CONTINUED INVESTIGATION INTO THE EPIDEMIOLOGY AND CONTROL OF FUSARIUM BULB* ROT OF GARLIC

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ABSTRACT

As in past years, inoculum of Fusarium roseum culmorum placed into soil with garlic seed at planting in 1985, incited preemergence plant loss and extensive seasonal bulb rot in 1986; however, the importance of holdover soil-borne sources of the fungus remains uninvestigated. In uninfested field soil, about 10 and 30% loss to fusarium bulb rot by harvest occurred from two separate seed sources that had received hot water + formalin treatment. The extent of symptoms in fields in which these seed lots were produced was not a good indicator of disease occurence in the seed lot plantings. Furthermore, in identical test plantings in western Oregon and California. little fusarium bulb rot developed in either of these same seed lots. Bulbs infected at harvest, including those with and those without symptoms or signs of the fungus but considered intact enough to pass into seed lot storage, continued to decay for several months until bulbs and cloves were

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Note: Previous reports have described this disease as "basal" rot; however, it recently became known that the onion basal rot organism (<u>Fusarium oxysporum</u>) may infect garlic and cause mild disease under certain conditions. Thus, to avoid future confusion, basal rot should be reserved for <u>F. oxysporum</u> diseases of all <u>Allium</u> species. We have switched to "bulb" rot for disease of <u>Allium</u> species caused by Fusarium roseum. totally decayed, or until extensive drying caused the fungus to become inactive. Many bulbs and cloves were shown to harbor the inactive fungus, either anywhere on the clove if partial rot was present, or in the clove stem plate if no signs or symptoms were present. Fungal isolations from cloves indicated that 50% of seedborne fusarium could survive the standard commercially hot water + formalin seed treatment utilized for stem and bulb nematode. Benlate and Mertect seed treatments prevented fusarium bulb rot from developing in the field from seedborne sources, and reduced the disease developing from soil infestation.

INTRODUCTION

Fusarium basal rot was first identified in 1976 in the Salinas Valley of California, inciting extensive bulb decay in a commercial garlic field. The causal agent, Fusarium roseum culmorum also is able to attack roots and other underground seedling structures of wheat, barley, and oat, but the specialized strain which damages garlic can be much more damaging to garlic than the fungus generally is to cereals. Most \underline{F} . roseum culmorum isolates from soil or cereals do not have the capacity to attack garlic in this manner. Garlic isolates may decay stem plates, covering leaf sheaths and cloves at all stages of garlic growth. Garlic seed growers and commercial growers have been concerned with the impact, spread, and control of this new disease. Losses have occurred every year in central Oregon since the disease was first noted. In 1983, losses in central Oregon garlic seed fields were widespread and disrupted the seed supplies to the commercial industry in California. In other years, losses in central Oregon have been less, but substantial in some fields. In most years, other parts of Oregon, California, and Nevada report less damage in the field than central Oregon.

Trials were established in 1984 and 1985 at the Madras field of the Central Oregon Experiment Station to investigate questions of epidemiology and control of this disease. These studies complement similar disease studies in California and in the Willamette Valley of Oregon. Results from previous trials in 1984-85 are reported in the Irrigated Crops Research in Central Oregon - 1986, Special Report 780.

The objectives of the 1985-86 central Oregon field research were:

1. To gather epidemiological evidence to help determine the role of seed and soilborne inoculum in fusarium disease spread and development. Various typical control measures were utilized to help in making these distinctions, as were several different seed lots selected from fields with differing incidence of fusarium disease in 1984-85 (continuing objective).

- Determining the effectiveness of various standard control measures on fusarium diseases (continuing objective).
- 3. To determine the post-harvest impact of fusarium on garlic seed held in storage (new objective).

MATERIALS AND METHODS

Bulbs from two different sources of a Basic American Foods virus-infected 'California Late' clone were collected at harvest in the summer/ fall of 1985, and stored in King City until treatment. Seed lots were selected on the basis of fusarium incidence, but also were chosen for the high probability of freedom of stem and bulb nematode Ditylenchus dipsaci infection. Trials 1 and 4 included seed labeled "Cassaca", from a field in California in which fusarium disease incidence was very high in 1984-85. Trials 2 and 3 included seed labeled "Nevada", from a field in Nevada in which no fusarium or very little fusarium symptoms were observed in 1984-85. Bulbs were cracked under commercial conditions, and hot water and formalin treatments for the stem and bulb nematode control, if done, were treated on the day of, or within 24 hours of cracking as described below. Previous studies indicated that this treatment for nematodes affected the incidence and severity of fusarium disease, perhaps making it worse.

All hot water and formalin clove treatments were made in King City, California, using 50-gallon, temperature controlled, experimental treatment tanks. Nematode eradicative treatments involving the University of California method of 30 minutes at 100 degrees F, followed by 20 minutes at 120 degrees F, both with 1% Formalin, followed by a 15-minute cold dip, and finally followed by drying at 90 degrees F were the standard treatment. Certain variations on this treatment with respect to temperature and time in Trial 1 are shown in Table 1. For additional treatments against fungal diseases such as penicillium seed rot, botrytis disease and fusarium disease, fungicides were added to the cold dip part of the seed treatments process. Formulations and rates of application were Benlate 50W and Mertect 340F as batch seed dips with 2 lbs a.i./100 lbs seed. In one trial, common household bleach (0.5% NaOC1) was added at a rate of 10 gal product/100 lbs seed in the same manner as fungicides. After treatment, all cloves were stored under ambient conditions until shipment to Madras, Oregon, in late September 1984.

F. roseum culmorum isolated from diseased garlic was grown in August and September 1985 on sterile barley. After air-drying, the decayed barley was ground and stored air dry. Twenty-five gm inoculum/plot was sprinkled by hand into open seedlines just before planting.

Cloves for individual treatments were counted by hand for each seedline and stored until planting. Clove weights were maintained as uniform as possible to reduce the influence of clove size. Seedlines were opened by hand using garden hoes. Individual trials were hand planted into two seedlines per bed, and then closed with a hoe. Plots were all single bed plots, 10 feet long, and separated by a 2-foot alley unless noted. All garlic was planted at 20 cloves per bed foot, on 36-inch beds center-to-center. All treatments were in randomized block design and data were analyzed by standard analysis of variance methods.

The field received 400 lbs/A 16-16-16 in September 1985. Residual nitrogen in the soil was about 8 lbs/Ac. In mid-April, 1986, 150 lbs/A of nitrogen as ammonium nitrate was applied. Standard chemical weed control was supplemented with some hand weeding. The field was watered by solid set sprinkler irrigation according to local requirements.

Notes were collected weekly during the season following emergence. After drydown of the crop, plants were dug from each plot on July 16, the soil was shaken from the roots and the plants were then laid on top of the plot bed section to dry further. Harvest data were collected on July 22. Plants were observed for disease, and bulbs weighed after roots below the stem plate and foliage higher than 2 cm above the bulb were removed. Bulbs were considered harvestable if stem plates and covering leaf sheaths were intact upon squeezing the bulb by hand. This harvestability criterion did not distinguish between non-infected and infected bulbs, but was developed to simulate commercial harvest practices in which only bulbs diseased enough to shatter during the harvest process are excluded.

After harvest, bulbs from Trials 3 and 4 were stored in open paper bags on wood pallets in a covered shed. No bulbs were layered greater than 10 cm deep in the bag. On August 29, 1986, stored bulbs were re-examined as follows: Class A bulbs had no apparent decay or signs of fusarium growth; bulbs were firm and intact on squeezing, and were not shriveled. Class B bulbs were "weighty", but had partial or general internal softness, sometimes with signs of fusarium growth somewhere on the bulbs. Class C bulbs were extremely light bulbs, usually with intact covering leaf sheathes, but sometimes with external signs of decay and deterioration of stem plate or leaf sheaths; almost always these were dry and brittle, and usually were slightly shriveled. There was no evidence of "nesting" of decay among bulbs within the stored lots. Bulbs from classes A and B were weighed and counted. Cloves were hand cracked from bulbs of each class and graded into subclasses as below. Class C bulbs were cracked or cut across the bulb through all cloves and inspected for similar subclasses.

Subclass 1 included cloves which were visually with no signs, symptoms, or defects. Subclass 2 included cloves which were considered intact, viable cloves at the time of inspection, but which had signs of limited <u>F</u>. roseum infection somewhere on the clove. Subclass 3 included cloves some of which were possibly viable at that time, but which were partially-to-extensively decayed with fusarium and which were not considered likely to grow if planted. Subclass 4 included cloves which were fully decayed with <u>F</u>. roseum or with organisms such as penicillium or aspergillus.

Seed from most A and B subclasses was split into fractions, with some fractions then receiving standard hot water/formalin seed treatment in a small, lab bench batch process. Isolations onto potato dextrose agar (PDA) were made from both treated and untreated cloves, some without surface disinfestation with 0.05% sodium hypochlorite. Both treated and untreated cloves from most classes were planted in the field in October 1986, for disease evaluation in 1987. Reinoculations into symptomless cloves were made with isolations to verify isolate pathogenicity.

Additional cloves were separated into various parts, including stem plates, protective leaf, storage leaf, and first leaves for the next growth period, to determine the common locations of seedborne fusarium and other fungi.

RESULTS

Fusarium trials are summarized in Tables 1 through 4. Stand was reduced in some treatments where inoculum was added to the seed line and where no fungicides were utilized. Stands also were reduced when the cold dip treatment was eliminated and where the hot water treatment of 115F was extended for 2 hour (Trial 1, Table 1). Stand also was reduced in Trial 2 and 3 for both untreated seed and standardly treated seed. With the 'Nevada' seed lot, extensive seedborne fusarium disease was present where Benlate and Mertect were not utilized. For the 'Cassaca' seed lot, some seedborne fusarium was present, but less than with the 'Nevada' seed lot. As in past studies, inoculum added to the soil of the seed bed planting lines effectively killed most of the garlic (Trials 2 and 3), although both fungicides greatly delayed and reduced the amount of disease from this inoculum source. Fusarium bulb rot symptoms were noted early on some plants as stunted growth, although these plants generally died within a week or two. Disease

symptoms continued to appear through the season. A few plants, pulled at various times, revealed stem and lower bulb decay, and usually a reddish color in the decay area (not purple, which is a normal response at certain times). No botrytis or other disease was seen, so all mid-season symptoms were assumed to be from fusarium bulb rot. At harvest, badly affected plants had pithy stem plates and usually some degree of leaf sheath and clove decay. Reddish discoloration was not always present at harvest.

For bulbs held in storage for 5 weeks, classes A and B were weighed and these weights are listed in Tables 3 and 4; Class C contributed no significant weight to the total (less than 0.01 kg, the lower limit of our field scales). Bulbs per class were counted. There was no evidence of "nesting" of decay within the stored lots, although it was evident that fusarium had been and still was actively rotting cloves within bulbs.

For 5.45 kg of class A bulbs from combined field treatments, subclass Al included 5.27 kg (97%) of the cloves, and combined subclasses A2 + A3 included 0.18 kg (3%) of the cloves, which were found partially-to-fully decayed with \underline{F} . roseum, usually just an occasional clove amongst good ones. Some class A bulbs had more extensive internal rot of numerous cloves, but exterior cloves and covering leaf sheaths were intact. No subclass 4 cloves were found among A bulbs at the date of inspection.

For 3.15 kg of Class B bulbs, all bulbs had several to all cloves in some process of decay to F. roseum and/or other organisms. Subclass B-1 included 1.58 kg (50%) of the cloves, subclass B-2 included 0.6 kg (19%) of the cloves, subclass B-3 included 0.73 kg (23%) of the cloves, and subclass B-4 included 0.73 kg (8%) of cloves. Subclass B-4 cloves were apparently decayed with F. roseum, but other organisms such as penicillium or aspergillus frequently were present in addition; these cloves were almost always enough decayed to be considered non-viable.

Class C bulbs were cracked or cut across the bulb through all cloves. No cloves were found fully intact; bulbs were hollow shells except that clove protective sheaths usually were intact. Mycelium of \underline{F} . roseum generally filled the clove positions, with some occasional presence of penicillium and aspergillus.

Table 5 summarizes the percentage by weight of cloves within each class and subclass.

A summary of isolations from treated and untreated cloves from various subclasses is shown in Table 6. <u>F. roseum culmorum</u> was reisolated in approximately equal proportions from both surface sterilized and on-surface sterilized seed. All subclasses contained \underline{F} . roseum, but with increasing amounts when signs of the fungus were greater. Even symptomless cloves, however, still continued 25-28% infection before hot water/ formalin seed treatment. Following this treatment, incidence of recovery of \underline{F} . roseum from cloves was reduced to 10-16%. Thus, hot water/formalin seed treatment reduced fusarium infection by about half. All \underline{F} . roseum culmorum isolates tested were capable of decaying symptomless garlic cloves when either wound inoculated into these cloves from PDA or even if mycelium from PDA was smeared onto clove stem plates without wounding. Results from planting out in the field of cloves hot water/formalin-treated will not be available until next year.

Some bulbs from the various classes were inspected into September and October. In general, decay appeared to have progressed, but more slowly as bulbs dried. It appeared that once cloves/bulbs dried enough, fusarium activity stopped; however, <u>F. roseum culmorum</u> still was isolated from all clove subclasses.

At about 8-10 weeks after harvest, one hundred each of class A cloves with and without hot water/ formalin treatment were sectioned into three parts: 1) stem plate section; 2) storage leaf section, and 3) first leaf piece. The stem plate section and the storage leaf section were plated on PDA, while the first leaf piece was kept in a humid Tupperware dish. Results show that 22% of the untreated cloves had <u>Fusarium</u> growth, and in all but two cases the <u>Fusarium</u> grew from the stem plate section, not the storage leaf. Thirteen percent of the treated cloves contained <u>Fusarium</u> growth. No <u>Fusarium</u> growth was observed in the first leaf pieces.

DISCUSSION

As in past years, bulb rot was incited through the season when inoculum of the garlic strain <u>F. roseum culmorum</u> was artificially infested into the soil. Disease resulting from this source was extensive at all stages, including preemergence; but it remains unknown how important soilborne sources are actually important in the field. Potentially, <u>F. roseum culmorum</u> may remain active in soil and survive various crop rotations, in effect lying in wait for the next garlic crop. Further investigation will be required to determine this. Mertect and Benlate seed treatments (but not common household bleach) greatly reduced the amount of disease from soil sources, but the perhaps excessive amount of inoculum utilized in these tests still caused significant disease loss.

As for the last several years, the soil into which these plots were placed had no history of garlic production. Theoretically the <u>F</u>. roseum culmorum garlic strain could have been in

the soil anyway, but the commercial seed lots planted around our plots have never had greater than a fraction of a percentage of loss to fusarium bulb rot. Presumably, then, this year's results showed that the fusarium pathogen could be brought in at high levels with infected garlic seed. Surprisingly, it proved difficult to relate the amount of disease on the seed crops grown in 1984-85 with the amount experienced in 1985-86 in our plots in central Oregon: plants grown from seed taken from the field with extensive fusarium losses in 1984-85 developed about 10% fusarium disease in 1985-86. Plants grown from the field with no observed fusarium in 1984-85 developed 30% or more fusarium disease by harvest in 1985-86. Storage losses continued following harvest. Several possible explanations for this apparent reversal expected in disease incidence exist: (1) our seed lots became mixed (we have excluded this possibility) (2) most badly infected seed decayed before harvest or before planting of the 1984-85 crop, whereas mildly infected seed passed through the system without decay (3) other seed health factors affected fusarium development on the two seed lots. It is notable that fusarium was very slight (about 1%) by harvest on the same seed lots grown in western Oregon and in California (data from other areas are not shown here). Thus, there seems additionally to be a strong environmental effect on disease expression. This fits with the general disease experiences in these areas: central Oregon has had greater losses to fusarium bulb rot than have other regions of western garlic production. These environmental factors are not understood but may involve growth stresses from overwintering cold or dryness which most other seed production areas do not experience to the same degree. Spring emergence tends to be a little lower in central Oregon than other locations, especially with known sources of reduced seed vigor. In these tests, the poor emergence when seed had been treated with higher temperatures and durations of hot water was not duplicated in western Oregon and in California, where emergence was equal for all hot water and time variations (data from other areas are not shown here).

F. roseum culmorum continued to be active during bulb drydown in storage; postharvest weights and bulb and seed clove condition were reduced significantly from the condition at harvest. Eventually, the disease became inactive on very dry bulbs and cloves, but the pathogen was found to be present on a significant number of cloves which would pass through into the next seed crop, either on cloves generally if partial rot was apparent, or inconspicuously in the stem plate of symptomless Standard hot water seed treatment alone eliminated cloves. only about one half of the clove infections. Why general surface contaminations of cloves with fusarium spores were not observed is uncertain. Mertect and Benlate each fully controlled the seedborne fusarium in field tests, thus incorporation of Benlate (which is registered for use against penicillium seed rot) is encouraged. Because seed lot infection levels may be obscure, fungicidal treatment of all seed to be

planted in central Oregon is recommended. Seed treatment for fusarium control in plantings in other areas in which the disease is less likely to be severe is a less certain consideration: Overuse of Benlate or any fungicide may select for resistance in a fungus; and such resistance to Benlate already has been noted in certain cases with penicillium on garlic seed. Registration of Mertect and screening of other fungicides for efficacy against fusarium also are recommended.

It is further recommended that an investigation be initiated into the survival of the garlic strain of \underline{F} . roseum culmorum in field soils, the amounts of the fungus in soil required to incite an economic level of disease, and the possible roles of alternate host crops (e.g. cereals).

Also, a general survey of seed lots for infection levels may prove useful.

Table 1: Fusarium Test #1, Madras, OR 1985-86 Cassaca Seed Source

Treatment ^b	Stand ^C (# Emerged) 4/7/86	% Stand 4/7/86	Symptoms	
1. Untreated	177	89	9	4.94
2. St+Ben	170	85	3	5.32
3. St+(125deg)	175	88	1	5.87
4. St+(130deg)	176	88	2	5.54
5. St+(130deg-CD)	119	60	2	3.67
6. St+(2hr,110deg)+Ben	183	92	1	4.94
7. St+(2hr,115deg)+Ben	157	79	0	4.85
8. St+(2hr,115deg-CD)+	Ben 115	58	2	3.65
X	159	80	2.4	4.85
CV	7,9	7.9	102.5	10.7
F-Test	**q	* *	**	**
LSD .05	18.5	9.3	3.6	0.74

Treatment Means and Stat Summary^a

- a. Means of 4 replications are rounded to nearest whole number (except for wts)
- b. St = standard seed treatment: 30 min at 100F, followed by 20 min at 120F (both with 1% Formalin), followed by 15-min cold dip. St plus parenthetical variations indicate time and/or temperature variations of the hottest water part of the standard treatment, and -CD means the cold dip was eliminated. Mer = Mertect and Ben = Benlate added to cold dip treatment water
- c. All plots planted 20 cloves/bed ft x 10 ft = 200 cloves/ plot

* & ** = significance at 5% and 1% levels, respectively

d.

Table 2: Fusarium Test #2, Madras, OR 1985-86 Nevada Seed Source

Treatment ^b	Stand ^C (# Emerged) 4/7/86			Harvestable Bulbs
1. Standard	154	77	46	2.54
2. St+Mer	191	96	2	4.45
3. St+Ben	190	95	5	4.57
4. St+FRC	145	73	55	1.95
5. St+Mer+FRC	188	94	20	3.31
6. St+Ben+FRC	187	94	17	4.13
X	176	88	24.2	3.49
CV	71	7.1	59.0	22.3
F-Test	**d	**	**	**
LSD .05	18.9	9.3	21.5	1.17

Treatment Mean and Stat Summary^a

- a. Means of 4 replications are rounded to nearest whole number (except for wts)
- b. St = standard seed treatment = 30 min at 100F, followed by 20 min at 120 F (both with 1% Formalin), followed by 15min cold dip. Mer = Mertect and Ben = Benlate added to cold dip treatment water. FRC = <u>Fusarium roseum culmorum</u> garlic strain placed into planting furrows
- c. All plots planted 20 cloves/bed ft x 10 ft = 200 cloves/
 plot
- d. * & ** = significance at 5% and 1% levels, respectively

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Column #	l Stand ^C # Emerged) 4/7/86	2 X Stand 4/7/86_	3 Z Plants with Fus Symptoms 6/18/86	4 Number of Hervestable Bulbs 7/22/86	bulbs as	6 Harvestable bulbs as Z of seeded	7 Wt (Kg) Harvestable Bulbs 7/22/86	8 Wt (Kg) Post-Harves 9/29/86		10 Storage Wt Loss As X of Harvest Wt	Clar Wt (Lg)	12 Arvest as A 2 Wt of Total	- C1	14 Harvest ASS B 2 Wt of Total	- Clas	2 01	17 Post-Ha Clas Number	s B	19 Post-Hai <u>Class</u> Number	вC
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I CV F-Test LSD .05	136 11 ₄ 0 21.9	68 11.0 ** 11.0	47 29.9 ** 20.5	68 30.1 ** 30.1	44 24.1 ** 15.5	34 30.1 ** 15.0	2.53 25.4 ** 0.94	1.71 28.3 ** 0.71	0.81 28.8 ** 0.34	28 29.5 ** 10.6	1.50 30.8 ** 0.68	85 14.7 ** 18.4	0.21 46.9 ** 0.14	15.9 83.2 NS NS	52 34.3 ** 26.4	75.7 16.4 NS NS	8.2 46.3 ** 6.0	14.0 63.8 NS NS	7.9 55.6 **	10.9 57.8 ** 9.3
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Table 3: Fusarium Test #3, Madras, OR 1985-86: Treatment Means and Stat Summary^a

c. All plots planted 20 cloves/bed ft x 10 ft = 200 cloves/plot d. * & ** = significance at 5% and 1% levels, respectively

a. Heans of 4 replications are rounded to nearest whole number (except for wts) b. St = standard seed treatment: 30 min at 100F, followed by 20 min at 120F (both with 1% Formalin), followed by 15-min cold dip. Her = Hertet, Ben = Benlate and NaOC1 = Sodium hypochlorite (common household bleach) added to cold dip treatment water.

Table 5: Grade of garlic cloves, as a percentage of the weight fraction within fusarium bulb rot class and clove decay subclass following postharvest storage

Bulb Rot Class	<u>Clove</u> 1	Decay Subclass 2	Within Bulb 3	Rot Class 4
Α	97%	(3%)	0%
В	50%	19%	23%	8%
С	0%	0%	95%	5%

Table 6: Frequency of isolation of <u>Fusarium roseum culmorum</u> (FRC) from garlic cloves of different bulb rot classes and clove decay subclasses, with and without hot water + formalin seed treatment.

BULB ROT CLASS AND CLOVE DECAY SUBCLASS	STANDARD HOT WATER & FORMALIN SEED TREATMENT	SURFACE STERILIZATION	NUMBER OF CLOVES FROM WHICH ISOLATIONS WERE ATTEMPTED	% OF CLOVES WITH FRC RECOVERED
A1	-	-	20	25
A 1		+	120	27
A 1	+	+	140	13
B1	-	+	25	28
B1	+	+	25	12
B2	-	+	10	50
B2	+	÷	10	20
B3	_	+	10	80
B3	+	+	10	50