Repeated Irrigation of Low Amounts of Germination Stimulants for Reduction of Sclerotia of *Sclerotium cepivorum*

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Introduction

Sclerotia of *Sclerotium cepivorum*, the Allium white rot fungus, specifically respond to Allium flavor and odor compounds that naturally volatilize away from Allium roots. Stimulants are specific to this fungus, and are all chemically related organic sulfur compounds (common structure 3C-S) (King and Coley-Smith 1968, Coley-Smith and King 1969) that dominate the unique flavor and odor of Allium species (Pruthi et al. 1959, King and Coley-Smith 1968, Coley-Smith and King 1969). With ideal soil temperature (range within 9-22°C [50-72°F]; optimum at 15-18°C [59-65°F]) and moisture in the range of good soil tilth (0.001-0.3Mpa; optimum at 0.01-0.03 Mpa), germination is high (Crowe and Hall 1980b) and continues as long as sufficient stimulants and nongerminated sclerotia remain in the soil (Crowe et al. 1980, Crowe, et al. 1994). When an onion or garlic crop is planted, roots ramify the entire soil profile and continuously leak such stimulants. As a result, during the growing season, the "old" sclerotial population disappears as sclerotia germinate (Crowe et al. 1980, Crowe et al. 1994). Once germinated, the fungus within each sclerotium is totally committed to infection of roots (or, if nearby, direct infection of bulbs), followed by formation of new sclerotia on rotting bulbs. Failing to grow into a bulb and reproduce results in death of the fungus, as it cannot grow or reproduce in any other way, such as on soil organic matter in soil or other types of plants (Scott 1956, Coley-Smith 1971, Crowe et al. 1980).

Garlic and onion crops continuously leak stimulants at low rates, and elicit near-total germination of the population of sclerotia in a very natural process (Crowe et al. 1980). The true concentration of stimulants in the soil from root leakage has not been determined, but presumably is quite low on a part per million (ppm) basis of either total soil volume. In the laboratory, nearly 100 percent germination response of *S. cepivorum* occurred in infested field soil treated with single doses of diluted garlic or onion juice under controlled conditions (Coley-Smith 1960, Crowe et al. 1980), although such dilutions of 100 to 1,000 times were almost certainly many times higher than natural root leakage.

Since the 1960's, it has been speculated that germination stimulants artificially applied to soil when Allium crops were absent could "trick" sclerotia into germinating, greatly reducing and possibly even eradicating the fungus (T. Kosuge, University of California, Davis, personal communication, 1974; Coley-Smith and Parfitt 1986). However, all earlier attempts failed to elicit high germination response in the field, and a series of researchers gave up this approach using either natural stimulants or petroleum-derived Diallyl disulfide (DADS), which is a major stimulatory component of natural garlic juice (Elnaghy et al. 1971; Merriman et al. 1980, 1981; Entwistle and Munasinghe 1981; Entwistle et al. 1983, Coley-Smith and Parfitt 1986). In 1986-1992, we succeeded (Crowe et al. 1994) where others failed by understanding that the germination response is

only highly efficient near the temperature and soil moisture optimum for the fungus. Timing of field applications depends on local soil temperature patterns, and on other farming constraints such as planting and harvesting of other crops. Where summer soil temperatures exceed 72°F, applications are restricted to fall or spring (or including winter in very mild regions), or to deeper soil layers still within the conducive temperature range. In our previous research field trials, when soil temperatures dropped below 45-50°F, sclerotia of *S. cepivorum* become dormant, but DADS becomes non-volatile, thus any residual DADS reactivated in the spring when temperatures warmed above 45-50°F. For single dosage applications in the field, a period of at least 2 months was required between soil temperatures of 50-72° (with as much time in the mid-range as possible) to achieve 98-99 percent germination response levels, discounting periods of time when soil temperature is colder. While not quantitative, we observed that the human nose could still detect Allium volatiles for 6-8 weeks after applications of high rates of stimulants, longer when soil temperature was very cool.

In our earlier work, rates were chosen based on reported soil residence times for DADS (Coley-Smith and Parfitt 1986). These guidelines were very rough because small amounts of added stimulants are impossible to analytically discern from the background of non-organic and organic but nonstimulatory sulfur compounds in soil (R. Dick, Department of Crop and Soil Science, Oregon State University, personal communication; I. Tinsley, Department of Environmental and Molecular Toxicology, Oregon State University, personal communication). In our earlier work, we initially intended to mimic natural root leakage by applying DADS in irrigation water, but without knowing what rates to choose, it was determined that with high rates the odor of concentrated DADS was too intense to work with in the open air. On the other hand, we found that the rates initially chosen elicited very high germination responses. As a result, we refocused on fine-tuning single applications, either by spraying the soil surface followed by immediate tillage and/or flood irrigation, or by shank injection. The effect of repeated lower rates was never investigated. We empirically determined that single doses of DADS at 5 l/ha (roughly 1.9 gal/acre) or higher, applied in the absence of an Allium crop, resulted in 98-99 percent germination of sclerotia (Crowe et al. 1994). When United Ag Products (UAP) took over commercialization of DADS beginning in about 1992, this was the base rate they used for their shank-injected applications.

Work reported here revisited the concept of repeated dosages of very low rates of application, using garlic juice. Several lines of evidence suggest that garlic or onion juices could be highly diluted and remain at least partially stimulatory. These include some limited lab testing (Crowe 1978; Crowe, unpublished data from 2000 to 2001), together with the fact that sclerotia in untreated soil samples can germinate at high rates simply if onions or garlic are present in the same room (F. Crowe, unpublished data). After all, in the field, sclerotia respond to regular root leakage of stimulants from onions and garlic, and onion and garlic odors are very difficult to detect in undisturbed field soil if care is taken to avoid breaking roots (which causes added odors to accumulate).

Our working hypothesis was that garlic may be effective at eliciting some germination down to levels detectable by the human nose. In a random test of six people in our

laboratory, half could detect the flavor/odor of garlic extracts diluted to 1 ppm (weight: volume). The other half could detect just above this dilution. On a weight to volume basis, one pound (4-5 bulbs) of macerated garlic blended with one pound of water, coarsely filtered and diluted with 1 million pounds of water equals roughly 16,000 ft^3 of water. For irrigation of 2 to 3 inches of such extract-treated water per acre, that one pound of garlic could treat 1.5 to 2.2 acres with 1-ppm garlic extract. This might be highly cost-effective if repeated application reduced sclerotia populations substantially. Increasing the concentration might still be cost effective, especially for cull product source with little (or negative) value and/or if the number of repeat applications can be reduced.

Materials and Methods

Preliminary soil assays demonstrated that the trial area used in 2006-2007 had a high, relatively uniform population of sclerotia formed on garlic in 2005. In the fall of 2006, winter wheat was planted into the trial area, at which time the population in the future trial area ranged from 250 to 1,000 intact, viable sclerotia/l of soil, and averaged approximately 500/l distributed to about 6 inches deep. Irrigated treatments were initiated soon after irrigation water was available in April 2007. The wheat was killed with Roundup® in April so that plots could be sprayed more conveniently. Weeds were similarly treated several times during May through October.

Freshly extracted commercial food-grade garlic juice was donated by The Garlic Company (Shafter, CA). The juice was received in 5-gal plastic buckets that were held refrigerated until use. The company indicated such storage results in minimal product degradation, and no apparent changes were noted as buckets were opened each 2 weeks of the summer. A fresh bucket was opened at each 2-week time of application, and a taste and odor dilution was sampled by the same 8 station staff. Detection of garlic odor and flavor occurred at 1 and 10 ppm by the same staff at each application period, half of the staff detecting at 1 ppm and the other half at 10 ppm.

Treatments were 0, 0.1, 1.0, 10, 100, and 1,000 ppm diluted garlic juice. There were four replications of each treatment structured into a randomized block experimental design. Plots were 10 ft wide by 30 ft long. There were four replications in a randomized, experimental block design. In addition, two additional (nonreplicated) plots were located 20 ft from the trial area and included a no garlic treatment and a 10,000 (1 percent) garlic juice treatment. All treatments were made at 2-week intervals from mid-April to mid-October 2007.

Applications were calculated as if the amount of garlic juice were distributed throughout 2 inches of irrigation water applied once every 2 weeks, but in practice the garlic juice was partially diluted and sprayed onto each plot just ahead of sprinkler application of 2 inches of water. In addition, Advantage® soil surfactant was included in each application to assist in product infiltration through the soil profile. A non-replicated plot of a very high rate of application was located outside the trial area, along with a non-treated plot. At each application, the non-garlic control application was applied first, followed by the

increasing rates. Treatments were all applied soon after dawn when there was no wind and when temperatures were coolest, to avoid both drift and evaporation of stimulants. Temperature was never above 60°F at time of application. Sprinklers were started immediately upon completion of the last spray application. The entire spray series was completed within 20 minutes on each date of application.

Two 12-core (from 1-inch-diameter soil tubes) subsamples were collected to 6 inches deep, and were bulked and assayed separately immediately pretreatment and monthly thereafter the day before treatment periods. At each assay period, sclerotia were counted and determined to be alive or dead. In previous studies, living sclerotia were germinable by stimulants if older than 1 year from production on the previous garlic crop (Crowe et al. 1980), thus "alive" is equivalent to "viable". Throughout the season, viabilities were not found to vary statistically between treatments ($P < 5$ percent), so an average percentage viability was calculated for each sampling date. As in the past, the coefficient of variation between such subsamples increased with lower inoculum densities, but the average number of sclerotia from the two subsamples still were considered sufficient to represent the recovery from each plot. The average number of sclerotia recovered from each plot was converted to a percentage of the average number found upon pretreatment sampling in April.

The soil assay involves sieving soil to the size of sclerotia and counting sclerotia amongst soil residue under a microscope. We noted if sclerotia were found in an active state of germination.

Soil temperatures were monitored under irrigated conditions at 4 and 8 inches deep from a USDA-managed weather station located at the same research center at Madras.

Results

Sclerotia recovered at each sampling date were observed to see if they were intact and viable. At the same time, sclerotia in the state of active germination were recorded; active germination is notable by a hyphal plug of mycelium emerging from a single point (sometimes two points) on the rind of a sclerotium. Prior to application of garlic treatments, no sclerotia were actively germinating in mid-April. From mid-May through mid-October, no germinating sclerotia were found in soil in any non-garlic plots. Beginning mid-May through mid-July, some sclerotia were found to be germinating in all garlic juice-treated plots, with increasing frequency of such recovery at higher rates of garlic juice application. This was clear evidence that garlic juice elicited some germination in all garlic juice treatments. However, no active germination was observed in any garlic juice treatment after mid-July.

Soil temperature data at 4 and 8 inches (10 and 20 cm) were collected by the automated AgriMet weather station located at the Central Oregon Agricultural Research Center. Soil temperatures were within the 50-72°F (10-22°C) range suitable for stimulated germination beginning in April and extending until early July 2007. For several weeks in early to mid-July, soil temperatures exceeded this range at both 4 and 8 inches, and

sclerotia of *S. cepivorum* would not be expected to germinate for some undetermined period of time thereafter, which was reported above. We expected germination to resume later in the summer as soil temperatures dropped back into the conducive range, but this was not observed. As a result, soil recovery data are shown below only for the period of April through July 2007; no changes were seen in population levels after July.

The number of viable, intact sclerotia was determined for each monthly soil sampling. Data for each plot were averaged for the two subsamples. Coefficients of variation were determined but are not reported here. As expected, coefficient of variation between duplicate samples was high, but determined to be acceptable. For each monthly sampling date, data were reported as:

1. Mean recovery of intact, viable sclerotia for each treatment for the four replications. These data are not shown because plot-to-plot recoveries are so variable, even in plots before any applications.
2. Mean percentages of the pretreatment recoveries converted to percentages of the mid-April recovery on a plot-by-plot basis (the primary transformation, see Table 1).
3. Mean percentages of the non-garlic treatment for each monthly sampling. This was a secondary transformation following the first transformation above, i.e., percentages of percentages (see Table 2). The validity of this transformation is discussed below.

Both tables reflect a drop in recovery of intact, viable sclerotia. Such declines were attributed to stimulated germination as observed for soil assay residue under the microscope. In general, there was a relative decrease in percentage recovery with respect to garlic juice concentration as shown in Table 1. The apparent increase in recovery in water-only treated check plots was not attributed to any real increase in sclerotial numbers due to either reproduction or splitting of aggregated sclerotia. Instead, the increase was attributed to increasing soil compaction that elevated the actual concentration of sclerotia in each fixed-volume soil sample. Such compaction was observed to occur as wheat roots decayed and as plots were irrigated during the season. The second transformation of data (Table 2) was done to correct for such apparent increases. When transformed, the untreated check plots were corrected to 100 percent for each sampling date (Table 2); the relative decline in recovery was more pronounced at increasing rates of application of garlic juice.

Data are not shown from the two remote plots. These were included only as additional checks in case plot-to-plot interactions were observed (e.g., if it appeared that drift might have resulted in some stimulated germination in main-trial non-garlic treated plots). In general, the same patterns of sclerotial germination were seen in these plots, although even more stimulated germination was observed at the very high rate of garlic juice application, and even lower recovery of intact, viable sclerotia was found from May to July.

Table 1. Mean percentage recovery of intact, viable sclerotia of *S. cepivorum* from plots treated with either water (check) or garlic juice at various concentrations (ppm). Each individual "check" plot automatically equals 100 percent prior to first treatment in mid-April 2007.

	Mid-April	Mid-May	Mid-June	Mid-July
check	100	115 a	118 a	131 a
0.1 ppm	100	99 a	73 b	73 bc
1.0 ppm	100	95 ab	85 ab	91 abc
10 ppm	100	87 ab	77 b	109 ab
100 ppm	100	98 a	65 b	77 bc
1,000 ppm	100	67 b	47 b	55 c

Table 2. Mean percentage recover of intact, viable sclerotia of *S. cepivorum* from plots treated with either water (check) or garlic juice at various concentrations (ppm). Each individual "check" plot adjusted to 100 percent prior to first treatment in mid-April 2007, then re-adjusted to 100 percent for each sample date at subsequent sampling times.

Mean per	Mid-April	Mid-May	Mid-June	Mid-July
check	100	100 a	100 a	100 a
0.1 ppm	100	86 a	62 b	56 bc
1.0 ppm	100	83 ab	72 ab	69 abc
10 ppm	100	76 ab	65 b	83 ab
100 ppm	100	85 a	55 b	59 bc
1,000 ppm	100	58 b	40 b	42 c

Discussion

We clearly were able to move diluted garlic juice to depths sufficient to stimulate sclerotia, as observed visually (actively germinating sclerotia) and by decline in relative recovery of intact, viable sclerotia. Thus, we are convinced that this trial proved that the concept of repeated application of inexpensive low levels of stimulants was sound. Over a few months, natural decline in sclerotial numbers or viability is minimal. Given the very high initial population levels present in plots, and only modest decline in actual numbers, none of these plots could be replanted to onions or garlic without much greater reduction in sclerotial numbers.

Most likely, many improvements and modifications could be implemented to improve on our results. We did not stratify sampling routinely to demonstrate that stimulants reached all depths equally well, but very limited sampling suggests that this occurred. We likely applied too many applications of soil wetting agent; fewer applications should be investigated.

It also is likely that results will vary greatly among soil types, most specifically regarding the infiltration characteristics that might promote or impede movement of stimulants applied in irrigation water. In addition, the type of irrigation would influence not only infiltration but also evaporative losses of stimulant components (especially if DADS was

used rather than diluted garlic juice). In this trial, garlic juice was not fully diluted in the irrigation water, but was applied somewhat concentrated to soil immediately before sprinklers were turned on. Whether this truly simulated fully diluted juice was assumed, but future work should address the uncertainty that our applications may have moved in a concentrated pulse ahead of most of the following irrigation water.

Crop management in our trial was unlike any commercial management, and irrigation by crop variations would need to be addressed in future work and by each farmer attempting to treat in this manner. As a proof of concept, we did not concern ourselves with this issue at this time.

We were surprised that stimulated germination was not observed to resume later in the summer as soil temperatures dropped well below 72°F (22°C) and well into the optimum range for stimulated germination. This delay in response could be an item for future investigation. In most summers, soil temperature in central Oregon would not exceed 72°F at any depth during the summer, so 2007 was considered rather hot. Soil temperature, of course, is one of the greater factors to consider in regions where soil temperatures routinely exceed 72°F during much of the growing season; any treatments such as those in this trial must be limited to fall, winter, and spring.

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