Identification of Characteristics Associated with Resistance to Root Rot Caused by *Aphanomyces euteiches* in Pea

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ABSTRACT

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Previous attempts to quantify resistance in pea to *Aphanomyces euteiches* have involved oospore counts in infected root tips and disease severity ratings to separate resistant from susceptible lines. In a controlled environment with known zoospore inoculum levels, differences in oospore production in infected roots, rate of lesion development in taproots, and zoospore germination in seedling exudates were evident in resistant and susceptible pea lines. Indirect enzyme-linked immunosorbent assay (ELISA) measurements of infected root tissue within a visible lesion revealed a positive, linear regression of lesion length on ELISA readings at 405 nm ($R^2 = 0.91$). Zoospore germination was reduced in seedling exudates from resistant but not from susceptible pea lines. Resistance in pea roots to *A. euteiches* is associated with reduced oospore production, pathogen multiplication, zoospore germination, and slower lesion development.

Additional keywords: *Pisum sativum*, root disease, root vigor

Common root rot, caused by *Aphanomyces euteiches* Drechs. f. sp. *pisi* W.F. Pfender & D.J. Hagedorn, is the most destructive soilborne disease of pea (*Pisum sativum* L.) worldwide (6). This disease has been a serious yield constraint in the Great Lakes area and the northeastern United States since the 1920s (7) and has been a recognized problem in the Pacific Northwest since 1985 (1,16).

Marx et al. (20) reported that resistance to common root rot is associated with three dominant alleles that control node length, flower color, and hilum color. Substitution of the recessive, horticulturally desirable alleles resulted in reduced resistance. Difficulties exist in identifying resistance in pea under field conditions due to interaction with other root-invading pathogens, the environment, and differences in virulence of *A. euteiches* isolates (19,22). However, resistance or tolerance to *A. euteiches* has been identified in pea with desirable or acceptable horticultural traits (3,5,9,10,11,17). Lewis and Gritton (18) reported that resistance to *A. euteiches* was quantitatively inherited with low heritability. Attempts have been made to quantify resistance to Aphanomyces root rot through counts of oospores in affected root tips (21), differences in plant weight of inoculated and uninoculated plants (15), and disease severity ratings of roots infected with *A. euteiches* in an aeroponic chamber (23). We reported the development of a polyclonal antiserum to measure differences in antigens produced by *A. euteiches* in resistant and susceptible pea lines (12). We will discuss the relationship of oospore production, rate of lesion spread, quantification of antigen within the lesion, and zoospore germination in exudates from resistant and susceptible pea seedlings. A portion of this work was reported earlier (13).

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MATERIALS AND METHODS

Pea line selection and inoculum production. Pea lines tested included the cultivars Dark Skin Perfection, Puget, and Bolero, the resistant PI accession 180693 (19), and USDA-ARS breeding lines 79-2022 (9), 86-2231 (10), 90-2079, and 90-2131 (11). The breeding lines 79-2022, 86-2231, 90-2079, and 90-2131 were resistant and(or) tolerant both in pure culture tests in the greenhouse (15) and in fields where Aphanomyces root rot is severe. For all tests, seeds were surface-disinfested with an alcohol–hydrogen peroxide dip described previously (14). Disinfested seed were placed on autoclaved germination paper, moistened with sterile glass-distilled water, and incubated at 21°C. Seedlings formed roots in 5 to 7 days. Test seedlings were chosen for uniformly straight primary roots approximately 5 to 6 cm in length.

Isolate SP-7 of *A. euteiches* (originally collected in a heavily infested field near Potlatch, Idaho), subcultured on cornmeal agar, was used to produce zoospores for inoculum (15). For all tests, inoculum concentration per milliliter was determined from a 50-ml aliquot shaken vigorously to induce zoospore encystment and counted with a hemacytometer.

Oospore production in roots. Five-day-old seedling roots of PI 180693 and cvs. Dark Skin Perfection and Bolero were inoculated by immersion of roots of five plants per pea line in a suspension of 7×10^4 zoospores per milliliter. Roots were exposed for 1 h and placed on premoistened germination paper. The root tip at time of inoculation was marked with a small black dot. Inoculated plants were incubated for 4 days. At harvest, roots were excised just below the cotyledons, placed on moistened filter paper, and put in the refrigerator overnight or until processed. Roots were cut into seven 1-cm sections from the excised area toward the root tip. Each equivalent centimeter section from each of five roots per line was macerated in a Sorval microblender at full speed for 1 min in 6 ml of glass-distilled water. After maceration, each sample was placed in a test tube, covered with Parafilm, and placed in the refrigerator until counted. One-ml aliquots from each sample were placed on a Hawksley nematode-counting slide and allowed to settle for 1 min. Numbers of oospores per sample were determined by examination with a compound microscope with 160× brightfield magnification. Oospores on the first 10 lines of the slide were counted for each replicate. Counts for each centimeter sample were performed five times. These were averaged and multiplied by 30 (number of lines per slide) to determine the number of oospores per centimeter sample. This was replicated three times for each line, and the test was performed twice.

Lesion development in seedlings. To measure root lesion development over time, seedlings with a primary root 5 to 6 cm long were inoculated by immersion of the root tip in a 0.25-ml drop of a suspen-

sion of 25,000 mobile zoospores per ml in a closed petri dish for 1 h. Each inoculated seedling was sandwiched between two sheets of autoclaved, premoistened germination paper, which was loosely rolled and placed in a 500-ml glass beaker containing 100 ml of sterile water. The inoculated seedlings were placed in an incubator set at 22°C with minimal fluorescent light (11.5 W/m^2 and 16-h day length). The length of the discolored, watery area on the taproot was measured to the nearest mm after a 3-, 6-, and 8-day incubation. Test seedlings were returned to the germination paper after each measurement and again incubated as described. Measurements were made on the same seedlings for all incubation times. This test was performed twice with 10 replicates per treatment. Data were subjected to covariance and collinearity analyses with the general linear models procedures of SAS (SAS Institute, Cary, NC) software.

Enzyme-linked immunosorbent assay (ELISA) measurements. A polyclonal antiserum to antigens of *Aphanomyces euteiches* (12) was used to determine the relationship between lesion size and amount of antigen produced in that lesion. The visible lesions on inoculated taproots of plants incubated 3, 6, and 8 days were cut into 2-cm sections. Uninoculated plants incubated 3, 6, and 8 days served as the control. Each root was marked at the root tip with indelible ink when the test was initiated. Tissue analyzed by ELISA included root sections 2 cm on either side of the ink mark. Each 2 cm of root tissue was excised, ground in CEP (carbonated coating buffer with egg albumin and PVP-40), and run in indirect ELISA as described previously (12). In each case, ELISAs were run the same day the roots were harvested. This test consisted of 10 replicates per pea line for inoculated and uninoculated plants, and was performed twice. Total lesion length of inoculated tissue was regressed on ELISA readings.

Zoospore attraction studies. Seedling exudates were collected as described previously (13) from germinating, non-fungicide-treated seeds of cv. Bolero, PI 180693 (resistant), and ARS breeding lines 79-2022 and 90-2131. Seed of each line was surface-disinfested, and exudates were collected after a 3-day incubation when root radicals were 2 to 3 cm long. Seedling exudates were aseptically filtered through a 0.45-µm filter and stored at 4°C until used. Numbers of zoospores that germinated due to seedling exudates were determined with an initial concentration of 2 × 10^5 motile zoospores per milliliter. A 1-µl aliquot of seedling exudate was taken up in a micropipette and immersed in the zoospore suspension contained in an autoclaved glass petri dish. Observations were made with a stereoscopic dissecting microscope with overhead illumination at 16×. Numbers of zoospores that germinated in five adjacent microscopic fields, beginning at the capillary tube mouth and proceeding into the micropipette, were recorded at 30 min and 1.5, 3, and 5 h. The control consisted of micropipettes filled with glass-distilled water and immersed in the zoospore suspension. This study was performed three times, with 10 micropipettes observed for each seedling exudate per pea line.

RESULTS

Significantly greater numbers of oospores were produced in the primary roots of the susceptible cvs. Bolero and Dark Skin Perfection than in roots of the more resistant PI 180693 (Fig. 1). Maximum numbers of oospores were produced in

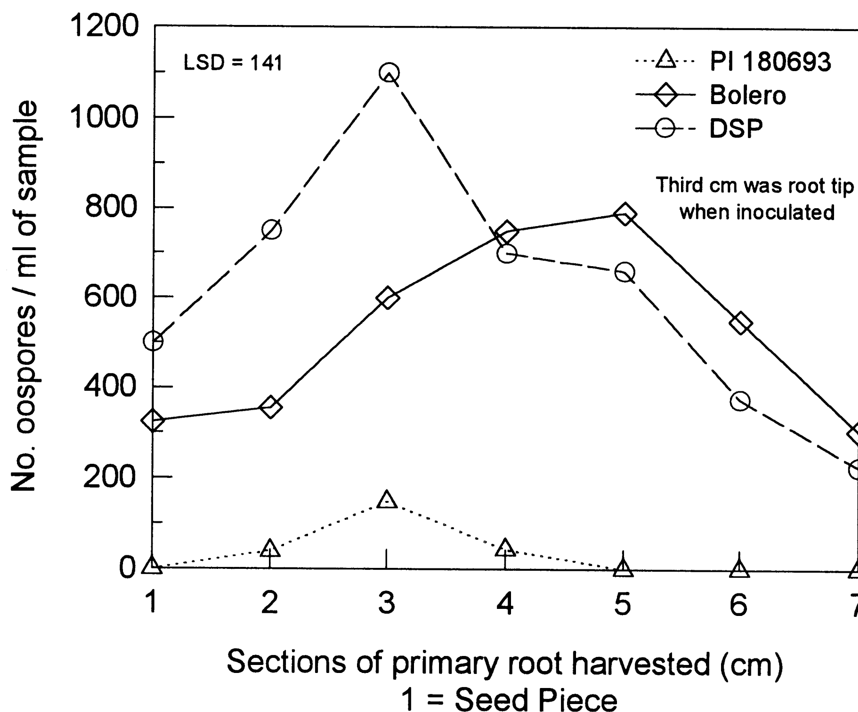

Fig. 1. Numbers of *Aphanomyces euteiches* oospores in roots of resistant and susceptible pea lines. Data are averages of three replicates of samples of 5 counts per cm performed twice.

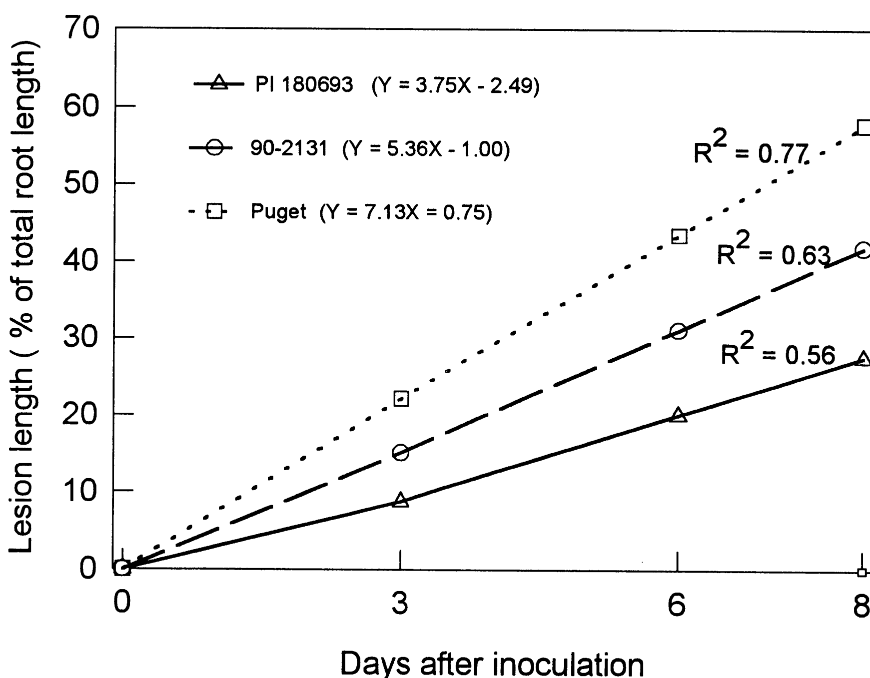

Fig. 2. Comparison of root growth and lesion length over time for resistant and susceptible pea lines inoculated with *Aphanomyces euteiches*.

Dark Skin Perfection roots in centimeter section 3, which had been the root tip at inoculation. In contrast, oospore numbers in taproots of Bolero were greater in sections 4 and 5 (1 and 2 cm of new root growth after inoculation). Oospore production in roots of PI 180693 was significantly lower in all root sections, but increased slightly in section 3.

Lesion spread occurred more slowly in inoculated roots of resistant than in roots of susceptible lines (Fig. 2). PI 180693 was the most resistant line used in this study; lesion length increase was the slowest, and the slope of the linear regression was the lowest on PI 180693. Breeding line 90-2131 was the next most resistant line, and cv. Puget was the least resistant.

Measurements from indirect ELISAs showed that antigens produced by *A. euteiches* did not increase as rapidly in roots of resistant vs. susceptible plants in host tissue encompassed within a given lesion. In addition to slower lesion development, *A. euteiches* did not grow as rapidly in resistant tissue as in susceptible tissue (Fig. 2). An R^2 value of 0.9 was obtained when comparing lesion size to ELISA readings with a linear regression.

Few zoospores germinated in exudates from PI 180693 compared with germination in exudates from the susceptible cv. Bolero (Fig. 3). Numbers of zoospores that germinated in exudates from the germ plasm lines 79-2022 and 90-2131 were between the numbers that germinated in exudates from the susceptible cv. Bolero and the resistant PI 180693. The relationship between numbers of zoospores germinated and time was linear for all pea lines tested.

DISCUSSION

Resistance to *A. euteiches* in pea appears to be associated with slower lesion development and pathogen multiplication. Previous research by Morrison et al. (21) demonstrated that fewer oospores were produced in excised root tips of resistant than of susceptible lines. Our work likewise demonstrated that fewer numbers of oospores were produced in inoculated roots of a resistant than of a susceptible pea line. This result indicates a reduced multiplication of the pathogen in invaded root tissue. This reduced rate of pathogen multiplication was further confirmed by a reduced rate of lesion spread and less antigen buildup in lesions on roots of resistant lines than on roots of the susceptible cv. Puget.

Our results showed a relationship between zoospore germination and resistance or susceptibility. Cunningham and Hagedorn (2) reported that zoospores of *A. euteiches* were attracted to both resistant and susceptible pea lines equally at the region of elongation. Our results, in which exudates were collected from germinating seeds, demonstrated clear differences in zoospore germination between resistant and susceptible pea lines. This difference can be partially explained by the presence of anthocyanin pigmentation in exudates from PI 180693, which are fungistatic to several genera, including *Aphanomyces* (8). However, 79-2022 does not contain anthocyanin pigmentation in the testae, and 90-2131 possesses the Pl gene for anthocyanin pigmentation only in the hilum (10). All three exudates reduced zoospore germination compared with exudates from the commercial cv. Bolero.

Resistance in pea to *A. euteiches* is probably due to more than one gene effect. It is unfortunate that no major gene for resistance to *A. euteiches* has been identified. Work by Lewis and Gritton (18) demonstrated progress in breeding for *Aphanomyces* resistance in peas with recurrent selection to effectively compile minor genes. We are also using recurrent selection, an extremely laborious and time-consuming approach.

In our breeding program, lines that produce >30% more root area per unit of time have been identified. We are now crossing these increased rooting lines with *Aphanomyces*-resistant lines to combine genes for slower lesion spread with increased rooting vigor and reduced oospore and zoospore germination. This strategy may result in an increased overall resistance to *Aphanomyces*. During the course of our investigations, we found variation among *A. euteiches* isolates for pathogenicity to pea breeding lines. This variability in virulence reflects the tenuous nature of resistance and the need to keep adding minor genes for resistance whenever they can be identified.

A decline in resistance to root infection was reported previously when plant vigor is reduced (4). Insect pests, environmental stress, and senescence are major factors in reducing plant vigor. The consequence of reduced plant vigor is the rapid spread of root-infecting fungi into uninfected areas of the root system. Consequently, the eventual control of Aphanomyces root rot must incorporate such factors as improved cultural practices, increased plant vigor, improved seed dressings (chemical and biological), and resistant cultivars.

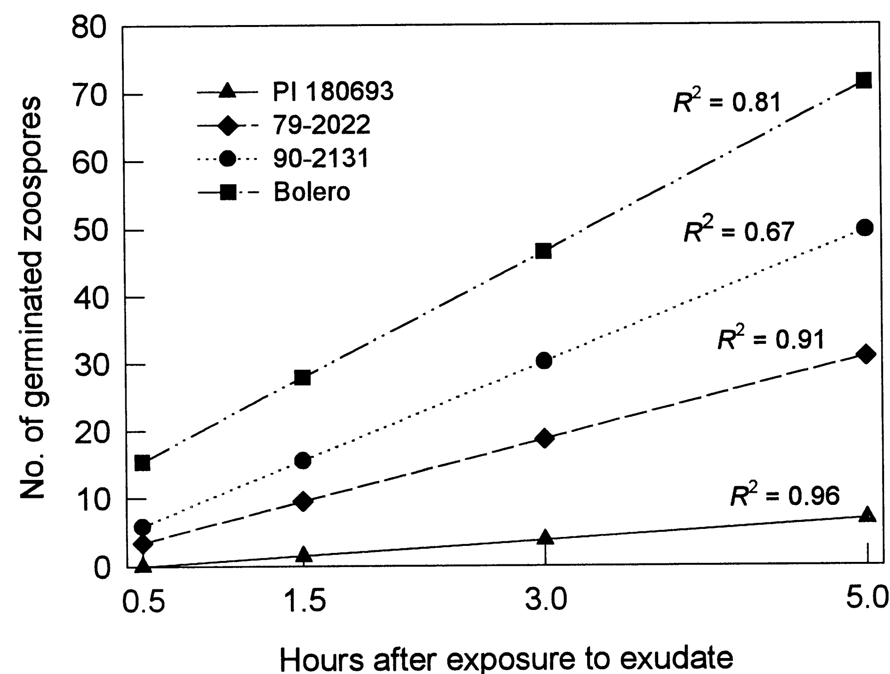

Fig. 3. Germination of *Aphanomyces euteiches* zoospores as affected by exudates from 3-day-old pea seedlings of cv. Bolero, PI 180693, and ARS lines 90-2131 and 79-2022.

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