Comparison of Isozyme and Virulence Diversity Patterns in the Bean Rust Fungus *Uromyces appendiculatus*

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We thank B. J. Ballantyne for providing some of the host lines and J. R. Stavely and J. R. Steadman for providing some of the isolates used.

Published as paper 16,941 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project 22-73, supported by U.S. Department of Agriculture Competitive Grant 58-5759-6-1.

Mention of companies or commercial products does not imply recommendation or endorsement by the University of Minnesota or U. S. Department of Agriculture over others not mentioned.

Accepted for publication 8 August 1989 (submitted for electronic processing).

ABSTRACT

Linde, D. C., Groth, J. V., and Roelfs, A. P. 1990. Comparison of isozyme and virulence diversity patterns in the bean rust fungus *Uromyces appendiculatus*. Phytopathology 80:141-147.

Fifty-five enzyme and protein stains were screened in three horizontal starch gel electrophoresis systems for usefulness as phenotypic markers for *Uromyces appendiculatus*, the bean rust fungus. Thirteen enzymes, representing 15 phenotypic markers, were found suitable for this purpose. Phenotypic diversity as detected by the 15 isozyme markers and virulence on 18 differential bean lines were compared for 27 geographically diverse isolates. Greater diversity was found for virulences than for isozymes. No relationship between geographic and phenotypic distance was found. Three phenotypic clusters based on virulence and isozyme data were identified by cluster analysis. The data support the hypothesis that *U. appendiculatus* is not a homogeneous species and also suggest that urediospores may occasionally fertilize pycnia in nature.

Uromyces appendiculatus (Pers.) Unger, the bean rust fungus, is characterized by a wide diversity of virulence phenotypes distributed in space and time (23,26). Consequently, resistance to bean rust has not been useful for long periods of time. Because of the virulence diversity, economic importance of the disease, and ease of manipulation in the greenhouse (15), *U. appendiculatus* was chosen as a model for studying fungal plant pathogen diversity (14). A better understanding of diversity in *U. appendiculatus* may lead to the development of new or improved breeding strategies to increase the durability of resistance to this and other important foliar pathogens.

The two kinds of markers most often used today in plant pathology to study pathogen diversity are virulences and isozymes. Differences in virulence are of obvious interest because of their economic importance, and they have been used widely as phenotypic and genotypic markers (1,19,28,31,37). Isozymes have been used increasingly in the study of fungal pathogen diversity (4,9,13,20,27). Of particular interest is a preliminary study made by Lu and Groth (21) on isozymic variation in 12 isolates of *U. appendiculatus*. Using polyacrylamide gel electrophoresis (PAGE), they screened 20 enzyme staining systems and identified 27 putative allozyme loci. Eighteen (in 12 enzymes) showed variation involving at least one isolate. Most variation existed among three isolates that rarely or never produced telia and the remaining nine telia-producing isolates. Lu and Groth (21) concluded that *U. appendiculatus* may not be a homogeneous species.

In this study, 27 geographically diverse isolates of *U. appendiculatus*, including nine of the 12 isolates used by Lu and Groth, were compared for both isozyme and virulence variation. Starch slab electrophoresis was substituted for the PAGE system because of the ease of comparison of isozyme patterns in slabs versus tubes, the generally simpler protocols involved, the larger maximum number of isolate × isozyme combinations that can be scored readily by one worker per day (90 versus 36 for PAGE), the availability of replication within gels, and the generally simpler and more definitive band patterns produced. The objectives of this research were to screen a large number of enzyme and protein staining systems with horizontal starch gel electrophoresis for their usefulness as phenotypic markers and to compare the patterns of variability as detected by virulences and isozymes.

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MATERIALS AND METHODS

Sources of isolates. Twenty-seven single-uredium isolates were used in this study. Their place of origin, host cultivar and bean type from which they were isolated, and the letter code assigned are listed in Table 1. The isolates were chosen to represent a broad geographic area. Progeny from three crosses among some of the isolates also were included because they had been scored previously for virulence and isozyme patterns.

Virulence rating and urediospore increase. The virulence phenotype for each isolate was determined with 18 differential bean (*Phaseolus vulgaris* L.) lines: Pinto 111, U.S. #3, Early Gallatin, Fleetwood, Golden Gate Wax, Aurora, Top Crop, B1349, Y6-71, 2C-120, 814, 765, UrB, UrE, UrJ, UrVer, Ur1, and Ur2. Six of the differential lines were chosen because of their ability to detect variability in other bean rust populations. Other lines were chosen randomly or because of their distinct avirulence fleck reaction. The Ur lines were included because they reportedly possess different resistance genes (3). Seed of the differential lines was germinated in vermiculite in steam-sterilized galvanized-steel flats. At the first primary leaf stage, two plants of each line were transplanted into steam-sterilized soil in 12.5-cm clay pots. Urediospores suspended in a lightweight volatile oil, Soltrol 170 (Phillips Petroleum Co., Bartlesville, OK), were sprayed onto the young plants. One pot containing a susceptible line always was included as a control. The oil was allowed to evaporate from the plants for at least 20 min before the plants were misted with water and placed in a galvanized-steel humidity chamber for at least 12 hr. One to four days after the pots were removed from the humidity chamber, 0.5 g of oxamyl (10% granular) was added per pot to control insects.

Two weeks after inoculation, the plants were rated and assigned reaction grades on a 0 to 9 scale (17) by the first author.

Inoculations were repeated on different days until consistent reactions were obtained twice in succession. After rating, plants inoculated with virulent isolates were pruned of new growth and saved for isozyme analysis. Urediospores of each isolate were collected by tapping the infected plants over aluminum foil, and the spores were stored in No. 00 gelatin capsules until needed.

Electrophoresis procedures. Electrophoresis samples were made from either fresh urediospores or urediospores that were desiccated over $CaSO_4$ for 3 days and stored frozen at −60 C in sealed plastic bags. Frozen urediospores were rehydrated at 80% RH for 45 min before germination. Germination buffer (0.01 M potassium phosphate and 10^{-5} M monobasic calcium phosphate dihydrate, pH 7.0, containing ethanol [500 μg/ml], Tween 20 [100 μg/ml], and β-ionone [5 μg/ml]) were added to triple-rinsed 125-ml flasks at a rate of 35 ml per 100 mg of urediospores. The flasks then were shaken on a wrist-action shaker for approximately 3 hr. Percent germination was estimated by examining a minimum of 100 urediospores. The buffer subsequently was removed, and the urediospores were divided into equal lots of at least 22.5 mg of germinated urediospores (dry weight basis). The lots then were placed into microcentrifuge tubes containing 1 drop of universal buffer (100 mM MES-potassium salt, 15% [v/v] ethylene glycol, and 2% [v/v] 2-mercaptoethanol adjusted to pH 6.8 with HCl) per 20 mg (dry weight) of total urediospores. The buffer and spores were mixed well and immediately frozen in liquid nitrogen. The samples for electrophoresis were stored at −60 C until used, generally within 2 mo.

Starch gels were poured the afternoon before electrophoresis. Occasionally, gels were poured the morning that the electrophoresis was performed and allowed to age 2 hr. Three types of electrophoresis systems were used: one continuous (histidine) and two discontinuous (lithium-borate and tris-citrate). Details of these systems are found in Table 2. Twenty-five grams of hydrolyzed starch (Connaught Laboratories Ltd., Willowdale, Ont.) were suspended in 83 ml of the appropriate gel buffer at room temperature, and 167 ml of boiling gel buffer was added to the suspension and mixed thoroughly. The solution then was degassed and poured into a horizontal mold comprised of four plastic bars greased with white petrolatum on a 180 × 265 mm piece of crown glass. The plastic bars were held in place on the glass plate with metal clips to form a 215 × 120 × 6 mm mold. Immediately after pouring, another glass plate was placed on top of this mold, and the excess starch solution was squeezed out.

After the gel had aged, the upper glass plate and plastic bars were removed. A vertical slice was made across the width of the gel 6 cm from the cathode end of the gel. The gels were chilled on ice for a minimum of 20 min.

Up to 12 urediospore electrophoresis samples were thawed in individual chilled mortars and ground thoroughly with a pestle to break the germ tubes. Extra universal buffer was added during the grinding so that there was adequate moisture to wet the 4 × 6 mm filter paper wicks that were placed in each mortar to absorb the urediospore extract. The wicks were dabbed dry on absorbent paper and placed side by side in the vertical slice made in the gel. Each sample was replicated once within the gel on each side of the center lane. In the case of the histidine gels, a single wick dipped in red food dye (FD&C Red #40 and #3 [Schilling brand, McCormick & Co., Inc., Baltimore, MD]) was placed in the center lane to serve as a tracking dye.

The gels, still on the lower glass plates, were placed on clear lucite boxes (22 × 13 × 7 cm) through which ice-cold water was circulated. For the tris-citrate and lithium-borate gels, a constant 200 V were applied for the first 20 min. The sample wicks then were removed, and electrophoresis was resumed at 300 V. Blue ice blocks, supported above the gels by side mold bars, were used as supplemental cooling. For the histidine gels, 25 constant mAmps were applied until the tracking dye had separated into its two distinct components, which occurred at about 20 min. The sample wicks then were removed, and electrophoresis resumed at 25 constant mAmps. Electrophoresis was terminated in all gels when the front or tracking dye had reached 90 mm.

After electrophoresis, the gels were sliced horizontally, and the three slices obtained per gel were stained. A total of 55 enzyme and protein staining systems (Tables 3–5) was screened in each of the three electrophoresis systems.

Data analysis. All isolates were compared pairwise for virulence ratings and isozyme patterns. Virulence ratings for two isolates were considered unambiguously different if: 1) one isolate had a virulence rating less than 3 or a mesothetic reaction of 1 to 4 and the other isolate had an average virulence rating of 3 or higher, 2) both isolates had average virulence ratings of 3 or above but the average rating of the two isolates differed by two or more rating units, or 3) both isolates had virulence ratings less than 3 or a mesothetic reaction but the ratings were not the same. Isozyme patterns were considered different if there was any band not in common between the two isolates. The number of similarities of each isolate with every other isolate pairwise was summed for both virulences and isozymes to calculate a simple matching similarity coefficient (35).

TABLE 1. Single-uredium isolates of *Uromyces appendiculatus* used in comparing isozyme and virulence diversity, letter code assigned to isolates, origin of isolates, and bean cultivar and type from which isolated

			Host	
Isolate	Code	Origin	Cultivar	Type
MX3-2	A	Mexico	Unknown	Dry
KW8-1	B	Minnesota	Kentucky Wonder	Green
P14-6	C	North Dakota	Unknown	Dry
S3-1[a]	D	Minnesota	Unknown	Navy
R32-1[a]	E	Maryland	Unknown	Green
KW7-1	F	Minnesota	Kentucky Wonder	Green
DREP2	G	Dominican Republic	Unknown	Dry
81M-36B	H	Nebraska	Unknown	Dry
AIS1[a]	I	Minnesota	Aurora	Navy
SWBR4	J	Unknown	Unknown	Unknown
PR1-2	K	Puerto Rico	Unknown	Dry
MD41	L	Maryland	Unknown	Green
MD67[a]	M	Florida	Unknown	Green
MD58	N	Dominican Republic	Unknown	Dry
U2-12	O	Minnesota	Unknown	Green
MX3-1	P	Mexico	Unknown	Dry
U1-4	Q	Minnesota	Unknown	Green
GER2	R	West Germany	Unknown	Unknown
DREP1	S	Dominican Republic	Unknown	Dry
S1-5	T	Michigan	Unknown	Navy
P14-3	U	North Dakota	Unknown	Dry
W73-2[a]	V	Wisconsin	Unknown	Green
P10-1	W	North Dakota	Pinto 111	Dry
P24-6	X	Minnesota	Agate	Dry
PW3-1	Y	P10-1 × W73-2	...	...
AP21-1	Z	DREP2 × 81M-36B	...	...
FT5-1	AA	DREP2 × 81M-36B	...	...

[a]This isolate does not form telia under the greenhouse conditions at St. Paul, MN.

TABLE 2. Tray and gel buffer components and pH values for three electrophoresis systems used to evaluate isozyme banding patterns in *Uromyces appendiculatus*

	Tray buffer		Gel buffer	
System	Components	pH	Components	pH
Histidine	0.4 M trisodium citrate, pH adjusted with 0.25 M citric acid (monohydrate)	8.0	4.6×10^{-3}M DL-histidine HCl, adjusted with NaOH	8.0
Tris-citrate	0.1 M NaOH and 0.3 M boric acid	8.7	7.5×10^{-4}M citric acid (monohydrate) and 3.8×10^{-3}M Trizma base	8.0
Lithium-borate	0.05 M lithium hydroxide and 0.19 M boric acid	8.5	0.065 M Trizma base, 9×10^{-3} M citric acid (monohydrate), and 9% tray buffer	8.2

Andansonian analysis based on the simple matching coefficients was carried out for both the virulence and isozyme data separately as described by Skerman (33). The order of isolates was determined by single linkage cluster analysis for the virulence data. Cluster analysis for both types of markers was done by the Proc Cluster procedure of SAS Institute, Inc. (29). The number of dissimilarities among isolates was used to create the distance matrix. Three types of unweighted, pair-group methods of cluster analysis were performed on the data: single linkage, average linkage, and centroid (35). Distance dendrograms were constructed with Proc Tree of SAS Institute, Inc. (29) and converted to percent similarity dendrograms. The data also were plotted as the number of isozyme patterns differing from the commonest of each enzyme among the 27 isolates over the number of virulent reactions (an average rating of 4 or higher) on the 18 differential lines for each isolate.

TABLE 3. Enzyme staining systems found useful for phenotypic analysis of *Uromyces appendiculatus*

Enzyme	E.C. number[a]	Stain[b]	Band pattern (R_f)	Bean rust isolates with band pattern	System of choice[c]
Acid phosphatase	3.1.3.2	M	.16,.22,.38	All	T
Esterase 1	3.1.1.1	B,M	.43	V	H
			.39,.43	E,Y	
			.39	All others	
Esterase 2	3.1.1.1	M	.38	All others	T
			.19,.38	E,F,H,K,N, V,W,Y	
			.19	U,X	
Esterase 3	3.1.1.1	M	.56	All others	T
			.56,.53,.50	E,Y	
			.50	V	
Fumarase	4.2.1.2	B	.36,.40	V	H
			.16–.40	E,Y	
			.16,.29	All others	
Glutamate dehydrogenase (NAD)	1.4.1.2	B	.06	G,M	L
			.05–.06	D,S,Z	
			.05	All others	
Glutamic-oxaloacetic transminase	2.6.1.1	B	.58	All	L or T
Hexokinase	2.7.1.1	B	.56,.62	All others	L
			.50,.56,.62	E,Y	
			.50,.62	V	
Malate dehydrogenase	1.1.1.37	B	.06,.10	All others	H
			.04,.06,.10	E,Y	
			.04	V	
Nucleoside phosphorylase	2.6.1.1	S	.36	S,V	H
			.33–.36	E,Y	
			.33	All others	
Peptidase	3.4.x.x	M	.27,.59	V	T or L
			.21,.27,.59	E,Y	
			.21,.59	All others	
Peroxidase	1.11.1.7	M	.27	All others	L
			.18–.27	E,Y	
			.18	V	
Phosphoglucose isomerase	5.3.1.9	B	.44	R,V	L
			.37,.40,.44	E,Y	
			.37	All others	
Phosoglucomutase	2.7.5.1	B	.40	V	H
			.19,.40	E,Y	
			.22	X	
			.19,.22	C	
			.19	All others	
6-phospho-gluconate dehydrogenase	1.1.1.44	B	.51	All others	H
			.49,.51	D	

[a]After the Nomenclature Committee of the International Union of Biochemistry (18).
[b]Staining system used: B = similar to Brewer (6); M = Micales et al (25); and S = Selander et al (30).
[c]Electrophoresis system used (Table 2): L = lithium-borate; H = histidine; and T = tris-citrate.

RESULTS

Of the 55 staining systems screened, 13 enzymes exhibited sufficiently resolved banding patterns and adequate activity to be useful as phenotypic markers. The 13 enzymes and the banding patterns

TABLE 4. Enzyme staining systems detecting some activity in germinated urediospores of *Uromyces appendiculatus* as shown by streaking and/or occasional bands observed

Enzyme	E.C. number[a]	Stain[b]
Aconitase	4.2.1.3	B,S
Aldolase	4.1.2.13	B,S
Aspartate dehydrogenase	1.4.3.x	S
Glucose-6-phosphate dehydrogenase	1.1.1.49	B
Glutamate dehydrogenase (NADP)	1.4.1.4	B
Glutathione reductase	1.6.4.2	M
Glyceraldehyde-phosphate dehydrogenase (NAD)	1.2.1.12	S
Glyceraldehyde-phosphate dehydrogenase (NADP)	1.2.1.1	S
Isocitrate dehydrogenase	1.1.1.42	B
Leucine aminopeptidase	3.4.1.1	B,S
Malic enzyme	1.1.1.40	B
Mannitol dehydrogenase	1.1.1.67	Sh
Mannitol-1-phosphate dehydrogenase	1.1.1.17	S
Menadione reductase	1.6.99.2	M
Shikimate dehydrogenase	1.1.1.25	V
Sorbitol dehydrogenase	1.1.1.14	Sh
Succinate dehydrogenase	1.3.99.1	B,Sh
Xanthine dehydrogenase	1.2.3.2	S
Buffalo black for proteins	...	Sm

[a]After the Nomenclature Committee of the International Union of Biochemistry (18).
[b]Staining system used: B = similar to Brewer (6); M = Micales et al (25); Sh = Shaw and Prasad (32); and S = Selander et al (30); Sm = Smith (34); V = similar to Vallejos (36).

TABLE 5. Enzyme staining systems detecting low or no activity in germinated urediospores of *Uromyces appendiculatus* as shown by no streaking or bands observed

Enzyme	E.C. number[a]	Stain[b]
Adenylate kinase	2.7.4.3	S
Alanine dehydrogenase	1.4.1.1	S
Alcohol dehydrogenase	1.1.1.1	S
Alkaline phosphatase	3.1.3.1	B,M,S
Catalase	1.11.1.6	B
β-galactosidase	3.2.1.23	S
Glucosyltransferase	2.4.1.11	S
β-glucuronidase	3.2.1.31	Sh
Glutamic-pyruvic transaminase	2.6.1.2	S
α- and β-glycerophosphate dehydrogenase	1.1.1.8	Sh
3-hydroxybutyrate dehydrogenase	1.1.1.30	S
Lactate dehydrogenase	1.1.1.27	S
Leucine dehydrogenase	1.4.3.2	S
Lysine dehydrogenase	1.4.3.x	S
Mannose phosphate isomerase	5.3.1.8	S
NADH diaphorase	1.6.4.3	B
NADPH dehydrogenase	1.6.99.1	M
Octanol dehydrogenase	1.1.1.73	S
Phosphoglycerate kinase	2.7.2.3	M
Ribonuclease	3.1.x.x	Sh
Superoxide dismutase	1.15.1.1	S
Threonine dehydrogenase	1.1.1.x	S
Oil red O for lipoproteins	...	Sm

[a]After the Nomenclature Committee of the International Union of Biochemistry (18).
[b]Staining system used: B = similar to Brewer (6); M = Micales et al (25); Sh = Shaw and Prasad (32); S = Selander et al (30); Sm = Smith (34).

observed for the 27 isolates are listed in Table 3. Esterase exhibited three putative genetic loci resulting in a total of 15 isozymic phenotype markers. Nineteen additional staining systems (Table 4) exhibited some activity as shown by streaking and/or occasional banding. These systems perhaps can be developed into useful markers by increasing the concentration of the soluble germinated urediospore extract or determining the right gel buffer-tray buffer system that allows more enzyme activity and production of distinct bands. Twenty-three staining systems (Table 5) exhibited no activity; that is, no bands or streaking were observed.

For an easy visual comparison of the ability of isozymes and virulence markers to define phenotypic differences, the Andansonian analyses for both virulences and isozymes were placed side by side in Figure 1. If the virulence and isozyme markers were equivalent in this respect, the two sides of the left-to-right, top-to-bottom diagonal of the figure should have similar patterns. Clearly, more phenotypic differences were detected with the virulence markers.

The dendrograms constructed with average linkage cluster analysis of the isozyme and virulence data are shown in Figure 2. Dendrograms based on single linkage and centroid cluster analysis also were constructed, but not shown, because they were very similar in appearance to the average linkage clustering dendrograms. Comparison of the dendrograms based on isozyme and virulence data shows again that much more diversity is detected with virulences.

A statistically rigorous method of identifying clusters does not exist (35). Clusters often are identified using common sense and intuition (2,12). On the basis of the isozyme dendrogram, it was judged that there are three distinct clusters. Cluster 1 consists of the isolate W73-2 (V); cluster 2 is composed of two isolates, R32-1 (E) and PW3-1 (Y); and cluster 3 is made up of the remaining 24 isolates. The number of clusters present in the virulence dendrogram is less obvious. Cluster 3 could be split into two clusters which will be referred to as subclusters 3A (isolates S, G, M, Z, I, and D) and 3B (isolates T, R, B, K, AA, H, Q, N, L, P, C, U, O, J, X, W, F, and A). (The potential split of cluster 3 was not evident in the centroid clustering dendrogram although it was in the single linkage clustering dendrogram). A problem with representing data in dendrogram form is that there is of necessity a considerable loss of information (10). Subclusters 3A and 3B are not as distinctly separated as they appear in the dendrogram. Of the 108 pairwise comparisons between the isolates in the two subclusters, five are similar at the 72% level, 13 are similar at the 67% level, and 51 are similar at least to the 56% level, the approximate baseline of the subclusters. It similarly can be argued that clusters 1 and 2 are distinct from cluster 3. Only one of the 72 pairwise comparisons between isolates in

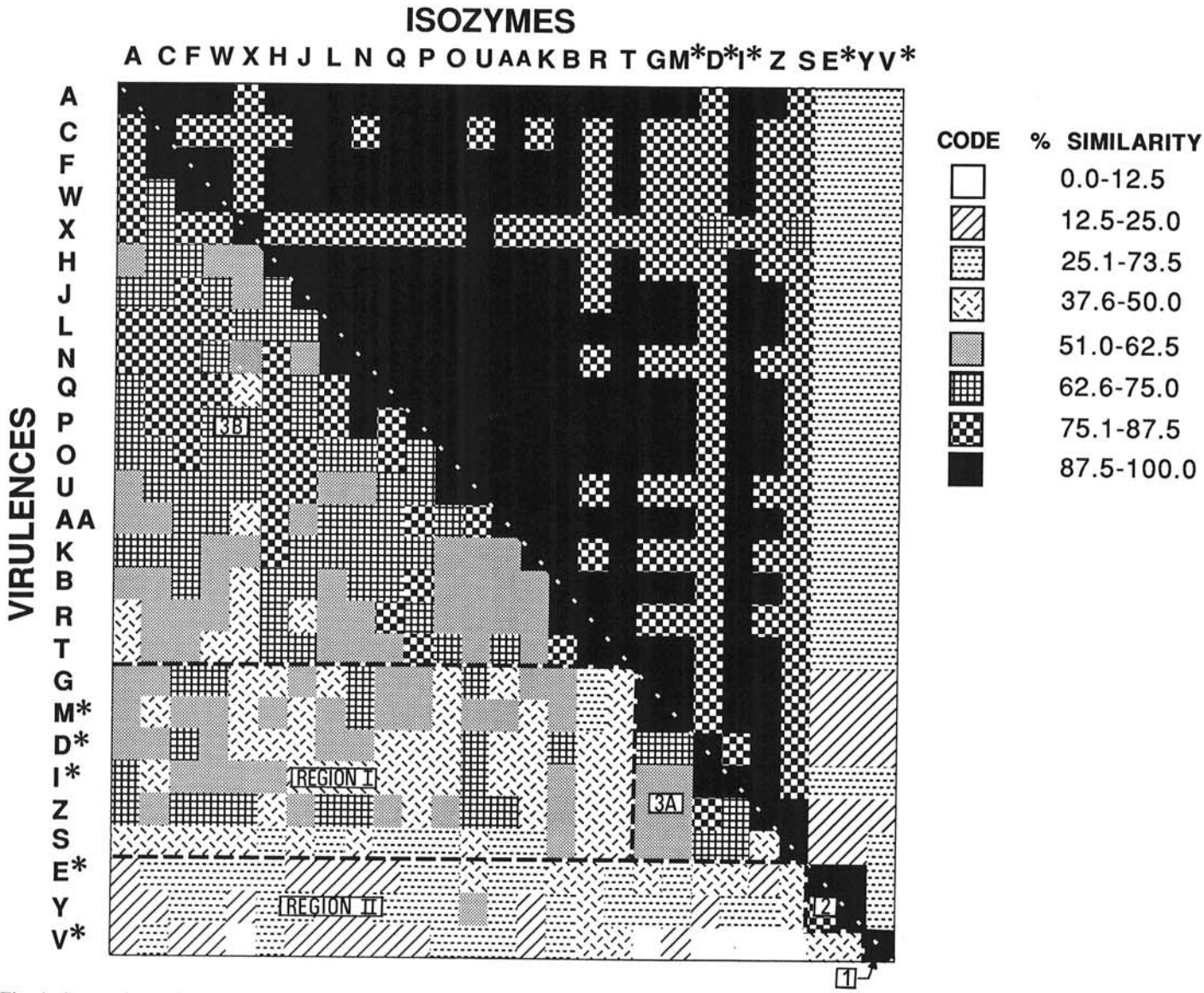

Fig. 1. Comparison of Andansonian analyses based on 15 isozyme markers and 18 virulence markers for 27 isolates of *Uromyces appendiculatus*. Isolates not forming telia under the greenhouse conditions at St. Paul, MN, are identified with asterisks.

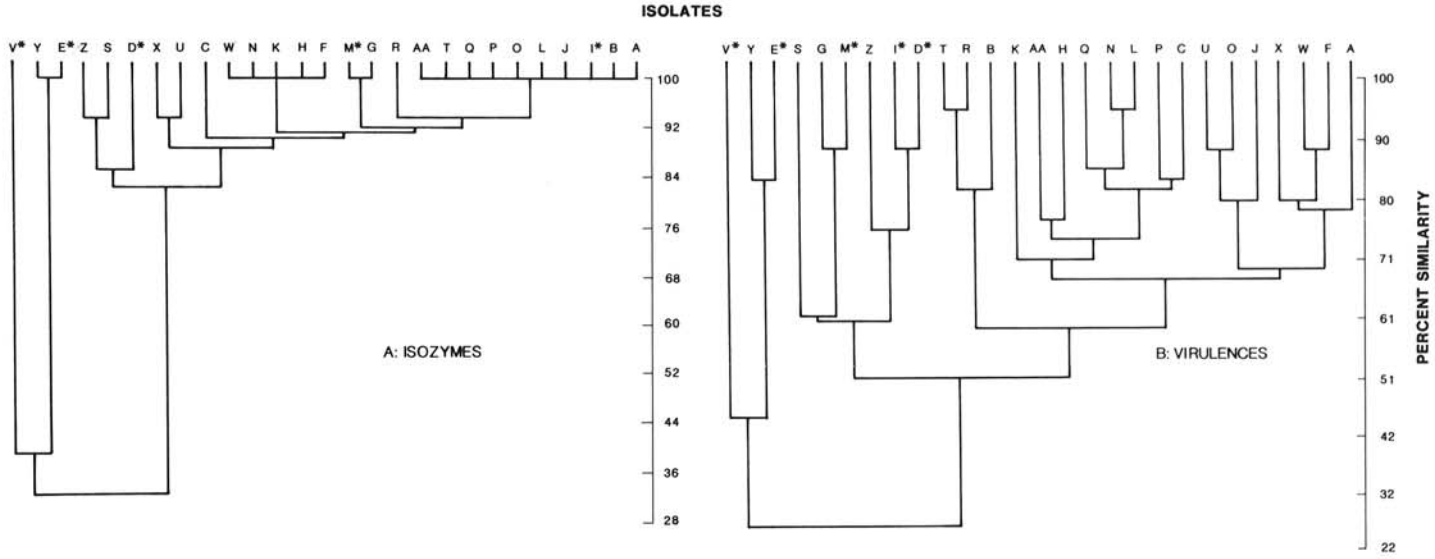

Fig. 2. Dendrograms of isozyme and virulence similarity coefficients for 27 isolates of *Uromyces appendiculatus* showing the result of average linkage cluster analysis. Isolates not forming telia under the greenhouse conditions at St. Paul, MN, are identified with asterisks.

clusters 1 and 2 with isolates in cluster 3 (isolates Y and U) is similar at the 50% level, which is approximately the baseline of cluster 3.

Such an approach also can be taken when viewing the Andansonian analysis of the virulence ratings (Fig. 1). Subclusters 3A and 3B grade into Region I which contains the pairwise comparisons between the isolates of these two subclusters. On the other hand, clusters 2 and 3 do not blend into Region II which contains the pairwise comparisons between the isolates of cluster 3 and the isolates of clusters 1 and 2. In Figure 3, it is clear that clusters 1 and 2 differ from cluster 3 because the isolates in clusters 1 and 2 are virulent on relatively few of the 18 differential lines.

DISCUSSION

These results conform to the pattern observed in other plant pathogenic fungi that virulence variation is greater than isozyme variation (5,7,8). The level of diversity observed is a function of the markers used and the population sampled (16). These results probably are biased to show more variation for virulence markers because, as previously mentioned, some of the differential lines were chosen on their ability to detect diversity in other bean rust populations. Nevertheless, with these sets of markers, much more diversity was detected with virulences. The high level of virulence diversity probably is caused in part by the multitude of resistance gene combinations present in *P. vulgaris*. Every differential line used in this study (several of which have been grown commercially) yielded at least one virulence rating difference for one or more bean rust isolate relative to its most similar differential line. The deployment of different resistance gene combinations in cultivars has placed some selection pressure on populations of *U. appendiculatus* to develop new virulence phenotypes. The low level of isozymic diversity in *U. appendiculatus* also may indicate that such diversity is selected against. Leung and Williams (20) argued that mutation in fungi with high reproductive potential such as *U. appendiculatus* should produce considerable enzyme variability if isozymes are neutral with respect to fitness. The low isozyme variability may suggest that most electrophoretic variants are unfit or that coadapted isozyme complexes exist.

The differences in the relative amount of diversity detected with isozymes and virulences did not influence the number of clusters identified or the isolates contained within clusters. The sample studied is small relative to the population existing in nature. Thus, the three clusters defined by virulences may not exist if the number of isolates examined was substantially larger.

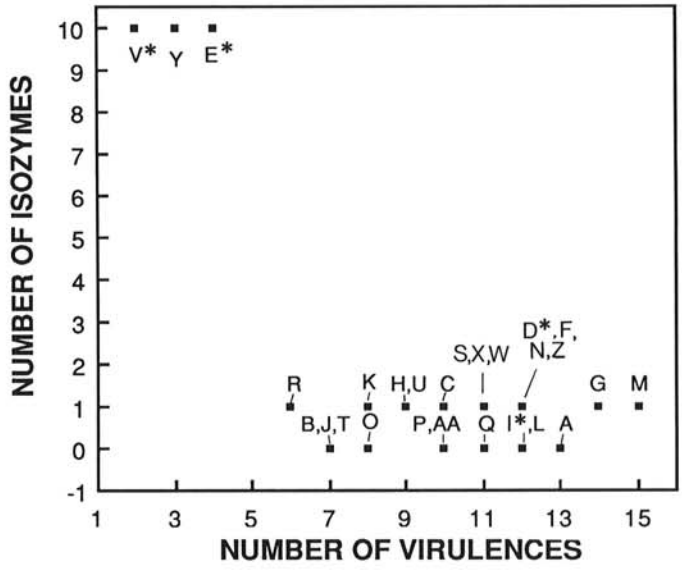

Fig. 3. Plot of number of uncommon isozyme patterns versus the number of virulence reactions (average rating of 4 or greater on a scale of 0 to 9) on 18 differential bean (*Phaseolus vulgaris*) lines for 27 isolates of *Uromyces appendiculatus*. Isolates not forming telia under the greenhouse conditions at St. Paul, MN, are identified with asterisks.

U. appendiculatus is notable because of its virulence diversity, and the gaps between the clusters defined by virulence patterns corroborate the clusters defined by isozyme patterns. Gene flow patterns are apparently affecting both types of markers. The data presented herein support the hypothesis of Lu and Groth (21) that *U. appendiculatus* is a heterogeneous species.

Lu and Groth proposed that *U. appendiculatus* is composed of two, possibly three, clusters. Their two definitive clusters correspond with clusters 1 and 3 of this study. One isolate they used, but not included in this study, was U2-1. Based on comparison with their isozyme patterns in PAGE, U2-1 would be a member of cluster 1. U2-1 and W73-2 (V) are "asexual" isolates which never or rarely (for U2-1) produce telia under the greenhouse conditions at St. Paul, MN. Their third possible cluster consisted of isolate S3-1 (D), which is also an asexual isolate. This isolate belongs in cluster 3 which contains primarily sexual isolates. The extra cluster identified in this study (cluster 2) is composed of two isolates, R32-1 (E) and PW3-1 (Y), not included in the study by Lu and Groth. R32-1 was isolated from nature,

and PW3-1 is a synthetic isolate produced by fertilizing pyncia of the isolate P10-1 (W) with urediospores of W73-2 (22). R32-1 and PW3-1 have the same putative heterozygous isozyme patterns for the 10 isozyme markers that differ in the parents of PW3-1 (W73-2 and P10-1). R32-1 and PW3-1 also have similar virulence phenotypes with only three of the ratings on the 18 differential lines differing slightly (2 versus 1 on Ur2, mesothetic versus 4.25 on UrB, and 1 versus 2 on B1349). One important difference between these two isolates is that PW3-1 will form telia, although slowly and in low yield, whereas R32-1 has never been observed to form telia. The close phenotypic correspondence between the natural isolate, R32-1, and the synthetic isolate, PW3-1, suggests that urediospores occasionally may fertilize pycnia in nature if cluster 1 isolates do not form telia in nature. Whether this is the case or not, cluster 2 appears to be the result of hybridization between isolates from clusters 1 and 3.

The three clusters represent gene pools that are isolated to some extent. The forces that maintain this structure are unknown. If some cluster 2 isolates do form telia like PW3-1, the question of what prevents them from hybridizing amongst themselves or with isolates of cluster 3 is raised. The resulting progeny would fill in the gaps between the clusters. If such hybridization occurs, selection acting on coadapted isozyme and virulence complexes could drive the F1 and subsequent generations toward clusters 1 and 3. Perhaps cluster 2 isolates are effectively asexual. Whether PW3-1 would have time to form telia in nature is questionable because it takes approximately 6 wk before telia form in the greenhouse. The ability to form telia under field conditions may be rapidly lost if it is not useful.

Lu and Groth (21) hypothesized that asexuality may be responsible for maintaining the separation between clusters 1 and 3. Asexual isolates such as W73-2 and U2-1 may have diverged from the main sexual group (cluster 3) and become adapted to a specialized ecological niche. In this survey of isolates, cluster 3 contained some asexual isolates (D, I, and M). This observation suggests that the sexual population may segregate for telia-forming ability. Isolates D, I, and M may be a part of the population in the beginning stages of diverging from cluster 3. Cluster 1, however, may be the result of convergence. No information on the host range of the 27 isolates outside of *P. vulgaris* was obtained.

Leung and Williams (20) found that climatic factors or geographic races of rice were partly responsible for isozyme diversity in *Magnaporthe grisea* (Hebert) Barr comb. nov., the rice blast fungus. Adaptation to climate or bean type does not appear to influence isozyme or virulence phenotype in *U. appendiculatus*. W73-2, which is very different relative to the other isolates in this study, was isolated from a field of green-type beans in the upper Midwest as were some of the most typical isolates for virulence and isozyme patterns. The four Caribbean isolates (G, K, N, S), two Mexican isolates (A, P), and one Old World isolate (R) are not particularly different for isozymes or virulences relative to most midwestern U.S. isolates. This finding contrasts with the analysis of Goodwin et al (13) who found geographical differentiation of isozyme patterns for populations of *Rhynchosporium secalis* (Oud.) Davis in northern California and Idaho. One possible reason for the lack of geographical differentiation in *U. appendiculatus* is the potential long-distance transport of urediospores. Indeed, W73-2 may have been transported from a southern tropical region because it is an apparently asexual isolate and thus could not have overwintered in Wisconsin. (Another possibility is that the population W73 may have been segregating for telia-producing ability.) Geographical differentiation probably is more likely to develop in *R. secalis* because its conidia, although smaller than urediospores of *U. appendiculatus*, are not designed for transport of any substantial distance because they are oblong, hyaline, thin walled, and water-splash disseminated (11).

The potential transport of bean rust urediospores from southern tropical areas means that breeders in the United States may need to breed against the entire population of *U. appendiculatus* in the northern half of the Western Hemisphere. In this survey, there was a wide range of virulence combinations. A continuum of virulence combinations probably exists through mutation or recombination in such a large geographic region. Gaps in the continuum could exist, however, in the form of virulence dissociations. This study suggests the presence of one such virulence dissociation. Very few isolates were virulent on the differential lines Ur1 and UrE (both putative, single-gene differential lines [3]), and none was virulent on both. Admittedly, the total isolate sample was quite small. Bulk urediospore collections from natural populations would be most efficient for identifying virulence dissociations. Urediospores from different populations would be inoculated onto a differential line. The subpopulation of urediospores produced would be used to inoculate a second differential line. Barring contamination problems between the first and second inoculations, potential virulence dissociations would manifest themselves by an avirulent reaction on the second differential line. Complete virulence dissociations, if identified, probably would be most useful in regions where disease pressure is relatively low. Such dissociations would be expected to be more durable in environments where the rate of pathogen adaptation is slower. In the tropics, where the disease pressure is higher, beans serve as an important source of protein, and product uniformity standards are less strict, rotating multivars might be a viable approach for disease control. McDonald et al (24) suggested using lines from advanced generations of composite cross populations as multivar components.

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