Isozyme Variability in *Alternaria solani* and *A. alternata*

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ABSTRACT

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Fifty-four isolates of *Alternaria solani*, the causal agent of early blight of potato, and 96 isolates of *A. alternata* from various hosts and geographic locations were examined for isozyme variability; starch gel electrophoresis was used for the analyses. Thirteen loci were identified in 10 enzyme systems. Thirty-five electrophoretic types (ETs) were detected. The most common ET had 47 isolates, whereas 18 ETs had only one isolate. The average genetic diversity for a given enzyme ranged from 0.000 to 0.763; the average genetic diversity was 0.500. The number of alleles detected per enzyme ranged from one to four; the average was 2.9. Cluster analysis divided the isolates into groups that almost completely corresponded to species. Only one isolate of *A. alternata* did not fit this pattern. Three enzymes differentiated the isolates according to species for all but one of the isolates. No significant correlation was found between isozyme phenotype and host or geographic origin of the isolates.

Alternaria solani Sorauer is the causal agent of early blight of potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), and other solanaceous crops (6). *A. solani* produces simple conidiophores bearing dark, multiseptate conidia. The conidia have long beaks and are usually borne singly on conidiophores in foliar lesions. The mycelium ranges in color from gray to black, and some isolates produce a dark orange-red pigment in culture (5).

A. alternata (Fr.) Keissl. is a related species but differs in some aspects from *A. solani*. In culture, *A. alternata* produces an aerial mycelium that appears fluffy and is lighter in color compared to mycelium of *A. solani*. The conidia of *A. alternata* are multiseptate, darkly pigmented, smaller, have shorter beaks, and are borne in chains rather than borne singly as in *A. solani*.

In addition to morphological differences, these two *Alternaria* species differ in their pathogenicity. *A. solani* is recognized as a true pathogen of potato, yet *A. alternata* is often regarded as a saprophyte or weak parasite (8). However, Droby et al (4) have shown *A. alternata* to be the causal agent of brown spots on potato foliage in Israel. *A. alternata* has also been cited as the cause of black pit disease on potato tubers in Israel (3). *A. alternata* can also be isolated from diseased tomato and pepper (20).

Previous research has provided evidence for the existence of physiological races of *A. solani*. Bonde (1) demonstrated that *Alternaria* isolates from potato tubers exhibited differences in colony morphology, sporulation capacity, and growth rates on artificial media. He also reported that differences in pathogenicity exist between isolates. In a later study, Henning and Alexander (7) obtained preliminary evidence for physiological differences in *A. solani* isolates inoculated onto tomato seedlings. These studies indicated differences between isolates of *A. solani* at a morphological, physiological, and pathogenic level; differences may also exist at a biochemical level.

The study of protein polymorphisms through isozyme analysis provides a powerful tool for assessing the genetic variability in plant pathogen populations. Measures of variability such as genetic distance and genetic diversity can be calculated from isozyme data. Leung and Williams (13) used enzyme polymorphisms and genetic distance to examine the degree of similarity between isolates of *Magnaporthe grisea*. Lu and Groth (15) used isozyme data to calculate the percentage of similarity between isolates of the bean rust fungus. In a related study, Linde et al (14) constructed dendrograms with cluster analysis to examine relationships between isozyme phenotypes and virulence for *Uromyces appendiculatus*. *A. solani* lacks a known sexual cycle, and, therefore, hybridizaton methods of studying its genetic variability are not possible. Isozyme analysis provides an ideal method of examining the level of genetic variability in these fungi.

The objectives of this study were to screen isolates of *A. solani* and *A. alternata* from different solanaceous hosts and geographic locations throughout the United States for isozyme activity, and then to determine if a correlation exists between species, host, or geographic location of the isolates and isozyme phenotype. Last, we sought to estimate the amount of genetic variation within and among *A. solani* and *A. alternata* isolates.

MATERIALS AND METHODS

Isolates were obtained from fresh leaf tissue, dried leaf samples, or infected potato tubers. Sections of plant material with early blight lesions were surfaced-disinfested in a 1% sodium hypochlorite solution for 1 min, rinsed in sterile distilled water, placed on acidified potato-dextrose agar (PDA) in petri plates, and incubated at 22 C with a 12-h daylength. Mycelia growing from the diseased tissue were hyphal-tipped and transferred onto plates containing V8 agar. The resulting isolates were incubated at room temperature until mycelial growth covered approximately half of the petri plate, usually within 5–7 days. Isolates then were identified as *A. solani* or *A. alternata* by spore and colony morphology. For this study, 54 *A. solani* and 96 *A. alternata* isolates were used for electrophoresis. Table 1 lists the number of isolates in each electrophoretic type, and the state of origin and the host range of the isolates. Eighty-eight single-spore *Alternaria* isolates and 62 hyphal-tipped isolates were used for electrophoresis. Single-spore and mass isolates were kept on V8 agar plates or slants for short-term storage and were preserved on silica gel for long-term storage (18).

We prepared *Alternaria* isolates for electrophoresis by inoculating 50 ml of sterile potato broth with four mycelial plugs. Flasks were shaken continuously at 125 rpm for 7–10 days. We then separated the mycelium from the broth by suctioning the broth through a Buchner funnel and adsorbing the mycelial pieces onto filter paper. The mycelium was washed with 5 ml of homogenization buffer (1.21 g of Tris, 0.292 g of EDTA, 38 mg

of NADP per 1 L of distilled water), ground with 1.5 ml of homogenization buffer in a hand-held glass homogenizer, and centrifuged for 5 min in a table-top centrifuge at 3,400 rpm. The supernatant was collected and frozen at −80 C until tested.

Electrophoresis and staining procedures were carried out according to Selander et al (19). Twenty enzymes were tested for activity in three buffer systems; only Tris-citrate, pH 8.0 (tray buffer) and Tris-citrate, pH 8.0, diluted 1:30 (gel buffer) were used throughout this study. All isolates were tested at least twice.

Electromorphs were measured as millimeters from the cathode. Relative mobilities were calculated with the most commonly occurring electromorph designated 100 (Table 2). Electromorphs then were assigned integral values; the electromorph that migrated closest to the anode was designated 1. Electromorphs were equated with alleles at corresponding enzyme loci. Only one band per isolate was detected at some loci. Where more than one band per isolate was detected, these bands did not migrate independently and were, thus, scored as a single electromorph and treated as a single allele. Abbreviations with only the first letter capitalized refer to loci coding for a given enzyme, and abbreviations in all capital letters refer to enzymes (16).

The data were analyzed by using the computer program, "ETDIV and ETCLUS" (T. S. Whittam, Dept. of Biology, The Pennsylvania State University, University Park) (19,23). Genetic diversity at each enzyme locus and a genetic distance matrix were calculated along with cluster analyses. The data were also analyzed according to species, host from which cultures were isolated, and geographical origin. Genetic diversity was calculated as $h = (1 - \Sigma x_i^2)(n/[n - 1])$, in which x_i = frequency of the ith allele at the locus, and n is the number of isolates in the sample. Isolates were classified into electrophoretic types (ETs) on the basis of unique combinations of alleles at each of 13 loci. The genetic distance matrix was generated by comparing the number of different alleles between all possible combinations of ETs and was subjected to cluster analysis, which grouped the ETs according to relatedness. From the genetic distance matrix and cluster analysis, a dendrogram was produced that represents a hypothetical phylogenetic tree indicating the average genetic divergence among isolates.

TABLE 1. Number of *Alternaria* isolates of each electrophoretic type (ET), and state of origin and host range of the isolates

ET[a]	Number of isolates within each ET	State of origin[b]	Host range
1	11	GA, ID, OR, PA	Potato, tomato
2	2	ME, VA	Potato, tomato
3	1	PA	Potato
4	4	PA	Potato
5	2	GA, PA	Potato, tomato
6	1	PA	Potato
7	2	GA, PA	Potato, tomato
8	4	GA, PA	Pepper, potato, tomato
9	1	OR	Potato
10	2	PA	Amaranthus, potato
11	3	GA, PA	Potato, tomato
12	1	PA	Potato
13	1	PA	Potato
14	1	CO	Potato
15	5	GA, ID	Potato, tomato
16	1	GA	Tomato
17	5	GA, PA	Potato, tomato
18	46	FL, GA, MN, NY, OR, PA	Pepper, potato, tomato
19	1	OR	Potato
20	1	PA	Potato
21	1	OR	Potato
22	1	PA	Potato
23	14	ID, PA	Potato
24	8	NY, PA	Potato
25	1	MN	Potato
26	3	OR	Potato
27	13	ID, ME, NY OR, PA	Potato
28	2	PA	Potato
29	1	CO	Potato
30	7	ME, PA	Potato
31	1	PA	Potato
32	1	PA	Potato
33	1	PA	Tomato
34	1	ME	Potato
35	1	DE	Tomato

[a] ETs 1–22 and 35 were all *Alternaria alternata*. Ets 23–34 were all *A. solani*.

[b] State abbreviations are DE = Delaware; GA = Georgia; ID = Idaho; ME = Maine; MN = Minnesota; NY = New York; OR = Oregon; PA = Pennsylvania.

RESULTS

Of the 20 enzymes tested, 10 produced activity that was clear enough to be scored. These enzymes were malate dehydrogenase (MDH; EC 1.1.1.37), peptidase (PEP; EC 3.4.x.x) (using phenylalanyl-*L*-leucine as a substrate), isocitrate dehydrogenase (IDH; EC 1.1.1.42), aconitase (ACO; EC 4.2.1.3), phosphoglucomutase (PGM; EC 2.7.5.1), glucose-6-phosphate dehydrogenase (G6P; EC 1.1.1.49), mannose phosphate isomerase (MPI; EC 5.3.1.8), mannitol-1-phosphate dehydrogenase (MPD; EC 1.1.1.17), glyceraldehyde-3-phosphate dehydrogenase (G3P; EC 1.2.1.12), and phosphoglucose isomerase (PGI; EC 5.3.1.9). Thirteen genetic loci were detected from the staining of these enzymes. PEP, MPI, and MPD each produced electromorphs indicative of two loci, which were subsequently scored and labeled as *Pe1* and *Pe2*, *Mp1* and *Mp2*, and *Md1* and *Md2*, respectively. The number of alleles per locus ranged from one for *G6p* to four for *Idh*, *Pe2*, *Pgm*, and *Md1* (Table 2). Two or three alleles were detected at most enzyme loci.

Of the 13 loci, 12 were polymorphic. *G6p* was the only monomorphic locus (Table 2). However, two loci were monomorphic within *Alternaria* species. *A. solani* isolates were monomorphic

TABLE 2. Number of alleles present, genetic diversity (h), and relative mobilities of alleles at 13 genetic loci for 10 enzymes tested on *Alternaria* isolates

Locus[b]	Number of alleles	h[c]	Relative mobility of allele[a] 1	2	3	4	Null[d]
Mdh	2	0.494	100/84[e]	73/61	...	...	...
Pe1	4	0.311	122/109	109/100	109/88	100/88	...
Pe2	3	0.501	100	70	...	...	0
Idh	4	0.654	180/160	120/100	100/84	52	...
Aco	2	0.481	115	100	...	...	...
Pgm	4	0.529	112	100	90	...	0
G6p	1	0.000	100	...	...	...	...
Mp1	3	0.592	118	100	...	...	0
Mp2	3	0.635	100	60	...	...	0
Md1	4	0.763	100	100/78	78	...	0
Md2	3	0.615	100	68	...	...	0
G3p	3	0.669	100	70	...	...	0
Pgi	2	0.252	129	100	...	...	...
Average	2.9	0.500					

[a] The most commonly occurring allele was designated 100, and other alleles were designated relative to the 100 allele on the basis of relative mobility.

[b] *Mdh* = malate dehydrogenase; *Pel* = peptidase 1; *Pe2* = peptidase 2; *Idh* = isocitrate dehydrogenase; *Aco* = aconitase; *Pgm* = phosphoglucomutase; *G6p* = glucose-6-phosphate dehydrogenase; *Mp1* = mannose phosphate isomerase 1; *Mp2* = mannose phosphate isomerase 2; *Md1* = mannitol-1-phosphate dehydrogenase 1; *Md2* = mannitol-1-phosphate dehydrogenase 2; *G3p* = glyceraldehyde-3-phosphate dehydrogenase; *Pgi* = phosphoglucose isomerase.

[c] h = Genetic diversity; see text for calculation of genetic diversity.

[d] Null allele scored as 0 relative mobility and as allele 0.

[e] More than one electromorph per isolate was detected. When electromorphs did not migrate independently, they were scored as a single allele.

at the *Pe1* locus. *A. alternata* isolates were monomorphic at the *Pgi* locus. At some loci, although several alleles were detected, one allele was predominant. For example, four alleles were detected at the *Pe1* locus, but allele 2 was predominant, occurring in 29 of the 35 ETs (Table 3). Alleles 4, 3, and 1 occurred in only one, three, and two ETs, respectively. For the *Pgm* locus, in which four alleles were also detected, allele 2 occurred in 23 of the 35 ETs.

The enzymes ACO, MDH, and IDH are useful in distinguishing between the two *Alternaria* species. For ACO, which has two alleles, all except two *A. solani* isolates have allele 2 and all but two *A. alternata* isolates have allele 1. For IDH, all *A. solani* isolates carry either allele 3 or 4, and all but three *A. alternata* isolates carry either allele 1 or 2. All *A. solani* isolates have allele 1 for MDH, whereas all but two *A. alternata* isolates have allele 2.

The genetic diversity of isolates for individual enzyme loci ranged from 0.00 for *G6p* to 0.763 for *Md1* (Table 2). Although the level of diversity varied widely overall, the genetic diversity at a given locus was greater than 0.400 for 10 of 13 loci. The average genetic diversity of the isolates for all enzyme loci was 0.500.

The isolates separated into 35 ETs (Table 1); the alleles characteristic of each ET are provided in Table 3. The ETs having the greatest number of isolates were ETs 1, 18, 23, and 27 with 11, 47, 14, and 13 isolates, respectively. Eighteen ETs had only one isolate. In several cases, isolates belonging to the same ET were isolates from the same source. ETs 1, 4, 8, 15, 17, 18, 23, 24, 26, 27, 28, and 30 each have isolates collected from a common plant or region. For example, the three isolates belonging to ET 26 were from the same region of Oregon. However, in most cases, other isolates not from a common region were also within the given ET. Six of the seven isolates belonging to ET 30 were isolated from potatoes grown on a single farm in Pennsylvania, but the seventh isolate was from a potato leaf from Maine.

Each ET was composed of isolates from only one species. Twelve ETs were found within the *A. solani* isolates, and 23 ETs were found within the *A. alternata* isolates. The mean genetic diversity was 0.423 for the *A. solani* population and 0.388 for the *A. alternata* population. The ETs divided into two clusters at a genetic distance of 0.375; ETs 1–22 made up one cluster, and ETs 23–35 made up the other (Fig. 1). Within each cluster, isolates were of a single species; the upper cluster consisted of *A. alternata* isolates, and the lower cluster consisted of *A. solani* isolates. The only exception to this was ET 35, which had only one isolate of *A. alternata* that more closely resembled the *A. solani* than the *A. alternata* isolates in isozyme variation (Fig. 1). In future references to this dendrogram, ETs 1–22 will be referred to as the *A. alternata* group, and ETs 23–35 as the *A. solani* group. The *A. alternata* group clustered at a genetic distance of 0.350, and the *A. solani* group clustered at 0.375. However, if ET 35, which had a single *A. alternata* isolate, is disregarded, the total genetic distance for the *A. solani* group was 0.288. The total genetic distance for all isolates was 0.688. At a genetic distance of 0.200, there are seven smaller clusters, four within the *A. alternata* group and three within the *A. solani* group. Four ETs, 21, 22, 34, and 35, each consisting of only one isolate and which were more distantly related, did not fall into any of these smaller clusters. Of the closely related ETs (genetic distance of 0.100 or less), several had isolates that were collected from the same host species. Six isolates from ET 23 and four isolates from ET 24 were collected from the same sample of potato tubers. All isolates belonging to ET 23 were collected in Pennsylvania, except one which was from Idaho. One isolate each from ETs 26 and 27 was collected from the same leaf.

TABLE 3. Alleles[a] detected at 13 isozyme loci for 35 electrophoretic types (ETs) of 150 isolates of *Alternaria solani* and *A. alternata*

ET	Number of isolates	Enzyme locus[b]												
		Mdh	*Pe1*	*Pe2*	*Idh*	*Aco*	*Pgm*	*G6p*	*Mp1*	*Mp2*	*Md1*	*Md2*	*G3p*	*Pgi*
1	11	2	2	1	2	1	2	1	2	2	3	2	2	1
2	2	2	2	1	2	1	2	1	2	2	2	2	2	1
3	1	2	3	1	2	1	3	1	2	2	1	2	2	1
4	3	2	3	1	2	1	2	1	2	0	0	2	2	1
5	2	2	2	1	1	1	2	1	2	0	3	2	1	1
6	1	2	1	1	1	1	2	1	0	2	3	2	2	1
7	2	2	2	1	1	1	2	1	1	2	1	2	2	1
8	4	2	2	1	1	1	2	1	1	2	3	2	2	1
9	1	2	2	1	3	1	2	1	1	0	0	2	2	1
10	2	2	2	1	2	1	3	1	1	2	1	2	2	1
11	3	2	2	1	2	1	2	1	1	2	1	2	2	1
12	1	2	2	1	2	1	1	1	1	0	0	0	0	1
13	1	1	2	1	2	1	2	1	1	2	1	2	0	1
14	1	2	4	1	2	1	2	1	1	0	1	2	0	1
15	4	2	3	1	2	1	2	1	1	0	3	2	1	1
16	1	2	2	2	2	1	2	1	0	1	3	2	1	1
17	5	2	2	1	2	1	2	1	1	2	3	2	1	1
18	47	2	2	1	2	1	2	1	1	2	3	2	2	1
19	1	2	2	1	2	2	2	1	1	0	0	0	0	1
20	1	2	1	1	2	1	2	1	1	0	3	2	2	1
21	1	2	2	1	3	1	2	1	1	0	0	0	1	1
22	1	2	2	1	1	2	0	1	0	2	0	0	1	1
23	14	1	2	2	3	2	2	1	2	1	2	1	1	1
24	8	1	2	2	3	2	2	1	2	1	1	1	1	1
25	1	1	2	2	3	2	3	1	2	0	2	0	1	1
26	3	1	2	2	3	2	3	1	2	1	2	1	1	2
27	13	1	2	2	3	2	2	1	2	1	2	1	1	2
28	2	1	2	2	3	2	1	1	2	0	2	0	0	2
29	1	1	2	2	3	2	2	1	1	0	1	2	0	1
30	7	1	2	2	3	2	2	1	2	0	1	0	0	2
31	1	1	2	2	3	2	1	1	2	0	1	0	0	1
32	1	1	2	2	3	1	1	1	2	0	1	0	0	1
33	1	1	2	2	3	1	1	1	2	1	2	0	1	1
34	1	1	2	2	4	2	1	1	2	0	2	0	1	1
35	1	1	2	0	3	2	1	1	1	0	3	0	0	1

[a] Alleles were numbered sequentially from the anodal end of the gel.
[b] Allele abbreviations as given in Table 2.

In several cases, all of the isolates belonging to an ET were from the same host species. The seven isolates belonging to ET 30 were all from potato leaves, as were the 13 isolates of ET 27 and the three isolates of ET 26. Thirty-three of the 47 isolates in ET 18 were collected from tomato leaf tissue. Of the 35 ETs, 22 had only isolates collected from potato, either from foliage or tubers. The isolates from potato tubers belonged to only three ETs; the nine pepper isolates also belonged to three ETs, and seven of these isolates belonged to a single ET. Pennsylvania, the location with the largest number of isolates, also had the largest number of ETs. The 58 isolates from Georgia separated into nine ETs; 37 of the isolates were in a single ET (ET 18). The three isolates from Florida belonged to a single ET. Of the 17 ETs with multiple isolates, only four had isolates from a single location.

DISCUSSION

The amount of variation that was found among *Alternaria* isolates was rather high when compared to the results of other studies. The percentage of polymorphic loci found in *Peridermium* populations ranged from 0 to 38.5%, the mean number of isozymes per locus ranged from 1 to 1.46, and the total genetic distance for all populations was approximately 0.475 (22). Lu and Groth (15) found that nine of 12 bean rust isolates differed in isozyme phenotypes by no more than 9%. In a later bean rust study, Linde et al (14) found that the most distantly related isolates in the study had approximately 36% similarity, but most isolates had similarity coefficients of 84% or greater. Of 20 loci detected by isozymes in *Phytophthora cinnamomi*, 10 were polymorphic, and the overall variation in these isolates from Australia and Papua New Guinea was very low (17). Kirby (11) found only 11% of the detected loci to be polymorphic in *Ustilago bullata* isolates. In addition, little isozyme variation was detected in populations of *Colletotrichum* (12). These studies all show relatively low amounts of variation in the populations examined. In contrast, the percentage of polymorphic loci detected in the *Alternaria* isolates examined was 92%. The average number of electrophoretic alleles detected per locus was 2.9, and the mean genetic diversity was 0.500.

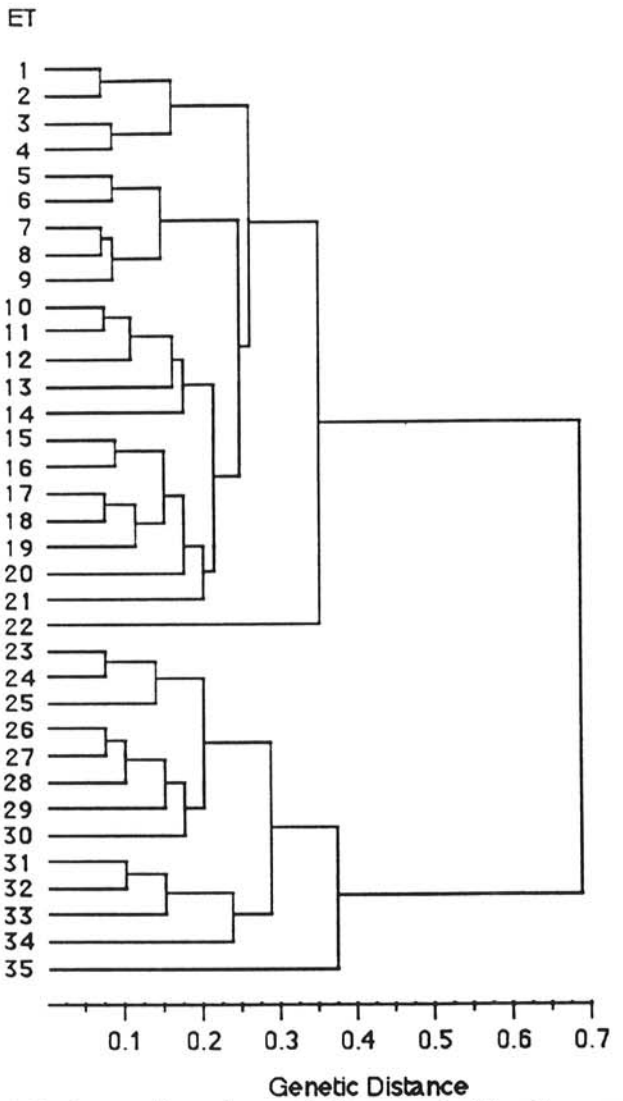

Fig. 1. Dendrogram illustrating genetic distances and relationships among electrophoretic types (ETs) of *Alternaria solani* (ETs 23–34) and *A. alternata* (ETs 1–22 and 35).

One explanation for the level of variation detected may be natural mutation. *A. solani* and *A. alternata* are capable of producing very large numbers of spores in a short period of time. This, combined with natural mutation rates, could lead to a relatively high level of diversity. Leung and Williams (13) have discussed isozyme variability in fungi with high reproductive capacity; they stated that mutations in these fungi could produce significant isozyme variation if isozymes are indeed neutral with respect to fitness. Lu and Groth (15) found that three *Uromyces* isolates incapable of producing teliospores and, thus, asexual, were not very closely related to a group of telia-producing isolates. In addition, they were less related to each other than the sexually reproducing isolates, which were very closely related.

Some studies using asexually reproducing populations have found little genetic variation (2). A proposed explanation has been that asexual populations are unable to exchange genetic material and, therefore, are restricted in the number of isozyme phenotypes that occur. This is in contrast to the results of our study, in which a considerable amount of variation was found. One way this might occur is through recombination, whether asexual or sexual. Asexual recombination can occur through the parasexual cycle, which, although it is unknown if it occurs commonly in *Alternaria*, may be a source of variation. A second route through which recombination might occur is through a sexual cycle. Although no sexual cycle is known for *A. solani*, teleomorphs for several other *Alternaria* species have been discovered (21).

Julian and Lucas (10), in a study involving several *Pseudocercosporella* species, found the most complex isozyme patterns on *P. anguioides*, a species with low pathogenicity. They postulated that this organism may be under less host-selection pressure than more pathogenic *Pseudocercosporella* species, which may be related to the more complex isozyme patterns. Leung and Williams (13), in comparing populations of the rice blast fungus, found the average genetic diversity for isolates from rice hosts to be 0.03. For isolates from a variety of nonrice hosts, the average genetic diversity was 0.21. In our study, the banding patterns produced by *A. alternata* were not necessarily more complex, but a greater number of ETs were found within *A. alternata* than *A. solani* isolates. One explanation for this may be that *A. alternata* is often found as a saprophytic organism on a wide variety of plants. *A. solani*, however, has a host range generally restricted to solanaceous crops. It is possible that there is less selection pressure on *A. alternata* because of a less restricted host range, which may account for the greater number of ETs. It is also possible that the saprophytic nature of *A. alternata* may force it to be adaptable to a more diverse range of hosts, which may subsequently lead to its being more diverse as a species. Another possibility that should be considered is that more *A. alternata* than *A. solani* samples were analyzed.

For three enzymes, ACO, MDH, and IDH, the alleles detected distinguished between the two species of *Alternaria* with only a few exceptions. Even though the dendrogram, which is only based on 13 enzyme loci, does not show an intermediate group between species, several isolates carried the allele characteristic of the other species. It may be that these isolates share additional characteristics such as pathogenicity with the other species. In many instances, both *Alternaria* species can be isolated from the

same foliar or tuber lesion. There were two instances in which isolates of the same species collected from the same lesion fell into different but closely related ETs. This indicates that multiple infections occurring within close proximity on the plant may allow for exchange of genetic material through asexual recombination processes.

There was almost a complete grouping of the isolates by cluster analysis according to species; a genetic distance of 0.688 was found between the two *Alternaria* species. This would seem to indicate that the species, although related, can be distinguished by enzyme polymorphism analysis. This is not surprising because there are differences in morphological, and possibly, pathogenic characteristics between the two species.

There was no obvious differentiation of the isolates either according to host or geographic origin by isozyme analysis. This may be significant because it may indicate that populations of *Alternaria* from various hosts and locations are not homogeneous, and the populations do not seem to be genetically isolated by host or by location. For example, ETs 1 and 2, which differ at only one locus, collectively contain isolates from six different states and two hosts. These statements cannot be conclusive, however, because of the limited sample numbers from several locations and hosts. A study by Hwang et al (9) shows that populations of *A. mali* within a geographic feature are more closely related than geographically separated isolates on the basis of esterase isozymes.

Through isozyme analyses of populations of *A. solani* and *A. alternata*, we have found that the two species can be distinguished, in particular by three enzymes, ACO, MDH, and IDH. The overall amount of variation found among the isolates was relatively high, although the species are relatively closely related. There was no clear differentiation of isolates according to host or location. In addition, the isozyme markers detected in this study may be useful in future studies involving *Alternaria*.

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