Efficacy of Germination Stimulants of Sclerotia of *Sclerotium cepivorum* for Management of White Rot of Garlic

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ABSTRACT

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The ability of soil-applied garlic powder and diallyl disulfide to stimulate germination of sclerotia of *Sclerotium cepivorum*, the cause of white rot of onion and garlic, was evaluated in four field trials. Because sclerotia germinate in response to exudation of specific volatile sulfides and thiols from allium roots, sulfides applied to the ground in the absence of an allium crop cause death of the sclerotia after they germinate and exhaust nutrient reserves. In this study, garlic powder and a synthetic garlic oil, diallyl disulfide, were incorporated into the soil in commercial fields naturally infested with *S. cepivorum*. Methyl bromide was included as a chemical control. Within 3 months after treatment, over 90% of the sclerotia died in the plots treated with the germination stimulants, which was similar to the reduction of viable sclerotia achieved with an application of methyl bromide. The degree of sclerotial mortality in plots treated with garlic powder at 112 kg/ha or more was almost equal to that achieved by diallyl disulfide at 0.5 ml/m² or methyl bromide at 448 kg/ha. Despite the efficacy of the stimulants and methyl bromide to reduce populations of sclerotia, the pathogen caused substantial root rot and yield losses in subsequent garlic crops planted about a year after soil treatment. However, germination stimulants have utility because the reduction of the vast majority of sclerotia in a field reduces the risk of spread of the pathogen to neighboring fields.

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum* Berk., is a continuing threat to worldwide allium production. An inoculum density of a few sclerotia in a liter of field soil potentially can result in crop failure (7) and no economical control measures currently exist. Furthermore, once a field is infested, it will remain so for at least 40 years and probably longer because sclerotia of the fungus remain dormant indefinitely in the absence of allium hosts (2). Loss estimates are difficult to ascertain because once white rot is identified in a field, growers are forced to grow other nonsusceptible (non-allium) crops. Hence, infested fields often are abandoned from further onion or garlic production.

S. cepivorum produces no functional spores. Instead, it propagates by the production of round, poppy-seed-sized sclerotia on the roots of decayed host plants. Sclerotia spread via mass movement of soil or water and especially on infested plant parts. Once introduced into an area,

S. cepivorum is moved gradually from field to field on contaminated equipment or planting materials; slowly, the production of garlic and onion in the entire region is threatened. Garlic culture is perhaps the principal mode of movement because it is propagated vegetatively, and garlic bulbs and cloves are sufficiently large that an infestation by mycelia or a few sclerotia might go unnoticed. The disease currently is spreading throughout western North America.

Traditional methods to manage white rot are either economically prohibitive or ineffective. Currently, one of the most effective methods of control is tarped fumigation with methyl bromide. This method may kill most of the sclerotia in a treated field, but it does not result in complete eradication. Therefore, retreatment has to be made on an ongoing basis because a few viable sclerotia remaining in a field can result in disastrous consequences for allium production. Clearly, treatment with methyl bromide is not cost effective and the material itself is scheduled to be phased out for use according to the Environmental Protection Agency, which initiated action under the Clean Air Act for a phase-out of chemicals threatening the ozone layer. Soil treatment with metam-sodium also reduces populations of sclerotia in the soil and may allow the production of a single allium crop after treatment; however, positive results have

been erratic and retreatment is necessary (1). Crop rotation is ineffective because the sclerotia are dormant in the absence of specific sulfides exuded from roots of allium crops (3).

The severity of white rot is related directly to the number of sclerotia in the soil at planting (7). Few sclerotia can result in great crop losses. For example, economic losses due to white rot can occur at inoculum densities as low as 0.1 sclerotium/liter of soil and populations above 10 sclerotia/liter of soil may cause near total crop loss. Populations near 1 sclerotium/liter of soil may cause crop losses between 30 to 60%. When a sclerotium germinates and infects an allium root, mycelia grow upward toward the bulb, eventually destroying it (6). The fungus also spreads from plant to plant, increasing disease incidence within the same growing season.

White rot is a disease limited to allium crops. The fungus successfully colonizes only allium plants and sclerotia germinate only in response to exudation by allium roots (2,4). These exudates contain alkyl and alkenyl-L-cysteine sulphoxides, which are metabolized by the soil microflora to yield a range of volatile thiols and sulfides that activate the dormant sclerotia (3,11). The specific reaction between sclerotia and sulphoxides or their breakdown products suggests a possible use of these sclerotial germination stimulants for managing white rot disease. If these thiols can be applied to the ground in the absence of an allium crop, the sclerotia may germinate in the absence of a host. Mycelium from germinating sclerotia may persist for periods ranging from a few days to several weeks depending on the soil temperature, but then die after exhausting nutrient reserves and failing to infect a host plant. Early attempts to reduce the number of viable sclerotia in soil with artificial onion oil or plant extracts (8) or distilled allium oils (10) either failed, possibly due to suboptimum application methods, or the products were prohibitively expensive.

One natural sclerotial stimulant from *Allium* spp. is diallyl disulfide, which also is recoverable from distillation of petroleum (5,9). When distributed through the soil profile in the absence of an allium crop, this product forced 90 to 99% of the sclerotia to germinate (12). Because all the receptive sclerotia are influenced by germination stimulants, the total population of

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sclerotia in a field is susceptible to control with this method (7).

Garlic powder, a deregulated product from dehydrated garlic bulbs used in food processing, is another potential sclerotial stimulant. Being a natural plant product, it is readily available (especially in California, where most of the nation's garlic crop is grown), environmentally compatible, and a renewable resource. Another advantage for the use of garlic powder as a white rot management tool is worker safety. The purpose of this research was to investigate the potential use of garlic powder to reduce populations of *S. cepivorum* in infested commercial fields.

MATERIALS AND METHODS

Four trials were established in commercial fields naturally infested with *S. cepivorum*. A crop of onion or garlic had been produced in each field 2 years before each experiment was initiated. Each of the crops suffered extensive losses to white rot. Between the allium crop and application of the experimental treatments, non-allium crops were grown in each field. During this time, volunteer onion and garlic plants were removed from the fields. Ultimately, no volunteer allium plants were found in the field plots for at least 1 year preceding the experimental treatments. The experimental design in each trial was a randomized complete block with four replications. In two trials (Douglas, NV and Kern, CA; trial 1), the experimental units were 14 by 30 m. In the San Benito, CA trial and Kern, CA (trial 2), individual plots were 6 by 6 m. Data were collected from the center 6 by 15 m in the former trials or the center 3 by 3 m in the latter two trials. In all cases, plots were separated by a 6-m border to prevent cross contamination of the volatile compounds. Treatments included a nontreated control, standard-grade garlic powder (the purest commercial product made by drying and granulating fresh garlic) applied at 56, 112, or 224 kg/ha, second-grade garlic powder (which has more stem and leaf tissue than the standard grade) applied at 224 kg/ha, an effluent from a garlic-processing plant applied at 500 liter/ha (active ingredient unknown), diallyl disulfide (DADS) at 0.5 ml/m², and methyl bromide (98% methyl bromide, 2% chloropicrin) at 224 kg/ha applied under tarp by a commercial applicator. Not all trials received all treatments. The garlic powder was mixed with sand to facilitate application with a tractor-pulled fertilizer applicator (Douglas, NV and Kern, CA; trial 2) or a hand-held fertilizer applicator (San Benito and Kern, trial 1); DADS was diluted with water and applied with a pressurized pesticide applicator. At the Nevada and Kern trial 2 sites, each application, except the methyl bromide treatment, was followed by disking to incorporate the materials. At the San Benito, CA and Kern, CA (trial 1) sites, treatments

were incorporated manually with the aid of rakes.

At the Douglas, NV site, half of the garlic powder and half of the DADS were applied on 16 October 2000; the other half of each treatment was applied on 13 April 2001. In the San Benito, CA site, garlic powder was applied once on 13 April 1998. At the Kern, CA (trial 1) site, half of the garlic powder and DADS was applied on 19 October 1999; the other half was applied on 7 March 2000. Methyl bromide was applied under tarp by a commercial

applicator on 19 October 1999. At the Kern, CA (trial 2) site, all applications were made on 26 February 1998.

Soil populations of sclerotia were determined on the initial date of soil treatment and at approximately 3-month intervals thereafter. On each sampling date, a composite sample of 10 1-cm-diameter subsamples per plot was collected from the soil surface to 15 cm deep. From each sample, 500 cc of soil was directly assayed (or air-dried at room temperature prior to assay) by wet soil sieving (1). After siev-

Fig. 1. Numbers of sclerotia of *Sclerotium cepivorum* in 500 cm³ of soil sampled immediately before treatments were applied (the first sampling date in each trial) and periodically thereafter. Each bar represents the standard error of the mean. In the Douglas County, NV trial, both industry standard and export grades of garlic powder were used at 224 kg/ha. The San Benito County trial included only the standard grade of garlic powder. DADS = diallyl disulfide applied at 0.5 ml/m².

ing, soil residue was refrigerated until observation. To test for sclerotial viability, a small sample of sclerotia (generally 10 or less) was disinfested with mild bleach (0.05% NaOCl) for 30 s to rid the sclerotial surface of superficial contaminants and plated on water agar amended with 25 ppm streptomycin to observe sclerotial germination.

At the Douglas, NV site, garlic was planted on 15 November 2001 to evaluate disease control in the spring of 2002. At

the San Benito County site, garlic was planted on 10 December 1998. In Kern County, garlic in trials 1 and 2 was planted on 24 November 2001 and 18 December 1998, respectively. Harvest dates were 12 June 2002, 5 June 1999, 15 May 2002, and 22 May 1999 for the Douglas, San Benito, and Kern trials 1 and 2, respectively. At harvest, plants were lifted gently for examination of the root systems. White rot severity was evaluated on a scale of 0 to 6, where 0 = 0%, 1 = 7% (1 to 14%), 2 =

21% (15 to 35%), 3 = 50% (36 to 64%), 4 = 79% (65 to 85%), 5 = 93% (86 to 99%), and 6 = 100% of the root system decayed. The percentages of decayed root systems of a minimum of 40 individual plants from each plot were recorded. After the tops of the plants were cut off, bulbs from the center 3 m of bed within each plot were weighed.

The relative increase or decrease of sclerotia by soil treatments was determined by analysis of covariance (least square means) of the difference between initial and final sclerotial populations. Disease severity and yields were analyzed by analysis of variance. Means were separated according to the Tukey-Kramer multiple comparison test (SAS version 8.1; SAS Institute, Cary, NC).

RESULTS

In all trials, populations of soilborne sclerotia of *S. cepivorum* were relatively high at the initiation of the experiments. All treatments caused a general reduction in numbers of sclerotia, beginning about 2 months after soil treatment (Figs. 1 and 2). Mortality of sclerotia in plots treated with garlic powder, DADS, or methyl bromide generally was over 90% by the end of the sampling period. Based on the ratio of populations of sclerotia about 1 year after soil treatments to pretreatment populations, sclerotial viability was reduced significantly by all treatments (Table 1). There were no significant differences between the reduction of sclerotia caused by garlic powder and that caused by DADS or methyl bromide. The effluent, however, was less effective than DADS or methyl bromide (Table 1).

Plant health was negatively affected by white rot, regardless of treatment. Relative to the percentage of healthy roots of plants grown in nontreated soil, the garlic powder treatment improved root health in only one of the four trials (Table 2). DADS significantly improved root health in two of the three trials. Methyl bromide significantly improved root health in both of the trials in which it was included. However, the severity of white rot in the methyl bromide-treated soil, as well as in the plots that received the other treatments, was high. The effluent did not improve root health. DADS significantly increased garlic yields relative to the nontreated control in two of three trials (Table 3). Methyl bromide significantly increased yields in both of the trials where it was included. In one of the four trials, yield was improved by the high rate of garlic powder relative to the nontreated plants. All yields were low compared with yields expected in a field with no history of white rot (data not presented).

DISCUSSION

All germination stimulants of *S. cepivorum* sclerotia included in these experi-

Fig. 2. Numbers of sclerotia of *Sclerotium cepivorum* in 500 cm³ of soil sampled immediately before treatments were applied (the first sampling date in each trial) and periodically thereafter in two locations. Each bar represents the standard error of the mean. The effluent was a waste product from a garlic processor applied at 500 liter/ha. DADS = diallyl disulfide applied at 0.5 ml/m². Methyl bromide was applied at 448 kg/ha.

ments (i.e., garlic powder, the effluent from a garlic processing plant, and DADS) reduced populations of sclerotia. DADS and the higher rates of garlic powder approached the effectiveness of methyl bromide, which reduced sclerotial populations by more than 90%. The low rate of garlic powder apparently was less effective than the other treatments. The efficacy of the different commercial grades of garlic powder was similar, indicating that, should this management practice be adopted in fields known to be infested, inexpensive grades of garlic powder can be used. However, despite the effectiveness of all treatments to reduce sclerotial populations, the amount of disease in subsequent garlic crops was very high. This supports the view that relatively few sclerotia in the soil can result in extensive crop loss. Even at the highest rate, garlic powder increased yields significantly in only one of the four trials. DADS and methyl bromide were more consistent, but yields in those plots were low compared with expected yields in fields not infested with *S. cepivorum*. Comparable yields in fields free of *S. cepivorum* might be expected to be in the order of 10 times the yield obtained in the highest-yielding plots. Hence, it appears that, although all treatments, including the higher rates of garlic powder, caused extensive sclerotial mortality, adequate numbers of sclerotia survived to reduce garlic yields to levels near those in the nontreated plots. Repeated applications of germination stimulants over the course of two or more years may provide levels of mortality that might allow profitable crop production. Alternatively, the benefits from sclerotial stimulants may need to be augmented with fungicide applications. In preliminary trials, some fungicides as seed treatments or in-furrow drenches provided excellent control of white rot (R. M. Davis, unpublished). Although fungicides may protect a crop during a growing season, they also may prevent further build-up of sclerotial populations by reducing sclerotial formation on the current crop. Because fungicides would not cause sclerotial mortality in the soil beyond the treated area, sclerotial germination stimulants may be an important part of a sustainable effort to manage white rot.

In the Central Valley, where most of the garlic and onion production occurs in California, the number of fields infested with sclerotia of *S. cepivorum* has increased yearly (R. C. Ehn, unpublished). Although, 10 years ago, there were about a dozen known infested fields, currently, there probably are over 50. Other important production areas in California include Santa Clara and San Benito Counties, Salinas Valley, and the Tulare area, where numerous fields are infested to varying degrees. Garlic is no longer a major crop in most of these areas due, in part, to the occurrence of white rot. This re-

search indicates that germination stimulants have practical use in reducing sclerotial populations in infested fields and that their use would reduce the risk of

movement of sclerotia to other fields by farm implements, flooding, or animals. Thus, DADS and garlic powder are effective substitutes for methyl bromide to limit

Table 1. Effect of germination stimulants and methyl bromide (MeBr) on the ratio of sclerotial populations of *Sclerotium cepivorum* about 1 year after soil treatments to pretreatment populations

Treatment	Trial ^y			
	Douglas Co., NV	San Benito Co., CA	Kern Co., CA, trial 1	Kern Co., CA, trial 2
None	0.87 a	0.97 a	0.89 a	0.77 a
Garlic powder				
Standard grade				
56 kg/ha	...	0.28 b	0.27 bc	...
112 kg/ha	...	0.07 b	0.05 bc	...
224 kg/ha	0.09 b	0.02 b	0.06 bc	0.08 b
Export grade				
224 kg/ha	0.17 b	...	...	...
Effluent (500 liter/ha)	...	...	0.50 b	...
DADS (0.5 ml/m ²) ^z	0.12 b	...	0.03 c	0.04 b
MeBr (448 kg/ha)	...	...	0.03 c	0.09 b

^y Each value is the mean of four replications. Separation of means is based on analysis of covariance (least square means) of the difference between initial and final sclerotial populations. Means in each column followed by the same letter are not significantly different according to the Tukey-Kramer multiple comparison test, $P \leq 0.05$.

^z DADS = diallyl disulfide.

Table 2. Effect of germination stimulants and methyl bromide (MeBr) on root rot caused by *Sclerotium cepivorum* in garlic planted about 1 year after treatment

Treatment	Healthy root system in each trial (%) ^y			
	Douglas Co., NV	San Benito Co., CA	Kern Co., CA, trial 1	Kern Co., CA, trial 2
None	3.0 a	11.6 a	6.0 a	19.8 a
Garlic powder				
Standard grade				
56 kg/ha	...	22.7 b	7.8 a	...
112 kg/ha	...	29.0 b	2.3 a	...
224 kg/ha	7.9 a	27.5 b	4.7 a	29.2 a
Export grade				
224 kg/ha	3.4 a	...	...	...
Effluent (500 liter/ha)	...	...	3.4 a	...
DADS (0.5 ml/m ²) ^z	41.8 b	...	7.1 a	67.5 b
MeBr (448 kg/ha)	...	...	45.0 b	80.0 b

^y Each value is the mean of four replications. White rot severity was evaluated on a scale of 0 to 6, where 0 = 0, 1 = 7, 2 = 21, 3 = 50, 4 = 79, 5 = 93, and 6 = 100% of the root system decayed. Percentages of healthy root systems of individual plants (means of a minimum of 20 plants per plot) are presented. Means in each column followed by the same letter are not significantly different according to the Tukey-Kramer multiple comparison test, $P \leq 0.05$.

^z DADS = diallyl disulfide.

Table 3. Effect of germination stimulants of sclerotia of *Sclerotium cepivorum* and methyl bromide (MeBr) on yield of garlic (kg/m) planted about 1 year after treatment

Treatment	Trial ^y			
	Douglas Co., NV	San Benito Co., CA	Kern Co., CA, trial 1	Kern Co., CA, trial 2
None	0.08 a	0.59 a	0.62 a	0.61 a
Garlic powder				
Standard grade				
56 kg/ha	...	0.76 a	0.48 a	...
112 kg/ha	...	0.83 a	0.52 a	...
224 kg/ha	0.08 a	1.20 b	0.62 a	0.65 a
Export grade				
224 kg/ha	0.10 a	...	...	...
Effluent (500 liter/ha)	...	...	0.45 a	...
DADS (0.5 ml/m ²) ^z	0.22 b	...	0.58 a	0.80 b
MeBr (448 kg/ha)	...	...	1.13 b	0.85 b

^y Each value is the mean of four replications. Means in each column followed by the same letter are not significantly different according to the Tukey-Kramer multiple comparison test, $P \leq 0.05$.

^z DADS = diallyl disulfide.

spread of white rot. Because of the large garlic dehydrator industry in the Central Valley of California, garlic powder is a readily available stimulant that may play a role in continuing viability of the industry (approximately 14,973 ha of garlic with a total value of \$261 million and 14,569 ha of onion with a total value of \$144 million in about 27 counties). The entire industry in the state is threatened by white rot, and sclerotial stimulants such as garlic powder and DADS may help reduce the rate of spread of the disease.

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