

ERADICATION OF THE WHITE ROT FUNGUS FROM INFESTED SOIL BY  
STIMULATION OF SCLEROTIA IN THE ABSENCE OF HOST CROPS.

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ABSTRACT

A single application of di-allyl di-sulfide (DADS), a stimulant of germination of sclerotia of the white rot fungus (*Sclerotium cepivorum*), was irrigated into furrows of an infested field which had been planted to bluegrass seed. Pre-treatment populations of sclerotia in the soil were estimated, then compared with those present one month and one year later. After one month many sclerotia were actively germinating or had germinated already. After one year, no sclerotia were found in beds receiving various rates of product, neither were sclerotia found in water-only treatments and border beds to the treated areas. The population of sclerotia in field soil more than 20 ft away from the test area remained measurable during this period. Odors of the product were detected in soil and air space in the test area for several months and may have stimulated sclerotia away from the points of application. Bluegrass production apparently was unaffected. These results confirm that DADS and/or related compounds might be utilized to reduce soil populations of the white rot fungus, and possibly to eliminate the pathogen from field soils at times when Allium crops are absent.

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INTRODUCTION

Sclerotia of the white rot fungus may lie dormant in soil for years until stimulated by sulfur compounds naturally emitted as gaseous vapor from roots of onions or onion relatives. In small, closed test systems, 100% of sclerotia may respond to stimulation. Stimulation is optimal at near 65°F, and is restricted to the temperature range between 48-75°F. Germination of sclerotia is optimal at intermediate soil moisture levels. Prolonged saturation promotes sclerotia decay. The pathogen has been reported unable to grow

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and reproduce on plants other than Allium species or on soil organic matter.

Using a naturally-infested field site in central Oregon, we attempted to irrigate into the soil profile a commercial, petroleum-derived source of di-allyl di-sulfide (DADS), one of the stimulants found garlic. This was done in an attempt to rapidly reduce soil populations of the white rot fungus in the absence of onions or other Allium species. We presume that repeated applications will be necessary to reduce populations sufficiently for successful recropping of susceptible crops. If the amount and cost of material are low enough, and the delivery system is inexpensive and non-disruptive to other cropping, treatments could be repeated as necessary.

Initially we chose to test various concentrations of DADS applied in single irrigation. In doing so, we hoped to determine an appropriate working range of concentrations, to determine the approximate number of applications which might be required commercially, and to determine if the oil-based product even could be effectively moved through the soil profile with irrigation water.

#### METHODS

Field site selection: The field relebted was near Madras OR. Based on preliminary analyses, the most highly infested area was selected for location of test plots. The field was in bluegrass seed production since fall 1982, and was furrow irrigated.

Experimental design: There were five replications of each treatment, in a randomized block design. Data collected were: 1) pre-treatment inoculum densities from soil collected from each plot on October 13, 1987, 2) 1-month post-treatment inoculum densities from soil collected from each plot on November 17, 1987, and 3) 1-year post-treatment inoculum densities from soil collected from each plot on November 28, 1988.

Population estimation: From plots, two sub-samples of twenty composited 1-in dia x 6-in deep cores were taken uniformly along the tops of beds. Care was taken not to sample from furrow sides or bottoms, so as not to disturb normal irrigation water infiltration of the bed profile. Additional soil samples were collected from alleys, beds between plots, and from areas 20 or more ft outside the test area. Cores were bulked, air-dried, coarsely ground and mixed well, and stored air-dry at room temperature. Samples were assayed by wet-sieving through screens, and observing

residue under a binocular microscope. Sclerotia were collected, forced to grow and identified. Inoculum density was expressed as the number of sclerotia per 100 mo (cc) soil.

Plot design and DADS treatments: Treatment was initiated on October 15, 1987. At that time, daytime soil temperature at four inches in the bed was 49-58°F. Plot irrigation treatments were into the three furrows bordering two 20-ft long bed sections. Bed sections were separated along the row by dammed 3-ft alleys which were unirrigated, and which were left dammed over the winter. Side-by-side, plots were separated by two additional bed sections, and the furrow between these beds was irrigated with water only at the time of plot treatment. DADS was 70% product, obtained from Phillips Petroleum. Because the bulk of the impurities were related compounds, also thought to be stimulatory, concentrations were developed based on total product. In addition to a water-only treatment, treatments were four 10-fold dilutions from a maximum dosage of 1% (v/v). Dosage calculation was based on the estimated total water held at saturation in the soil within 8 in from the top of the beds. Water already in the soil profile was included in the calculation. DADS, along with 20 ml non-ionic wetting agent was mixed with 4 gal water in buckets and applied uniformly to each 20-ft furrow section. No wetting agent was applied with water-only controls. Separate buckets were used for each rate of application. As soon as this amount had infiltrated the soil (approx. 1 min), the furrows were filled three times with additional water, totalling 195 gal per plot. The water source was normal ditch water. Water was dispensed directly into all three plot furrows at a time from the ditch, using a submersible pump, a hose and a PVC manifold.

Following the test irrigation, dams were left in place over the winter. No additional irrigations were made commercially or experimentally to the field in 1987 or 1988. In March 1988, dams were removed and the field was irrigated commercially, including all furrows through plot areas.

"Garlic" odors: DADS smells much like garlic. Observations were periodically made of odors in the air around plots and from soil samples as a measure of determining the persistence of treatments.

Kentucky bluegrass growth: Visual observations were made on the condition of Kentucky bluegrass plants in the first month after treatment and during 1988.

## RESULTS

Inoculum densities from outside the test: The average number of sclerotia per 100-ml soil from samples taken from

outside the test area was 1.75 in August 1984, 1.37 in August 1987, and 0.75 in Dec 1988 (Figure 1). The population may have naturally dropped slightly during this period, but Figure 1 primarily shows that the field remained measurably infested during the test period.

Inoculum densities from within the test area: Plots averaged 2.28 sclerotia per 100 ml in October 1987, prior to treatment. The plot area was selected because it initially was higher than the field average. One year after treatment (Dec 1988), no sclerotia were recovered from soil collected from any plots; a total of 5,000 ml soil was assayed (25 samples, two sub-samples each). Figure 2 shows average pre-treatment and 1-yr post-treatment inoculum density from both within and outside the test area. Additionally, soil was collected from five inter-plot borders and from alleys. One year after treatment, no sclerotia were found from four of these samples (2,000 ml soil). One sclerotium was found in 500-ml soil, from one sample taken from 3-5 ft outside the plots.

Pre-treatment and one-month post-treatment inoculum densities are shown for each treatment in Figure 3. Notably, inoculum density significantly increased over pre-treatment levels during this period for applications of 10, 100 and 1000 ppm DADS. Many sclerotia were found in a state of germination in soil from these treatments. Furthermore, numerous objects which clearly were the old shells of sclerotia already germinated/decayed were present in soil from these treatments. Not surprisingly, little activity was noted in the first month from the water-only treatment and from the DADS concentration of 10,000 ppm (1%). Allyl sulfide is known to be inhibitory at concentrations above this amount.

"Garlic odors": The immediate atmosphere surrounding the entire test area smelled strongly of garlic for at least one month. In the field it proved impossible to determine by smell if low rates of application were odoriferous due to the intense odors emanating from nearby plots at higher rates. A faint garlic odor could be detected in the air around the plots even during March of 1988, 4-5 months later. At the time of 1-month post-treatment soil sampling for sclerotia population estimation, a few additional cores were collected from each treatment and placed in sealed metal cannisters. After various testers' had not smelled garlic for several days, cannisters were opened and tested for garlic odor, beginning with the water-only check, and thereafter with increasing treatment concentration. No garlic odor was detected in samples from the water-only check or from the lowest DADS treatment (10 ppm). For progressively increasing concentrations, garlic odor was present and increasingly strong. By March, 1988, faint garlic odor still was present in soil taken from the highest concentration treatment.

Kentucky bluegrass growth: No affect was noted on bluegrass plants growing in plots in 1987 or 1988. Except in one case where an oily scum separated from water in the furrow of the highest concentration. This coated and quickly killed seedling bluegrass plants growing in the furrow. Seedlings in furrows of other treatments of lesser concentration showed no effect. Plants growing from the top of the bed in high-concentration treatment showed no effect.

#### DISCUSSION AND CONCLUSIONS

Based on highly active germination one month after treatment within the treated area, and inability to recover sclerotia from treated areas after one year, we were able to stimulate sclerotia to germinate using DADS applied in a commercially equivalent manner with irrigation water. We drew this conclusion by comparing the response of populations within the test area with the response of populations from areas away from the test area. Populations away from the test area remained measurable for sclerotia of the white rot fungus, whereas within the test area sclerotia either were eradicated, or at least reduced to a level well below our ability to detect them. Based on other studies in the western United States, 1 or more sclerotia per 100 ml soil is sufficient inoculum to result in the loss of 90-100% of fall-planted garlic or onions, or the same proportion of onions spring-planted in areas with a cool summer. Populations below 1 sclerotium per 1000 ml soil would result in less than 10% plant loss. It proves difficult to measure populations below 1 per 1000 mls soil (about 1 qt), but the post-treatment populations within the test area fell to well below this level; no sclerotia were found from 30 samples and over 50 separate assays (7,000 mls soil). Disease-loss relationships have not been determined for regions with other climates and cropping systems. Spring-planted onions in the Treasure Valley might be expected to suffer less loss, because high soil temperatures might limit fungal germination and growth during the summer, however, white rot could be active on summer onions in this area during the spring and fall.

It is likely that DADS influenced sclerotia to germinate in nearby untreated plots and alleys within 5 ft from the application site. Because of the equal response among all treated and untreated plots, any potentially differential germination response to various rates of application was obscured. Without detailed knowledge of manner of movement of stimulant within soil and air, we cannot make conclusions concerning dosage and time responses of application of this product in the field. Clearly, continued testing, with design modifications, is called for to further test rates,

methods of application, influence of wetting agents, and influence of soil type.

Although detailed data was not collected on effects of DADS on bluegrass plants, no significant effect was noted from casual observations. Because our treatments were to the furrows rather than directly onto plants via overhead irrigation, additional data are required to determine if DADS applied with sprinkler irrigation might affect crop plants.