Variation in Pathogenicity and Genotype Among Single-Zoospore Strains of *Aphanomyces euteiches*

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ABSTRACT

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The role of asexual reproduction in the production of pathogenic and genotypic variation in *Aphanomyces euteiches* was investigated. Variation was studied among three groups of 18 single-zoospore progeny of *A. euteiches* derived from each of three single-zoospore parental strains. Pathogenicity was assessed by evaluating disease severity (DS) on roots of five pea lines possessing different levels of resistance to *Aphanomyces* root rot and of a susceptible cultivar of snap bean and alfalfa. None of the single-zoospore progeny incited significantly higher DS levels than

their parental strain on any of the seven hosts; however, 3 or 4 of the 18 progeny in each group incited significantly lower DS than their parental strains. The host range of the progeny either decreased or remained the same as compared with parental strains. Genotypic variation was assessed with randomly amplified polymorphic DNA (RAPD) analysis. Polymorphic RAPD markers that distinguished parental and progeny strains were detected within two of the three groups of strains with two of four RAPD primers used. Of 76 total RAPD markers that were detected among all strains in all groups, four (5%) were polymorphic. The polymorphic markers were not associated with the pathogenic variation.

Additional keywords: diversity, legumes, oomycete.

Aphanomyces euteiches Drechs. is a soilborne plant pathogen that infects a variety of legume species (16). Pea (*Pisum sativum* L.) is the crop that appears to be the most seriously damaged by *Aphanomyces* root rot, but alfalfa (*Medicago sativa* L.), snap bean (*Phaseolus vulgaris* L.), and red clover (*Trifolium pratense* L.) may also be damaged severely. *A. euteiches*, classified in the kingdom Chromista, division Oomycota, is a phenotypically and genotypically heterogeneous species. Different strains having selective host ranges on several legume species and cultivars have been characterized with pathogenicity and genotype studies (7,9,12). The intraspecific variation within *A. euteiches* may at least partially explain why host resistance to this pathogen has been difficult to obtain. Additional information on the characteristics of diversity within this pathogen should enhance the development and deployment of disease-resistant legume cultivars and contribute to understanding the population biology of this organism.

Although intraspecific variability has been identified in *A. euteiches*, the source and mechanism by which the variability is generated is assumed, but has not been defined experimentally. Sexual recombination and somatic variability via asexual production of zoospores may be two sources of variation (15). Variation among single-zoospore strains has been reported for Oomycota. The most extensive studies of intraspecific variation have focused on *Phytophthora sojae* (a homothallic species) and *P. infestans* (a heterothallic species). Rutherford et al. (19) reported variation in virulence among single-zoospore isolates of *P. sojae* to the extent that some zoospore progeny had a different race classification than the parental strain. Pathogenic variation among single-zoospore cultures of *P. infestans* has also been reported (2,3,11). The source and mechanisms of change were not reported; however, Caten (3) suggested "cytoplasmic changes" may lead to variation.

Somatic mutation or segregation, expressed as pathogenic variation among zoospores, has also been reported as a possible means by which diversity is generated in *A. euteiches* (9). Holub et al. (9) compared pathogenicity characteristics among single-zoospore progeny from one broad-host-range parental isolate. They reported somatic segregation for decreased virulence among the progeny using five legume species. Very little else has been reported on the generation of variation in *A. euteiches*. Furthermore, the role of sexual recombination in generating variation in *A. euteiches* is not understood. *A. euteiches* appears to reproduce homothallically, and the occurrence or importance of heterothallic reproduction is unknown. The lack of consistent and controlled germination of oospores in culture is a formidable barrier to investigating traits of potential progeny of crosses.

This investigation addressed the extent and basis of variation in *A. euteiches* that is expressed through asexually reproduced zoospores. Single-zoospore progeny from three different parental strains of *A. euteiches* were studied, and pathogenicity traits were investigated using pea, bean, and alfalfa cultivars. Genotypic variation among the progeny was investigated with random amplified polymorphic DNA (RAPD) analysis (5,21,22). The main objective of this study was to determine if *A. euteiches* generates pathogenic and genotypic variation through production of zoospores.

MATERIALS AND METHODS

***Aphanomyces* cultures.** Three parental strains, P134(1), AeMM187(1), and AOP46(1), derived from single zoospores, were chosen to represent pathogenic variability and different hosts and locations of origin (Table 1). Eighteen single-zoospore progeny were generated from each of these parental strains by inducing zoospore production as described by Mitchell and Yang (14). Zoospores were spread on an agar medium (17) and single germinating zoospore cysts were subcultured. The single-zoospore progeny are labeled P134(1-X), in which X is between 1 and 23; AeMM187(1-Y), in which Y is between 2 and 20; and AOP46(1-Z), in which Z is

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TABLE 1. Pathogenicity of three parental strains of *Aphanomyces euteiches* and 18 single-zoospore progeny from each parental strain on five pea cultivars (P1 to P5), alfalfa (A), and bean (B)

Parent	Origin of parent		No. of single-zoospore progeny	No. of zoospore progeny pathogenic on different hosts ^a						
	Host	Location		P1	P2	P3	P4	P5	A	B
P134(1)	Pea	Wisconsin	18	17	16	15	17	17	0	0
AeMM187(1)	Pea	Minnesota	18	0	0	0	18	18	15	15
AOP46(1)	Alfalfa	Wisconsin	18	0	0	4	17	17	16	0

^a Pathogenic strains incited disease severity levels greater than 3.0 on a scale of 0 to 5. Nonzero values indicate that parental strains are pathogenic on particular hosts.

between 2 and 28. All strains were maintained on corn meal agar (Difco Laboratories, Detroit) at 4°C.

Pathogenicity testing. All parental and single-zoospore progeny strains of *A. euteiches* were tested for pathogenicity on five pea cultivars, snap bean, and alfalfa (Table 2). Two previously characterized strains, MF-1(1) and GB7(1), that infect alfalfa and bean, respectively, were included as pathogenicity controls (7,12). Waxed, 160-ml paper cups (7.5 × 6.5 cm) containing vermiculite were sown with 3 seeds of bean or pea, or 8 to 10 seeds of alfalfa. Seeds of alfalfa were sown in combination with host-selective *Rhizobium meliloti*. Experiments were conducted in a greenhouse maintained at 24°C ± 2°C with a 12-h light/dark cycle. Seven days after sowing seed, 3,000 zoospores were injected with a pipetter 1 cm below the surface of vermiculite near seedlings in each cup. Cups were flooded at the time of inoculation and watered as needed to keep the vermiculite moist. Plants were harvested 14 days after inoculation, and disease severity (DS) and biomass loss were evaluated. DS (4) was determined visually using a 0 to 5 scale, in which 0 = no macroscopic symptoms; 1 = a few small, discolored spots on roots; 2 = minor discoloration covering only part of the root system; 3 = over 90% of the root system brown or yellowed, but no symptoms on the epicotyl/hypocotyl; 4 = all of the root system brown or soft and the epicotyl/hypocotyl shriveled or brown; and 5 = plant dead. DS values ≤3.0 were considered a nonpathogenic interaction. Below this DS level, there was minor or no root discoloration and no clear evidence of successful root infection, and attempts to isolate *A. euteiches* were unsuccessful. Ten pea and five alfalfa plants with characteristic symptoms of *Aphanomyces* root rot were examined microscopically for the presence of oospores. The experiment was organized as a split plot design with strain as the main plot factor and cultivar the subplot factor. Three replicate cups were used for noninoculated controls and for each combination of strain and plant host. The entire experiment was repeated once. Sets of whole pea and bean plants inoculated with the parental strains and nine progeny from each parental strain were dried for 8 days at 24°C, and dry biomass was determined. Percent loss of biomass of inoculated versus noninoculated dry plants was calculated to measure the effect of disease on whole plant growth. DS and percent loss values among strains were evaluated with analysis of variance, and means were compared using Fisher's least significant difference test (SAS; SAS Institute, Inc., Cary, NC).

Polymerase chain reaction amplifications and RAPD assays.

Aphanomyces strains were grown at 22°C with a 12-h photoperiod in peptone-glucose broth (14) amended with 40 µg of rifampicin per ml (Sigma Chemical Co., St. Louis) for 6 days. DNA was extracted (18), and DNA concentrations were measured with a fluorometer (model TKO 100; Hoefer Scientific Instruments, San Francisco, CA) and standardized for all strains prior to RAPD analyses. Four decanucleotide primers with arbitrary sequences obtained from the University of British Columbia, set 100/1, were used for RAPD analysis (Table 3). The primers were selected based on band clarity and numbers of total and polymorphic bands produced in previous studies with strains having different pathogenicity characteristics on pea, bean, and alfalfa (12). DNA from all parental and progeny strains of *A. euteiches* was amplified using each of the four primers. Each 25-µl amplification reaction contained 15 ng of primer,

TABLE 2. Code, characteristics, and source of pea (*Pisum sativum*), alfalfa (*Medicago sativa*), and bean (*Phaseolus vulgaris*) used for pathogenicity tests

Code	Host spp.	Line/cv.	Relative level of resistance/tolerance ^a	Source
P1	Pea	MN 313	High	D. Davis, Univ. of MN (4)
P2	Pea	MN 314	High	D. Davis, Univ. of MN (4)
P3	Pea	K902079	Moderate	J. Kraft, USDA (10)
P4	Pea	W18904	Moderate	E. Gritton, Univ. of WI (8)
P5	Pea	Little Marvel	Low	Burpee Seed Co., Warminster, PA
ALF	Alfalfa	Saranac	Low	Public variety
BEAN	Bean	Early Gallatin	Low	Rogers Bros. Seed Co., Stanton, MN

^a Resistance/tolerance to root rot caused by *Aphanomyces euteiches*.

2 mM MgCl₂, 2.5 ng of genomic DNA, and 0.75 units of *Taq* DNA polymerase. Amplifications were conducted in a Tempcycler II (Coy Laboratory Products, Inc., Grass Lake, MI) programmed for one cycle of 94°C for 30 s; 46 cycles of 94°C for 40 s, 36°C for 1 min, and 72°C for 2 min; and one cycle of 72°C for 7 min. Amplified samples (11 µl) were loaded into composite gels made with 0.6% agarose (Fisher Scientific Co., Pittsburgh) and 0.44% Synergel (Diversified Biotech, Boston) in 1× TAE buffer (40 mM Tris, 1.3 mM EDTA, and 0.11% glacial acetic acid; pH 8.0), and electrophoresis was conducted at 6.8 V/cm for about 2.2 h. RAPD banding patterns were analyzed as binary data representing the presence or absence of bands. Clear, intense bands were included in the analysis, while faint, inconsistent bands were excluded to improve reproducibility of results. Reproducibility of RAPD banding patterns was tested by amplifying DNA from strain P134(1) 14 times and by reamplifying DNA once and comparing resulting banding patterns from 55% (10 out of 18) of all single-zoospore progeny strains from each set. This replicated subset included all strains that contained polymorphic bands.

RESULTS

Pathogenicity comparisons. Noninoculated control plants remained healthy with DS = 0. Strains MF-1(1) and GB7(1), used for positive pathogenicity controls on alfalfa and bean, incited DS levels of 3.3 ± 1.1 and 3.5 ± 0.6 on these hosts, respectively. Inoculated plants developed characteristic symptoms of *Aphanomyces* root rot, and all roots examined contained typical oospores. Two to four single-zoospore strains were identified in each group that incited significantly lower ($P = 0.05$) levels of DS than the parental strain (Table 4). No single-zoospore progeny strains were identified that incited significantly higher DS levels on any host than their parental strains (Figs. 1 to 3). Zoospore progeny that incited a significantly lower DS than the parental strain were detected on all plant hosts used except P3 (Table 4). The host range of some single-zoospore progeny was reduced compared with parental strains; however, none of the progeny had a host range greater than the corresponding parental strain on the seven host cultivars (Table 1). Some strains that may appear to be much different than the parental strain are not significantly different. These data also illustrate the different relative levels of resistance to *Aphanomyces* root rot among the five pea cultivars, with

TABLE 3. Random amplified polymorphic DNA (RAPD) primers used for analysis of single-zoospore strains of *Aphanomyces euteiches* and the number of bands and RAPD genotypes detected in each group (A, B, and C) with each primer

UBC ^a primer code	Primer sequence (5' to 3')	Mean number of bands amplified per strain (standard deviation)	Number of polymorphic bands within groups of single-zoospore strains			Total number of bands among all strains in groups			Number of RAPD genotypes in groups		
			A ^b	B ^c	C ^d	A ^b	B ^c	C ^d	A ^b	B ^c	C ^d
2	CCTGGGCTTG	5.3 (0.6)	0	0	0	5	6	5	1	1	1
12	CCTGGGTCCA	6.3 (0.6)	1	0	1	6	6	7	2	1	2
31	CCGGCCTTCC	7.3 (2.5)	0	0	0	5	7	10	1	1	1
33	CCGGCTGGAA	6.3 (2.3)	1	0	1	9	5	5	2	1	2

^a Code for primer sequence from the University of British Columbia.

^b 18 single-zoospore strains in the P134(1-X) group.

^c 18 single-zoospore strains in the AeMM187(1) group.

^d 18 single-zoospore strains in the AOP46(1-X) group.

TABLE 4. Disease severity (DS) incited by parental strains and single-zoospore progeny (1-X) of *Aphanomyces euteiches* that incited significantly different ($P = 0.05$) DS than the parental strain on one or more of five pea lines/cv. (P1 to P5), alfalfa (Alf), or snap bean (Bean) in two replicated experiments^a

Strain	DS ^{b,c}													
	P1		P2		P3		P4		P5		Alf		Bean	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
P134(1-8)	2.6	3.6	3.7	2.0	4.2	3.8	3.7*	3.6*	5.0	3.6	1.7	1.3	1.3	1.4
P134(1-18)	3.7	0.7	3.7	1.0	4.5	0	4.6	0.5	5.0	0.6	0.8*	0*	1.3	0.4
P134(1-21)	3.3	2.7	2.9*	3.0*	4.0	3.7	3.7*	3.2*	3.8	3.6	2.0	1.5	1.3	1.7
P134(1) (exp. 1)	4.0	4.0	4.0	4.1	4.6	4.3	4.6	4.7	4.6	4.7	2.2	2.8	2.8	1.4
LSD ^d	0.8	0.9	0.6	1.0	0.7	1.3	0.9	0.8	0.6	0.7	0.4	0.8	0.8	0.8
AeMM187(1-3)	0.3	0.3*	0.7	1.0	2.1	1.3	4.4	4.5	4.4	3.7	2.5*	2.3*	2.8	1.7
AeMM187(1-12)	0.6	0.4*	0.4*	0*	1.2	0.3	4.0	4.4	4.3	4.7	3.1	2.8	3.4	2.6
AeMM187(1-15)	2.0	0.2	0.8	0	2.8	0.4	3.7*	2.5*	4.1	2.8	3.0	3.0	2.3*	1.9*
AeMM187(1-17)	1.6	0.8	1.2	1.0	2.2	0.8	3.7*	3.3*	4.3	3.9	2.8	3.3	3.3	2.6
AeMM187(1)	1.0	1.7	0.7	1.1	2.2	2.0	4.2	4.5	3.7	4.5	2.6	3.2	1.8	3.2
LSD (exp. 1)	1.0	0.6	0.5	0.5	1.0	0.9	0.7	0.8	0.6	0.9	0.5	0.5	0.7	1.3
AOP46(1-2)	2.6	2.7	1.8	1.0	2.4	2.0	2.9	4.5	3.1	4.5	2.8	3.2	2.7*	1.1*
AOP46(1-4)	1.4*	0.8*	0.5	1.0	2.9	2.7	3.7	2.4	3.8*	3.9*	2.8	2.1	2.1	2.1
AOP46(1-10)	2.0	2.1	1.6	1.0	2.3	2.8	3.9	4.2	4.3*	4.0*	3.0	3.0	1.6	1.5
AOP46(1-28)	0.5	2.1	0.5	1.0	0.5	3.3	0.8*	3.9*	0.3	4.4	0	2.7	0	1.8
AOP46(1)	2.6	2.5	1.1	1.5	2.3	3.1	4.6	4.8	4.6	4.8	2.6	3.2	2.3	2.9
LSD	0.8	0.9	0.6	1.0	0.7	1.3	0.9	0.8	0.6	0.7	0.4	0.8	0.8	0.8

^a 18 single-zoospore progeny from each of the three parental strains (P134(1), AeMM187(1), and AOP46(1)) were tested, and only those that incited significantly different DS on at least one host than the parental strain are shown.

^b DS is based on root infection using a 0 to 5 scale, in which 0 is healthy and 5 is dead. All DS data represent the mean from three replications.

^c Values marked with an * represent strains that incited significantly different DS ($P = 0.05$) than the parental strain on a given plant host in each of two experiments.

^d Least significant difference.

P2 expressing the highest level of resistance, followed by P1, P3, P4, and P5.

Dry biomass of selected sets of inoculated and noninoculated plants were compared. Whole plant biomass was reduced in inoculated versus noninoculated plants for most treatments. Biomass loss values ranged from 0 to 45%. However, most results were highly variable because of nonuniform growth habits of the plants (data not shown). Because of the variability, no significant differences ($P = 0.05$) in percent loss of biomass between parental and progeny strains was detected. Regression analysis revealed linear relationships between DS values and percent loss on all pea cultivars. For the groups of P134(1-X), AeMM187(1-Y), and AOP46(1-Z) zoospore progeny, the mean and standard deviation for the coefficients of determination (r^2) values were 0.46 (0.28), 0.81 (0.12), and 0.64 (0.22), respectively.

RAPD analysis. Genomic DNA from all parental and single-zoospore progeny strains was used in single-primer reactions to identify monomorphic and polymorphic amplified fragments ("markers"). Most markers were monomorphic in the groups of strains that consisted of a parental strain and 18 single-zoospore progeny (Table 3, Fig. 4). RAPD analysis with the four primers detected polymorphic markers within the P134(1-X) and AOP46(1-Z) groups of single-zoospore progeny. Polymorphic markers were not detected in the AeMM187(1-Y) group. There were 25, 24, and

27 RAPD markers detected in the P134(1-X), AeMM187(1-X), and AOP46(1-Z) groups of strains, respectively, and 8, 0, and 7% of these markers were polymorphic. Primers 2 and 31 detected no polymorphisms and, hence, only one 'genotype', whereas, primers 12 and 33 detected polymorphisms and one or two 'genotypes' in each group of strains. Thus, primers 12 and 33 could potentially be used to distinguish particular strains. Primer 12 produced one marker band from strain P134(1-12) and another from strain AOP46(1-15), which distinguished these progeny from their parental and sibling strains. Primer 33 produced one marker from strain P134(1-7) and another from strain AOP46(1-27), which distinguished these progeny from their parental and sibling strains. Polymorphic markers were not detected in progeny that differed significantly from the parental strain in DS. Thus, no polymorphic markers were associated with the pathogenic variability detected on the seven plant cultivars. Replicated RAPD analysis of 55% of all strains, including all strains that exhibited polymorphisms, revealed the same bands in both replications and demonstrated that the RAPD banding patterns were reproducible.

DISCUSSION

A. euteiches is a variable species with regard to host range (1,7,9, 13,20). Its population biology is poorly understood, and the relative

Fig. 1. Number of single-zoospore progeny strains from *Aphanomyces euteiches* parental strain P134(1) that incited different levels of disease severity (DS) on pea cultivars P1 to P5, alfalfa, and bean. The horizontal bar on each plot represents the mean and standard deviation of DS for P134(1). Data represent the mean from two experiments with three replications each. DS is based on severity of root infection using a 0 to 5 scale.

importance of asexual versus sexual reproduction for the generation of intraspecific variability is not known. Because of difficulties in germinating sexually produced oospores of *A. euteiches*, the occurrence and function of heterothallic crossing remains unknown. This is the first study to investigate variation and stability among single-zoospore strains from more than one parental strain of *A. euteiches* and to directly estimate genotypic variation. It demonstrates that asexual reproduction generates low levels of pathogenic and genotypic variability among zoospore progeny. Interpretations of these data must consider that a relatively

Fig. 2. Number of single-zoospore progeny strains from *Aphanomyces euteiches* parental strain AeMM187(1) that incited different levels of disease severity (DS) on pea cultivars P1 to P5, alfalfa, and bean. The horizontal bar on each plot represents the mean and standard deviation of DS for AeMM187(1). Data represent the mean from two experiments with three replications each. DS is based on severity of root infection using a 0 to 5 scale.

small number of single-zoospore strains were studied compared with the numbers that may be produced by single zoosporangia (e.g., up to 300) and, moreover, by single strains and populations (16). As discussed by Rutherford et al. (19), experimental logistics limit the number of strains that can be studied and compared. Regardless, the sample numbers were high enough in this study to detect genotypic and pathogenic variation among single-zoospore progeny.

A key hypothesis underlying this study was the following: asexual reproduction can produce progeny strains originating from

Fig. 3. Number of single-zoospore progeny strains from *Aphanomyces euteiches* parental strain AOP46(1) that incited different levels of disease severity (DS) on pea cultivars P1 to P5, alfalfa, and bean. The horizontal bar on each plot represents the mean and standard deviation of DS for AOP46(1). Data represent the mean from two experiments with three replications each. DS is based on severity of root infection using a 0 to 5 scale.

single zoospores that vary in pathogenicity traits from parental strain(s) and that have abilities to overcome new sources of host disease resistance. These new genotypes of *A. euteiches* may then undergo selection pressure by host species or cultivars with new sources of resistance, thus shifting the population structure towards the new pathogen genotypes. Although DS varied between some parental and single-zoospore progeny strains on individual host species, all the significant pathogenicity changes detected in this study were in the direction of decreased DS on given plant cultivars as compared with the parental strain. No progeny incited significantly ($P = 0.05$) greater DS than the corresponding parental strain on any host. Several progeny in all three

single-zoospore groups incited significantly lower DS than the parental strain on at least three of the seven plant hosts, and some progeny lost pathogenicity on particular hosts. In addition, the trend was for many progeny strains to incite lower, although insignificant, levels of DS than the mean DS incited by the parental strain. In no case was the host range expanded for progeny beyond that of the parental strains, but in some cases it was reduced.

Caten (2) reported zoospore variability in *P. infestans*, but suggested it is not an important source of new fungal genotypes that overcome novel sources of resistance. This study and a previous study (9) with *A. euteiches* suggest that the same may be true for *A. euteiches*. This study contrasts with a study by Holub et al. (9) that used one cultivar each of pea, alfalfa, and bean, and did not directly study genotypic traits. They (9) studied pathogenic variation among single-zoospore progeny from one wide-host-range parental mass isolate. They also reported that many single-zoospore progeny were less pathogenic than the parent culture. In both this and Holub et al. (9) studies, the trend towards lower pathogenicity of progeny compared with parental strains was more pronounced on pea than on alfalfa or bean cultivars.

Another goal of this study was to determine if pathogenic variation was based on genomic mutations or rearrangements rather than variable gene expression or adaptive physiological traits. RAPD analysis has been used successfully for similar studies (5) and was used to detect variable genetic markers in this study. The RAPD analysis revealed genetic variability within two of the three groups of single-zoospore progeny. The polymorphic RAPD markers detected, however, were not found to be associated with the strains that exhibited pathogenic variation. The results suggest that genetic variation is generated through asexual reproduction, but it has not been confirmed that the pathogenic variation is due to genotypic change. Testing of additional single-zoospore progeny and using additional primers would increase the chance of detecting putative genetic loci that are related to pathogenic variability.

The detection of variable RAPD markers with a relatively small number of primers and single-zoospore progeny suggests that genetic change may occur in *A. euteiches* during asexual reproduction. It is unknown if any of the variable genetic markers detected are associated with pathogenicity or any functional genes. The pathogenic variability detected, however, suggests that changes occurred in genes or gene expression related to pathogenicity. Considering that pathogenicity is often a polygenic trait and the spontaneous mutation frequency per gene in fungi is estimated at 1×10^{-6} (6), it seems likely that the variation detected in single-zoospore progeny is due to a mechanism other than spontaneous mutation. The genetics of inheritance in *A. euteiches* are poorly understood, and no genetic studies with this pathogen have been done to provide information to explain the results. Studies of *P. infestans* may provide some clues to understanding the biology of *A. euteiches*. Caten (3) suggests that individual mycelia of *P. infestans* are composed of a mixture of different cytoplasmic genotypes that are normally available for selection. Leach and Rich (11) suggest that heterokaryosis and parasexuality may play a role in generating variability among single zoospores of *P. infestans*.

A. euteiches is a variable organism and may have the capacity to adapt to selection pressures through asexual reproduction. Variability among single-zoospore cultures must be considered when conducting any laboratory, field, or disease-resistance study with this pathogen. In particular, care must be taken when choosing single-zoospore cultures for specific investigations. Additional studies are needed to elucidate the genetics and variability in this organism if we hope to understand its population biology and structure and ultimately to develop successful methods to control *Aphanomyces* root rot diseases.

Fig. 4. Random amplified polymorphic DNA banding patterns generated with primers 2 (top panel) and 12 (bottom panel) using total genomic DNA from subsets of strains from three groups of single-zoospore strains. The first and last lanes in the top and bottom panels are DNA size markers with the top five visible bands having sizes of 2.6-, 1.6-, 1.2-, 0.7-, and 0.5-kb pairs. (Top) Lanes 2 to 10 are single-zoospore progeny strains P134(1-X), in which X is 1, 2, 5, 7, 10, 12, 13, 17, and 22. Lane 11 is parental strain P134(1). Lane 12 is P134(1-23). Lanes 13 to 21 are single-zoospore progeny strains AeMM187(1-Y), in which Y is 2, 3, 5, 6, 9, 12, 13, 14, and 19, and lane 22 is parental strain AeMM187(1). Lanes 24 to 32 are single-zoospore progeny strains AOP46(1-Z), in which Z is 2, 3, 7, 10, 13, 14, and 27. Lane 33 is parental strain AOP46(1). Polymorphic bands appear in lanes 7 and 29 slightly above the 0.7-kbp marker. (Bottom) Lanes 2 to 11 contain the same strains as appear in lanes 2 to 11 in the top panel. Lanes 12 to 21 contain the same strains as appear in lanes 13 to 22 of the top panel. Lanes 22 to 31 contain the same strains that appear in lanes 24 to 33 of the top panel, except lane 29 contains AOP46(1-15). Lane 32 is strain AOP46(1-15), and lane 33 is a water (no DNA added) control.

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Erratum

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In the manuscript entitled "Variation in Pathogenicity and Genotype Among Single-Zoospore Strains of *Aphanomyces euteiches*" by D. K. Malvick and J. A. Percich (Phytopathology 88:52-57), Figure 4 and caption are as follows

Fig. 4. Random amplified polymorphic DNA banding patterns generated with primers 12 (top panel) and 2 (bottom panel) using total genomic DNA from subsets of strains from three groups of single-zoospore strains of *Aphanomyces euteiches*. The first and last lanes are DNA size markers (M) with the top five visible bands having sizes of 2.6-, 1.6-, 1.2-, 0.7-, and 0.5-kb pairs. (Top) Lanes 1 to 10 are single-zoospore progeny strains P134(1-X), in which X is 1, 2, 5, 7, 10, 12, 13, 17, 22, and 23. Lanes 11 to 19 are single-zoospore progeny strains AeMM187(1-Y), in which Y is 2, 3, 5, 6, 9, 12, 13, 14, and 19. Lane 20 is parental strain AeMM187(1). Lanes 21 to 30 are AOP46(1-Z), in which Z is 2, 3, 5, 7, 12, 13, 14, 15, 18, and 27. Lane 31 is parental strain AOP46(1), and lane 32 is a water (no DNA added) control. Polymorphic bands appear in lanes 6 and 28 slightly above the 0.7- kbp marker. (Bottom) Lanes 1 to 10 contain the same strains that appear in lanes 1 to 10 of the top panel. Lane 11 is P134(1-23). Lanes 12 to 20 contain the same strains that appear in lanes 11 to 19 of the top panel. Lanes 21 and 23 to 30 contain the same strains that appear in lanes 21 to 30 of the top panel. DNA was not amplified in lane 22. Lane 31 is strain AOP46(1-28). Lane 32 is parental strain AOP46(1).