EFFECT OF FLOODING ON SCLEROTIA OF THE ALLIUM WHITE ROT FUNGUS (*Sclerotium cepivorum*)

Final Report from experimental field trials 1992-1993 and 2004-2005

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Abstract

Sclerotia of the Allium white rot fungus (*Sclerotium cepivorum*) produced in the laboratory were mixed with field soil in Tulelake, California in micro-plots arranged in a randomized experimental design with four replications. Survival of sclerotia was monitored in soil that was nonflooded or flooded for 1 or 2 years. Micro-plots were irrigated periodically when not flooded. Data are reported here from two separate trials, 1992-1993 and 2004-2005. Soil temperature at 7.5 cm (6 inches) ranged from 2°C (35°F) in spring/fall to 22°C (73°F) in mid-summer; soil was frozen much of the winter. Sclerotial body integrity and ability to grow remained near 100 percent in plots that were irrigated but never flooded over the 2-year period 2004-2005. For nonflooded plots in 1992-1993, approximately 30 percent decline in survival occurred rapidly in early 1992, but recovery stabilized thereafter for the remainder of the 2-year period. Some sclerotia may have been incompletely formed prior to burial in 1992. Only a small percent of newly formed sclerotia survived first-year flooding in 1992 or 2004 or 2 years of flooding in 2004-2005; no sclerotia survived 2 years of flooding in 1992-1993. In contrast, for sclerotia immersed in irrigated soil for a year during 1992 or 2004, 80 percent survived flooding in 1993 or 2005. These results suggest that newly formed sclerotia matured in irrigated soil such that their survival was enhanced under later flooded conditions. While survival of older, naturally formed sclerotia was not studied, these results suggest good potential for high reduction or eradication of the white rot fungus from soils in cool regions if the soil is flooded soon after harvest of an infected Allium crop. Flooding may be less effective if delayed.

Introduction

Sclerotium cepivorum reproduces and survives in soil as poppy-seed-sized sclerotia. Sclerotia have a black rind, which is leathery when wet. The rind is tough, shrinking and swelling as sclerotia wet and dry in soil. While persistent for many years under normal field environments, these sclerotia will germinate or die if the sclerotial rind is damaged. Incompletely formed sclerotia do not persist when formed on decaying Allium tissue (Andrew Entwisle, personal communication, and F. Crowe, personal observation).

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).

In the western United States, disease incidence in full-season Allium species may be 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.

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 50-80 percent compared to 10 percent annual decline in nonflooded soil (Banks and Edgington 1989). Nevertheless, winter flooding was insufficient to reduce Allium white rot disease incidence to commercially acceptable levels in a single flooded winter.

The impact of multiple years of winter flooding, and of flooding when water temperature is warm, has not been fully evaluated under field conditions. An initially high inoculum density declined to less than 0.1 sclerotia/l soil (but short of total eradication) after 21 weeks of continuous summer-fall 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 on field experiments to further evaluate the survival of sclerotia of *S. cepivorum* during summer flooding in a high, cool desert environment near Tulelake, California and Klamath Falls, Oregon. Summer flooding of reclaimed lakebeds in this region is feasible and might be justified commercially if combined control of diseases, weeds, nematodes, and insect pests could be achieved. Allium white rot is a component of the local disease complex in this region.

Methods and Materials

Sclerotia of *S. cepivorum,* produced on sterile oats in the laboratory, were mixed with 115 ml noninfested Tulelake volcanic soil (20 percent organic matter), 1,000+/-45 sclerotia (in 1992) and 800+/-35 (for 2004). 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 10 (in 1992) or 8 (in 2004) per 19-l micro-plot bucket, in a circular 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 presence of the nylon did not affect flooding or sclerotial behavior in buried chambers (Crowe and Carlson 1994).

Micro-plot buckets were replicated four times for each treatment in a fully randomized experimental design. The trials were located in a turfed area at the University of California Intermountain Research and Extension Center (1,231 m; 4,040 ft). 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).

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. All weeds were removed as seedlings from the micro-plots, and irrigation appropriate to maintain the turf was applied, approximately 3 cm each 2 weeks.

A double bucket arrangement allowed for micro-plots to be either flooded or irrigated with normal soil drainage. Flood and nonflood episodes were initiated by moving the inner buckets to either perforated or nonperforated 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: In the first trial, flooding was initiated May 15, 1992 and terminated March 10, 1993. In the second trial, flooding was initiated May 24, 2004 and terminated March 1, 2005.

Flood 2 years: In the first trial, flooding was initiated May 15, 1992 and terminated November 30, 1993. In the second trial, flooding was initiated May 24, 2004 and terminated November 5, 2005.

Second-year flood only: In the first trial, flooding was initiated May 10, 1993 and terminated November 30, 1993. In the second trial, flood treatment was initiated March 15, 2005 and terminated November 5, 2005.

Nonflooded: For the first trial, chambers for all nonflooded plots were placed in perforated outer buckets May 15, 1992 and received seasonal sprinkler irrigation May 15 to November 30, 1992 and May 10 to November 30, 1993, along with the year-round low natural precipitation for this arid region. For the second trial, nonflooded plots received sprinkler irrigation June 1 to November 30, 2004 and March 1 to November 5, 2005, along with natural low precipitation.

Soil temperatures and precipitation were recorded by an automated University of California weather station, at 15 cm (6 inches) soil depth from irrigated turf adjacent to the micro-plot trial area. Additional temperature recording units were placed 15 cm deep into flooded plots during 2005.

One chamber was removed from all micro-plot buckets within 1 week of burial, just prior to initiation of first flooding. This sample served as the pretreatment recovery baseline measurement of sclerotia for all future comparisons. In the 1992-1993 trial, remaining canisters in each micro-plot bucket were sampled on an irregular schedule established separately for each treatment (see data graphs). In the 2004-2005 trial, remaining chambers in each micro-plot bucket were removed every 2 months, in July, September, and November of each year. For each sampling date, chambers were recovered randomly from each micro-plot bucket. Chambers and soil were frozen until assayed 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 the 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 (water 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. Records also were kept for sclerotia that proved viable for *S. cepivorum* but from which other microorganisms also grew (e.g., bacteria, fungi).

Prior to burial in soil in 1992 and 2004, sclerotia were 100 percent viable.

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

Data were expressed as (a) the mean number of recovered intact sclerotial bodies, and (b) percent viability of intact sclerotia. Additional data were calculated as (c) the mean number of intact viable sclerotia recovered. Means and standard deviations for each of these are shown along with graphs of data in Figures 1 through 4. Analysis of variance among treatments was determined for synchronous sampling dates in 2004-2005, but is not shown. In general, wide spacing among graphed data is indicative of statistically significant differences (5 percent). For asynchronous sampling dates in 1992-1993, analysis of variance within a sampling date usually was not possible.

Nonflooded treatment (Fig. 1): In 1992, 1 week after initial burial of approximately 1,000 sclerotia per canister, between 900 and 1,000 sclerotia were recovered from all canisters. During the first few months of burial, the number of sclerotial bodies recovered from nonflooded plots fell to around 700 intact sclerotia and viability declined to around 75 percent. Further recoveries for the nonflooded treatment stabilized at about this level, slowly declining over the 2-year period through 1993. In 2004, 1 week after initial burial of approximately 800 sclerotia per canister, essentially all 800 were recovered with full (100 percent) viability. Further recoveries through the 2-year period were consistently similar, with no measurable attrition. It seems likely that perhaps 25 percent of the sclerotia buried in the first trial may have been incompletely formed and decayed quickly, as this did not occur in the second trial. Alternatively, the closer packing of sclerotia in the first trial may have led to higher attrition due to predation or pathology.

Flooded first year only (Fig. 2): In 1992, the number of intact sclerotial bodies recovered slowly declined, but the viability of intact sclerotia rapidly declined. By November 1992, viability was only 2 percent. Further recovery of chambers from these plots under irrigation during 1993 indicated that the number of intact sclerotia further declined slowly and that germination of these sclerotia remained very low. Apparent increase in viability does *not* reflect reproduction or changes in viability per sclerotium, but rather demonstrates that some sclerotia retained full viability as intact while the background of dead sclerotial bodies finally rotted away, i.e., only that higher proportion of remaining intact bodies were viable. In 2004, the number of intact sclerotial bodies recovered declined more rapidly than in 1992, but the percentage viability declined more slowly than in 1992. The calculated graph of intact viable sclerotia is similar for both 1992 and 2004, but survival was somewhat higher for 2004 than 1992, which again may reflect an initially more vigorous population of sclerotia in 2004.

Flooded 2 years (Fig. 3): For plots flooded 2 years, the trends seem to be simple extensions of first year flooding (Fig. 2). Sclerotial bodies recovered stabilized somewhat, but viability continued to fall. At the end of 1992, no intact viable sclerotia were recovered. At the end of 2005, a very few intact, viable sclerotia were recovered.

Flooded second year only (Fig. 4): For plots that were flooded beginning a year after burial of canisters, recovery of sclerotia in the first year under irrigation was (as expected) comparable to recovery for nonflooded plots under irrigation. However, during flooding in the second year, the number of sclerotial bodies recovered did not much decline, nor did the viability of sclerotia drop greatly. In addition, declines measured for both parameters were delayed until mid-season, compared to first-year flooding. For viability, this is better seen in Figure 4 for 2005, because some data were excluded from1993 because of technical problems in the laboratory. For second-year-only flooding in 1993 and 2005, the number of calculated intact viable sclerotia was perhaps only 20 percent less than the initially stabilized population in nonflooded soil, in contrast to over 99 percent reduction for first-year-only and 2-year flooding.

Growth of other fungi or bacteria from sclerotia was rare from nonflooded plots, but increased slightly for sclerotia recovered from flooded plots (a few percent at most, data not shown). For sclerotia recovered from flooded plots, other microorganisms sometimes grew from intact viable sclerotia, but this still was only a few percent.

Average daily soil temperatures at 15 cm were 5-16°C for spring and fall for 1992 and 1993. Mid-summer soil temperatures were 19-23°C for 1992 and 15-19°C for 1993. For 2004, spring and fall soil temperatures were 10-13°C for May and October, and 4.5° for mid-November; mid-summer soil temperature reached 21°C.

Discussion

In previous studies, for sclerotia recovered from "normal" field soil, 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. For these flooding trials, our measure of viability simply was whether sclerotia retained the ability to grow on agar. We did not determine whether living sclerotia retained an ability to germinate by stimulation. Conceivably, the ability to respond to germination stimulants could be at least temporarily altered during flooding. It may be interesting to discover whether sclerotia that survive flooding retain normal behavior when Allium crops are replanted.

In this study, numerous intact and normal-looking sclerotial bodies were recovered that did not test viable. Rotting of dead bodies generally took a number of months. It has been previously noticed for sclerotia killed by soil fumigation that dead sclerotial bodies may take up to a year to decay. Intact but dead, they continue to shrink and swell with normal soil wetting, retain their normal internal appearance and rind integrity and few if any other microorganisms grow from them following 2-min surface sterilization with 0.5 percent sodium hypochlorite. On the other hand, sclerotia killed by hot water may have damaged rinds and immediately are invaded by other fungi and bacteria (F. Crowe, unpublished data). Of course, simply nicking the rind of living sclerotia stimulates a

germination response. While rind integrity may not be directly related to viability, rind disruption leads directly to either growth of *S. cepivorum* or to invasion and decay of the sclerotium.

The population of sclerotia used in 2004-2005 appears to have been more tolerant to flooding compared to the population used in 1992-1993. Both were grown in the laboratory, and flooding was initiated within a week was dampened at the start of the field trials. Whether these lab-grown sclerotia respond similarly to those that form more naturally on garlic or onions in the field is of concern. Our initial assumption was (and is) that newly formed sclerotia behave similarly in soil irrespective of where they were formed, even though the microbiological associations with sclerotia that arise on infected Alliums that naturally decay in soil initially are absent from lab-grown sclerotia. The early attrition seen in our 1992 sclerotial population more likely was either partially damaged during production (e.g., screening to size them), or the rinds for some may have been incomplete to start with (e.g., premature harvest). Our best guess is that the sclerotia used in these first and second trials represent the range of expected responses to flooding in naturally infested fields.

We measured high survival for sclerotia that were flooded after being in soil for a year, and low survival for sclerotia flooded soon after being formed. This strongly suggests that sclerotia continued to mature in nonflooded soil. If so, this has not previously been described and should be investigated further. More practically, as a white rot management strategy; flooding should be implemented as soon after sclerotia form as possible. In the single commercial field that was flooded in central Oregon in 1990, flooding was initiated immediately at harvest (June) and very few sclerotia could be recovered after flooding (June-November). Soil temperatures in central Oregon are substantially similar to those in the Tulelake region of California.

Previous studies that reported rapid loss of sclerotia of *S. cepivorum* in flooded soil at higher temperatures utilized recently formed sclerotia taken directly from the laboratory (Crowe and Hall 1980). Such studies probably should be repeated using sclerotia with diverse soil associations. If summer flooding can successfully reduce 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 of effective treatment likely could be reduced; in a very warm area, perhaps a single, short flooding period might achieve full eradication.

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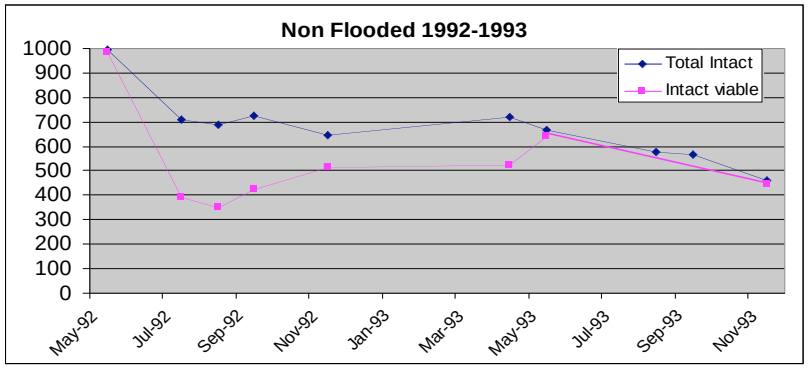
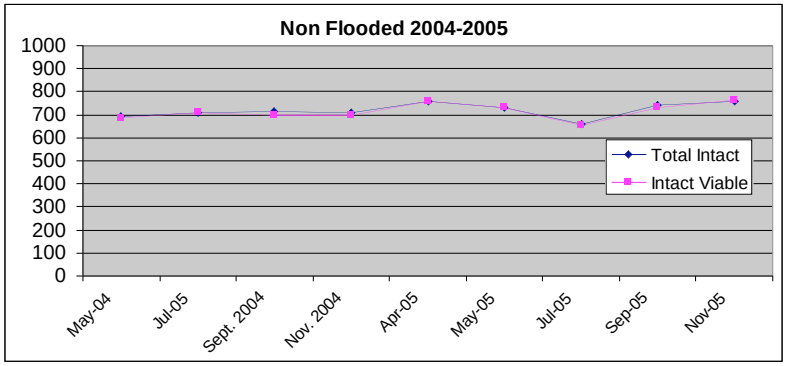
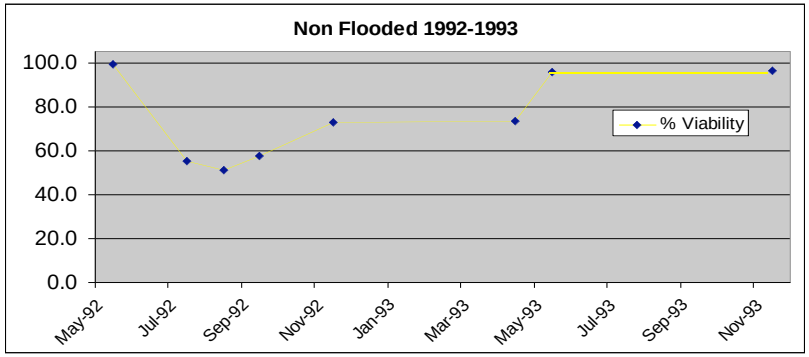
Figure 1. Means and standard deviations for total, intact sclerotial bodies, intact viable sclerotia, and percent viability of sclerotia of *Sclerotium cepivorum* recovered from *non-flooded* micro-plots at Tulelake, California, 1992-1993.

1992-1993	May	July	Aug	Sept	Nov	April	May	Sept	Nov
Total intact	995	710.5	687	727.5	643.1	717.8	667.3	568.3	459
St dev	*105*	*45.2*	*109.3*	*81.6*	*162.3*	*100.8*	*91.4*	*124.3*	*48.7*
Intact viable	986.7	391.6	347.9	422.5	515.3	525.6	641.4	-	442.4
St dev	*110.6*	*51.0*	*70.5*	*102.9*	*112.9*	*111.8*	*110.3*	-	*56.2*
% Viability	99.2	55.4	50.8	57.5	73.0	73.3	95.8	-	96.5
St dev.	*0.8*	*9.3*	*7.5*	*8.7*	*12.1*	*11.6*	*4.0*	-	*7.4*

2004-2005	May	July	Sept	Nov	April	May	July	Sept	Nov
Total intact	694	707.3	716.3	706.5	759.5	729.3	658.8	740.5	759.5
St dev	*16.8*	*46.2*	*10.7*	*15.5*	*79.6*	*63.5*	*95.7*	*21.4*	*13.9*
Intact viable	684.3	707.3	699.1	699.2	759.5	729.3	652.3	733.1	764.5
St dev	*22.0*	*46.2*	*20.3*	*15.9*	*79.6*	*63.5*	*97.9*	*27.1*	*13.9*
% Viability	98.6	100	97.6	99.0	100	100	99.0	99.0	100
St dev.	*1.0*	*0*	*1.9*	*1.2*	*0*	*0*	*1.2*	*1.2*	*0*







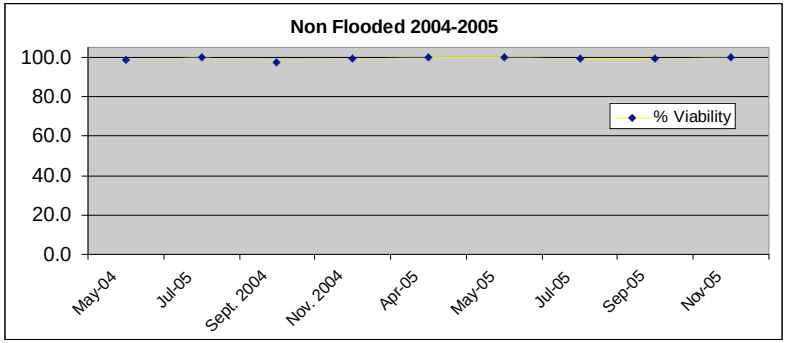
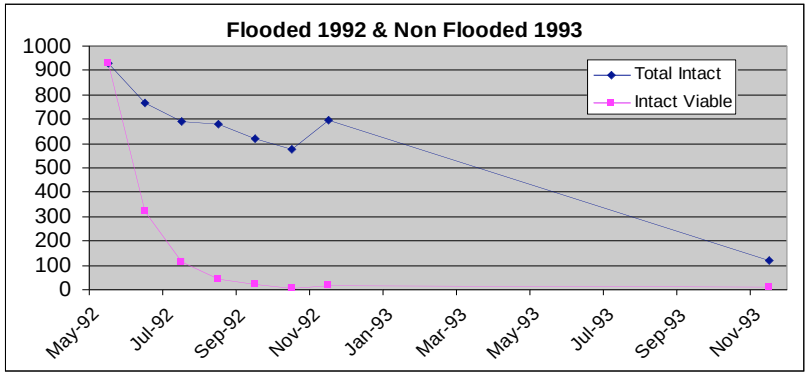
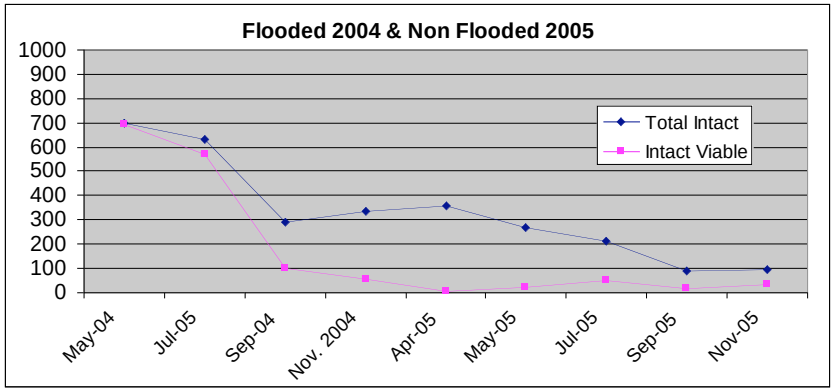


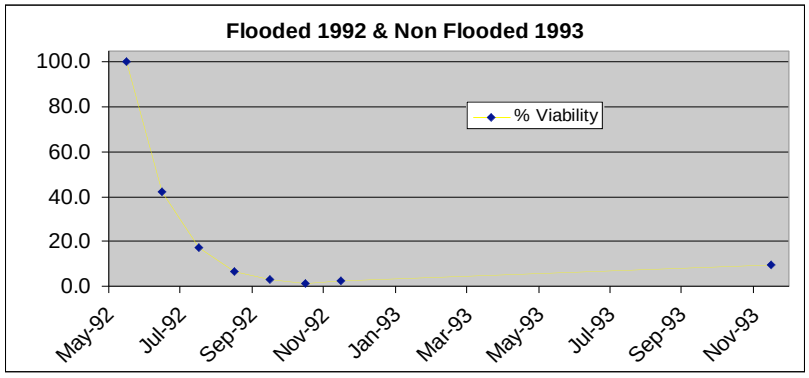
Figure 2. Means and standard deviations for total, intact sclerotial bodies, intact viable sclerotia, and percent viability of sclerotia of *Sclerotium cepivorum* recovered from micro-plots *flooded first season* of 2-year burial at Tulelake, California, 1992-1993 and 2004-2005.

1992-1993	May	June	July	Aug	Sept	Oct	Nov	Nov
Total intact	927	768.8	688.5	680.3	620	578.5	695.	120.3
St dev	*117.6*	*101.5*	*64.2*	*75.3*	*118.7*	*180.4*	*73.0*	*38.9*
Intact viable	927	323.3	115.4	43.6	19	7.4	15.6	10.7
St dev	*117.6*	*83.6*	*21.3*	*20.4*	*21.9*	*8.7*	*17.1*	*9.6*
% Viability	100	42.1	17.1	6.7	2.9	1.3	2.1	9.3
St dev.		*9.1*	*4.6*	*3.3*	*2.8*	*1.6*	*2.1*	*5.9*

2004-2005	May	July	Sept	Nov	April	May	July	Sept	Nov
Total intact	700.0	629.3	292.5	335.5	360.0	268.0	214.3	88.0	93.3
St dev	*9.8*	*22.9*	*200.4*	*171.5*	*145.5*	*164.2*	*49.5*	*65.8*	*90.1*
Intact viable	693.7	570.1	98.8	54.7	6.3	19.9	52.5	15.9	35.1
St dev	*13.0*	*44.6*	*56.6*	*70.0*	*4.7*	*15.4*	*38.3*	*4.9*	*22.1*
% Viability	99.1	90.6	33.8	14.8	1.5	7.7	23.9	24.7	45.7
St dev.	*1.0*	*7.5*	*5.6*	*17.4*	*1.0*	*4.2*	*13.7*	*14.0*	*17.8*







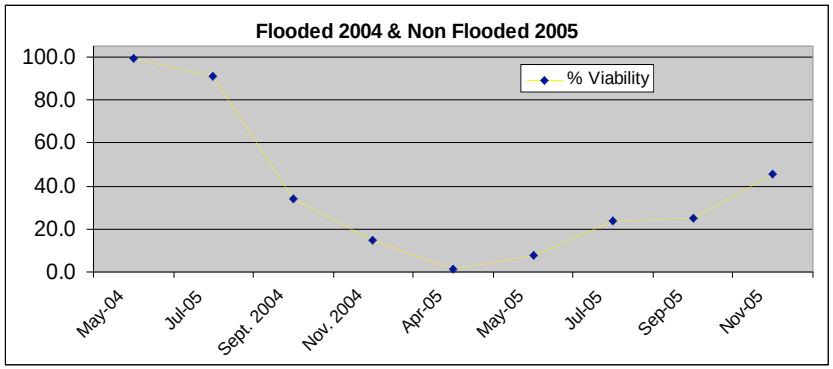
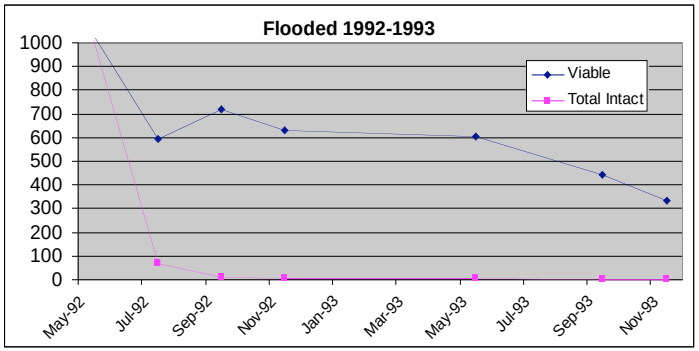
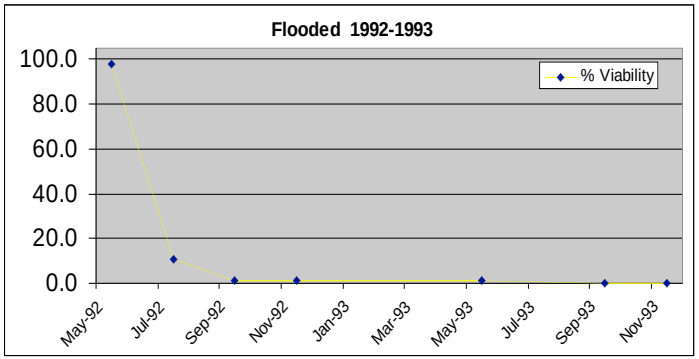


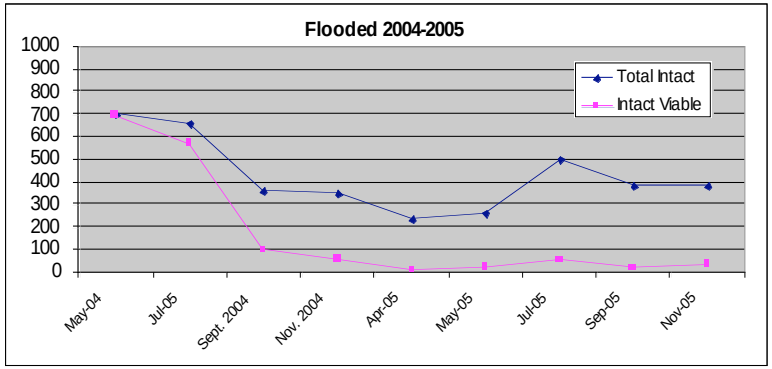
Figure 3. Means and standard deviations for total, intact sclerotial bodies, intact viable sclerotia, and percent viability of sclerotia of *Sclerotium cepivorum* recovered from micro-plots *continuously flooded* over the 2-year burial at Tulelake, California, 1992-1993 and 2004-

1992-1993	May	July	Sept	Nov	May	Sept	Nov
Total intact	1030.3	594.5	718.5	628.8	606	442.8	333.3
St dev	*120.2*	*119.5*	*150.6*	*21.7*	*69.8*	*139.3*	*196.4*
Intact viable	1009.1	65.1	10.2	7.6	6.9	0	0
St dev	*121.3*	*57.8*	*12.9*	*9.6*	*9.0*	*0*	*0*
% Viability	97.9	10.4	1.3	1.3	1.3	0	0
St dev.	*0.8*	*7.6*	*1.6*	*1.6*	*1.6*	*0*	*0*





2004-2005	May	July	Sept	Nov	April	May	July	Sept	Nov
Total intact	697	658.3	358.8	347	234.8	262.8	501.8	381	378.3
St dev	*27.6*	*17.1*	*102.3*	*108.7*	*131.5*	*85.6*	*72.6*	*70.9*	*134.9*
Intact viable	684.4	658.3	139.2	42.7	9.4	15.7	28.2	0	1.3
St dev	*23.7*	*20.6*	*45.6*	*13.8*	*16.1*	*18.7*	*43.0*	*0*	*2.7*
% Viability	98.2	94.2	33.8	13.8	2.6	6.1	5.1	0	0.5
St dev.	*2.1*	*4.0*	*18.0*	*7.7*	*3.9*	*8.3*	*7.5*	*0*	*1.0*



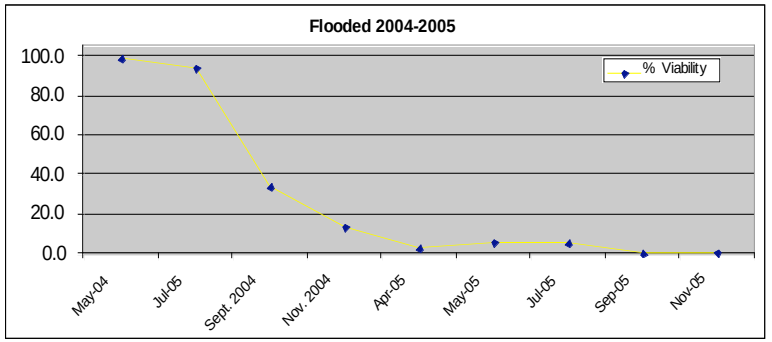


Figure 4. Means and standard deviations for total, intact sclerotial bodies, intact viable sclerotia, and percent viability of sclerotia of *Sclerotium cepivorum* recovered from micro-plots *flooded second year* of a 2-year burial at Tulelake, California, 1992-1993 and 2004-2005.

1992-1993	May	May	June	Aug	Oct	Nov
Total intact	931.3	772.8	605.5	703.5	694.7	515
St dev	*54.5*	*34.5*	*71.4*	*98.3*	*69.4*	*58.1*
Intact viable	916	582.7	-	-	670	512.7
St dev	*63.0*	*340.5*	-	-	*85.1*	*55.8*
% Viability	98.3	74.6	-	-	96.3	99.6
St dev.	*2.4*	*43.1*	-	-	*2.8*	*0.8*

2004-2005	May	July	Sept	Nov	April	May	July	Sept	Nov
Total intact	691	698.2	609.3	684.3	721.5	754.3	769.8	681.8	652
St dev	*9.6*	*40.7*	*12.2*	*34.4*	*43.1*	*69.9*	*54.5*	*23.8*	*54.1*
Intact viable	678.6	698.2	587.4	684.3	717.6	750.1	758.1	545.3	502.7
St dev	*23.7*	*43.2*	*25.7*	*34.4*	*40.7*	*66.1*	*57.4*	*85.0*	*70.6*
% Viability	98.2	99.2	96.4	100	99.5	99.5	98.5	80.1	77.0
St dev.	*2.5*	*1.7*	*3.6*	*0*	*1.0*	*1.0*	*1.0*	*13.2*	*8.1*