EFFECTS OF HOT WATER AND FORMALDEHYDE SEED TREATMENTS ON SCLEROTIA OF THE WHITE ROT FUNGUS

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ABSTRACT

All garlic cloves infected at harvest with the white rot fungus (Sclerotium cepivorum) decayed while in storage. The white rot fungus was not recovered from any decayed trashy material in this seedlot other than sclerotia. Nevertheless some sclerotia of the white rot fungus survived traditional hot water-formaldehyde seed treatment processes. Those surviving were aggregated within decayed cloves, which might pass through the mechanical processors associated with cracking and seed treatment. Reduction in time and/or temperature of the treatment solutions allowed partial survival even of unaggregated sclerotia. It remains unclear if sclerotia surviving hot water treatments are fully competent.

Hypothetical Question: May the garlic industry feel secure that the standard Lear-Johnson seed treatment, and possibly milder variations of it, will eradicate the white rot fungus from a seed lot.

METHODS

In the summer of 1987, a bag of garlic infected with the white rot fungus (Sclerotium cepivorum) was collected in Monterey County CA, and shipped to the University of California Tulelake Field Station in Tulelake, CA. It was held as per normal seed storage until January, 1988. In January, 1988, the stored garlic was separated into several components: 1) loose sclerotia and 2) fully-decayed cloves in which abundant sclerotia were aggregated together with clove tissue and mycelium ("aggregates"), these varied from 0.5 mm to 1.5 mm in various irregular cross sections and were calculated to contain from 4,000-25,000 sclerotia each and 3) cloves which appeared normally developed and uninfected (no signs of the fungus on either stem plates or covering leaf sheaths).

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An attempt was made to find cloves on which the fungus appeared to be present either as partly decayed covering leaf sheaths, partly-decayed storage leaves or partly-decayed stem plates, but none were found -- it appeared as if all active mycelium present at harvest had progressed in storage to fully decay any cloves which had been infected. Bulbs were identified which had entirely decayed, but most had only certain cloves decayed, and these apparently were fully-decayed into either loose sclerotia or aggregates. If additional cloves became infected during storage, these had fully decayed by January.

The garlic was hand cracked and all variations of the above components of the infested/infected seedlot were placed separately within loose single-layer bags of cheesecloth and suspended and treated (in Tulelake) as listed in Table 1. All treatments utilized a single laboratory hot water bath containing 40 liters of treatment solution. All treatment solutions were prepared on site, using tap water with or without laboratory sources of formalin. Once equilibriated, treatment solutions maintained the listed temperatures within 1 degree Fahrenheit. This was true even after bags were immersed in the solution -- at the most, less than 250 g of material was present in any solution at one time. Even though only one bath was utilized, practice in removing and then adding set amounts of additional treatments solutions prepared with lab heating devices allowed solution type and/or temperature to be adjusted in less than 30 seconds. Some treatments were run simultaneously, and all treatments were completed within 3 hours. Following treatment, all bags were drained on paper towels on a lab bench overnight, then stored in open paper bags. The next day, all treated material was transferred to the lab of the Central Oregon Experiment Station in Redmond, OR. At that time, all materials appeared air-dry, with no free surface moisture.

Materials were cultured within 7-8 days following seed treatment. Treated materials were divided into four lots of each type and plated onto both plain agar (WA) and potato dextrose agar (PDA), following surface sterilization for 2 minutes with 0.05 percent sodium hypochlorite. From both intact and decayed bulbs, pieces of covering bulb leaf sheathes, clove protective leaves and stem plates were plated with or without surface sterilization. Additionally, intact and apparently uninfected cloves from bulbs with nearby decayed cloves were similarly cultured for the white rot fungus. Sclerotia on WA were cracked with forceps and on PDA they were not. Aggregates were separated into sclerotia from their surfaces and sclerotia from their centers. Following the experiments, all active white rot fungus, and sclerotia were destroyed.

Analysis of variance was developed for data from the four lots of each material type cultured after treatment. The validity of such analysis is discussed below.

RESULTS

No Sclerotium cepivorum grew from any living or decayed garlic tissue on either medium with or without surface sterilization. Roughly 40 pieces of each material described above were plated and intact cloves were cultured from several locations in addition to stem plates. Other organisms cultured from these materials included a typical range of bacteria and other fungi, but these fungi were not predominated by Trichoderma and Penicillium as reported below for sclerotia.

Two hundred sclerotia were plated from each of four treatment lots. Sclerotia plated on PDA were difficult to assess for germination, due to abundance and rapid advance of other organisms present, especially bacteria, and Trichoderma and Penicillium fungi, and also due to irregular germination of S. cepivorum. Such confirmation problems have been found in other studies and is the reason that the use of WA with cracking was developed. Cracking elicits simultaneous growth of the white rot fungus. WA discourages aggressive competitive growth, and encourages formation of characteristic spermatia-like structures, for easy identification of S. cepivorum. The same other contaminating organisms also grew from many sclerotia placed on WA, but did not seem to interfere with growth of S. cepivorum from the same sclerotium. A presumption was made that if S. cepivorum did not grow from a sclerotium, then it was not alive within that sclerotium at the time of culturing. If S. cepivorum grew from a sclerotium, the sclerotium was rated as germinable. These presumptions proved reliable in previous studies in which statistical comparisons were made between sclerotia plated on various growth media and in various manners compared with sclerotia naturally stimulated to germinate in soil (1). Although not listed within Table 1, the amount of contamination due to the above organisms was far less on treatments A (i.e. water at room temperature) and F (i.e. the standard Lear-Johnson treatment) than on any "intermediate" treatment. Detailed notes on degree of sclerotium contamination were not developed.

Sclerotia on the surface of aggregates behaved similarly to loose sclerotia. The percentage germination for aggregated and unaggregated (loose) sclerotia for the various seed treatments is listed in Table 1. Most sclerotia (85-89 percent) were alive and germinable with water treatment at room temperature. Progressive reduction in germinability was achieved with progressive harshness of treatment, both higher temperatures and longer durations. Maximum effect

was achieved with the Lear-Johnson treatment, both with and without 1 percent formalin. For each treatment, sclerotia in the center of aggregates survived better than loose sclerotia, and approximately 20 percent of sclerotia near the center of aggregates survived the Lear-Johnson treatment, with and without 1 percent formalin.

DISCUSSION

The study can be criticized in at least one major technical way; because there was inadequate decayed garlic in the stored lot to provide many aggregates and decayed cloves, replications of each treatment series were not conducted. In other words, each listed treatment was only prepared once, with all the sets of garlic materials immersed in each one. Statistics presented in Table 3 thus were developed on only "pseudo-replications", wherein the treated material were divided into four batches after treatment. Done properly, each separate treatment would have been prepared four separate times, since the solutions and temperatures series are what were under investigation. Future investigations should provide true replication of treatment solutions.

Clearly, each increase in duration and temperature decreased the survival of the white rot fungus. At intermediate treatments, there was substantial survival of the pathogen, but also substantial occurrence of other micro-organisms within the sclerotia. Conceivably, sclerotia in which substantial bacteria, Trichoderma, and Penicillium developed might have been less effective in eliciting white rot disease, but this was not determined. Conservatively, this can not be assumed, since previous studies have indicated that contamination of living sclerotia can exist which still allows sclerotia to be able to germinate and infect Allium crops (1). However, additional testing may show that, following hot water treatment, 1) sclerotia from which contaminating organisms are abundant, are in fact greatly incapacitated from causing disease, and/or 2) with longer storage, these sclerotia might become fully decayed by the contaminating organisms.

With treatments E and F, the Lear-Johnson temperature and duration series without and with 1 percent formalin, respectively, there was little contamination of plates with other organisms. Presumably, most contaminants also succumbed to these more severe treatment conditions.

There was roughly 20 percent survival of S. cepivorum within the interior of aggregates. Presumably, this survival reflected reduced heat and/or moisture penetration into the interior of aggregates.

Loose sclerotia might pass through the seed treatment process held within leaf sheaths and protective leaves, and between doubled cloves, etc. Aggregates might have substantial enough mass to simulate cloves during the cracking, blowing and seed treatment processes. In either case, the bodies of sclerotia might pass through a seedlot and become planted in the field. With the Lear-Johnson treatment, the primary risk would be from aggregates, since loose sclerotia and those directly contacting the treatment solution would die. Less severe seed treatments might allow even loose sclerotia to survive through the seed treatment process. Formalin, at least within the usage in this study, did not increase the effectiveness of the Lear-Johnson temperatures and durations. [In other unpublished studies, it has been shown that formaldehyde solutions can kill sclerotia, but that temperature and duration of treatment is critical.]

[Note: in discussion with Mike Davis, Univ. Calif. Davis, survival might even be greater if sclerotia were tested for survival immediately following seed treatments. In the study above, there was a week's delay between treatment and culturing. The other organisms found in sclerotia in Oregon might have developed aggressively during this post-treatment period. Future investigation might further clarify if a time delay effects sclerotia survival following treatment.]

REFERENCES

1. Crowe, F.J., D.H. Hall, A.S. Greathead, and K.G. Baghott. 1980. Inoculum density of Sclerotium cepivorum and the incidence of white rot of onion and garlic. Phytopathology 70:64-69.

Table 1. Effect of Garlic Seed Treatments on Germinability of Sclerotia of Sclerotium Cepivorum recovered from garlic affected by white rot disease

Trt.	Temp, Time	% Germinability	
		Unaggregated[a] sclerotia	Aggregated[b] sclerotia
A. Water	62F, 20 min.	85	89
B. Water	100F, 30 min.		
Water	62F, 10 min.	20[c]	76[c]
C. Water	120F, 10 min.		
Water	62F, 10 min.	14[c]	30[c]
D. Water	120F, 20 min.		
Water	62F, 10 min.	18	20
E. Water	100F, 30 min.		
Water	120F, 20 min.		
Water	62F, 10 min.	0	18
F. 1% formalin	100F, 30 min.		
1% formalin	120F, 20 min.		
Water (Lear-Johnson)	62F, 10 min.	0	24
LSD ($P \leq 0.05$), all treatments[d]		--------11--------	

a Sclerotia already loose within fully decayed cloves. Results were similar for sclerotia recovered from the exterior of aggregates.

b Sclerotia held en masse (4,000-25,000 per clove) by dried mycelium and garlic tissue within partially-decayed, dried cloves. Data here are shown only for those from the interior of aggregates.

c Bacteria, Trichoderma and Penicillium grew extensively from most sclerotia from these treatments.

d LSD ($P \leq 0.05$) calculated according to Duncan's new multiple range test. Technically, this analysis is invalid, since hot water seed treatments were not replicated, and treated lots of sclerotia taken from each treatment were divided into pseudo-replications.