FURTHER INVESTIGATION OF FLOODING AS A MEANS OF ALLIUM WHITE ROT CONTROL, 2004

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Abstract

This investigation repeats and extends flooding trials previously conducted in 1992 and 1993 at Tulelake, California, in which survival of sclerotia of the Allium white rot fungus (*Sclerotium cepivorum*) in flooded soil was monitored for two different single seasons or for one double season period, and compared to sclerotia recovered from irrigated but unflooded soil. Data reported here are from 2004, the first season of the current 2-year study, and will be incomplete until the end of 2005. In 2004, as was found in 1992, the number of intact, viable sclerotia declined in flooded plots by about 93 percent (7 percent survival). In 2004, nearly 0 percent decline (100 percent) survival was measured from nonflooded plots. The number of intact, viable sclerotia was calculated by multiplying the total intact sclerotial bodies recovered by the percentage of intact bodies that were viable. In 2004, the total intact sclerotial bodies declined approximately 50 percent by September. Viability declined nearly 70 percent by September and nearly 90 percent by November, a similar pattern to 1992. Sclerotial decay previously was found to be highly temperature dependent. Soil temperature at 15 cm (6 inches) between late May and mid-November ranged from 2.2°C (36°F) to 22°C (72°F) but did not drop below 10°C (50°F) until late October 2004. Soil temperature for much of the summer of 2004 was 6°C (11°F) warmer than the summer of 1992.

Introduction

Sclerotium cepivorum reproduces and survives in soil as sclerotia. While thought to be quite persistent under normal field environments, these sclerotia may die under flooded soil conditions. Temperature plays a major role in this response. In the laboratory, survival of sclerotia of *S. cepivorum* after 3 weeks was nil in continuously saturated soil at constant 24°C or above, whereas survival after 3 weeks increased progressively to 95 percent at constant 6°C in continuously saturated soil. In the same investigation, survival also increased as the soil matrix potential decreased away from saturation, e.g., “wet” but not saturated allowed higher survival (Crowe and Hall 1980).

Winter flooding was implicated in poor survival of sclerotia in muck soils in British Columbia (Leggett and Rahe 1985). Extended winter flooding in muck soils in eastern Canada, with soil temperatures predominantly below 5°C, reduced sclerotial populations in the field by 80 percent compared to 10 percent decline in unflooded soil (Banks and Edgington 1989). In neither Canadian example was winter flooding sufficient to reduce Allium white rot disease incidence to commercially acceptable levels in only a single flooded winter.

In the western United States, except for a few areas where the growing season occurs at elevated soil temperatures restrictive to *S. cepivorum*, disease incidence in full season

Allium species is unacceptably high if inoculum density at planting is greater than 0.1 sclerotia/l soil. Unfortunately, inoculum density in infested fields in this large region commonly ranges between 1 and 1,000 sclerotia/l soil (Crowe et al. 1980; Crowe, unpublished data). Therefore, white rot control by reduction of initial inoculum would require a decline of at least 10 times in the least infested fields, and much more commonly would require a reduction of between 100 to 10,000 times. Control practices based on inoculum reduction are further limited because inoculum density may resurge to high levels from very low levels within a single season due to prolific reproduction of sclerotia on the few plants decayed during recropping of Allium species (Crowe et al. 1980). Thus, without full eradication, continued retreatment may be required between Allium crops, which may be economically unfeasible if the treatment is expensive.

The information reviewed above suggests that a single season of winter flooding likely will remain inadequate for white rot disease control in most temperate climates. The impact of multiple years of winter flooding, and of flooding when water temperature is warm, have not been fully evaluated under field conditions. An initially high inoculum density (greater than 100 sclerotia/l soil based on widespread and rapid death of plants [Crowe et al. 1980]) declined to less than 0.1 sclerotia/l soil after 21 weeks of continuous summer flooding of a naturally infested commercial field in the cool, high desert production region near Madras, Oregon (700 m, 2,297 ft). Flooding was initiated in late June. Soil temperature remained at 20-25°C for several months, gradually falling to 10°C by the end of fall when flooding was terminated (Crowe and Debons 1992). The resultant inoculum density likely would allow commercial recropping without significant disease loss in many situations, but failure to eradicate all sclerotia indicates that white rot disease would have recurred on a few plants and sclerotial populations would have rebuilt (Crowe et al. 1980). Promising results from summer flooding also may have been achieved in the high altitude (2,000 m) production region north of Mexico City, an area warmer than central Oregon or Tulelake in spite of the high altitude (E. Redondo, personal communication).

We report here results of experiments in progress to determine survival of sclerotia of *S. cepivorum* during summer flooding at Tulelake, California (1,231 m, 4,040 ft at the University of California [UC] Intermountain Research and Extension Center). Also in the high desert, summer flooding of reclaimed lakebeds is feasible and might be justified commercially if combined control of diseases, weeds, nematodes, and insect pests could be achieved.

Data reported here are for the first year of a 2-year trialwhich is a repeat of a 2-year trial conducted in 1992 and 1993 at Tulelake (Crowe and Carlson 1994). In 1992, the number of initial intact, viable sclerotia at the beginning of flooding in April declined by about 50 percent early in the season. This suggests that about half of the initial sclerotia were incomplete or weakened, resulting in their rapid decay. Thereafter, for the duration of the experiments, sclerotial numbers in nonflooded plots remained somewhat stable, except for a peculiar and unexplainable temporary decline in recovery of intact, viable sclerotia mid-season of 1993 that recovered to initial levels by the end of 1993. This suggests some sampling or other irregularity during 1993 (see below). In contrast, for plots flooded the first season (1992), the number of intact, viable sclerotia regularly declined,

ending in November with less than 1 percent reported survival from initial levels. Upon reanalysis of the data, we currently believe this should have been about 3 percent survival. In plots flooded two seasons (1992 and 1993), the second year survival fell to 0 (no intact, viable sclerotia were recovered). However, plots flooded for the single season of 1993 behaved oddly: the number of initial, intact sclerotia in flooded plots at the beginning of the 1993 flooding season declined by about 95 percent in the first half of the flooding season, but reportedly rebounded to near-initial levels by the end of the flooding season. This suggested either a strong recovery in viability, or reproduction of sclerotia. However, either possibility (or both) would be contrary to all published literature and experience. Most likely, data from the1993 flooded and nonflooded plots were flawed by mislabeling, mishandling of plots or canisters, misrecording of data, etc. As a result, in addition to requiring more flooding data in order to develop guidelines for commercial controls, we wished to repeat the 1992-1993 experiments to determine whether in a second trial sclerotia behaved as expected (e.g., as per 1992 single-year flooding and 1992-1993 2-year flooding) or as was recorded for the flooded and single season nonflooded plots of 1993.

The only differences between the 1992-1993 and 2004-2005 trials were:

1. The 1992 trial was flooded beginning in April, whereas the 2004 trial was flooded beginning in late May. Both starting points were early enough so that sclerotia were exposed to the warmest summer periods.
2. The 1992-1993 trials were flooded from April to October, with no winter flooding. Thus, each 1-year trial was flooded from spring through fall, and the 2-year flooding plots were flooded for two spring-through-fall periods. The 2004-2005 trial was revised so that flooding would occur continuously for the 2-year period (i.e., June 1, 2004 through November 1, 2005) and the two 1-season flooding trials would extend from June 1, 2004 through November 1, 2004 and March 1, 2005 through November 1, 2005. In the early 1990's over-winter flooding of commercial fields was not considered likely, whereas in recent years this would be possible.

Methods and Materials

Approximately 1,000 sclerotia of *S. cepivorum,* which had been produced on sterile oats in the laboratory, were mixed with 115 ml non-infested Tulelake volcanic soil (20 percent organic matter). This soil was placed in 60-mm-inner-diameter by 60-mm-high PVC chambers that were closed at the ends with nylon mesh (0.2-mm gap between strands). The mesh was held in place with a 4-mm-high PVC ring, which fitted tightly into the ends of the chambers. Chambers were buried 8 per 19-l micro-plot bucket, in a circle arrangement with all chambers roughly equidistant from the nearest neighbors. Nylon-covered ends were oriented up and down, with the upper end 10 cm below the soil surface. Buckets were filled within 10 cm from the top with noninfested Tulelake soil. Previous research indicated that the nylon did not affect flooding or sclerotial behavior (Crowe and Carlson 1994).

Micro-plot buckets were replicated four times for each treatment in a fully randomized experimental design. The trial was located at the UC Intermountain Research and Extension Center, Tulelake, California. Treatments included nonflooded experimental controls and various flooding treatments. Soil in all micro plots was dampened (not flooded) at the time of burial (micro-plot establishment) in mid-May 2004.

Buckets for nonflooded controls were perforated at the bottom to allow drainage, and these buckets were irrigated periodically during the May through November growing season to provide typical seasonal fluctuating soil moisture for agricultural soils. As all weeds were removed as seedlings from the micro plots, irrigation frequency was somewhat less than for cropped fields in the region.

Two outer buckets were buried side-by-side for each treatment replicate. The bottom of one of the pair of outer buckets was perforated to allow drainage. All inner buckets were perforated at the start of the experiment. Flood and nonflood episodes were initiated by moving the inner buckets to either perforated or non-perforated outer buckets as appropriate for the treatment. At the initiation of flooding, water was added until it remained at the upper lip of the buckets, 10 cm above the soil. This level was maintained through the flooded season by periodically adding water if sprinkler irrigation was insufficient (see below).

First year flood only: Flood treatments were initiated June 1, 2004 and terminated March 1, 2005.

Flood 2-years 2004-2005: Flood treatments were initiated June 1, 2004 and will be terminated November 1, 2005.

Second-year flood only: Flood treatment was initiated March 1, 2005 and will be terminated November 1, 2005.

Nonflooded: Canisters for all nonflooded plots were placed in perforated outer buckets to allow drainage and received only seasonal sprinkler irrigation (approximately weekly from May through September) and natural precipitation.

Soil temperatures and precipitation were recorded by an automated UC weather station. Soil temperature was measured at 15 cm soil depth from irrigated turf adjacent to the micro-plot trial area. Additional temperature recording units will be placed into flooded plots in 2005 to determine whether soil temperature readings from the weather station truly represent the temperature in flooded plots. Overhead irrigation was sufficient to maintain the irrigated lawn in which the buckets are buried, but was not recorded for 2004.

One chamber was removed from all micro-plot buckets within 1 week, just prior to initiation of the first flooding, which began on May 24, 2004. This sample served as the pretreatment recovery of sclerotia for all future comparisons. Thus, seven of the eight chambers in each micro-plot bucket were available for sampling after May 2004. In

2004, plots were sampled every 2 months, in July, September, and November. For each sampling date, chambers were recovered randomly from each micro-plot bucket. Chambers and soil were frozen until assay at Oregon State University Central Oregon Agricultural Research Center (OSU-COARC) in Madras, Oregon.

Soil was assayed and sclerotia were tested for viability as per Crowe et al. (1980). Briefly, sclerotia were concentrated from soil by size (sieving through screens) and by density (flotation on a sucrose solution). Remaining soil residue was observed under a binocular microscope. The number of sclerotial bodies remaining intact upon light manipulation was counted. If more than 60 intact sclerotial bodies were counted, then 60 selected at random were tested for viability on unamended agar. If 60 or fewer were counted, then all intact bodies were tested for viability. Sclerotia were washed, surface disinfected for 2.5 minutes in 0.5 percent sodium hypochlorite, cracked using forceps, and placed on unamended Bactoagar (Difco) in sterile Petri dishes to induce growth. Sclerotia that developed characteristic mycelial growth and clumps of microconidia in the agar were identified as those of *S. cepivorum*. Sclerotia were observed for such growth and development for 3 weeks, after which they were determined to be nonviable and presumed to be dead.

The number of recovered intact sclerotial bodies was determined by direct observation under the binocular microscope of soil residue of the same size and buoyancy as sclerotia. Candidate bodies were lightly manipulated with fine tweezers to assure integrity of the rind. The number of viable sclerotia recovered per plot was determined by multiplication of the proportion that were viable times the total number of intact sclerotial bodies recovered.

Results below are means of four replications for each sampling date, with plots arranged in a randomized block design in the field. Statistical analyses were analysis of variance, (ANOVA) using general linear model, PROC GLM, of SAS version 9.1 (SAS Institute, 2002). Treatment means were separated by Fisher's protected least significant difference (LSD) test.

Results

The mean number of recovered intact sclerotial bodies per treatment is shown in Figure 1. Mean percentage viability is shown in Figure 2. The mean number of viable sclerotia recovered per plot (Fig. 3) was determined by multiplication of the proportion that were viable times the total number of intact sclerotial bodies recovered.

In May 2004, 1 week after treatment implementation and canister burial but before plots were flooded, viability of sclerotia recovered was nearly 100 percent for all plots (Fig. 2). Nearly 700 sclerotial bodies, almost all viable, were recovered from the first canisters recovered from all plots (Fig. 1). The roughly 30 percent difference between the estimated 1,000 infested sclerotia and 700 initially recovered is attributable to both estimate error and rapid decay of nonviable lab-grown sclerotia. (Note that in 1992, this drop from estimated to actual was close to 50 perecent.) The number of intact, viable

sclerotia is shown in Figure 3, and is essentially equal to 700 for the first (preflood) sample date. The approximately 700 intact, viable sclerotia represent an initial average inoculum density of 467 sclerotia/100 ml soil, a value sometimes encountered in highly infested areas of naturally infested fields (Crowe et al. 1980), but which is less than the density of sclerotia commonly remaining where host plants with white rot are left to decay.

For treatments not flooded during 2004 (i.e., Flood 2005 only and Unflooded), sclerotia continued to be recovered intact and highly viable from May through November (Figs. 1-3). Few degraded sclerotial bodies were observed from nonflooded plots. The small drop in mean recovery in September for plots in the *Flood 2005 only* treatment probably was due to sample handling problems, as recovery from the same plots in November was the same as measured for May and July and was comparable to the Non-flooded plots.

From plots flooded longer than 2 months (i.e., Flood 2004 only and Flood 2-years 2004-2005), recovery of intact bodies declined about 50 percent compared to unflooded plots (Fig. 1). Percentage viability (Fig. 2) fell regularly by mid-season for flooded plots, but did not decline for nonflooded plots. This resulted in a net recovery of intact, viable sclerotia that declined during the season in flooded plots, ending in November with 42 to 55 intact, viable sclerotia in flooded plots, or about 93 percent decline (7 percent survival).

Average daily soil temperature (detail of data not shown here) increased from about 13°C (55°F) in late May, to near around 21°C (70°F) during July through mid-August, then declined gradually to 10°C (50°F) by mid-October, 4.5°C (40°F) by mid-November, and down to around freezing during December 2004-January 2005. Irrigation and precipitation data are not shown for 2004.

Discussion

In previous studies, for sclerotia recovered from "normal" field soil, treated and handled as above during and after recovery and assay, it was earlier determined that the frequency of growth on agar after surface sterilization and cracking was equivalent to the frequency of sclerotia that could be stimulated to germinate by Allium host exudates and to infect Allium roots (Crowe et al. 1980). Thus, if a sclerotium was found to be alive and able to grow, this was equivalent to being able to germinate and infect Allium roots in field soil. In this flooding investigation, we have not determined yet whether sclerotia that are alive after flooding retain the ability to germinate by stimulation. Conceivably, the ability to respond to germination stimulants could be at least temporarily altered during flooding. This will be determined during 2005.

Data from the first year of flooding (2004) of the 2-year trial closely follow what was found in the first year of flooding in the 2-year trial of 1992-1993. No unusual or unexpected findings were observed as discussed in the introduction for flooding and nonflooding plots in 1993. Decline of sclerotial bodies and viability in 2004 was similar to that observed in 1992. Data from 1992 were reported incorrectly (Crowe and Carlson

1994), in that a lower initial value (between 400 and 500) should have been used to estimate later survival. As a result, first-year decline in flooded plots was closer to 97-98 percent rather than over 99 percent. Thus, the 2 years are comparable.

Based on data from this experiment still in progress but nearing completion, seasonal flooding has potential as a control treatment for Allium white rot. Whether one season will be sufficient for economic replanting of onions may depend on the initial starting inoculum density and length of flooding period. Some disease would recur after one season's flooding, and higher soil populations of the white rot fungus would reestablish. Full eradication over 2 years (as achieved in 1992-1993) may be a more worthy target. More will be known after the trial is complete later in 2005.

If summer flooding successfully reduces inoculum of *S. cepivorum* to acceptable levels in cool regions such as central Oregon and northern California, it should be more successful in warmer regions. The duration for effective treatment likely could be reduced; in a very warm area, perhaps a single flooding period might achieve full eradication.

Acknowledgment

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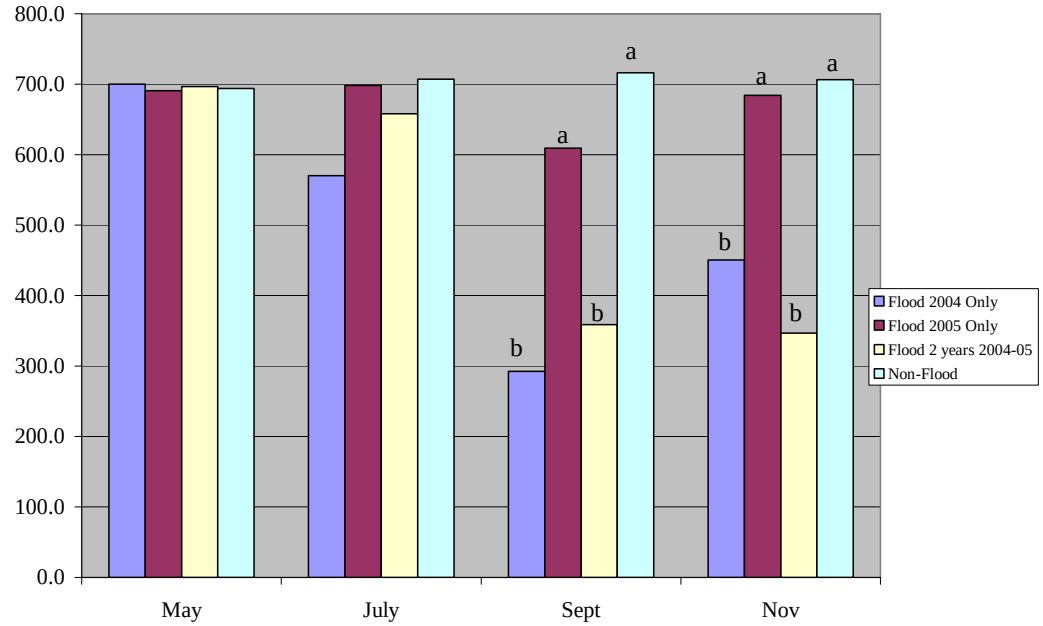

Figure 1. Total intact sclerotia recovered during 2004 in the flooded trial at Tulelake, CA.

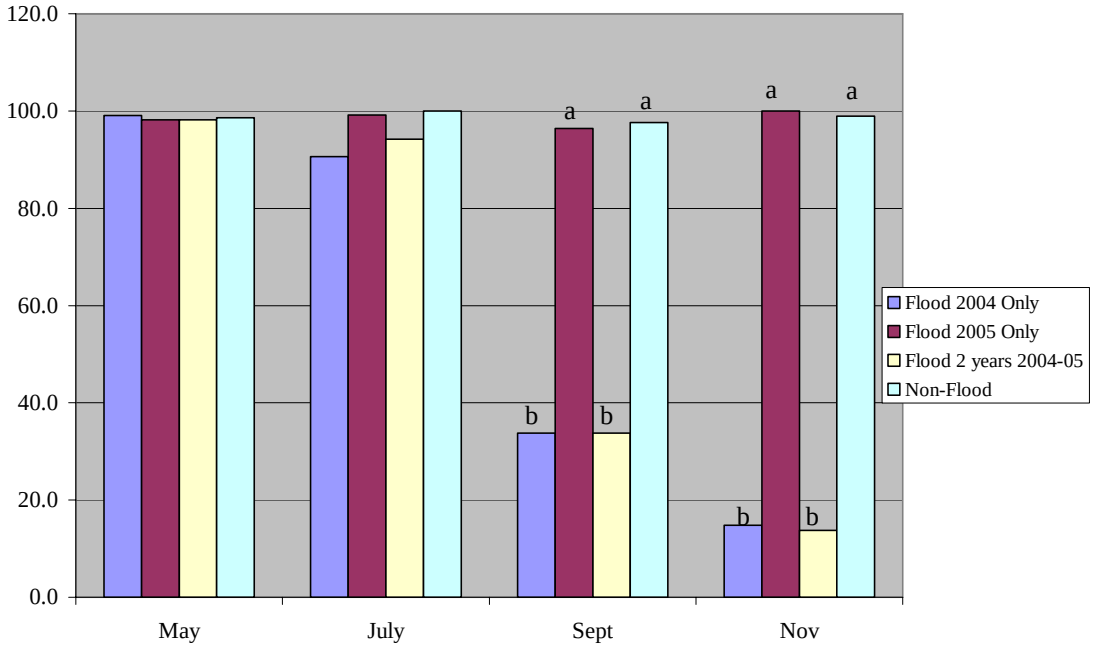

Figure 2. Percent viability of sclerotia recovered during 2004 in the flooded trial at Tulelake, CA

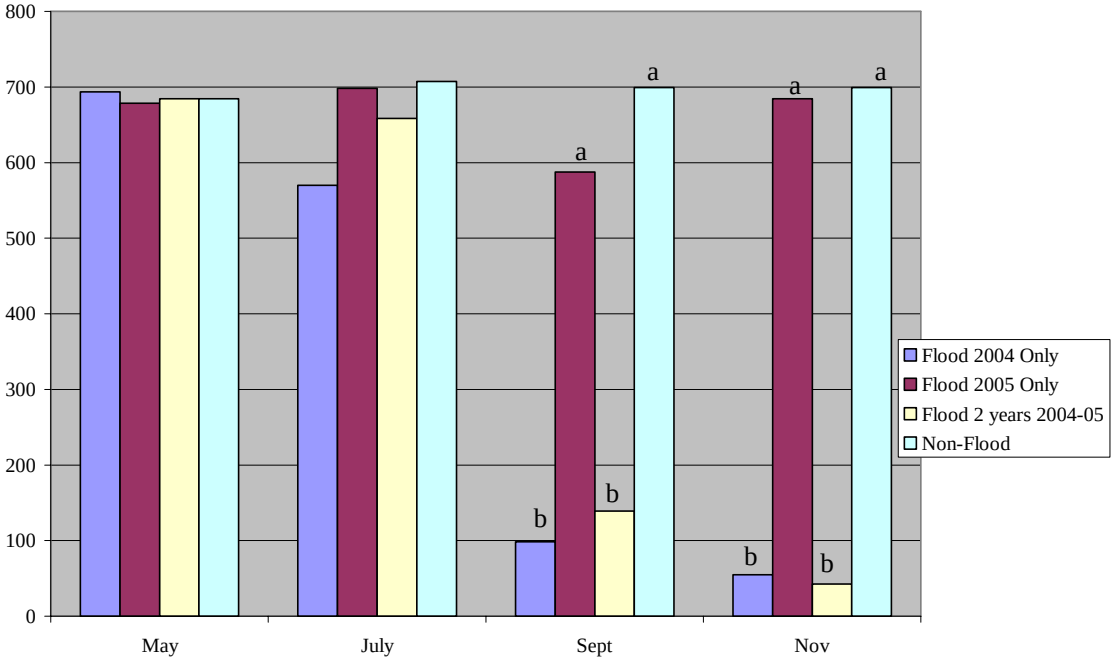

Figure 3. Total intact, viable sclerotia recovered druing 2004 in the flooded trial at Tulelake, CA.

*Means with unlike letters are significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.