Evaluate Application of Diallyl Disulfide During a Cover Crop for Management of Allium White Rot

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Introduction

White rot caused by *Sclerotium cepivorum* Berk. is a major disease of Allium crops worldwide, including California, Oregon and Washington. Commercial Allium varieties with adequate resistance to this disease are not yet available despite considerable efforts in searching for resistance. The pathogen *S. cepivorum* attacks the root system of Allium plants and causes yield declines, plant death, or decay of bulbs during storage. At the end of the growing season, the pathogen produces a great number of poppy seed-sized sclerotia on infected tissue. Populations of just a few sclerotia in a liter of soil can potentially cause severe disease and result in crop failure. In the absence of an Allium host crop, the sclerotia can remain dormant and survive in the soil for many years. In California, where 50,000 acres of garlic and onion valued at more than $300 million are grown each year, Allium production is continually challenged by white rot. Despite the extremely restrictive seed certificate program, more and more commercial fields become infested with *S. cepivorum*. Control of white rot in Allium crops is technically and economically difficult because sclerotia can survive in soil for a very long time in the absence of host plants, and a very low sclerotium density is sufficient to cause considerable loss. Once a field is infested, it is generally considered unsuitable for garlic or onion production for up to 40 years. In the San Joaquin Valley, 14,000 acres of land were infested by the pathogen from 1994 to 2007 and are no longer suitable for production of garlic and onion. As a result of white rot and foreign competition, the total harvested acreage of U.S. garlic production has steadily decreased 33 percent from 38,000 acres in 1998 to 25,440 acres in 2008 (USDA NASS).

Control measures for this disease have been mainly targeted at reduction of the soil-borne sclerotium densities. One of the most effective methods is fumigation with methyl bromide, but it is not cost effective and has been phased out due to its harmful environmental effects. In the United States and Canada, dicarboximide fungicides (iprodione and vinclozolin) were used to control Allium white rot, but their effectiveness declined due to enhanced microbial degradation in the soil (Walker et al., 1986). Tebuconazole (Folicur) was recently approved for white rot in Allium crops although it is potentially phytotoxic to germinating onions (Fullerton et al., 1995). A number of alternatives to fungicides have been investigated for white rot management. These methods include the use of sclerotia germination stimulants such as diallyl disulphide (DADS®), solarization, biological control agents, and incorporation of plant residues.

Sclerotia of *S. cepivorum* lie dormant in the soil in the absence of an Allium crop. Diallyl disulfide (DADS) exuded from the roots of an Allium crop stimulates the sclerotia to germinate. When DADS is applied to the soil in the absence of an Allium host, the germinated sclerotia exhaust nutrient reserves and die (Merriman et al., 1980; Entwistle et al., 1982; Crowe et al., 2007; Davis et al., 2007). High temperature was also found to accelerate the decay of sclerotia of *S. cepivorum* (Crowe and Hall, 1980). When soil temperature in commercial fields was increased to 24.6 ~ 28.8^{o}C by covering with a 50-µm polythene film, sclerotial viability decreased 46.7 percent to 91.3 percent compared with the control (McLean et al., 2001). In studies conducted in

Spain and Mexico, solarization consistently reduced viable inoculum density in soil and provided good control of white rot in garlic (Melero-Vara et al., 2000; Ulacio-Osorio et al., 2006). The use of *Brassica* spp. and related plants as cover, rotation, or green manure crops for managing soilborne diseases has received increased attention in recent years. Toxic compounds released from decomposition of cruciferous residues can penetrate, weaken and thereby predispose sclerotia to parasitism by competitive saprophytic microflora (Smolinska and Horbowicz, 1999; Smolinska *et al*., 2002). Increased soil microbial activities associated with cover crops other than isothiocyanate production may also be important in the reduction of soilborne diseases and increase in yields (Cohen et al., 2005; Smolinska, 2000). Biological soil disinfestation (BSD), achieved by incorporating easily decomposable organic materials into moist 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 (Goud et al., 2004; Mattner et al., 2008; Momma, 2008). Ulacio-Osorio et al. (2006) found that both incorporation of broccoli and solarization alone significantly reduced viable sclerotium density of *S. cepivorum* and increased yield of garlic while incorporation of broccoli in combination with soil solarization resulted in the greatest reduction in number of viable sclerotia, the lowest white rot incidence, and the highest garlic yield.

In a previous study supported by CAGORAB, it was found that the viability (tested on water agar) decreased sharply after 2 weeks of solarization, but this decrease was not observed on late sampling dates, after the temperature dropped in the fall. There was no significant decrease in white rot incidence for the following garlic crop in those plots. Therefore, the short time heat from solarization seemed to not kill the majority of sclerotia. Instead, it might have conditioned the sclerotia into a "dormant status"—which become active again after temperature dropped, similar to findings by Gerbrandy (1989), in which burial in higher temperatures (5 and 10^{o}C) made sclerotia germinate slowly. Since solarization reduced disease in other studies, it is reasonable to hypothesize that prolonged heat treatment will eventually kill majority of sclerotia of *S. cepivorum*.

Therefore, it is proposed to determine the effects of heat treatments on germination and survival of sclerotia of *S. cepivorum* in the laboratory, and to study the effect of DADS application, cover crop, solarization and combinations of them on the survival of sclerotia in the field and the effects of these treatments on progress of white rot in the subsequent garlic crop.

The hypothesis is that sclerotia of *S. cepivorum* can be either active (more vulnerable to stresses and attacks by other microbes) or dormant (more resistant). With a better understanding of the biology of germination and survival of sclerotia, conditions can be created to accelerate the decay of sclerotia in commercial fields. The ultimate goal is to develop an environmentally friendly strategy for management of white rot in Allium crops in the western United States. It is widely reported that high soil temperature enhances the effects of cover crop and incorporation of plant debris on suppression of soilborne diseases; this is why it is desirable to include solarization in this study for comparison. However, as the soil temperature in central California often reach 25^{o}C during the summer, solarization may not be necessary for California garlic and onion growers to adapt this technique. If so, the cost of white rot management can be significantly reduced. Although DADS® and solarization may be prohibitively expensive, with site specific management, the means of control may not need to be applied to the entire field. If

the results from this study demonstrated that combination of cover crop/rotation crops and application of DADS® can bring the soilborne sclerotia level down sufficiently, many California fields infested with *S. cepivorum* may become suitable for garlic and onion production within a few years while income can still be made from growing other crops.

The specific objectives of this study are (1) to quantify the effects of DADS®, cover crops, incorporation of plant material and various combinations of these approaches on the viability of *S. cepivorum* sclerotia in soil; and (2) to compare influence of these management systems on white rot disease levels, yield and quality in the subsequent garlic crop.

Materials and Methods

A field trial was conducted in a field infested with sclerotia of *S. cepivorum* at the Central Oregon Agricultural Research Center in Madras, Oregon. Seven treatments were arranged in a randomized complete block design with 5 replications:

1) Untreated control -the field was left fallow, not covered during the spring and summer;
2) Oat was planted on May 1st, and oat residues were incorporated into soil on July 16th;
3) Mustard was planted on May 1st and mustard residues were incorporated into soil on July 16th;
4) DADS® applied on May 1st, oat was planted immediately after. Oat residues were incorporation into soil on July 16th;
5) DADS® applied on May 1st, mustard was planted immediately after. Mustard residues were incorporation into soil on July 16th;
6) DADS® applied on May 1st, oat was planted immediately after. Oat residues were incorporation into soil on July 16th then covered with 4 mil film until September 17th;
7) DADS® applied at 0.6 gal/acre on May 1, mustard was planted immediately after. Mustard residues were incorporated on July 16th then covered with 4-mil film until September 17th.

Each plot was 10 ft by 10 ft in size. DADS® was applied at 0.6 gal/acre via spraying at 40 gal water per acre just before planting on May 1. Oat (Monida White Oat) and mustard (Southern Giant Curled Leaf from Wilbur-Ellis, Culver, Oregon) seeds were broadcast at 55 lbs/acre and 20 lbs/acre, respectively, and then tilled into the soil immediately. The cover crops were fertilized once with 1:1 mixture of 30-0-6 and 40-0-0 on May 11th, and irrigated as needed. Due to herbicide damage caused by drift, we planted additional seeds on June 8th. Residues of the two cover crops were redistributed to make them uniform among plots, incorporated in on July 16th by running rolling tiller twice along different directions then covered with a clear 4-mil plastic film. Edges of the film were sealed by burying with dirt. Five soil cores of 1-inch diameter and 6-inch depth was collected from each plot monthly until incorporation of crop, and then after removal of the covering plastic film on September 17th. The five cores were mixed then a 250 cc subsample was drawn for assay of sclerotia. Soil was blended for 15 seconds and sclerotia concentrated from soil by size (wet sieving through screens) and by density (flotation on water and a sucrose solution). Remaining soil residue with sclerotia was collected and observed under a binocular microscope. The sclerotial bodies remaining intact were retrieved and enumerated. If more than 50 intact sclerotia were retrieved, 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 retrieved, then all intact sclerotia were tested for viability. Sclerotia were washed, surface-disinfected for 2.5 minutes in 1.0 percent sodium hypochlorite, rinsed three times with sterilized water, cracked using forceps, and placed on water agar plates to induce growth. Sclerotia that develop characteristic mycelial growth and clumps of microconidia in the agar were identified as viable sclerotia. Sclerotia not germinated in three weeks were considered to be dead.

Results and Discussion

The soil temperature at a 2-inch depth fluctuated between 70 and 80^{o}F in plots without plastic film on most of days after incorporation of cover crop residues until September 8 when the temperature started to drop down to the range from 60 to 70^{o}F (Fig. 1 upper). In the plots covered with the soil temperature followed a similar trend, but fluctuated wider between 75 to 100^{o}F early and then dropped down the range from 65 to 75^{o}F at the end. The soil temperature difference between the plots covered with plastic film and those without coverage fluctuated between 6^{o}F and 16 to 18^{o}F, from night to day time during the early stage, then narrowed down to as small as 2^{o}F to the end (Fig. 1 lower). These results together with those from last year suggested timing is critical to achieve a high soil temperature for solarization in central Oregon. The ideal period would be from middle July through August.

The sclerotium density started very high at the beginning of this trial, ranged from 110 to 200 sclerotia per liter soil (Fig. 2). Once again, no significant effect of DADS® on sclerotia of *S. cepivorm* was observed. Different from last year, this year DADS® was incorporated into the soil after spaying it onto the soil surface, but the same results were observed as the last year. Also, no significant reduction in number of viable sclerotia was observed by any treatment, cover crop in combination with solarization. The reasons behind this remained unclear. Extremely high sclerotium density may be one reason. Other possible reasons include also production of new sclerotia on garlic residues left from the last year, the special status of sclerotia in which sclerotia are irresponsive to the stimulation of DADS®, and etc.

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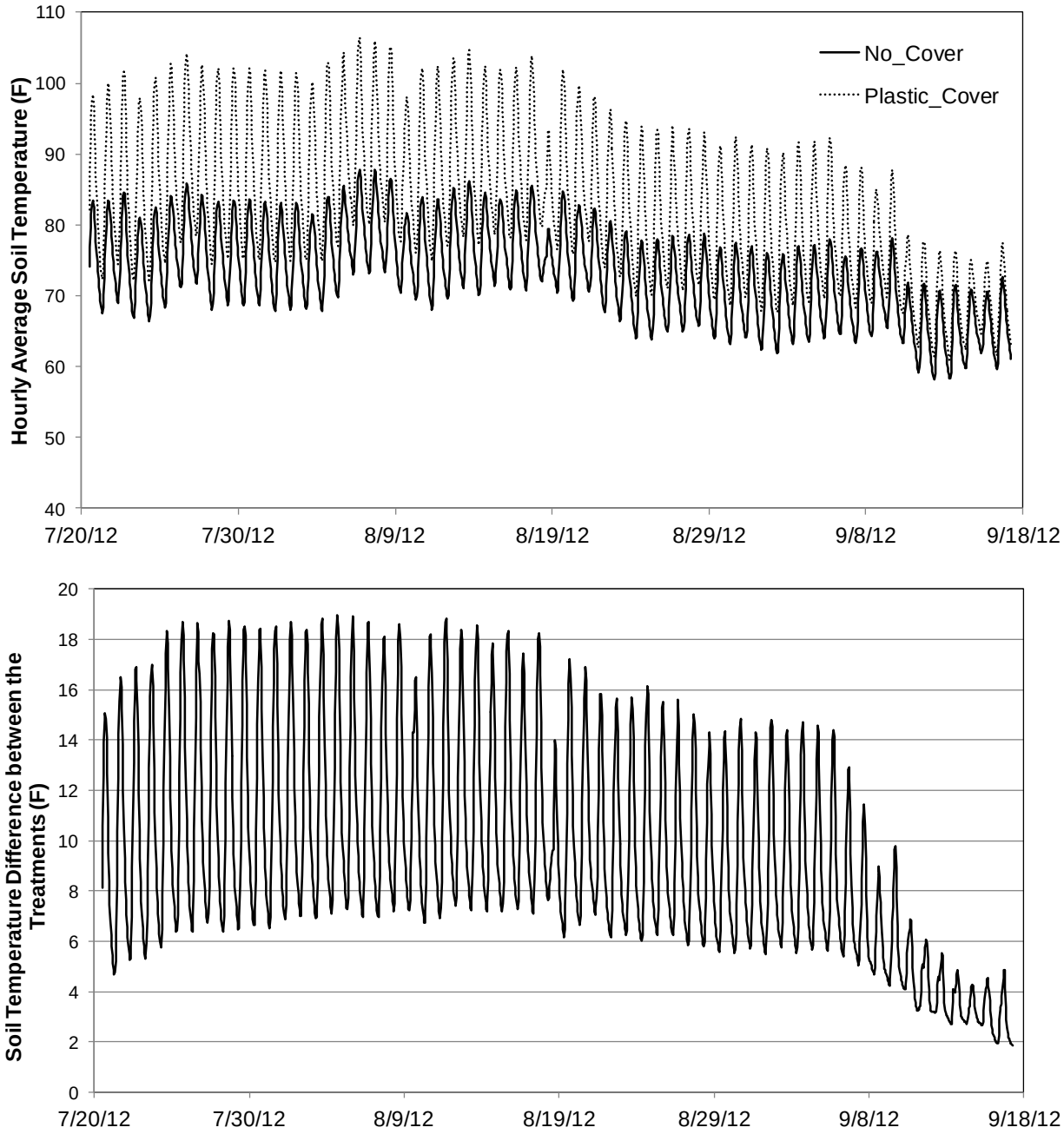

Fig. 1. Hourly averages of soil temperature in plots with and without plastic coverage (upper) and hourly soil temperature difference between the two treatments (lower). The soil temperature was measured with a Campbell soil temperature sensor 107 at 2-inch depth in two plots from each treatment.

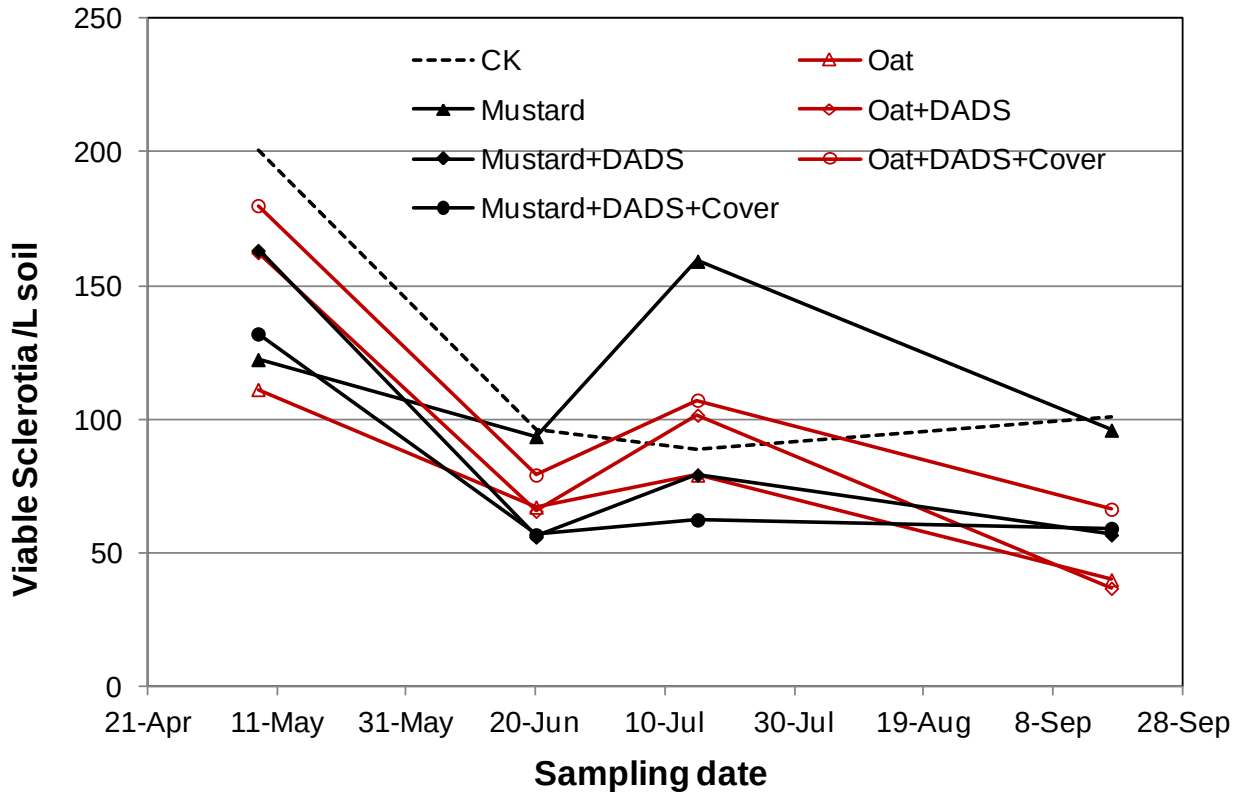

Fig. 2. Number of viable sclerotia of *Sclerotium cepivorum* per liter soil from the top 6-inch profile collected from plots subjected to different treatments, combinations of DADS®, cover crops and solarization.