Aeroponics Chambers for Evaluating Resistance to Aphanomyces Root Rot of Peas (*Pisum sativum*)

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ABSTRACT

Rao, A., Gritton, E. T., Grau, C. R., and Peterson, L. A. 1995. Aeroponics chambers for evaluating resistance to Aphanomyces root rot of peas (*Pisum sativum*). Plant Dis. 79:128-132.

An aeroponics system was used for evaluating resistance of peas to *Aphanomyces euteiches*. Five pea genotypes were tested for resistance to two isolates of *A. euteiches* at concentrations of 0 to 10,000 zoospores per milliter. Pea genotypes reacted differently to isolates of *A. euteiches*. Genotype 86-2231 demonstrated the lowest amount of disease and Dark Skin Perfection the highest. Isolate 467 produced consistently higher disease scores than isolate FB1. Inoculum concentration and isolate × concentration effects were significant. This study provides evidence that an aeroponics system can be useful in evaluating the reaction of peas to *A. euteiches*.

Common root rot, caused by *Aphanomyces euteiches* Drechs., is one of the most destructive diseases of peas (*Pisum sativum* L.) in the northeastern and Great Lakes states in the United States (2,9,20,30). The disease has been reported in North America, Europe, Australia, New Zealand, and Japan (27,30). *A. euteiches* can attack peas in all stages of growth, causing a severe rotting of the root, cortex, and epicotyl that results in stunted seedlings, yellow and wilting leaves, or dead plants (26,30). Serious damage in peas caused by Aphanomyces root rot was reported as early as 1925 in the United States (5,14,15), and the disease has been considered a limiting factor in pea production in many pea-growing areas since that time (18,28,38). In the Great Lakes and northeastern states, annual losses caused by the disease have been estimated at 10% (30).

One strategy for the control of *A. euteiches* root rot of peas is to avoid growing peas in heavily infested fields. The pathogen can survive in the soil for over 10 yr (15). Hosts other than peas probably contribute to the longevity of this pathogen in soil; *A. euteiches* has been recovered from alfalfa (*Medicago sativa* L.), faba or broad bean (*Vicia faba* L.), snap bean (*Phaseolus vulgaris* L.), red clover (*Trifolium pratense* L.), and subterranean clover (*Trifolium subterraneum* L.) (6,7,13,31,32). Host range studies have aided in controlling the pathogen through crop rotation.

This research was supported by the College of Agricultural and Life Sciences, Hatch project 142-D480, University of Wisconsin-Madison, and the Midwest Food Processors Assn.

Accepted for publication 4 November 1994.

Although there has been progress in identifying resistance to this disease (4,8), currently there are no commercially available disease resistant pea cultivars. Resistance has been reported to be linked genetically with three undesirable "wild type" genes conditioning tall plants, colored flowers, and colored seed (23). It has been difficult to identify superior disease resistance when evaluating pea germ plasm and cultivars because of the polygenic nature of resistance and the variability encountered in tests. Variability exists within *A. euteiches* for pathogenicity and virulence (2,6,22,35), and pea genotypes also express different reactions to isolates of *A. euteiches* (2,22,35; C. R. Grau; *unpublished data*).

Several methods have been developed for evaluating resistance to Aphanomyces root rot including use of an environmental shift technique and a modified environmental shift technique (33,34), dipping roots in a zoospore suspension (10), and testing under variable factors (19,20,21). These methods all involve growing pea plants in solid media, which makes it difficult to observe the roots. Morrison et al (25) inoculated excised root tips to determine the resistance of peas to *A. euteiches*. This technique requires great procedural control for reliable results. Since the root is the primary site of infection by the pathogen, evaluation of resistance to Aphanomyces root rot requires careful examination of pea roots. Therefore, methods of facile observation of roots without damaging them are necessary for evaluating this root disease.

Several approaches in which plants are grown in nonsolid media have been used in studies on plant root diseases. Hydroponics has been employed in evaluating plant diseases (16), but Veitenheimer's (36) attempt to screen for resistance to *A. euteiches* in peas using hydroponics was not satisfactory. Aeroponics as a technique for growing plants was reported as early as 1922 (1). This system, in which roots of plants are suspended and misted with a nutrient solution, has been of interest to many researchers (29). Zobel et al (39) utilized a simple, inexpensive aeroponics system for nodulation studies in peas, soybeans, and faba bean. Carter (3) developed a water vapor device to facilitate examination of roots of pineapple plants after infestation by mealy bugs. Hendrix et al (11) conducted root disease research in wheat using mist chambers. Wagner and Wilkinson (37) successfully applied an aeroponics system for investigating Phytophthora root and stem rot of soybean. To our knowledge, no attempt has been made to use an aeroponics system for evaluating disease resistance in pea.

The objectives of this work were to investigate the potential of an aeroponics system for evaluating resistance of peas to Aphanomyces root rot, study pathogenicity and virulence of isolates of *A. euteiches* on peas, and test for differential reactions of pea genotypes to isolates of *A. euteiches*.

MATERIALS AND METHODS

Aeroponics chambers. The aeroponics chambers used in this study (Fig. 1) were provided by L. A. Peterson, Department of Horticulture, University of Wisconsin-Madison. Design and operation of the aeroponics system were described by Peterson and Krueger (29). Peas were grown in the system on a misting schedule of 3 sec at 10-min intervals with modified Hoagland's solution (12). A very fine mist in a solid cone pattern provided for excellent root coverage. The system used nonrecirculating nutrient solution to eliminate infection by secondary inoculum. Root environment temperatures were 24–28 C.

Pea genotypes. Lines Minnesota 108 (MN 108) (4) from the University of Minnesota, and 86-2231 (17) from Washington State University and the U.S. Dept. of Agriculture, Agricultural Research Service, have previously been identified as possessing a high degree of resistance to *A. euteiches*. WI 8902 (8) from the University of Wisconsin-Madison has exhibited a somewhat lower level of resistance. The moderately susceptible cultivar CSC 8221 was provided by Nunhems Seed Co., Lewisville, ID.

The susceptible line Dark Skin Perfection (DSP) has been widely grown in pea production regions. These five were chosen for testing pea genotype reactions to *A. euteiches* using the aeroponics system.

Seed germination. Pea seeds were dusted with captan fungicide (Captan: 50% WP, 49% N-Trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) and planted in Jiffy potting mix (Jiffy Products of America Inc., West Chicago, IL) in sterilized wooden flats in the greenhouse. Seven-day-old seedlings were carefully pulled from the Jiffy mix, rinsed with distilled water to remove particulates, and transplanted into aeroponics chambers. Each chamber contained one plant each of the five pea genotypes in a 20-L container with a 2.5-cm-thick foam plastic cover. Plants were supported by compressible polyurethane plugs coated with stopcock grease to prevent absorption of nutrient solution.

Isolates, inoculum production, and inoculum concentration. Isolate 467, collected in Wisconsin, was recovered from pea; isolate FB1, collected in Manitoba, Canada, was recovered from faba bean. Both isolates have been previously studied for their variation in virulence (6). Zoospores of *A. euteiches* were produced using a modified technique of Mitchell and Yang (24). Four 5-mm-diameter mycelium-agar plugs cut from the colony margin of a vigorous culture were transferred to a sterile 125-ml flask containing 25 ml of peptone glucose broth (20 g of peptone and 5 g of glucose per liter of distilled water) and incubated at room temperature (approximately 24 C) for 3 days. Mycelial mats were washed three times with an autoclaved lake water/distilled water mixture at 2-hr intervals. Zoospores were released 12–16 hr after final rinse and density was determined by a hemacytometer. The desired concentrations of 100, 1,000, and 10,000 zoospores per milliliter were obtained by diluting the original zoospore suspension with sterile distilled water.

Inoculation method. Three days after transplanting seedlings into aeroponics chambers, roots were inoculated with zoospores of *A. euteiches*. In the first experiment, the foam plastic covers that contained the plants were removed from the aeroponics chambers and the roots dipped into the zoospore suspension of the desired concentration. The covers were returned to the plant containers immediately after inoculation. In the second experiment, roots were soaked in the inoculum for 15 min.

Pea root rot rating system. Pea root rot rating scores were recorded 14 days after inoculation, when the most susceptible plants were showing severe disease symptoms and the most resistant plants were showing only slight symptoms. MN 108 served as a resistant check and DSP as a susceptible check. The root rot rating system used in this study was:

1. Root: Healthy.
   Epicotyl: Healthy.
2. Root: Mostly white, but with feeder roots pruned.
   Epicotyl: Slight coloration, but firm and unshrunken.
3. Root: Feeder roots pruned, taproot discolored but firm.
   Epicotyl: Discolored and shrunken, but firm.
4. Root: All roots discolored and soft.
   Epicotyl: Discolored and soft.
5. Root: Most of root system disintegrated.
   Epicotyl: Rotted through, or nearly so; plant dead.

Experimental design. The first experiment was a split-plot arrangement of a randomized complete block design with three replications. Isolates were whole plots, inoculum concentrations subplots, and pea genotypes sub-subplots. Following analysis of the first experiment, in which the block (replication) effect was not statistically significant, it was felt the use of a split-plot arrangement of a completely randomized design with three replications for the second experiment would more precisely detect differences in the treatments. Combinations of isolates and concentrations were assigned as whole plots, and pea genotypes were subplots. Data from root rot rating scores were analyzed through analysis of variance and Fisher's protected LSD test for mean comparisons using the SAS GLM procedure (SAS Institute, Cary, NC). Inoculum concentration was considered a random effect, isolates and pea genotypes were treated as fixed effects. All F tests were performed according to expected mean squares.

RESULTS

First experiment. Highly significant differences were found for inoculum concentrations and pea genotypes (Table 1). Isolate 467 consistently caused higher disease scores than isolate FB1 (Table 2), averaging 3.2 over genotypes and concentrations compared with 2.3 for FB1. However, this difference was not always statistically significant. Disease severity increased with increasing inoculum concentration for each isolate (Table 2). However, the overall root rot rating score for the inoculum concentration of 1,000 zoospores per milliliter did not differ from 10,000 zoospores per milliliter. A significant isolate × concentration interaction occurred. Isolate 467 at concentrations of 100 and 1,000 zoospores per milliliter produced significantly more severe disease than isolate FB1 did, but the difference between isolates was not significant at 10,000 zoospores per milliliter.

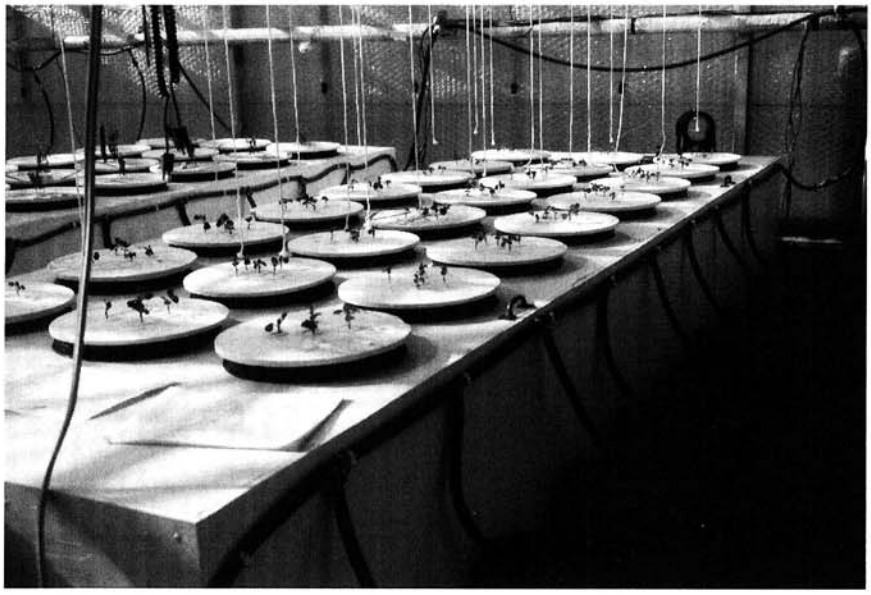

Fig. 1. The aeroponics system used for evaluating reaction of peas to *Aphanomyces euteiches*.

Table 1. Analysis of variance for root rot ratings of five pea genotypes inoculated with two isolates of *Aphanomyces euteiches* at four inoculum concentrations (experiment 1)

Source of variation	df	Mean square
Replications	2	0.908
Isolates	1	25.208
Reps × isolates (error a)	2	1.633
Concentrations	3	55.114[y]
Isolates × concentrations	3	4.792[z]
Reps × isolates × concentrations (error b)	12	1.107
Genotypes	4	3.289[y]
Isolates × genotypes	4	0.969
Concentrations × genotypes	12	0.812
Isolates × concentrations × genotypes	12	0.441
Error c	64	0.565

[y]Significant at the 0.01 probability level.
[z]Significant at the 0.05 probability level.

Pea genotypes differed in reaction to *A. euteiches* (Table 2 and Fig. 2). MN 108 and 86-2231 possessed a similar level of resistance. CSC 8221 and DSP did not differ in susceptibility, and they expressed significantly more severe disease symptoms than MN 108 and 86-2231 did. WI 8902 was intermediate between resistant and susceptible lines, and did not differ from all others.

Second experiment. Highly significant differences were found for inoculum concentration, isolate × concentration, and pea genotype (Table 3). All inoculum concentrations of *A. euteiches* differed in disease severity, with values of 1.0, 1.7, 2.5, and 3.0 for concentrations of 0, 100, 1,000, and 10,000 zoospores per milliliter, respectively. The average root rot rating scores with isolate 467 at concentrations of 100, 1,000, and 10,000 zoospores per milliliter were always significantly higher than with isolate FB1 (Fig. 3). Line 86-2231 expressed the lowest overall root rot rating score of 1.6, but was not significantly more resistant than MN 108 at 1.8 and CSC 8221 at 2.0. DSP at 2.4 expressed the most severe disease symptoms. WI 8902 at 2.3 did not differ significantly from CSC 8221 and DSP.

Significant isolate × genotype and concentration × genotype interactions were detected in this study (Table 3). When challenged with isolate FB1, MN 108 and 86-2231 were grouped together with low disease scores and WI 8902, CSC 8221, and DSP were grouped together with higher disease scores (Fig. 4). When tested with isolate 467, the grouping changed to 86-2231 and CSC 8221 with low disease scores and MN 108, WI 8902, and DSP with higher scores. Disease severity increased in all pea genotypes with increasing inoculum concentration (Fig. 3). The range in disease severity over genotypes with isolate FB1 was less at a concentration of 100 zoospores per milliliter than at 1,000 and 10,000 zoospores per milliliter. At both 1,000 and 10,000 zoospores per milliliter with FB1, MN 108 and 86-2231 had low and similar disease ratings, whereas CSC 8221, WI 8902, and DSP had high and similar ratings. The two higher concentrations were better for differentiating reactions of pea genotypes to *A. euteiches*. Isolate FB1 gave a larger range in disease severity among pea genotypes than did isolate 467 (Fig. 3) even though the former isolate was less virulent.

Table 2. Mean root rot ratings for five pea genotypes inoculated with two isolates of *Aphanomyces euteiches* at four inoculum concentrations (experiment 1)

Concentration (zoospores/ml)	Isolate	Pea genotype						
		MN 108	86-2231	WI 8902	CSC 8221	DSP	Mean[v]	Mean[w]
0	FB1	1.0	1.0	1.2	1.5	1.2	1.2 a[x]	
	467	1.0	1.0	1.0	1.0	1.0	1.0 a	
	Mean	1.0	1.0	1.1	1.3	1.1		1.1 a[y]
100	FB1	1.2	1.2	1.5	1.0	2.3	1.4 a	
	467	2.5	3.2	2.8	3.0	4.0	3.1 b	
	Mean	1.8	2.2	2.2	2.0	3.2		2.3 b
1,000	FB1	2.0	1.8	3.0	3.8	4.0	2.9 b	
	467	4.0	4.0	4.7	4.7	4.0	4.3 c	
	Mean	3.0	2.9	3.8	4.3	4.0		3.6 c
10,000	FB1	3.2	2.3	4.5	4.3	4.0	3.7 bc	
	467	4.2	4.3	4.5	4.8	4.7	4.5 c	
	Mean	3.7	3.3	4.5	4.6	4.3		4.1 c
	Host mean	2.4 a[z]	2.4 a	2.9 ab	3.0 b	3.2 b		

[v] Means of isolate × concentration.
[w] Mean of inoculum concentration.
[x] Means with same letter in column are not significantly different. LSD ($P = 0.05$) = 0.84.
[y] Means with same letter in column are not significantly different. LSD ($P = 0.05$) = 0.59.
[z] Means with same letter in row are not significantly different. LSD ($P = 0.05$) = 0.57.

Table 3. Analysis of variance for root rot ratings of five pea genotypes inoculated with two isolates of *Aphanomyces euteiches* at four inoculum concentrations (experiment 2)

Source of variation	df	Mean square
Isolates	1	20.513
Concentrations	3	21.322[x]
Isolates × concentrations	3	3.052[x]
Rep (isolates × concentrations) (error a)	16	0.174
Genotypes	4	2.572[x]
Isolates × genotypes	4	0.703[y]
Concentrations × genotypes	12	0.466[y]
Isolates × concentrations × genotypes	12	0.241
Error b	63[z]	0.204

[x] Significant at the 0.01 probability level.
[y] Significant at the 0.05 probability level.
[z] Degrees of freedom reduced by 1 because of missing value.

Fig. 2. Disease severity of five pea genotypes inoculated with *Aphanomyces euteiches*. The roots of the two plants showing relatively light discoloration are MN 108 and 86-2231; roots of the other three plants exhibit the darkening characteristic of susceptibility (experiment 1).

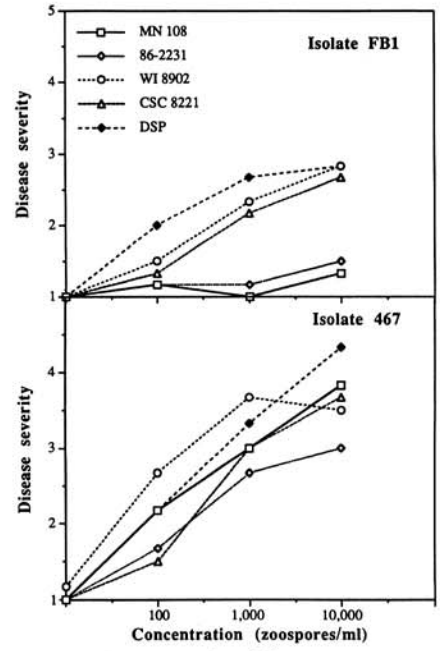

Fig. 3. Disease severity of five pea genotypes inoculated with two isolates of *Aphanomyces euteiches* at four inoculum concentrations (experiment 2).

DISCUSSION

Our results indicate the aeroponics system is useful in investigating pea plants infected with *A. euteiches*. The aeroponics system not only provided a favorable environment for plant growth, but also offered proper conditions for plant infection and disease development. The misting time and interval between misting can be precisely controlled. Composition of the nutrient solution can be easily changed at different plant growth stages by switching to canisters containing a solution with the desired nutrition. Specific root environment temperatures can be obtained using an air conditioner. Furthermore, the aeroponics system enables both continuous observation of the roots, without damaging them, to determine changes in disease severity over time, and also the obtaining of clean samples for scoring root rot ratings, without interference from soil or other particles.

Both experiments demonstrated the aeroponics system was effective in identification of pea genotypes differing in degree of resistance to the pathogen, with genotype 86-2231 consistently expressing the lowest disease symptoms and DSP the highest. This is in agreement with previous greenhouse studies (C. R. Grau, *unpublished data*). Higher disease scores were measured for isolate 467 than for isolate FB1. However, the effect of isolate on disease severity was statistically insignificant.

Results from the second experiment of this study provide evidence that pea genotypes may react differentially to isolates of *A. euteiches*. Each pea genotype tended to develop more severe disease when inoculated with isolate 467 than with isolate FB1. In a previous study, isolate FB1 produced a similar amount of disease on lines MN 108 and 86-2231 (C. R. Grau, *unpublished data*). Our study is consistent with this finding. However, isolate 467 caused more severe disease symptoms on MN 108 than on 86-2231 (Fig. 4). The most severe symptoms induced by both isolates were obtained in DSP. No difference in disease symptoms was observed between WI 8902 and CSC 8221 against isolate FB1, but WI 8902 developed significantly higher disease severity than CSC 8221 did against isolate 467.

The differential reactions of five pea genotypes to isolates of *A. euteiches* in this study support previous work that variation in virulence exists within *A. euteiches*. Beute and Lockwood (2) designated Race 1 and 2 based on virulence patterns of 15 isolates of *A. euteiches* on six pea cultivars. Sundheim (35) designated Race 3, 4, and 5 to differentiate 14 isolates of *A. euteiches* collected in Norway. Manning and Menzies (22) found that most isolates present in New Zealand could be assigned to Race 5 of Sundheim. Screening resistance in peas is complicated by the differential reactions of pea genotypes to races of *A. euteiches*, so it is important to choose proper isolates of *A. euteiches* to differentiate pea genotypes.

Evaluation of resistance of peas to this pathogen also is affected by inoculum concentration. It appeared that isolate FB1 better separated resistant genotypes from susceptible ones at the higher concentrations of 1,000 and 10,000 zoospores per milliliter than at 100 zoospores per milliliter. Isolate FB1 was also more effective than isolate 467 at these two higher inoculum concentrations (Fig. 3). It was noted that isolate FB1 tended to be less virulent than isolate 467. A question arising from this study is whether less virulent isolates of *A. euteiches* should be used to better differentiate for resistance among pea genotypes.

The aeroponics system can aid researchers in studies of pathogenicity of the fungus and host-pathogen reactions, and also in screening pea germ plasm for resistance to *A. euteiches*. In addition, the system should be useful in evaluating and selecting resistant progeny from crosses, and this can facilitate breeding programs. Finding that pea genotypes differ in disease resistance as expressed in the aeroponics system is in agreement with results obtained in the field (E. T. Gritton, *unpublished data*). The results of this study demonstrate the usefulness of an aeroponics system in evaluating resistance to *A. euteiches* in peas.

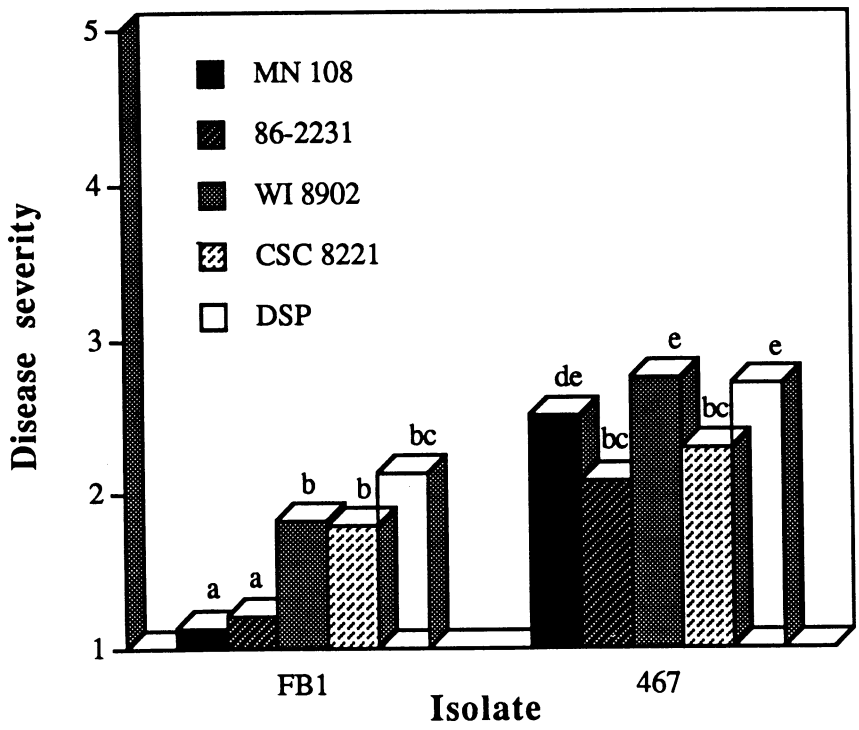

Fig. 4. Reaction of five pea genotypes to two isolates of *Aphanomyces euteiches* (experiment 2).

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