USE OF STIMULANTS OF SCLEROTIAL GERMINATION TO MANAGE INOCULUM DENSITY OF *Sclerotium cepivorum* AND TO CONTROL WHITE ROT OF ONIONS AND GARLIC*

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Abstract

For five field trials throughout western North America, with treatments replicated three or more times in randomized block experimental designs and in diverse soils naturally, and highly infested with sclerotia of *Sclerotium cepivorum,* the numbers of assayed sclerotia were reduced by as much as 98-99 percent in U.S. test plots treated once with 75 percent (plus related and active impurities) diallyl disulfide (DADS), a germination stimulant. All rates of application utilized, 0.56, 5.6, and 56 gal/a, were effective. DADS was less effective at the lower rates in a single Mexican trial. In untreated plots, there was measurable but limited natural reduction in sclerotial recoveries. In two of these trials, other chemically-related germination stimulants gave comparable results to diallyl disulfide, but dimethyl sulfoxide showed only moderate stimulatory effect. Both fall and spring applications of DADS were effective when timed to maximize the stimulus:response period within the temperature range of 48-70°F, based on the daily maximum soil temperature at 4 inches. Following fall application in regions where temperatures dropped below 48°F, the stimulus:response stopped, then resumed after rising above 48°F in the spring, with little or no reduction in net activity. Little or no further germination continued beyond a 2-2.5 month period within this temperature range, presumably due to enhanced loss of volatile stimulants at higher temperatures, and due to elevation of soil temperature out of the stimulus:response range. In all trials, soil moisture was managed to retard loss of volatile stimulants from the soil, to retain a biologically active stimulus:response system, and (in most trials) to initially move stimulants through the soil profile after application. For two trials, in which

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applications of DADS were repeated in a second year, no sclerotia were recovered after the second application, which correlated with a low probability of loss of Allium crop plants based on earlier studies. When these plots later were planted with garlic, 90-100 percent of the plants in untreated plots developed white rot, and 0-10 percent became diseased with white rot in treated plots. In the trial with the best control over possible sources of re-contamination following treatment, no white rot occurred in any twice-treated plots at any rates of application. A sixth trial area not conducive to soil assay was directly planted to onions after comparable single and double applications of germination stimulants, with comparable results to the two trials replanted with garlic.

Introduction

Sclerotia of the white rot fungus *(Sclerotium cepivorum)* remains dormant in the absence of *Allium* plants. For several weeks or months following scierotial formation, germination is restricted by an unidentified mechanism of constitutive dormancy (1, 2). Following this period, germination of sclerotia may be triggered by a number of specific volatile sulfides and thiols, which are breakdown products of amino acids peculiar to this genus, and which are released into the soil from roots (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 17, 18, 19 -- see 7, 18, and 19 for reference to specific compounds).

Germination in soil is stimulated between about 10-22°C (50-72°F), and may be restricted to the spring and fall months in some regions (8, 13, 23). The fungus itself has a somewhat broader range of temperature tolerance once mycelium is actively growing (13, 23). Germination of white rot sclerotia is optimal at intermediate soil moisture levels (-300 millibars, or about field capacity). Very wet soil (greater than -13 millibars, near saturation) inhibits germination, possibly by preventing volatilization and distribution of stimulants, and prolonged saturation promotes sclerotia decay. Too low moisture (less than -3 bars, quite dry) inhibits both stimulated germination and growth of the pathogen (13), and may promote loss of stimulants from the soil. Although the mechanism of stimulated germination is not well defined, limited germination partly may be a result of rapid volatility of the stimulants and their loss from the soil system before stimulation can be effective (8). Following germination, the fungus dies off without significantly reproducing if the bulbs of a host plant fail to become infected (2, 13, 14, 22). A small number of sclerotia (well below replacement level) form on host roots, and a low frequency of small, secondary sclerotia may form near to sclerotia (22). Neither of these sources are of much importance. In test systems in the lab and greenhouse, in small, highly-controlled field tests, and from observations of field situations, the level of such stimulated germination may be nearly 100 percent (2, 8, 13, 14, 22). Most test systems have utilized sclerotia and stimulants in full or partial confinement. Data from Coley-Smith and Parfitt (8) are the best to use for dosage-response-time in relation to temperature and seasonality in the field.

In the field, mycelium from sclerotia may infect roots up to 1-2 cm away, and successfully decay bulbs from sclerotia located 30 cm below stem plates (12). Abundant bulb infection was shown to result from secondary spread of mycelium among root systems (12, 14).

Initial infection occurred from sclerotia located at various depths, as well as from direct infection of single plants by shallowly-located sclerotia at high inoculum density (12, 14). Further, bulb decay occurred later when secondary infection was dominant, and earlier when direct infection was dominant. Post-season inoculum densities from a wide range of pre-season inoculum densities were similar, which was attributed to the relative amount of host biomass available for reproduction of sclerotia at the time of bulb infection (12, 14).

In addition to natural products, germination stimulants can also be recovered from petroleum. Diallyl disulfide (DADS), a primary flavor ingredient of expressed garlic juice and a primary breakdown product of the amino acid allylcysteine sulfoxide (alliin), which leaks from garlic roots, is one commonly available petroleum-derived stimulatory compound. Relatively pure DADS, applied by injection or irrigation at rates between 0.25 and 12.5 g to 1 m^2 plots in the field in England influenced as many as 90-99 percent of sclerotia to germinate through the soil profile. Activity was sharply reduced in summer months due to rapid loss of DADS from the soil, and was "postponed" during winter months when DADS became non-volatile (8), and during which time sclerotia would have been dormant (13). Very high concentrations of DADS may be inhibitory to germination (22), which further points out that the stimulation mechanism remains poorly defined.

In Australia, in an attempt to develop a commercialized field-scale treatment, artificial onion oil (a proprietary food-grade mixture of diallyl disulfide, di-isopropyl disulfide, allyl isothiocyanate, allyl alcohol, garlic oil, and nut oil) was injected into the soil with a commercial fumigation device at various rates and depths at one month prior to planting of onions. For single applications of several rates, this resulted in 77-91 percent reduction in sclerotia and reduced yield losses due to white rot from 57 percent to 13 percent (16, 20, 21). Actual rates of application were not determined due to the unknown concentrations in the basic product. This approach to control of white rot was not further investigated due to erratic control under annual cropping conditions, lack of availability of a well-defined and inexpensive stimulatory product, and by re-direction of the principle investigator into other endeavors (personal communication, P. Merriman). Data in the western United States (14) indicate that for commercially-acceptable control of white rot, most natural field populations of the pathogen would need to be reduced by 1,000-100,000 times. Reduction by only 90 percent, or less, as in Australia, would be insufficient. Also, based on conversations with P. Merriman, it is likely that timing of application may not have been carefully coordinated with the optimal responsiveness of the pathogen.

We wished to determine whether germination stimulants might be successfully applied to naturally infested commercial fields, such that infested fields might be replanted with onions or garlic without risk of economic crop loss. The basis for evaluation of treatments was the magnitude of drop in viable numbers of sclerotia based on pre- and post-application soil assays, and whether sclerotial numbers could be driven below the approximate economic threshold established for white rot under conducive temperature regimes for several regions of the western United States (14, and see Figure 1). In the field trials described below, we initially wished to determine the response achieved by single applications of a wide range of

product amounts and in diverse regions and soil types, in order to better plan future control strategies and costs.

Materials and Methods

Soil sampling/assay. Soil sampling and assay was as per Crowe, et al (14). At each time of sampling two sub-samples, each composed of twenty 12 x 1-inch diameter soil cores, were taken randomly from each field trial plot. For samples taken from recently treated soil, sclerotia frequently were in the process of germination, so samples were processed within two days or air dried at room temperature with the assistance of fans and held in a dry state, in order that the sample reflect the germination status at the time of sampling. For each sub-sample, 500-m1 soil were assayed for sclerotia. Soil was washed through 60- and 20-mesh screens to concentrate the size fraction of soil containing sclerotia (approx. 0.5 mm dia). Residue retained on the 60-mesh screen was discarded, and organic matter from the residue from the 20-mesh screen was separated from more dense soil particles by sucrose flotation. The organic residue from each sub-sample was observed under a dissecting microscope. Objects that appeared to be sclerotia of *Sclerotium cepivorum* (smooth, predominantly round, and of the appropriate texture and shade of black) were classified into the following categories: (a) Non-germinated -- rind intact, body firm, no germinating condition, (b) Germinating -- with a plug of characteristic whitish mold emerging from a point on the rind, (c) Sclerotia that probably germinated (or possibly decayed) much earlier -- only the full or partial shell of the rind present, or (d) Sclerotia that probably were decayed or parasitized -- rind not firm to touch with forceps, sometimes with moldy growth of other organisms present. The presence or absence of very small sclerotia were noted to determine the abundance of sclerotia that may have formed secondarily (22). Viability of intact bodies thought to be sclerotia of *S. cepivorum* was determined by plating surface sterilized bodies on water agar following cracking of the rind (14). Inoculum density of plots was estimated by totaling the number of viable sclerotia from 500m1 sub-samples. Inoculum density was expressed as the number of intact, viable sclerotia/liter of soil.

Inoculum density was determined from each plot in each trial just prior to treatment with germination stimulants. After application, inoculum density was reassessed monthly until no further response was seen in plots treated with germination stimulants.

Selection of trial areas. Field trials were located in several (not all) regions of western North America in which white rot disease was of high commercial interest, either as a well-established or emerging concern (Table 1). Because of the diversity and expanse of the territory involved, a local cooperator (county extension agent, extension specialist, or similar interested cooperator) was required for certain aspects of management of trial areas. In each area selected, several fields with a well-established history of white rot disease were sampled preliminarily for inoculum density determinations. (The single exception was near Salem, Oregon, on muck soil with nearly 100 percent organic matter, from which sclerotia of *S.*

cepivorum were difficult to assay.) In general, areas within fields were chosen if an area of the field large enough to contain a field trial was generally infested. Inoculum density above 15 sclerotia per liter of soil was preferred, so that inoculum densities before and after treatment would be more reliably evaluated.

Germination stimulants. Most materials tested for their ability to stimulate sclerotia of *S. cepivorum* in the field were obtained from Phillips 66 Company, Bartlesville, OK. Diallyl disulfide (DADS) was used in all trials. DADS 75 percent included 10 percent diallyl sulfide, 10 percent diallyl trisulfide, 1 percent pentane and 6 percent higher order, related sulfur materials (e.g. diallyl pentasulfide and hexasulfide). DADS 90 percent included mostly diallyl trisulfide as the remainder component.

Other Phillips materials used in some trials included 98 percent di-N propyl disulfide (DNPDS), including 2 percent related compounds, and "POLY", which was a mixture of related components produced by removing most of the DADS by distillation. "POLY" included 20-40 percent DADS., 30-69 percent diallyl trisulfide, 10-30 percent diallyl tetrasulfide, 0-2 percent diallyl sulfide, 0-5 percent diallyl pentasulfide, 0-0.2 percent pentane, and 0-2 percent related higher order sulfides. All components for the above products were known or believed to be stimulatory to sclerotia of *S. cepivorum* based on the active C-C-C-S structure common to each compound (7,17, 18, 19).

In some trials, dimethly sulfoxide (DMSO from the Sigma Chemical Co.) also was used. DMSO is related to the above compounds and it is relatively inexpensive. Even though it did not have the common C-C-C-S structure, sulfoxides can convert to disulfides, sometimes rearranging in the process (7, 17, 18). It did not seem to have been specifically tested for stimulated germination of *S. cepivorum* in previous investigations (7, 17, 18, 19). No known germination stimulants were identified as minor components of the DMSO.

Product application. All products were tested at three rates of application: 0.56, 5.6, and 56 gallons per acre. The amount of product per plot area was premixed using an equal amount of non- ionic wetting agent to facilitate mixing, brought to a total volume of either 1 gallon or 5 gallons with water for application to the plot area. In tests not reported here, a range of commercial non-ionic wetting agents had no effect on germination of sclerotia, nor any effect on stimulated germination with DADS. Therefore, no wetting agent was added to untreated check plots. In preliminary tests, surface irrigation following application of DADS to the soil surface moved the detectable odor of DADS to 10-12 inches deep in the soil profile, and sclerotia were stimulated to germinate at that level. In all tests reported here, premixed products were applied to the plot area with watering sprinkler cans, followed by (a) sprinkler irrigation, (b) flood irrigation, (c) rototillage to 10 inches followed by sprinkler irrigation, or (d) rototillage to 10 inches followed by flood irrigation. The time between application and irrigation was minimized to reduce loss of stimulant by volatilization. The time between application and irrigation ranged from 5 to 30 minutes.

Experimental design. All field trials were randomized block designs with four or more replications per treatment, unless otherwise indicated. Plots ranged from 8 x 12 ft to 12 x 12 ft in size. Plots were separated by at least 15 ft in all trials. Data was evaluated by analysis of variance.

Soil temperature and moisture considerations. Products were applied either in the fall or spring, based on soil temperature at 4 inches. Fall applications were made as close as possible to when the daily maximum soil temperature remained below 70°F for several days. Spring application coincided with maximum daily soil temperature rising above 48°F for several days. Soil temperature was monitored weekly during the period of germination activity.

In addition to irrigation immediately following product application, irrigation was further provided (unless there was rainfall) to maintain an active stimulus:response (13). General guidelines were that plots were irrigated with 1-2 inches water whenever soil moisture fell below field capacity during the period when soil temperatures were conducive for stimulated germination. The actual number of irrigations varied among trials.

Weed control. Weeds were eliminated from plots using commercially available herbicides on an as-needed basis. Plots were not tilled or otherwise disturbed during weed control.

Other plot management. In all field trials, traffic of all types was discouraged during the course of the experiments, to prevent movement of untreated soil into plots. In flooded plots, any washing of soil over the surface between plots was prevented by the dikes used to contain the flood irrigation, but in sprinkler irrigated plots, this potential source of contamination was not prevented. For trials that were carried over for second year application or for re-planting, soil in plots was spaded and/or rototilled as needed to encourage product infiltration and normal root development. Care was taken not to move soil among plots during these operations.

Results

Dates of application. Madras: Treated October 15, 1987. Walla Walla: Treated October 10, 1989, and again October 25, 1990. Nampa: Treated April 14, 1990, and again April 10, 1991. Five Points: Treated November 19, 1990. San Miguel de Allende: Treated December 2, 1990. Dates of treatment at Salem were not compiled as of this report.

Qualitative observations of movement through soil of germination stimulants. Initial movement of germination stimulants through soil was evaluated in 1987-88 near Madras, OR. Products were applied as indicated into dammed furrows in plots located in a furrow-irrigated field planted with Kentucky bluegrass. Immediately after application, furrow sections were filled and water was allowed to infiltrate downward and across formed beds, then this was repeated. Soil cores were taken from the tops of beds later in the same or following day. Strong odor of DADS was present at the center of beds in all soil depths to 10-12 inches. It was not determined whether the odor was due totally to bulk movement of

DADS into the lower profile sampled, or to volatile re-distribution of DADS, but based on this and observations of germination of sclerotia at 10-12 inches, we considered there to be few barriers to movement of DADS with irrigation water.

Qualitative observations of recovered sclerotia.. Sclerotia behaved similarly in all trials. In all trials, no sclerotia were found clearly decayed or parasitized at any time of recovery. No sclerotia were found in a germinating state in any untreated plot, and spent rind shells characteristic of recent past germination were rare. After one month, some sclerotia were found intact, others germinating (hyphal plug emanating from single point on rind), and others seemingly well past active germination (only rind shells remaining). For fall-treated plots, there typically was only one month of activity before temperatures dropped below 45°F and all further activity stopped, until soil temperature began to consistently rise above 45-50°F in the spring, at which time sclerotia continued to be found in a germinating state for another 1.5-2 months. For both fall and spring applications, active germination continued until either (a) no intact sclerotia could be recovered, or until (b) about 2.5-3 months of conducive soil temperatures had passed [excluding the time period of winter dormancy], when no more stimulant odor could be detected. For spring treatments, the 2.53 month period of activity occurred prior to soil temperatures rising generally above 70°F when the stimulus:response system would become inactive.

Curiously, during the first month following application of all known germination stimulants (but not DMSO), the number of intact sclerotia frequently rose by 50-150 percent in many plots, even though there were abundant germinating sclerotia and spent shells present. This effect was inconsistent among replications. This might be explained if there was a brief period of reproduction of sclerotia during this first month. Even though this was not expected, nor is this supported by any observations in the general literature, such applications would not simulate any natural field condition where stimulants would leak continuously at low rates from host roots. With DADS and "POLY" materials, the numbers of intact sclerotia declined greatly during the second month, whether such a possible reproductive phase occurred or not. With DNPDS, the same apparent rise in intact sclerotia occurred during the first month in two separate tests. In one of the tests (Walla Walla), the second month pattern was similar to that seen with DADS and "POLY" materials -- germination continued and few intact sclerotia were recovered after 2.5 months. However, in the other test in which DNPDS was included, even though germination did continue, decline in number of intact sclerotia were not as great, so that a the difference in final inoculum density in DNPDS treatments was only intermediate between untreated plots and DADS or "POLY" treated plots. In untreated plots, no elevation in intact sclerotia was ever seen in the first month. In DMSO-treated plots, there was a lesser amount of active germination in the first month than in plots treated with DADS, "POLY", or DNPDS, and there was no elevation of intact sclerotia.

In no recoveries from any plots did there seem to be any preponderance of very small sclerotia suggestive of secondary sclerotia (22). Nevertheless, the issue of apparent reproduction described above requires further investigation.

Pre- and post-treatment recoveries -- DADS rate comparisons. Data presented here are for inoculum densities after all activity of germination stimulants had terminated 3 months after treatment. For brevity, data supportive of the qualitative observations described above after one-two months is not presented here.

All data recovery of intact, viable sclerotia at Nampa, Walla Walla, and San Miguel are shown in Table 2. For each plot, each post-treatment recovery was expressed as a percentage of the pre-treatment recovery (Table 3). Percentages for each replication were then averaged to determine the means presented in Table 2. Analysis of variance was evaluated using these percentages.

At Walla Walla, a few days after initial treatment in the fall of 1989, heavy precipitation caused overground movement of water. Surface water passing over certain treated plots was observed to then pass over an adjacent untreated plot over 25 ft away. At the one month post-treatment sampling, some stimulated germination was observed, presumably due to low dosage of stimulant carried in the surface water. This effect did not persist into the following spring. In Walla Walla, additional plots external to the main trial area had been included for other reasons. In these external plots, no loss of sclerotia from stimulated germination occurred, nor did this seem to occur in the untreated plots in the other replications of this trial. For these reasons, the data from the affected replication was substituted with data from one of the external untreated plots.

In all trials, DADS treatments at all rates lowered the recovery of viable, intact sclerotia well below the level of natural attrition in untreated plots. Performance was better in the two U.S. trials than in the Mexican trial. Reasons for this difference in performance are not known, but the great distance between the project leader and the cooperators in Mexico, together with the language difference, did create some difficulties, and possibly some confusion, in plot management.

Pre- and post-treatment recoveries -- comparisons among various germination stimulants. Data are presented here similar to the above section. Data for a separate trial at Walla Walla from the one above, and from the trial at Five Points are also included in Table 2. Data for DADS 75% and DADS 90% were nearly identical, so the means shown on Table 2 are averaged data for the two treatments. Recoveries in DADS treatments were similar to those found for other U.S. trials.

"POLY", containing some DADS but with elevated proportions of less volatile stimulants, performed at least as well as the more purified DADS products.

Recovery of intact sclerotia from plots treated with DNPDS was slightly higher in the Walla Walla trial than recoveries from plots treated with DADS products, but a simple LSD test did not separate the means of DNPDS treatments from DADS and "POLY" treatments. Future analysis using Duncan's Multiple Range test might possibly find the performance of DNPDS significantly different at Walla Walla. In the trial at Five Points, however,

recoveries of intact sclerotia from DNPDS treatments was quite high in comparison to DADS treatments. The number of intact sclerotia recovered from the low rate was not statistically different from the untreated check, and the number recovered from the high rate was substantially higher than that found with other known stimulants. Note in the qualitative discussion above, however, that there occurred substantial stimulated germination in all DNPDS plots, and that one-month activity was quite high, whereas there was no germination in the untreated check plots. Again, this data may be explained if substantial early reproduction occurred, followed by an inconsistent level of continued stimulated germination in the second month of biological activity.

Plantin • of selected field trials and disease evaluation. Based on failure to recover sclerotia at field trials at Walla Walla and Nampa in 1991, the plots were prepared for planting in the fall of 1991. Soil was hand spaded and tilled. Virus-free garlic cloves that had been sized were hand planted 3 inches deep in rows spaced. At Walla Walla, clove size was 2.9 g ± 0.1 g, and 115 cloves per five 11 ft rows spaced 1 ft apart were planted on September 25, 1991. At Nampa, clove size was 3.07g ± 0.1 g, and 117 cloves per nine 12 ft rows spaced 1 ft apart were planted on September 23, 1991. The total number of cloves planted per plot was 575 and 1,056 at Walla Walla and Nampa, respectively. Again, care was taken to not move infested soil into plots during plot preparation or planting. Plots were irrigated as before, once in the fall of 1991, and from spring through early June of 1992. Plots were fertilized with 200 lb/a N as ammonium nitrate in the spring of 1992. Stand counts were taken beginning at emergence until the stand stabilized. Disease evaluations were conducted periodically during the spring and summer of 1992 based on top symptoms of white rot, and were confirmed by gently removing some soil from around the base of some plants. As bulbs matured, irrigation was discontinued, and bulbs were allowed to mature during June, 1992, at both trials. Harvest was on June 30 and July 1, 1992, at Nampa and Walla Walla, respectively. All non-diseased bulbs were recovered, counted, and weighed.

Some fall emergence occurred in all plots, but no plants were determined to be lost to factors other than white rot disease. No symptoms of white rot were observed in the late fall/early winter of 1991. Final stand counts included plants that died early in the season. Mean final stand counts at Nampa were 919, 938, 931, and 939 at Nampa, and 437.0, 454.7, 477.0, and 486.3 plants per plot for treatments of 0 (untreated), 0.56, 5.6, and 56 GPA DADS, respectively. At Walla Walla, plants in untreated plots showed early symptoms of white rot even during emergence. Analysis of variance indicated no significant differences in final stand at either Nampa or Walla Walla ($p < 0.05$), despite the trend of less stand where germination stimulants were not treated. Appearance of first white rot symptoms in 1992 at Nampa were in late April, and there was no suggestion of pre-emergence stand loss in stand count data. Excluding any pre-emergence losses, Table 4 shows the expected percentage disease loss based on pre-plant inoculum densities in the fall of 1991, the actual percentage disease loss recorded at harvest in 1992, and the weight of the harvested bulbs from each plot. Statistical differences among plots is not shown, but differences between treated and untreated plots were highly significant ($p < 0.001$) with respect to percentage disease and harvested weight. There were no differences among rates of application of DADS with

respect to either parameter, except that the small (1%) percentage of white rot in the low rate of application at Nampa was difference from the absence of white rot (0%) at medium and high rates of application ($p < 0.05$)

Trials in a muck soil near Salem, Oregon. In this area, muck soil could not be assayed for sclerotia. Trials areas were established in 1990, 1992, using DADS 75 percent, and in 1992 using treatments with DADS 75 percent, DNPDS and "POLY" . Onions were planted in the spring of 1990 following both fall and spring treatments. Data is not well summarized at this time, but results from DADS treatments in 1990, from the same rates of application as in other trials, were similar to the trials above, which were planted with garlic. Onions will be planted in the spring of 1993 in order to determine the effect of the 1992 treatments.

Discussion

In general, we greatly reduced the inoculum density in all field plots in which DADS was used, although reduction was not as much in the Mexican trial as in the U.S. trials. In field trials with germination stimulants, we were careful to eliminate or avoid all sources of interference with product efficacy:

a. We avoided fields in which white rot recently occurred, by at least 1/2 year, to avoid constitutive dormancy of sclerotia.

b. We timed applications at the earliest fall temperature (when temperatures were falling) at which the fungus became responsive, or similarly timed the spring application (when temperatures were increasing).

c. We irrigated over treated soil to help delay loss from soil by volatilization, and to assist retention of volatile germination stimulants in the soil.

d. We avoided re-planting a susceptible crop too soon after application of germination stimulants. Based on soil recoveries, it is likely that this time would need to be at least three months of active stimulatory period. For fall application, this three-month minimum would need to be extended by the period in which soils remained below 10°C during the winter.

f. Where possible, we monitored populations prior to re-planting of onions or garlic, in order not to plant until populations have dropped below the assumed economic thresholds (14)

In our field trials, we obtained excellent results with surface applications of DADS between 0.5-5.0 ml,/m^2 product. Less product likely would be less effective (8). It remains uncertain why the results from the single application in Mexico was less effective than in the U.S. trials. Results from small plot trials in England were comparable to our U.S. data (8). More field trials in other parts of the world in which white rot occurs would be desirable.

At Walla Walla and Nampa, we applied twice over a two-year period. Initial populations ranged as high as 100 sclerotia/mL soil in some plots. Based on 98-99 percent drop in population after one treatment, and failure to recover sclerotia at all in repeated soil assays after two treatments, we may have lowered populations by 10,000 times after two treatments. We did lower inoculum below our ability to detect sclerotia with our soil assay.

Our decision to treat twice was based on the expected losses from the remaining one percent after one treatment, assuming that 1 percent was randomly distributed. Randomly distributed, this one percent would be expected to incite higher than acceptable plant loss, based on Figure 1 (14). More recent data from Coley-Smith (personal communication) and from our test results at Salem, Oregon, in which some plots only received one application of stimulant, suggested that this decision could have been conservative. Coley-Smith found as we did that about 1 percent of the population remained after one treatment conducted optimally. However, he further determined that all surviving sclerotia were at or near 30 cm depth (12 inches). Other data (12) indicates that sclerotia at this depth only contribute slightly to the total disease loss. At both Nampa and Walla Walla, no sclerotia could be recovered from repeated soil sampling following the second application, for samples collected to 30 cm.

Little white rot occurred in 1992 in Nampa at the lowest rate of application, and none occurred at medium and high rates of application of DADS. At Walla Walla, no white rot occurred during the first half or more of the spring, although as many as 15 percent of the plants became infected with white rot by harvest. Crowe and Hall (12) found that such late season loss is suggestive of infections that occurred very deep in the soil profile, perhaps 10-12 inches deep. The delay was a combination of the extra time for roots to penetrate to those depths, combined with the time for mycelium to grow upward on the root system to stem plates. No symptoms arise until stem plates become infected. It seems likely that neither of the two applications at Walla Walla were sufficient to stimulate all sclerotia to germinate at that depth. This argument fits with data from Coley-Smith in England (personal communication) who failed to stimulate all sclerotia at 10-12 inches using surface-applied DADS. Most likely, more efficient means of application would surmount this deficiency. White rot at Nampa seemed to appear later in 1992 than it did at Walla Walla. Conceivably, with a longer season, more disease may have appeared at Nampa if deeply-placed intact sclerotia remained there also. In a short disease season, root infections by these sclerotia may not have had time to appear.

The apparent reproduction that occurred during the first month after application with any products containing either DADS or DNPDS was not expected. This phenomenon is not reported elsewhere and begs confirmation. In general, *S. cepivorum* has been found incapable of reproduction on soil organic matter (1, 2, 14, 23) and only limited reproduction has been observed in onion or garlic roots (1, 2, 14, 22). The rates of application used for DADS and DNPDS would not be expected to provide enough nutrient base for reproduction, nor did the size of a significant fraction of intact sclerotia apparently drop during this period

(although this was not specifically measured), which would have suggested formation of secondary sclerotia. If secondary sclerotia formed, they were not of the very small sclerotial diameters indicated in other reports (22), although such reports generally have observed secondary sclerotial formation in the absence of any exogenous nutrient supply.

DNPDS seemed to perform almost as well as DADS in the Walla Walla trial, but clearly under-performed in the Five Points trial. On the other hand, first month activity of DNPDS was very similar to that of DADS. This suggests that the apparent failure of DNPDS was more likely due to behavior of the product and pathogen later during the three month period of activity, rather than failure to stimulate sclerotia. Details of the stimulus:response, and of apparent reproduction, seems necessary to determine in future investigations.

The reduction in intact sclerotia in plots treated with DMSO also was unexpected, even though activity was much less than with other products. In the first month following treatment, the level of stimulation directly observed under the microscope was very modest in comparison to products containing DADS or DNPDS. DMSO was only used at the highest rate of application for all products. It seems likely that there would be even less stimulatory activity at lower rates.

Based on manufacturer information, the low and medium rates used for DADS and DNPDS might cost no more than many products used in agriculture. At this time, the product manufacturer and a potential product developer are presenting a chemistry package to the U.S. Environmental Protection Agency for determination of the regulatory procedures required to register these products for pesticidal use against onion and garlic white rot. Future research on the generality of our results, and on improvement in formulations and application procedures, awaits the EPA's response, which will determine the potential developmental costs for the manufacturer and product developer.

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Table 1. Trial areas and soil types in which germination stimulants were tested, 1987-92, for management of inoculum of the onion white rot fungus, Sclerotium cepivorum.

TRIAL AREA (nearest town)	SOIL TYPE	PRE-TREATMENT INOOCULUM DENSITY*	
		Mean	Range
		(viable sclerotia/liter soil)	
Madras, OR (Jefferson Co.)	Madras loam.	22.8	10-81
Milton-Freewater, OR (Umatilla Co.)/Walla Walla, WA (Walla Walla Co.)	Oliphant silt loam	58	15-146
Nampa, ID (Canyon Co.)	Nampa sandy loam	17.6	2-62
Salem, OR (Marion Co.) Five muck	100% organic	undetermined	
Points, CA (Fresno Co.)	Panoche sandy loam	53	6-161
San Miguel de Allende, Gto., Mexico	undetermined	19	35-186

* Average for all plots

Table 2. Percentage recovery of intact, viable (non germinated) sclerotia of Sclerotium cepivorum from plots treated or not treated with germination stimulants, after three months within the temperature range suitable for stimulated germination*.

trial	when	untreated	DADS					MSO	
		percentage recovery of intact, non-germinated sclerotia							
		products and rates (CPA) applied							
			FOLY		DNPDS				
location	applied	check	0.56	5.6	56	5.6	56	5.6	56	56	LSD.05
Walla Walla	fall. 1989	96.2	3.1	1.2	1.2						17.8
Walla Walla (retreated)	fall. 1990	63.3	0	0	0						8.2
Nampa	spring. 1990	91.8	12.3	0	1.2						34.4
Nampa (retreated)	spring. 1991	80.0	0	0	0						47.4
San Miguel	fall. 1990	90.4	32,9	5.5	3.1						15.5
Walla Walla	fall, 1990	9.8		1.8	2.7	0	1.1	6.5	4.4	51.7	16.2
Five Points	fall, 1990	0.7		1.1	2.0	2.0	0.5	80.2	30.0	49.6	29.9

Percentages based on initial pre-treatment inoculum density of viable sclerotia just prior to initiation of field trials.

Table 3. Individual plot inoculum densities of sclerotia of Sclerotium cepivorum before and after treatment with diallyl disulfide (DADS). Data are from trials located near Walla Walla, Washington, and Nampa, Idaho. Initial treatment was in October, 1989, and April, 1990, respectively, and plots were retreated in the same months a year later. Final inoculum density was determined in the late spring of 1991 for both trials. This data, or comparable data from other trials, was used to convert to percentage changes in inoculum density as shown in Table 2.

location	Walla Walla				Nampa			
gpa DADS	**0**	**0.6**	**5.6**	**56**	**0**	**0.6**	**5.6**	**56**
plot replication number								
1	21/47	0/100	0/70	0/31	8/8	0/25	0/6	0/3
2	25/70	0/38	0/60	0/42	31/59	0/2	0/5	0/3
3	32/113	0/15	0/21	0/146	8/16	0/4	0/5	0/41

Inoculum density is the number of viable sclerotia recovered per liter of soil per plot. Numerator is the inoculum density after two applications of germination stimulants. Denominator is the inoculum density prior to any treatments.

Table 4. White rot disease (Sclerotium cepivorum) data from field trials near Nampa, Idaho, and Walla Walla, Washington, planted with garlic in September, 1991 and harvested in June, 1992. Expected disease in 1989-90 was based on inoculum density prior to application of germination stimulants, and expected disease in 1992 was based on inoculum density present at the time of planting.

	Walla Walla				Nampa			
gpa DADS	0	0.6	5.6	56	0	0.6	5.6	56
Expected % disease Pre -trt, 1989-90	93	85	87	88	73	63	52	57
Expected % disease Post - trt, 1991-92	72	0-10	0-10	0-10	56	0-10	0-10	0-10
Actual % disease 1991-92 garlic crop	99	16		11	65	2	0	0
Harvest wt (Kg/plot) 1991-92 garlic crop	0	7	7	8	20	49	51	53

a. Based on Fig. 1 and Table 2. 4/90 & 4/91.

b. WW treated 10/89 & 10/90; Nampa treated

c. Spring stand counts at Walla Walla were 445 -502 plants per 10 m2 plots; at Nampa were 870-1001 plants per 20 m2 plots. Disease data include only bulbs infected with the onion and garlic white rot fungus.

Figure 1. Yield loss Vs. inoculum density relationship for onion and garlic white rot disease (Sclerotium cepivorum) in the Western U.S. Data are from Crowe, et al (14), supplimented with unpublished data. All data are from California, except for data from Nampa, Idaho.