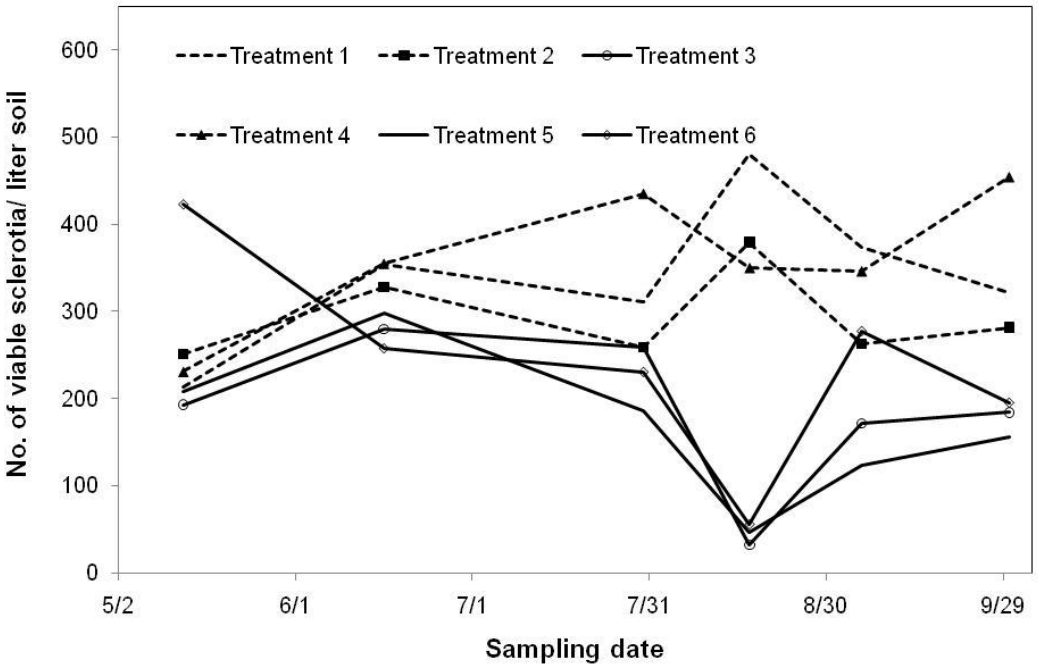

Figure 1. Number of viable sclerotia of *Sclerotium cepivorum* per liter top 6 inch soil in plots subjected to different treatments: 1) Untreated control; 2) DADS; 3) Solarization; 4) Incorporation of fresh cut oat; 5) BSD; and 6) DADS followed by BSD.

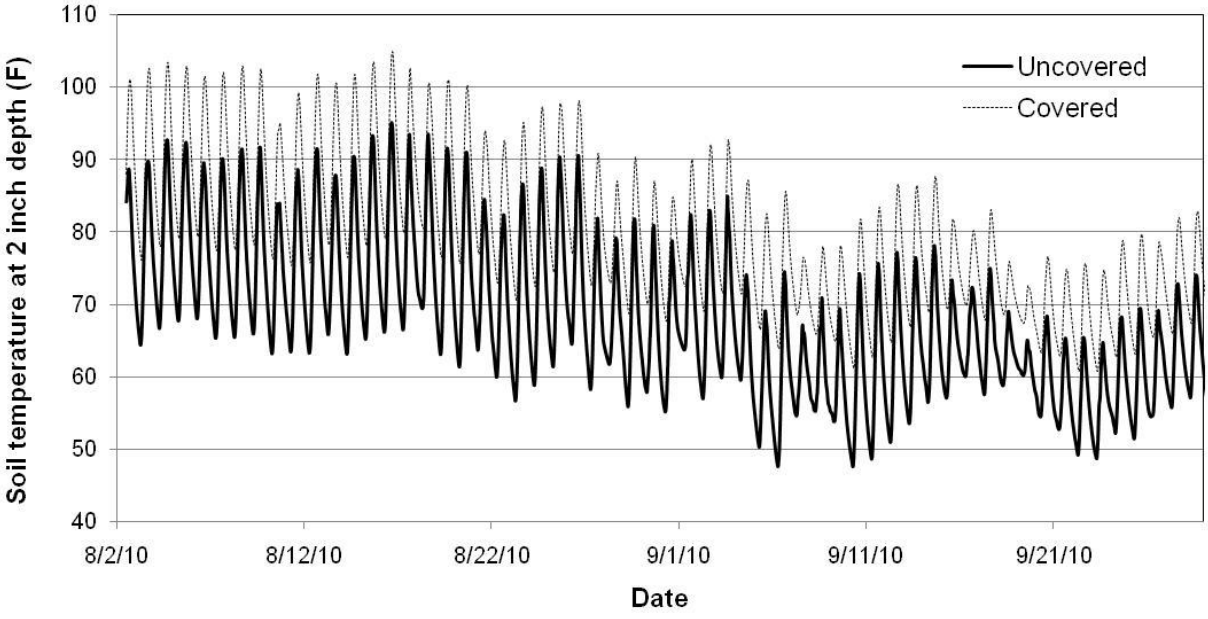

Figure 2. Half-hourly averages of soil temperature at 2 inch depth in plots covered with a 2-mil clear polyethylene film and in uncovered plots.