Pest Management Grants Final Report

Contract No. 97-0227

Use of a Natural Product to Stimulate Sclerotial Germination of *Sclerotium cepivorum* for the Control White Rot of Onions and Garlic

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Abstract

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum*, is a worldwide threat to *Allium* production. No economical control measures currently exist and once a field is infested, it will remain so indefinitely since sclerotia of the fungus remain dormant in the absence of *Allium* plants. Hence, infested fields are often forever abandoned from further onion or garlic production. Sclerotia germinate only in response to root exudation of specific volatile sulfides and thiols. If these sulfides can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be “tricked” into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for a while, then die after exhausting nutrient reserves. One material that may act as a sclerotial germinating stimulant is garlic powder used in the food processing industry. In this study garlic powder was incorporated into the soil at three rates. Periodically thereafter, the number of viable sclerotia in the soil dropped significantly in the treated plots, approaching the degree of sclerotia mortality achieved with an application of methyl bromide. Being a natural plant product, garlic powder is a readily available, environmentally compatible, renewable resource that should pose no problem to worker safety.

Executive Summary

White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum*, is a worldwide threat to *Allium* production. The disease is extremely serious on these crops - an inoculum density of a single sclerotium in a liter of field soil can potentially result in crop failure 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 since sclerotia of the fungus remain dormant indefinitely in the absence of *Allium* plants. Hence, infested fields are often forever abandoned from further onion or garlic production.

Sclerotia germinate only in response to exudation by *Allium* roots. 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. The specific reaction between sclerotia and sulphoxides or their breakdown products suggests a possible use of these sclerotial germination stimulants for controlling white rot disease. If these thiols can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be “tricked” into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for periods ranging from a few days to several weeks depending on the soil temperature, then die after exhausting nutrient reserves. Garlic powder, a deregulated product from dehydrated garlic bulbs used in food processing, may be a sclerotial stimulant.

The experiments were conducted in two grower fields, one in Kern County and one in San Benito County. The experimental design was a randomized complete block design with four replications per treatment. Experimental units were 20X20 ft. Data was collected from the center 10X10 ft. Plots were separated by a 20-ft. border to prevent cross contamination of the volatile compounds. Treatments included a nontreated control, garlic powder applied at 50, 100, or 200 lbs/acre, diallyl disulfide at 5 mls/m^2 (5.3 gal/acre), an effluent product from a garlic processing plant, and methyl bromide at 200 pounds per acre. All treatments were applied February 26, 1998 in Kern County and the garlic powder treatments were applied April 3, 1998 in San Benito County.

Soil populations of sclerotia were determined at the time of soil treatment and at approximately two-month intervals thereafter. On each sampling date, a composite sample of 10, 1 cm-diameter core subsamples per plot were collected from the soil surface to 10 inches deep. From each sample, 500 cc of soil was directly assayed (or air-dried at room temperature prior to assay) by wet soil sieving. After sieving, soil residue was frozen until observation and viability testing. Sclerotia were identified and plated on water agar amended with 25 ppm streptomycin to observe the proportion from which the fungus grew following disinfestations with mild bleach (0.05% NaOCl) to rid the surface of sclerotia of superficial contaminants.

The number of viable sclerotia in the soil treated with garlic powder was significantly reduced. At the high rates (100 or 200 pounds of garlic powder per acre), the degree of sclerotia mortality achieved approached that achieved with an application of methyl bromide. Diallyl disulfide was equally effective. The effluent reduced sclerotia numbers, but not to the degree of the other treatments. Being a natural plant product, garlic powder is a readily available, environmentally compatible, renewable resource that should pose no problem to worker safety. This work is applicable to a range of soil types and cultural practices.

Body of Report

Introduction White rot of onion and garlic, caused by the soilborne fungus *Sclerotium cepivorum* Berk, is a worldwide threat to *Allium* production. The disease is extremely serious on these crops - an inoculum density of a single sclerotium in a liter of field soil can potentially result in crop failure 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 since sclerotia of the fungus remain dormant indefinitely in the absence of *Allium* plants (Coley-Smith, 1959). Loss estimates to this disease are difficult to ascertain because once identified in a field, growers are forced to grow other, nonsusceptible (non-*Allium*) crops. Hence, infested fields are often forever abandoned from further onion or garlic production.

The white rot fungus produces no functional spores. Instead, it propagates only by the production of round, poppy seed-sized sclerotia produced on the roots of decayed host plants. Sclerotia spread in mass movement of soil or water, on animals (at least theoretically), and especially on infested plant parts. Once introduced into an area, *S. cepivorum* is gradually spread on contaminated equipment or planting materials, and slowly the production of garlic and onions in the entire region is threatened. Garlic culture is perhaps the principal mode of movement since it is propagated vegetatively, and garlic bulbs and cloves are sufficiently large that an infestation might go unnoticed. In any case, the disease is spreading throughout western North America. An example of the potential losses this disease poses to all production areas is the Salinas Valley in California, which at one time was a major producer of *Allium* crops. As white rot spread, garlic and onion production became less and less profitable, and the industry largely moved to the Central Valley of California. Today, the number of infested fields in the Central Valley is growing every year, despite efforts to prevent further introduction into the area and predictions that the weather was too warm to support growth of the fungus. The disease is now a recognized threat to the largest garlic and onion production area in the world.

Traditional methods to control white rot are either economically prohibitive or ineffective. Currently, the only effective method of control is tarped fumigation with methyl bromide. This method may kill 99% or more sclerotia in the soil, but does not result in complete eradication. Therefore, retreatment has to made on an ongoing basis since very few viable sclerotia remaining in a field can result in disastrous consequences in *Allium* production. Because retreatment with methyl bromide may be necessary, its use may not be cost effective. Moreover, the material itself is scheduled to be phased out for use in a few years according to the U.S. Environmental Protection Agency, which initiated action under the Clean Air Act for a phase out of chemicals threatening the ozone layer by the year 2001. 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, but positive results have been erratic and retreatment is necessary (Adams and Johnston, 1983). Metam-sodium also poses a degree of risk to the environment since it too is a general biocide. Crop rotation is ineffective since the sclerotia are dormant in the absence of specific sulfides exuded from roots of *Allium* crops (Coley-Smith, 1960).

The severity of white rot is directly related with the number of sclerotia in the soil at planting (Crowe et al., 1980). Surprisingly few sclerotia can result in great crop losses. For example, economic losses to white rot can occur at inoculum densities as low as 0.1 sclerotium/liter of soil yet in many infested fields populations may be as high as 200 or more sclerotia/liter of soil. Populations above 10 sclerotia/liter of soil may cause near total crop loss, and populations near 1 sclerotia/liter may cause crop losses between 30-60%. When a sclerotium germinates and infects an *Allium* root, mycelia grow upward toward the bulb, eventually destroying it (Crowe and Hall, 1980). The fungus also spreads 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 (Coley-Smith, 1959). 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 (Coley-Smith, 1960). The specific reaction between sclerotia and sulphoxides or their breakdown products suggests a possible use of these sclerotial germination stimulants for controlling white rot disease. If these thiols can be applied to the ground in the absence of an *Allium* crop, the sclerotia may be "tricked" into germinating. In the absence of a host, the mycelium from germinating sclerotia persist for periods ranging from a few days to several weeks depending on the soil temperature, then die after exhausting nutrient reserves. Early attempts to reduce the number of viable sclerotia in the soil with artificial onion oil or plant extracts (Elnaghy et al., 1971) or distilled *Allium* oils (Merriman et al., 1980) 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 is also recoverable from the distillation of petroleum (Coley-Smith and Parfitt, 1986 and Entwistle et al., 1982). Recent research demonstrated that diallyl disulfide distributed through the soil profile in the absence of an *Allium* crop in an infested field forced 90-99% of the sclerotia to germinate (Crowe, unpublished). This degree of germination resulted in disease control that is similar to control achieved with methyl bromide fumigation. Near-eradication of sclerotia of *S. cepivorum* was achieved at numerous locations in Washington, Idaho, California, Mexico, and Oregon between 1987-92 using diallyl disulfide and other petroleum-derived germination stimulants. In two field trials where initial populations ranged as high as 150 sclerotia/liter of soil, the numbers of sclerotia were reduced below the limit of detectability using rates as low as 5 gallons of diallyl disulfide per acre. After garlic was planted, nearly no white rot occurred in the twice-treated plots (once in each of two succeeding years) compared to near total losses in untreated plots.

The specific germination response to these stimulants can be as high as 100% (Coley-Smith, 1960 and Sommerville and Hall, 1987). Germinated sclerotia grow only 1-2 cm through the soil and die within two weeks if infection of roots or bulbs is not successful. When infested fields are planted with *Allium* crops, root exudates incite a germination response that includes all the receptive population of sclerotia within the soil and around the sphere of influence of the *Allium* roots (Coley-Smith, 1986). Thus, the total population of sclerotia in a field is susceptible to control with germination stimulants (Crowe et al., 1980).

Garlic powder, a deregulated product from dehydrated garlic bulbs used in food processing, is another sclerotial stimulant. In preliminary tests garlic powder effectively reduced sclerotia viability (Crowe, unpublished). Being a natural plant product, it is a readily available, environmentally compatible, renewable resource. Another advantage for the use of garlic powder as a white rot management tool is worker safety. It is the objective of this research to provide efficacy data on garlic powder as a sclerotial stimulant for the control of white rot in two locations in California using uniform protocols

Materials and Methods The experiments were conducted in two grower fields, one in Kern County and one in San Benito County. The experimental design was a randomized complete block design with four replications per treatment. Experimental units were 20X20 ft. Data was collected from the center 10X10 ft. Plots were separated by a 20-ft. border to prevent cross contamination of the volatile compounds. Treatments included a nontreated control, garlic powder applied at 50, 100, or 200 lbs/acre, diallyl disulfide at 5 mls/m^2 (5.3 gal/acre), an effluent product from a garlic processing plant, and methyl bromide at 200 pounds per acre. All treatments were applied February 26, 1998 in Kern County and the garlic powder treatments were applied April 3, 1998 in San Benito County.

Soil populations of sclerotia were determined at the time of soil treatment and at approximately two-month intervals thereafter. On each sampling date, a composited 10, 1 cm-diameter core subsamples per plot were collected from the soil surface to 10 inches deep. From each sample, 500 cc of soil was directly assayed (or air-dried at room temperature prior to assay) by wet soil sieving. After sieving, soil residue was frozen until observation and viability testing. Sclerotia were identified and plated on water agar amended with 25 ppm streptomycin to observe the proportion from which the fungus grew following disinfestation with mild bleach (0.05% NaOCl) to rid the surface of sclerotia of superficial contaminants. A garlic crop was planted following treatment to evaluate the benefits of disease control, if any, from the soil stimulants. This part of the project is in progress.

Results:

Populations of sclerotia in soil at both field sites were high. In Kern County, all treatments significantly reduced sclerotia numbers (Figure1). Thereafter, sclerotia counts were most effectively reduced by 100 or 200 pounds of garlic powder, diallyl disulfide (DADS), and the methyl bromide. There were no significant differences among these treatments. In San Benito County, results were similar (Figure 2). The two high rates of garlic powder were generally more effective in reducing the number of sclerotia in the soil than three low rates.

Discussion: When applied at 100 or 200 pounds per acre, garlic powder reduced the inoculum density of soilborne sclerotia of *Sclerotia cepivorum* as effectively as methyl bromide, currently the only effective method of control. Fumigation, however, does not result in complete eradication (although 99% or more sclerotia in the soil may be killed). Therefore, retreatment has to be made on an ongoing basis since very few viable sclerotia remaining in a field can result in disastrous consequences in *Allium* production. Because retreatment with methyl bromide may be necessary, its use may not be cost effective. Moreover, the material itself is scheduled to be phased out for use in a few years according to the U. S. Environmental Protection Agency, which initiated action under the Clean Air Act for a phase out of chemicals threatening the ozone layer by the year 2001. Garlic powder applied as a control of white rot approaches the degree of sclerotia mortality achieved with an application of methyl bromide. Being a natural plant product, garlic powder is a readily available, environmentally compatible, renewable resource that should pose no problem to worker safety. In this study, diallyl disulfide also effectively reduced sclerotia populations. The effluent from the garlic processing plant was relatively ineffective, although the degree of sclerotia mortality was significant.

Summary and Conclusions: In this study, germination stimulants of sclerotia of *Sclerotium cepivorum*, including garlic powder, diallyl disulfide, and an effluent from a garlic processing plant, were incorporated into the soil in replicated plots in two commercial fields naturally infested with the fungus. The experimental design in each case was a randomized complete block design with four replications per treatment. Experimental units were 20X20 ft. Data was collected from the center 10X10 ft. Plots were separated by a 20 ft. border to prevent cross contamination of the volatile compounds. Treatments included a nontreated control, garlic powder applied at 50, 100, or 200 lbs/acre, diallyl disulfide at 5 mls/m^2 (5.3 gal/acre), an effluent product from a garlic processing plant, and methyl bromide at 200 pounds per acre. All treatments were applied February 26, 1998 in Kern County and the garlic powder treatments were applied April 3, 1998 in San Benito County.

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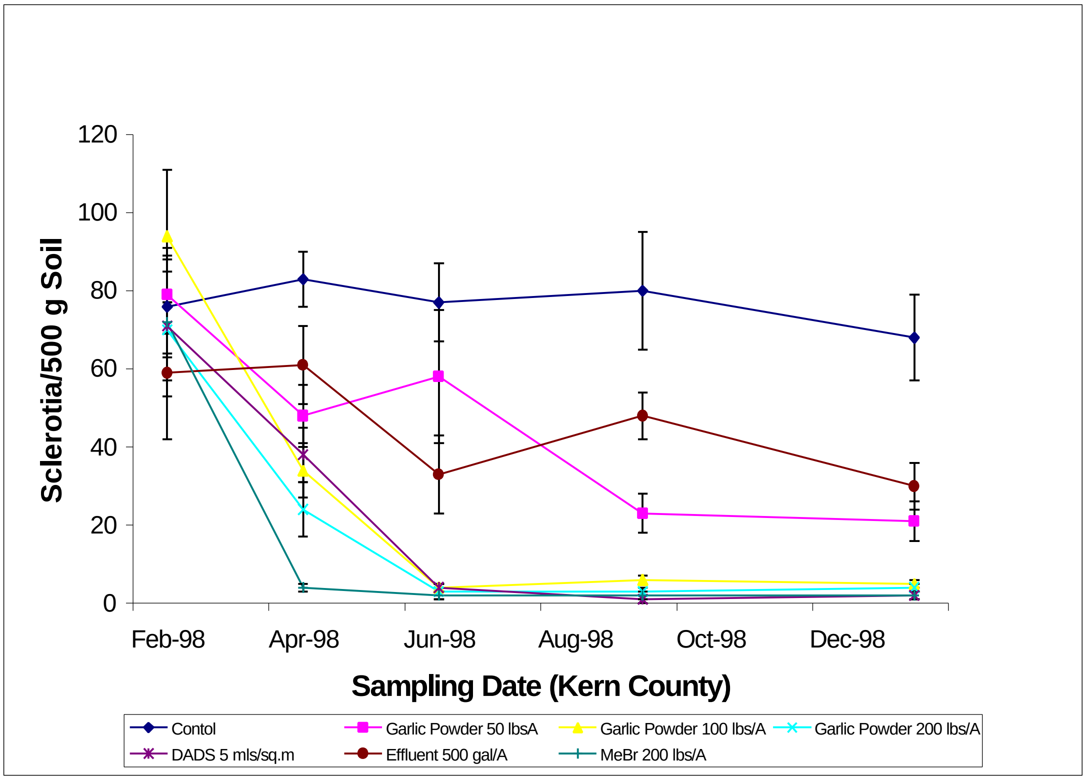
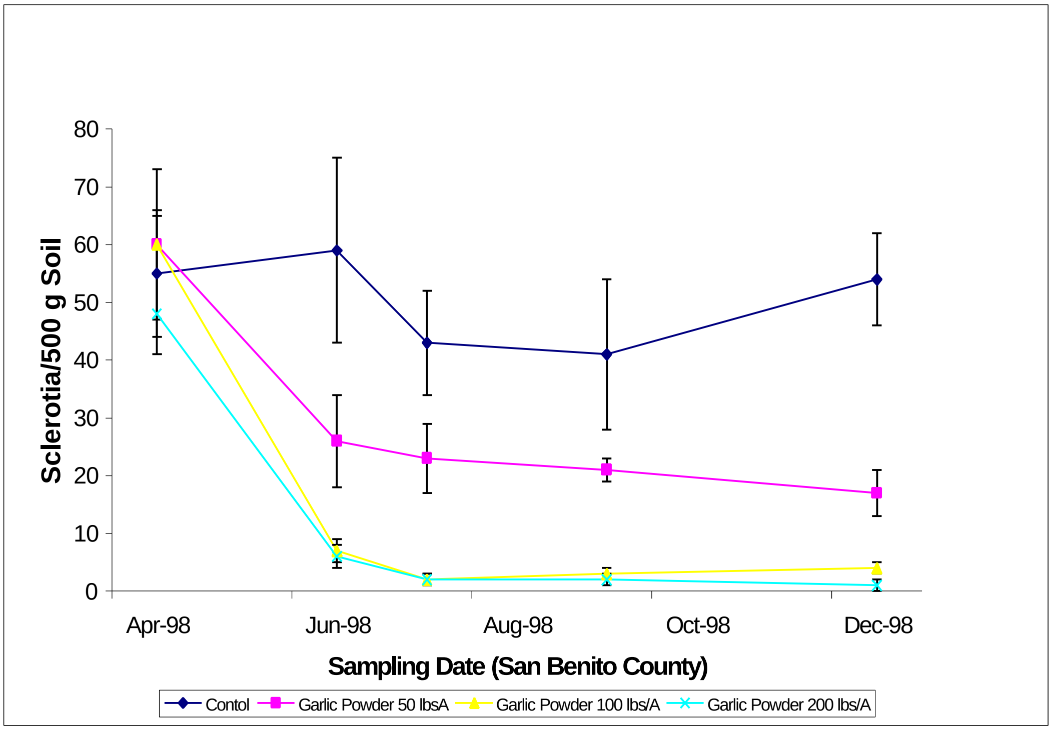
Appendix

Figure 1. Effect of sclerotia stimulants on numbers of viable sclerotia of *Sclerotium cepivorum*, Kern County



The vertical bars indicate the standard errors of the means.

Figure 2. Effect of sclerotia stimulants on numbers of viable sclerotia of *Sclerotium cepivorum*, San Benito County



The vertical bars indicate the standard errors of the means.