Laboratory Evaluation of Pink Root and Fusarium Basal Rot Resistance in Garlic

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ABSTRACT

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Laboratory methods for screening pink root (*Pyrenochaeta terrestris*) resistance and Fusarium basal rot (*Fusarium oxysporum* f. sp. *cepae*) in onion were applied to garlic (*Allium sativum*). Seven clones of diverse origin were screened, and evidence of resistance was observed. Symptoms of both diseases were least severe in PI 493112 (from Poland) and PI 493106 (from Austria). PI 493118 (from Poland) was pink root-resistant but *Fusarium*-susceptible. Phenotypic variation between and within the clones tested suggests the possibility for resistance selection in garlic.

Pink root and Fusarium basal rot are important diseases of cultivated alliums. Screening techniques have been devised and the germ plasm evaluated for sources of resistance in onion. Similar screening has not been reported for garlic.

Pyrenochaeta terrestris (Hansen) Gorenz, J. C. Walker, & Larson causes pink root disease on a wide range of plants including cucumbers, carrots, spinach, strawberries, and about 50 gramineous species (13,24,26); however, onions, garlic, and shallots are most susceptible (3,20,25). The disease has been observed in various parts of the United States (14) and other countries (3) where it causes large economic losses in onion field production (4,15) and infected roots serve as entry points for infection by *Fusarium oxysporum* f. sp. *cepae* (16). Pink root is soilborne and remains viable in the soil for many years.

The development of pink root-resistant cultivars has proven more practical than soil treatment (17). Many onion breeding programs have been directed toward developing pink root-resistant material (8,15,17–19). Resistance has been characterized in onions (15,17,19), *Allium fistulosum*, *A. ampeloprasum*, *A. schoenoprasum* (20), and *A. cepa* subsp. *ascalonicum* (7,26).

Basal rot of onions caused by *F. oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *cepae* (Hanz.) Snyd. & Hans. is economically important in field production and storage and has been reported in many countries (9,16,27). The fungus may infect onions in all stages of growth. On seedlings, the infection may be expressed as preemergence or postemergence damping-off and infected stem plates. The fungus penetrates the stem plate and the bases of older leaves (21). Leaves curve, then turn yellow and wilt; roots die and the bulbs rot (10,22).

Resistance in onions to basal rot has been reported (9,11,16,21,23); however, the genetic basis of resistance is still unknown. Some of the resistant onion cultivars are derivatives of the interspecific hybrid *A. cepa* × *A. fistulosum* (1). Efficient methods have been developed to test for resistance to Fusarium basal rot (2,21), and a high positive correlation has been noted between greenhouse and field results (21).

Both pink root and basal rot attack garlic, causing field and storage losses with symptoms similar to those in onions (20). Our research was initiated to investigate the possibility that variation for resistance to these two diseases occurs in the germ plasm of garlic. Laboratory testing was performed using onion screening techniques on a broad geographic range of garlic clones.

MATERIALS AND METHODS

The technique used for screening garlic for pink root resistance was that developed for selection of resistant onion seedlings by Gorenz et al (6) and modified by Nichols (17) and Nichols et al (18). The Fusarium basal rot screening technique used was adapted from the onion screening method developed by T. Weinman, M. J. Palmer, and P. H. Williams, University of Wisconsin, Madison (*personal communication*). *P. terrestris* isolate PHW 387 and *F. oxysporum* f. sp. *cepae* isolate PHW 593 were obtained from P. H. Williams, Department of Plant Pathology, University of Wisconsin, Madison.

P. terrestris was maintained on sterile soil at 4 C, transferred to potato-dextrose agar (PDA), and grown for 3 days at 24 C. Mycelial pieces were transferred to PDA plates (24 C) for increase. After 11 days, 10 plugs (each 10 mm in diameter) were placed in 800-ml bottles with 200 ml of Czapek's broth. Bottles were laid on their sides and incubated at room temperature. The broth was shaken three times per week to break the mycelial mat. After 4 wk, the mycelium and broth were blended three times for 30 sec at low speed in a Waring Blendor, then 200 ml of inoculum and 1,400 ml of distilled water were mixed with 23.5 kg of sterile silica sand.

The Fusarium basal rot fungus was stored at 4 C in sterilized soil and was increased on PDA at 24 C. Mycelial plugs were transferred to potato-dextrose broth and grown at 26 C for 5 days on a rotary shaker. The mycelium and broth were comminuted for 2 min at low speed on a Waring Blendor and the mixture was centrifuged 10 min at 3,400 rpm to wash the spores. The spore-containing pellet was resuspended in distilled water. Spore concentration was determined with a hemacytometer, and the spore suspension was mixed with sterile sand to establish inoculum levels at 10^5 spores per gram of sand.

In the laboratory screening, seven garlic (*A. sativum* L.) clones from five countries, 20 cloves each, and USDA onion (*A. cepa* L.) lines, 50 seeds each (resistant and susceptible checks), were evaluated. The garlic clones tested were PI 493105 from Turkey, PI 493106 from Austria, PI 493112 and 493118 from Poland, PI 493115 from Czechoslovakia, and PI 493119 and 493124 from Brazil. The onion lines tested were B2329 (pink root-resistant), B2399 (pink root-susceptible), B6701 (Fusarium basal rot-resistant), and B2923 (Fusarium basal rot-susceptible), all from USDA. Garlic cloves and onion seeds were planted 1 cm deep in infested sand in metal pans, which were placed in a Wisconsin soil-temperature tank held at 20 C under fluorescent light (120–150 quanta). For pink root resistance screening, representative resistant and susceptible onion lines included were in the flag stage when the tank temperature was raised to 24 C. For Fusarium basal rot resistance screening, onion seedlings included were 1 cm tall when the tank temperature was raised to 28 C. The plants were kept moist with water and fertilized with full-strength Hoagland's solution 1 day each week.

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RESULTS AND DISCUSSION

Nineteen days after inoculation, symptoms on garlic roots were not observed, whereas symptoms on the susceptible onion lines were clearly readable and most susceptible seedlings were dead. Symptoms on garlic plants were apparent 35 days after planting in infested sand. The pink root disease symptoms were graded into five classes (Table 1) on the basis of root pinking and basal plate rot as described by Nichols (17) and Nichols et al (19). Fusarium basal rot symptoms were classified on a similar scale on the basis of root and basal plate rot.

Variation in disease response within bulbs was minimal. Four cloves from the same bulb replicated in random locations in a testing pan spanned at most two consecutive disease classes for each bulb tested for both diseases (i.e., cloves from one bulb were placed in classes 1 and 2 or 2 and 3, etc.). Cloves from the same bulbs were all symptomless (mean disease severity score of 1) when grown in uninoculated sand at 20, then 24 C (pink root conditions), whereas fewer than 10% of uninoculated plants grown at 20, then 28 C (Fusarium basal rot conditions), showed some symptoms (mean disease severity score of 1.4).

Variation in disease response among clones was sizable for both diseases. The pink root test results are given in Table 1. Plants with yellowish brown leaf tips were observed at rates of 95, 23.5 and 11% in PI 493119, 493124, and 493115, respectively. Leaf symptoms were not observed in the other clones or the onions. All garlic clones had some pink root symptoms. The most susceptible plants were found in PI 493105, 493115, and 493119. The most tolerant were PI 493106, 493112, and 493118. The disease response was unusual for PI 493124 because cloves and roots were frequently symptomless, yet leaf damage was common.

Mean disease severity scores for pink root ranged from 1.8 to 3.2 over different garlic clones and suggested significant differences in disease resistance among clones (Table 1). Analysis of variance confirmed this suggestion because the *F*-test value variation among clones was highly significant ($P<0.01$).

Fusarium basal rot test results are listed in Table 2. All clones tested were susceptible to *F. oxysporum* f. sp. *cepae* to some extent, but phenotypic differences were evident. PI 493112 was most resistant, with 20 and 40% of plants in classes 1 and 2, respectively. These values compare favorably with the *Fusarium* resistance exhibited in the resistant onion line B6701 used as a standard. Leaf symptoms were not closely associated with root and bulb symptoms. Typically, plants of PI 493106 and 493124 had symptomless roots and bulbs and green leaves, whereas plants with badly diseased roots and bulbs but with green leaves occurred in PI 493112 and 493118.

Mean disease severity scores for Fusarium basal rot ranged from 2.5 to 3.5 among garlic clones. The *F*-test value from analysis of variance indicated significant variation among clones at $P=0.05$ but not at $P=0.01$.

Though untested, plants in classes 1–3 could probably survive in infested fields, but yield reductions may occur. The phenotypic variation observed among

Table 1. Symptoms of *Pyrenochaeta terrestris* infection on garlic and onion under laboratory conditions

Garlic clones and onion lines	Leaf evaluation		Root and basal plate evaluation (number of plants in each disease class)[y]					Garlic mean disease severity score[z]
	Green leaves	Leaves with brown tips	1	2	3	4	5	
Garlic								
PI 493105	20	0	4	10	4	2	0	2.2 ab
PI 493106	20	0	4	14	1	1	0	2.0 a
PI 493112	19	0	4	8	7	0	0	2.2 ab
PI 493115	16	2	0	4	12	2	0	2.9 bc
PI 493118	20	0	5	9	5	0	1	2.2 ab
PI 493119	1	19	2	0	10	8	0	3.2 c
PI 493124	13	4	7	7	3	0	0	1.8 a
Onion								
B2329	50	0	50	0	0	0	0	···
B2399	0	50	0	0	0	20	30	···

[y] Disease classes: 1 = without symptoms, 2 = less than 10% pink roots, 3 = 10–50% pink roots with up to 10% rotted basal plates, 4 = more than 50% pink roots with 10–30% rotted basal plates, and 5 = completely rotted roots and more than 30% rotted basal plates.
[z] Weighted mean of disease classes. Values in columns followed by different letters are significantly different ($P=0.05$) according to Tukey's HSD test.

Table 2. Symptoms of *Fusarium oxysporum* f. sp. *cepae* in garlic and onion under laboratory conditions

Garlic clones and onion lines	Leaf evaluation		Root and basal plate evaluation (number of plants in each disease class)[y]					Garlic mean disease severity score[z]
	Green leaves	Leaves with brown tips	1	2	3	4	5	
Garlic								
PI 493105	0	20	0	6	14	0	0	2.7 ab
PI 493106	2	18	2	0	17	0	1	2.9 ab
PI 493112	9	11	4	8	3	4	1	2.5 a
PI 493115	0	20	0	9	11	0	0	2.6 a
PI 493118	7	13	2	0	13	4	1	3.1 ab
PI 493119	0	20	0	1	18	0	1	3.1 ab
PI 493124	2	18	2	5	4	0	9	3.5 b
Onion								
B6701	15	35	15	11	8	0	16	···
B2923	0	50	0	0	30	10	10	···

[y] Disease classes: 1 = without symptoms, 2 = up to 10% rotted roots, 3 = 10–30% rotted roots with up to 10% rotted basal plates, 4 = completely rotted roots and 10–30% rotted basal plates, and 5 = completely rotted roots and more than 30% rotted basal plates.
[z] Weighted mean of disease classes. Values in columns followed by different letters are significantly different ($P=0.05$) according to Tukey's HSD test.

and within garlic clones suggests that garlic clones may be genetically variable for disease reaction. All garlic in commercial production today is asexually propagated, but recent developments suggest the possibility of routine sexual reproduction and consequent incorporation of new genetic variation into cultivated garlic (5,12). It is also possible that nongenetic factors account for the apparent resistance. Selection for resistance within clones will test these alternatives. It is necessary to compare laboratory screening results with field testing to determine whether field performance can be predicted from laboratory results.

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