CONTINUED INVESTIGATION OF FLOODING AS A MEANS OF ALLIUM WHITE ROT CONTROL

Fred Crowe and Harry Carlson

Abstract

Results are reported from an investigation initiated in 1992, part of which will be completed in 1994 and part of which was extended based on data presented below. Flooding for a single season from spring through fall at Tulelake, California, reduced recovery of viable inoculum (sclerotia) of *Sclerotium cepivorum* by 98 and 96 percent for 1992 and 1993, respectively, based on mid-summer sampling of sclerotia buried in flooded microplots. Following single-season 1992 flooding, inoculum remained low through 1993. Following single-season 1993 flooding, however, inoculum increased to 1993 pre-flooding levels. In additional plots which were flooded for the spring-fall period in both years, no surviving inoculum was found by mid-summer of the second season, nor was any found subsequently after treatment ended. In non-flooded microplots, inoculum also decreased during the summer months, but this decline was partially offset by an apparent increase in the fall and/or spring in each of 1992 and 1993. In spite of seasonal fluctuations in recovery of viable sclerotia in non-flooded plots, there was a downward trend in inoculum survival each year (30 percent decline in numbers after one year, and a 54 percent decline by the end of the second fall). Average daily soil temperatures at 15 cm were 5-16° C for spring and fall for both years, 19-23° C (average for the area) for summer 1992, and 15-19° C (much cooler than average) for summer 1993.

Introduction

Sclerotium cepivorum reproduces and survives in soil as sclerotia. While thought to be quite persistent under normal field conditions, 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 three weeks was nil in continuously saturated soil at constant 24° C or above, whereas survival after three weeks increased progressively to 95 percent at constant 6° C in continuously saturated soil. In the same investigation, survival also increased as soil matric potential decreased away from saturation (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 non-flooded soil (Banks and Edgington, 1989). In neither Canadian example was the decrease in inoculum during winter flooding sufficient to reduce Allium white rot disease incidence to commercially acceptable levels

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/liter soil.

Unfortunately, inoculum density in infested fields in this large region commonly ranges between 1 and 10,000 sclerotia/liter soil (Crowe, et al., 1980; Crowe, unpublished). Therefore, white rot control by reduction of initial inoculum would require a decline of at least 10 times in the least highly infested fields, and much more commonly would require a reduction by between 100 to 100,000 times. Control practices based on inoculum reduction are further limited because inoculum density may return 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 re-treatment may be required between Allium crops. This may be economically unprofitable.

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. Inoculum density of less than 0.1 sclerotia/liter soil was estimated to have survived 21 weeks of continuous summer flooding of a highly infested commercial field in the cool production region of central Oregon (700 m altitude). During this period, which began in late June, flooded soil temperature remained at 20-25° C for several months, gradually falling to 10° C by the end of fall when flooding terminated (Crowe and Debons, 1992). Such a result likely would allow commercial recropping without significant disease loss in many situations. Promising results from summer flooding also may have been achieved in the high altitude (2,000 m) production region north of Mexico City (E. Redondo Juarez, personal communication).

We report here results of experiments in progress to determine survival of sclerotia of *S. cepivorum* during summer flooding at Tulelake, California. In this high altitude (1,200 m), desert region of reclaimed lake beds, summer flooding is feasible and might be justified commercially if combined control of onion white rot, sclerotinia diseases of various crops, and other diseases, weeds, nematodes, and insect pests could be achieved. Presumably, if summer flooding successfully reduces inoculum of *S. cepivorum* to acceptable levels in cool regions such as central Oregon and northern California, it may be even more successful in warmer regions, and the duration for effective treatment might be reduced.

Methods and Materials

Pathogen and Plot Preparation

All soil used in the trial was pre-mixed field soil determined by field history and sampling to be free of the white rot fungus. Approximately 1,000 sclerotia of *S. cepivorum,* which had been produced on wound-inoculated onions 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 x 60-mm-high PVC chambers which 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 nine per 19-liter microplot bucket, with eight chambers located around a central ninth chamber, all chambers roughly equidistant from the nearest neighbors.

Nylon covered ends were oriented up and down, the upper end at 10 cm below the soil surface. Buckets were filled within 10 cm from the top with non-infested Tulelake soil..

Microplot buckets were replicated four times for each treatment. The trial was located at the University of California's Intermountain Research & Extension Center, Tulelake, California. Treatments included non-flooded experimental controls, and various flooding treatments. Soil in all microplots was dampened (not flooded) at the time of burial microplot establishment in April, 1992. One chamber was removed from all microplot buckets one month later, just prior to initiation of the first flooding period in May, 1992. This sample served as the pre-treatment recovery of sclerotia for all future comparisons. Another chamber in each microplot bucket is reserved for recovery in November of 1994, at which point the experiment will be completed. Thus, seven chambers of the nine chambers in each microplot bucket were available for sampling after May, 1992, and before November, 1994.

Soil temperatures were recorded by a completely automated University of California weather station, at 15 cm soil depth from irrigated turf adjacent to the microplot trial area.

Flooded and Non-flooded Treatments

Buckets for non-flooded controls were perforated at the bottom to allow drainage, and these buckets were irrigated periodically during the April-October growing season to provide typical seasonal fluctuating soil moistures for agricultural soils. As all weeds were removed as seedlings from the microplots, irrigation frequency was somewhat less than for cropped fields in the region. To determine whether the nylon chamber end coverings affected survival, an additional experimental control treatment was included in which the nylon was carefully removed from the chambers during burial. During excavation, chambers without nylon again were handled carefully so as not to lose infested soil.

In the 1992 flooding treatment, an inner microplot bucket, perforated at the base, was placed inside a second non-perforated bucket. At the initiation of flooding, water was added until it remained at the upper lip of the buckets, 10 cm above the soil. The flooded level was maintained between 1-10 cm through the flooded season by periodically adding water. Flooding was initiated in May and terminated in October by removing the outer bucket. Six chambers were recovered monthly through continuous seasonal flooding during 1992. Soil in the remaining perforated bucket was irrigated with drainage in 1993 as for the non-flooded treatments. A seventh chamber was recovered in November, 1993. Microplots in this treatment will continued to be managed in 1994 as for 1993, with the final chambers to be recovered in November, 1994.

In the 1993 flooding treatment, the buckets holding the chambers were perforated and in 1992 were irrigated with drainage as for the non-flooded controls. Beginning in April, 1993, the perforated buckets,were placed into non-perforated buckets, and these microplot buckets were flooded as for the 1992 flooding treatment. After the winter of 1993, the outer non-perforated bucket was removed, and the perforated bucket is being irrigated with drainage as for the non-

flooded control. Seven chambers per bucket were removed during 1993, leaving the last chamber to be recovered in November, 1994.

In the 1992-1993 flooding treatment, the chambers were contained in a perforated bucket, which was placed into a non-perforated bucket and flooded for 1992 as above. The non-perforated bucket was removed at the end of 1992, but was replaced prior to resumed flooding in 1993. At the end of 1993 flooding, the outer non-perforated bucket was removed, and the perforated bucket is being irrigated with drainage as for the non-flooded control through 1994. Three chambers were removed from each bucket during 1992, and four were removed during 1993, leaving the last chamber to be removed in November, 1994.

Recovery and Assessment of Sclerotia

From each sampling time, chambers were recovered randomly from one of the nine available locations in microplot buckets. Soil in chambers was air-dried with the chambers intact. Chambers were held in the laboratory until soil was processed. Chambers recovered between May, 1992, and November, 1993, were stored air-dried until the winter of 1993-94. Chambers from storage were selected at random and the soil was assayed between December, 1993, and February, 1994.

Soil was assayed and sclerotia were tested for viability as per Crowe, et al., 1980. Briefly, sclerotia were concentrated from soil by size by sieving through screens and by density by flotation on a sucrose solution. Remaining soil residue was observed under a binocular microscope. The number of sclerotial bodies remaining intact upon light manipulation were 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 disinfested for 2.5 min in 0.5 percent sodium hypochlorite, cracked using forceps, and placed on unamended Bactoagar (Difco) in sterile Petri dishes to induce growth. Sclerotia from which characteristic mycelial growth, plus from which clumps of microconidia formed in the agar, were identified as those of *S. cepivorum*. Sclerotia were observed for such growth and development for three weeks, after which they were determined to be non-viable, presumed to be dead.

Results

Recovery of Sclerotia

Table 1 shows the months during which chambers were recovered from various treatments. All recoveries were during the first week of the month shown. Results below are means of four replications for each sampling date.

In May, 1992, just prior to treatment implementation, viability of sclerotia recovered was 98.1 percent. An average of 920 viable sclerotia (range 810-1,080, standard deviation 63) were recovered from all chambers. This represents an average inoculum density of 800 sclerotia per

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 in the volume where host plants are decayed with white rot.
The number of recovered intact sclerotial bodies were 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 intact bodies recovered generally declined during the course of the study. The proportion of sclerotia viable was consistent within treatment sampling data, but was not consistent among treatments or across sampling dates with treatments. We have observed in this and other studies (Crowe, unpublished) that sclerotial bodies may take some time (months) to decay after death. From plots flooded longer than two months, an increasing number of sclerotia were found to be degraded. The number of viable sclerotia recovered per plot was determined by multiplication of the proportion which were viable times the total number of intact sclerotial bodies recovered. Chambers from the non-nylon treatments were sampled on a slightly different but overlapping schedule from the schedule for the nylon treatment. Where these sampling dates coincided, the sclerotial recoveries were very similar, and the temporal pattern of data from plots with and without nylon was similar in all respects. Thus, these data were merged for presentation. The mean numbers of recovered, viable sclerotia from non-flooded plots are shown in Figure 1. The mean number of viable sclerotia was initially high in the spring of 1992, dropped by 62 percent during the summer of 1992, increased during the fall of 1992 and spring of 1993, dropped by 90 percent from spring 1993 level during the summer of 1993, and increased again in the fall of 1993. None of the mean values later in the study exceeded the initial value determined in May, 1992, and there appeared to be a gradual decrease over the years in spite of seasonal fluctuations.

The mean numbers of viable sclerotia recovered from plots flooded in 1992, but not flooded subsequently, are shown in Figure 2. The mean number of viable sclerotia dropped by 65 percent in the first month, and by 98 percent for the season (May-November, 1992). Follow-up sampling in November of 1993 suggested that the population had stabilized at about 1 percent of the original number.

The mean numbers of viable sclerotia recovered from plots flooded in 1993, but not flooded at other times, are shown in Figure 3. The mean number of viable sclerotia recovered without flooding at the beginning of 1993 (prior to flooding) was 37 percent less than a year earlier, and comparable to the non-flooded treatment. During flooding in 1993, the mean number of viable sclerotia dropped by about 96 percent by early summer, but then increased by fall to a value comparable to that found in the spring of 1993.
The mean numbers of viable sclerotia recovered from plots flooded in 1992 and 1993, but not subsequently, are shown in Figure 4. The 1992 pattern is similar to that shown for 1992 flooding above, reflecting a great decrease in population. In 1993, no viable sclerotia were recovered after summer, and no apparent resurgence in numbers as was seen in the non-flooded treatment or in the treatment only flooded in 1993. It seems as if all sclerotia were killed during this treatment.

Soil Temperatures

Average daily soil temperatures were determined for 15 cm soil depth in irrigated, non-flooded soil located adjacent to microplots. Spring and fall soil temperatures for both 1992 and 1993 typically ranged from 5-16° C, typical for the region. Summer temperatures in 1992 ranged from 19-23° C, typical for the region. Summer temperature in 1993 were near record lows for the region, ranging from 15-19° C, with a brief mid-summer spike to 23° C. Winter soil temperatures for both years were typical and were low enough for soil water to remain frozen for much of the winter.

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 the frequency of sclerotia which were able to 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 did not determine whether sclerotia which were alive after flooding retained the ability to germinate by stimulation. Conceivably, the ability to respond to germination stimulants could be at least temporarily altered during flooding.

Based on data from this experiment still in progress but nearing completion, seasonal flooding has potential as a control treatment for Allium white rot. During single-season flooding in 1992, with a relatively average summer for this cool region, sclerotial populations were decreased by about 98 percent. In 1993, with a near record cool season for this region, single-season flooding reduced sclerotial populations by 96 percent, but the population appeared to increase later in the season, reverting to pre-flooding levels for this treatment. Flooding for a two-season period may have eradicated sclerotia from the treatment plots. The cost of single full-season flooding in central Oregon, including lost cropping opportunity costs, was estimated to be similar or somewhat less than the cost of tarped methyl bromide fumigation (Ed Macy, central Oregon farmer, personal communication). In warmer regions where the flooding period might be shortened or where labor and water cost structures are substantially different, flooding may be more affordable. In addition to treatment costs, whether summer flooding for one or two seasons is economically justified will depend on the level of white rot control required, and possibly how many other pest problems might be reduced in the process.

The non-flooded data support the concept that populations gradually decline over years (Leggett & Rahe, 1983; Crowe, et al., 1980), and contradict the concept that populations are static over years (Coley-Smith, 1959; Coley-Smith, et al., 1990).

It is intriguing that we found apparent increase in population at times during the season. We recall no other report for *S. cepivorum* in which within-season variation has been determined to

the extent reported here. In most of our own past investigations where inoculum density was assessed periodically from test plots or commercial fields, we have tended to sample either in the spring or fall, or yearly at about the same time of year, so we would not have seen these fluctuations in recovered viable sclerotia. Apparent reproduction by *S. cepivorum* in our plots seemed to coincide with cool temperatures of fall and spring. The data seem to suggest that such apparent reproduction was greater in the cooler 1993 than in the warmer 1992. If these data reflect real field variations, they challenge the accepted concept that *S. cepivorum* does not reproduce in field soil in the absence of a pathogenic association with Allium species (Scott, 1956). In our plots, there would have been little or no living plant food base available.

We ourselves question whether we have recorded real within-season reproduction, and are taking some efforts to examine other explanations/interpretations of our data. We are, however, among those who find it difficult to accept that individual sclerotia can survive in field soil for 20, 30 or 40 years as members of static or gradually declining populations which can incite high disease incidence after such long periods of time. [We do accept, of course, that even a few surviving sclerotia can incite substantial disease (Crowe et al., 1980.)] As have others, the senior author has at times looked for (but not found) special conditions which might allow reproduction of *S. cepivorum* in soil without an Allium food base. Could it be that some sclerotia of *S. cepivorum* are reproduced each season in the absence of white rot disease on Allium species, even if populations tend to decline overall in most years without this host? Might there even be special conditions in some years which allows a net population increase? Perhaps these data give us a clue.

References

Banks, E., and L.V. Edgington (1989) Effect of integrated control practices on the onion white rot pathogen in organic soil. *Canadian J. Plant Pathology* 11, 268-272.

Coley-Smith, J.R. (1959) Studies of the biology of *Sclerotium cepivorum* Berk. III. Host range, persistence and viability of sclerotia. *Ann. Appl. Biol.* 47, 511-518.

Coley-Smith, J.R., Mitchell, C.M., and C.E. Sansford (1990) Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli. Plant Pathology* 39, 58-69.

Crowe, F.J. and J. Debons (1992) Effect of in-season flooding on white rot of garlic and survival of *Sclerotium cepivorum. Phytopathology* 82, 1108.

Crowe, F.J. and D.H. Hall (1980) Soil temperature and moisture effects on sclerotium germination and infection of onion seedlings by *Sclerotium cepivorum. Phytopathology* 70, 74-78.

Crowe, F.J., Hall, D.H., Greathead, A.S. and K.G. Baghott (1980) Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. *Phytopathology* 70, 63-69.

Leggett, M. and J. Rahe (1985) Factors affecting the survival of sclerotia of *Sclerotium cepivorum* in the Fraser Valley of British Columbia. *Ann. Appl. Biol.* **106,** 255-263.

Scott, M.R. (1956) Studies of the biology of *Sclerotium cepivorum* Berk. I. Growth of the mycelium in soil. *Ann. Appl. Biol.* **44,** 576-583.

Table 1. Recovery dates for buried chambers (nine chambers per rnicroplot) containing sclerotia of *S. cepivorum* in various non-flooding and flooding treatments at Tulelake, California, 1992-1994

	Sampling Time (Mo/Yr)								
Treatment	1	2	3	4	5	6	7	8	9
Non-Flooded	5/92*	8/92	11/92	4/93	8/93	11/93	5/94	8/94	11/94
Flood 92	5/92#	6/92	7/92	8/92	9/92	10/92	11/92*	11/93	11/93
Flood 93	5/92*	5/93#	6/93	7/93	8/93	9/93	10/93	11/93*	11/94
Flood 92-93	5/92#	7/92	9/92	11/92*	5/93#	7/93	9/93	11/93*	11/94

* = begin drainage period
# = begin flooded period

Figures 1 and 2. Figure 1 shows means and standard deviations for the number of intact, viable sclerotia of *Sclerotium cepivorum* recovered from chambers buried in non-flooded microplots during 1992 and 1993. Figure 2 shows means and standard deviations for the number of intact, viable sclerotia of *S. cepivorum* recovered from chambers buried in microplots flooded from spring through fall of 1992 (shaded period), and from the same microplots held without flooding since October, 1992.

Fig. 1. Non-Flooded

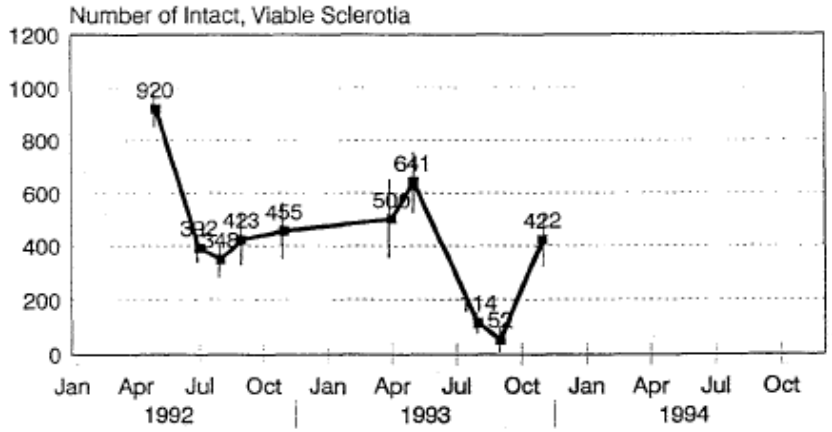
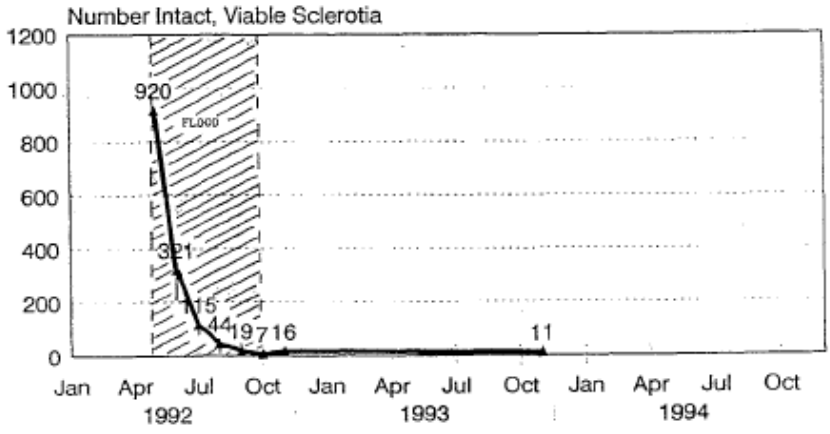


Fig. 2. Flood 1992 Season Only



Figures 3 and 4. Figure 3 shows means and standard deviation for the number of intact, viable sclerotia of *Sclerotium cepivorum* recovered from chambers buried in microplots irrigated but with drainage through 1992, then flooded from spring through fall during 1993 (shaded period). Figure 4 shows means and standard deviations for the number of intact, viable sclerotia of *S. cepivorum* recovered from chambers buried in microplots flooded for two periods from spring through fall (shown by shading) during 1992 and 1993.

Fig. 3. Flood 1993 Season Only

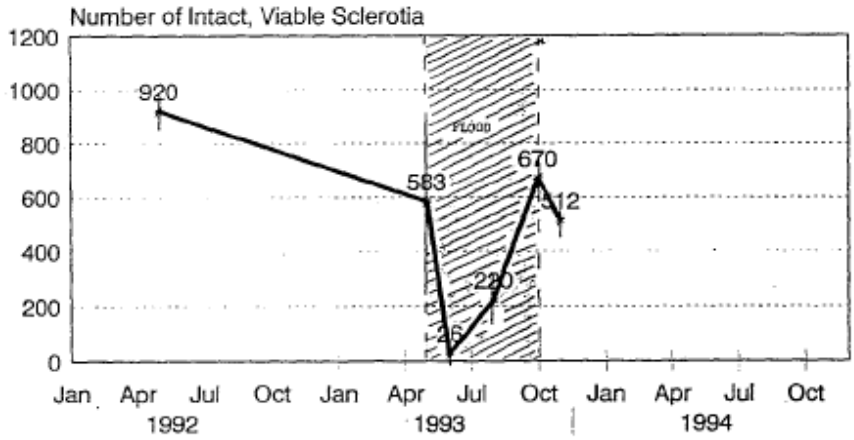


Fig. 4. Flooded 1992 & 1993