Etiology

Isozyme Comparisons for Identification of *Colletotrichum* Species Pathogenic to Strawberry

M. R. Bonde, G. L. Peterson, and J. L. Maas

First and second authors: Research plant pathologist and support scientist, respectively, USDA, Agricultural Research Service, Foreign Disease-Weed Science Research, Fort Detrick, Bldg. 1301, Frederick, MD 21702; third author: research plant pathologist, USDA, Agricultural Research Service, Fruit Laboratory, BARC-West, Beltsville, MD 20705.

We gratefully acknowledge the following for generously supplying *Colletotrichum* isolates for this study: T. Barksdale, retired, USDA-ARS, BARC-W, Beltsville, MD; J. M. Dingley, deceased, Department of Scientific and Industrial Research, Auckland, New Zealand; P. Gunnell, Department of Plant Pathology, University of California, Davis; C. M. Howard, Agricultural Research and Education Center, University of Florida, Dover; R. D. Milholland, North Carolina State University, Raleigh; N. O'Neill, USDA-ARS, BARC-W, Beltsville, MD; R. Rowe, Ohio State University, Wooster; B. J. Smith, USDA-ARS, Poplarville, MS; A. K. Watson, MacDonald College of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada; S. Wilhelm, retired, Department of Plant Pathology, University of California, Berkeley.

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Accepted for publication 6 August 1991 (submitted for electronic processing).

ABSTRACT

Bonde, M. R., Peterson, G. L., and Maas, J. L. 1991. Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. Phytopathology 81:1523-1528.

Presently there is uncertainty about the appropriate taxonomic classification of species of *Colletotrichum* infecting strawberry. Some workers consider *C. fragariae* part of the highly variable species *C. gloeosporioides*, whereas others feel they should be separate. The strawberry pathogens *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* were successfully distinguished by means of comparing isozymes for 12 enzymes and 14 putative isozyme loci. The intraspecific coefficients of similarity (CS) based on the 12 enyzmes and 14 loci were 1.00 for *C. acutatum* (maximum possible = 1.00), 1.00 for *C. fragariae*, and 0.80 for *C. gloeosporioides*. Intraspecific CS values indicated little variation in *C. acutatum* and *C. fragariae*; however there was considerable variation in *C. gloeosporioides*. The interspecific CS comparing *C. fragariae* and *C. gloeosporioides* was relatively low (0.42), suggesting *C. fragariae* and *C. gloeosporioides* are distinct species. The considerably higher CS of 0.77 when comparing *C. acutatum* and *C. gloeosporioides* suggests that they are more closely related. *C. coccodes* (= *C. atramentarium*) and *C. trifolii* also were included in the comparison. Neither exhibited intraspecific isozyme variation and further demonstrated the great potential for isozyme analysis to identify *Colletotrichum* species.

The taxonomy of *Colletotrichum* is in a state of confusion. Much of this confusion is centered on the species concept of *C. gloeosporioides* (Penz.) Penz. & Sacc.

Saccardo (25) in 1884 described conidia of this species as straight and cylindrical with rounded ends, 16–18 μm long $\times$ 4–6 μm wide. In 1957, von Arx (31) reduced 594 species names to synonymy with *C. gloeosporioides*. Von Arx, however, distinguished several pathogenic forms based on host specificity and distinctive cultural characteristics and retained them under their original names because they had no proven connection with the teleomorph, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk.

In 1931, *C. fragariae* A. N. Brooks was identified in Florida as the causal agent of strawberry (*Fragaria* $\times$ *ananassa* Duchesne) anthracnose (4), and in 1932 the cause of crown rot and wilt (5). Later, *C. fragariae* was shown to cause stolon, petiole, and

fruit lesions in addition to crown rot and summer wilt of strawberry throughout the southeastern United States (27).

Today uncertainty exists concerning the appropriate classification of *C. fragariae*. Some have considered *C. fragariae* part of the highly variable species *C. gloeosporioides* (12,29). Others contend *C. fragariae* should be considered a separate species (13,27).

This confusion is complicated further by reports that *C. acutatum* J. H. Simmonds (26), *C. dematium* (Pers.) Grove (1), and *Gloeosporium* species (15,27,30,32) also cause anthracnose of strawberry.

Smith and Black (27) recently compared morphological, cultural, and pathogenic characteristics of 24 isolates of *Colletotrichum* from strawberry. They considered *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* as three separate species. However, it is not clear how they differentiated the latter two species except that, of their isolates, only *C. fragariae* produced setae, and only *C. gloeosporioides* produced the *Glomerella* sexual stage. *C. acutatum* was easy to differentiate in their studies because the isolates grew slower on potato-dextrose agar than those of the other species. *C. acutatum* isolates also failed to produce lesions on wound-inoculated strawberry leaves. *C. acutatum* isolates generally produced distinctly fusiform conidia, whereas *C. fragariae* and *C. gloeosporioides* isolates produced cylindrical

TABLE 1. Isolates (and their sources) of *Colletrotrichum* spp. used in isozyme analysis[a]

Record	Species	Country	State	Source	Code
1	*C. acutatum*	USA	California	P. Gunnell	SS0042-1
2	*C. acutatum*	USA	California	P. Gunnell	SS0100-2
3	*C. acutatum*	USA	California	P. Gunnell	SS1-80-1
4	*C. acutatum*	USA	California	P. Gunnell	SS0062-2
5	*C. acutatum*	USA	Maryland	J. Maas	GL-S
6	*C. acutatum*	USA	California	P. Gunnell	Wolfskill
7	*C. acutatum*	USA	California	P. Gunnell	SS4-2a-1
8	*C. acutatum*	USA	California	P. Gunnell	SS8-4e-1
9	*C. acutatum*	USA	California	P. Gunnell	SS0009-1
10	*C. acutatum*	USA	California	P. Gunnell	SS5-4h-1
11	*C. acutatum*	USA	California	P. Gunnell	SS5-1a-1
12	*C. acutatum*	USA	California	P. Gunnell	SS3-1a-1
13	*C. acutatum*	USA	California	P. Gunnell	SS2-4k-1
14	*C. acutatum*	USA	California	P. Gunnell	SS0041-2
15	*C. acutatum*	USA	California	P. Gunnell	SS0037-2
16	*C. acutatum*	USA	California	S. Wilhelm	C-37-2-4
17	*C. acutatum*	USA	California	S. Wilhelm	C-37-2-3
18	*C. acutatum*	USA	California	S. Wilhelm	C-37-2-2
19	*C. acutatum*	USA	Mississippi	B. J. Smith	CA-Mil-2
20	*C. acutatum*	USA	Missouri	B. J. Smith	CA-Goff
21	*C. acutatum*	USA	N. Carolina	R. Milholland	NC-4
22	*C. acutatum*	New Zealand	...	J. M. Dingley	CA-1701
23	*C. acutatum*	USA	Mississippi	B. J. Smith	CA-Mil-1
24	*C. acutatum*	USA	Florida	C. Howard	C-206
25	*C. acutatum*	USA	Florida	C. Howard	C-202
26	*C. acutatum*	USA	California	P. Gunnell	SS3-3c-1
27	*C. acutatum*	USA	California	P. Gunnell	SS0102-1
28	*C. atramentarium*	USA	...	R. Bonde	ATCC 10902
29	*C. atramentarium*	USA	...	C. Boothroyd	ATCC 16991
30	*C. coccodes*	USA	California	T. Barksdale	C-140
31	*C. coccodes*	USA	California	T. Barksdale	C-139
32	*C. coccodes*	USA	Ohio	T. Barksdale	C-16
33	*C. coccodes*	USA	New York	T. Barksdale	NYTA
34	*C. coccodes*	USA	Louisiana	T. Barksdale	TRC
35	*C. coccodes*	Canada	...	A. K. Watson	AG-2
36	*C. coccodes*	USA	Idaho	R. Rowe	CP-241
37	*C. coccodes*	USA	Ohio	R. Rowe	CP-8
38	*C. fragariae*	USA	Louisiana	B. J. Smith	CF-1
39	*C. fragariae*	USA	Florida	C. M. Howard	H-192
40	*C. fragariae*	USA	Florida	R. Milholland	CF-14
41	*C. fragariae*	USA	Florida	C. M. Howard	CF-132
42	*C. fragariae*	USA	Louisiana	R. Milholland	CF-4
43	*C. fragariae*	USA	Florida	R. Milholland	NC-2
44	*C. fragariae*	USA	N. Carolina	R. Milholland	NC-14
45	*C. fragariae*	USA	Florida	C. M. Howard	C-205
46	*C. gloeosporioides*	USA	Maryland	J. Maas	GL-S-84
47	*C. gloeosporioides*	USA	N. Carolina	C. M. Howard	H-204
48	*C. gloeosporioides*	USA	N. Carolina	C. M. Howard	H-1984
49	*C. gloeosporioides*	USA	Maryland	J. Maas	C-1069
50	*C. gloeosporioides*	USA	Maryland	J. Maas	GL-D
51	*C. gloeosporioides*	USA	Florida	C. M. Howard	C-216
52	*C. gloeosporioides*	USA	Florida	C. M. Howard	C-215
53	*C. gloeosporioides*	USA	Florida	C. M. Howard	C-212
54	*C. gloeosporioides*	USA	Maryland	J. Maas	GL-S
55	*C. gloeosporioides*	USA	N. Carolina	R. Milholland	GC-10s
56	*C. gloeosporioides*	USA	N. Carolina	R. Milholland	GC-10
57	*C. gloeosporioides*	USA	California	R. Milholland	NC-7
58	*C. gloeosporioides*	USA	Florida	C. M. Howard	H-196
59–83	*C. trifolii*	USA	Worldwide	N. O'Neill	3-5

[a] Isolates 1–27 and 38–58 were isolated from infected strawberry plants or fruit; 28, 36, and 37 from potato; 29–34 from tomato; 35 from velvetleaf (*Abutilon theophrasti*); and 59–83 from alfalfa. All species designations for specific isolates are as given by the supplier.

conidia usually with one end rounded and the other pointed.

Characterization of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*, based on morphology of setae produced on strawberry leaf medium, has been reported recently (9,10). Other studies have attempted to differentiate strawberry-infecting species of *Colletotrichum* by gel electrophoresis of protein extracts (16,18). These studies generally support the treatment of these species as distinct entities.

Isozyme analysis is a technique presently being used by mycologists and plant pathologists to provide taxonomically useful data, identify unknown fungal isolates, "fingerprint" fungal strains, and determine the extent of genetic variation within and among related fungal populations (20). It is also being used to trace the geographic origins of pathogens, follow the segregation of genetic loci, and determine ploidy levels in life cycles of fungi (20).

Lenné and Burdon (16) surveyed four isozyme systems among *C. gloeosporioides* isolates from several populations of *Stylosanthes guianensis*, important tropical legumes. In their studies, isozymic diversity was limited to only a few phenotypes, which suggested to them that their fungal populations exhibited a high level of inbreeding or clonal reproduction. Isolates in our present study, however, were included from widely different regions to estimate the diversity of genotypic variation expressed in isolates pathogenic to strawberry irrespective of the geographic source of isolates.

Our objectives were to examine and define isozyme phenotype variability of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* isolates infecting strawberry. Isolates of *C. coccodes* and *C. trifolii*, which are not pathogenic to strawberry, were included for comparison. This information also should aid in developing a rapid and accurate diagnostic system of these and other species of *Colletotrichum*.

MATERIALS AND METHODS

Cultures. Twenty-seven cultures of *C. acutatum*, eight of *C. fragariae*, and 13 of *C. gloeosporioides* isolated from strawberry were obtained from the sources listed in Table 1. Five cultures of *C. coccodes* from tomato, two from potato, and one from velvetleaf, two isolates of *C. atramentarium*, and 25 isolates of *C. trifolii* (13 of race 1 and 12 of race 2) were also evaluated. Stock cultures were maintained either on V8 juice agar slants or agar plugs stored in a liquid nitrogen refrigerator (−196 C).

Preparing cultures for electrophoresis. Petri plates (90 × 15 mm) containing V8 juice agar (200 ml of unsalted V8 juice, 3 g of calcium carbonate, 800 ml of water, and 15 g of Bacto agar [Difco, Detroit, MI]) were seeded from stock cultures and incubated at 24 C for 3–5 days. Three 7-mm-diameter plugs were excised from the margin of each culture and used to inoculate 100 ml of filtered V8 liquid culture medium (V8 juice agar without the agar) in 300-ml Erlenmeyer flasks. The cultures were grown as shake cultures (125 rpm) for 4 days at 18 C, under continuous fluorescent light.

Mycelia then were collected by vacuum filtration. Approximately 1 cm^2 of mycelia was transferred to each of two or three 1.8-ml cryovials and immediately frozen in liquid nitrogen for later use in isozyme comparisons.

Frozen samples were removed from liquid nitrogen storage and thawed on ice. Extraction buffer (500 μl of 0.05 M Tris-HCl, pH 7.5) was added to each sample, and the mycelia was crushed with the aid of a 6-mm-diameter glass rod. Samples were refrozen in liquid nitrogen, removed, and rethawed on ice. The samples were again mixed and then centrifuged in the cryovials for 15 min in a refrigerated (4 C) centrifuge at 1,000 *g*. After centrifugation, the samples were maintained on ice.

Electrophoresis. The supernatant of each sample was absorbed into 3 × 10-mm paper wicks cut from filter paper (No. 470; Schleicher and Schuell Inc., Keene, NH). These were placed at the origin of 12% starch gels, and horizontal electrophoresis was carried out for approximately 3 h as described by Micales et al (20).

The gels each were cut horizontally into a stack of five gel slices, the top slice was discarded, and the four remaining slices were each individually stained for a specific enzyme while submerged in the appropriate solution of substrate and reagents at 37 C (20). Each isolate was tested at least twice per enzyme, and many isolates were tested several times to determine the variability within and among species. Reference isolates representing all the variability previously identified in each species were included when comparing species.

Genic nomenclature. Genic nomenclature follows that of Micales et al (20). Abbreviations with all capital letters refer to enzymes, and abbreviations with only the first letter capitalized refer to specific loci coding for the enzyme. Alleles at a particular locus were designated by the relative anodal mobility from the origin of their respective protein products. The designation for each allele was relative to the protein product of one allele (usually the most common) designated as 100. For example, Gpi-100 is the most common allele coding for glucosephosphate isomerase at the Gpi locus. Allele Gpi-118 is another allele at the Gpi locus coding for an enzyme molecule migrating 18% farther on the gel than the enzyme coded by allele Gpi-100.

Analysis of data. Analysis of data was performed with the use of the computer program "Allozyme" (R. Struss, University of Arizona, Tucson), which determines the coefficients of similarity (CS) according to Rogers (24).

According to this equation:

$$CS = 1 - D$$

$$D = \frac{1}{L} \sum_{i=1}^{L} \left[\frac{1}{2} \sum_{j=1}^{Ai} (Pijx - Pijy)^2 \right]^{1/2}$$

in which D = genetic distance, L = number of genetic loci, Ai = number of alleles at the ith locus, and $Pijx$ and $Pijy$ = frequency of the jth allele at the ith locus in population x and y, respectively.

RESULTS

Eleven gel buffer systems were used to test the presence of 63 enzymes. Of these enzymes, 13 were in sufficient quantities and able to be resolved (Table 2). One enzyme, malate dehydrogenase (MDH), produced results that could not be interpreted without further information on the genetics of the organisms. This enzyme was not used in calculating CS values. The other 12 enzymes provided a total of 14 putative isozyme loci; the alleles

TABLE 2. List of enzymes resolved, enzyme abbreviations, enzyme commission numbers, and best gel buffer for resolution

Enzyme (EC no.)	Abbreviation	Buffer system
Aspartate aminotransferase (2.6.1.1)	AAT	CA-8[a]
Cytochrome oxidase (1.9.3.1)	CTO	TC[b]
Esterase (3.1.1.1)	EST	R[c]
Glutamate dehydrogenase (1.4.1.2)	GDH	CA-8
β-Glucosidase (3.2.1.20)	β-GLU	R
Glucosephosphate isomerase (5.3.1.9)	GPI	C[d]
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6PDH	CA-8
Isocitrate dehydrogenase (1.1.1.42)	IDH	CA-8
Malate dehydrogenase (1.1.1.37)	MDH	TC
Mannitol dehydrogenase (1.1.1.67)	MADH	CA-8
Mannose phosphate isomerase (5.3.1.8)	MPI	CA-8
Phosphoglucomutase (2.7.5.1)	PGM	TC
Phosphogluconate dehydrogenase (1.1.44)	PGD	TC

[a] Buffer according to Steiner and Joslyn (28), 100 V, 3 h.
[b] Electrode buffer stock; 1.37 M Tris, 0.38 M citric acid; dilute 1:4 for anodal tray, 1:3 for cathodal tray. Gel buffer; 0.02 M Tris base, 0.005 M citric acid, 100 V, 3 h.
[c] Buffer according to Ridgway et al (23), 250 V, 3 h.
[d] Buffer according to Clayton and Tretiak (8), 200 V, 3 h.

detected at each locus are presented in Table 3. All CS values comparing within or between species are presented in Table 4, and representative isozyme phenotypes are shown in Figure 1.

C. acutatum (27 isolates) produced a total of three isozyme phenotypes with all variation occurring in MDH (Table 3). Omitting MDH in calculating CS values produced an intraspecific CS of 1.00 for *C. acutatum*, indicating little isozyme variation (Table 4). *C. fragariae* (eight isolates) exhibited a single phenotype (CS = 1.00), whereas *C. gloeosporioides* (13 isolates) had a total of four phenotypes with variation detected at nine isozyme loci and an intraspecific CS of 0.80 (Table 4). *C. coccodes* (= *C. atramentarium*), with a total of 10 isolates from three hosts, exhibited no isozyme variation. The 25 isolates of *C. trifolii* also were identical.

Interspecific CS values (Table 4) comparing *C. fragariae* with *C. gloeosporioides* were low (0.42). This value was similar to the CS comparing *C. fragariae* with *C. trifolii* (0.45), which are known to be morphologically and pathologically two distinct species, and *C. gloeosporioides* with *C. trifolii* (0.39), also separate species (Table 4). *C. gloeosporioides* compared with *C. acutatum* had a surprisingly high CS value of 0.77 (Table 4).

DISCUSSION

Of the 13 enzymes examined, 12 were easily analyzed, because each isolate generally produced a single band per zone of enzymatic activity in the gel (e.g., Fig. 1A). Each band was interpreted as the result of a single isozyme locus coding for the isozyme observed. Whether the organisms were haploid or diploid does not affect CS values when considering these 12 enzymes, because variability was not detected within an isolate (i.e., there were no double bands).

MDH was more difficult to interpret because a few isolates had two bands per zone in the gel (Fig. 1B). Possibly, the double-banded phenotype resulted from a secondary enzyme (perhaps caused by a post-translational change in the enzyme molecule). However, because each isolate consistently produced either a one- or two-banded phenotype and lacked several other characteristics of secondary enzymes (20) over many experiments, we believe the double bands were the result of multiple alleles at either one or two loci.

If the multiple alleles were the result of polymorphism at a single locus, and assuming the organisms are haploid, then presumably genetically different nuclei were present in individual isolates with two bands. Because at least four of the isolates with double bands are known to be derived from single conidia, the variation detected must reside in individual fungal cells (the conidia), perhaps the result of parasexuality.

The double bands also could be the result of the expression of two loci. MDH often is present in soluble plus mitochondrial forms (11). Individuals with one band for MDH may possess a null allele at the second locus.

TABLE 3. Distribution of phenotypes by isolate groups

Group	Loci														
	Aat	Cto	Est	Gdh	βGlu	G6pdh	Gpi	Idh	Madh	Mdh	Mpi-1	Mpi-2	Pgd	Pgm-1	Pgm-2
Colletotrichum acutatum I[a] (1)[b]	100	100	100	100	100	100	100	100	100	75	100	100	83	100	100
C. acutatum II (9)	100	100	100	100	100	100	100	100	100	100/75[c]	100	100	83	100	100
C. acutatum III (16)	100	100	100	100	100	100	100	100	100	100	100	100	83	100	100
C. coccodes (8)	100	128	100	122	100	88	104	183	120	100	100	100	125	86	57
C. atramentarium[d] (2)	100	128	100	122	100	88	104	183	120	100	100	100	125	86	57
C. fragariae (8)	100	85	98	133	108	100	118	116	120	50	100	116	100	93	128
C. gloeosporioides I[d] (6)	100	114	100	100	100	88	104	100	100	100/50[c]	100	100	83	100	100
C. gloeosporioides II (4)	100	114	100	100	100	88	104	116	100	50	100	100	83	100	100
C. gloeosporioides III (1)	100	114	100	100	100	100	100	100	100	100/75[c]	100	100	83	100	100
C. gloeosporioides IV (2)	100	114	98	133	100	100	118	116	120	50	0	0	125	100	100
C. trifolii (25)	110	57	53	100	116	111	136	166	100	100	115	130	158	100	100

[a] *C. acutatum* isolates were identical except for the Mdh locus where three phenotypes were identified.
[b] Number in parentheses is number of isolates in group.
[c] Double-banded phenotype. Data for Mdh was not used for calculating coefficient of similarity values in Table 4.
[d] *C. gloeosporioides* isolates separated into four phenotypes due to isozyme variation at 10 out of 15 loci.

TABLE 4. Average intra- and interspecific coefficients of similarity (CS) based on 12 enzymes and 14 putative isozyme loci[a]

Intra- or interspecific comparison	CS mean	SD	Number of isolate comparisons
Within *Colletotrichum acutatum*	1.00	0.00	351
Within *C. fragariae*	1.00	0.00	28
Within *C. gloeosporioides*	0.80	0.28	78
Within *C. coccodes*	1.00	0.00	28
Within *C. atramentarium*	1.00	0.00	1
Within *C. trifolii*	1.00	0.00	276
Between *C. acutatum* and *C. fragariae*	0.47	0.00	216
Between *C. acutatum* and *C. gloeosporioides*	0.77	0.16	351
Between *C. acutatum* and *C. coccodes*	0.45	0.00	216
Between *C. acutatum* and *C. atramentarium*	0.45	0.00	54
Between *C. acutatum* and *C. trifolii*	0.37	0.00	648
Between *C. fragariae* and *C. gloeosporioides*	0.42	0.14	104
Between *C. fragariae* and *C. coccodes*	0.46	0.00	64
Between *C. fragariae* and *C. atramentarium*	0.46	0.00	16
Between *C. fragariae* and *C. trifolii*	0.45	0.00	192
Between *C. gloeosporiodes* and *C. coccodes*	0.50	0.03	104
Between *C. gloeosporiodes* and *C. atramentarium*	0.50	0.04	26
Between *C. gloeosporiodes* and *C. trifolii*	0.30	0.02	312
Between *C. coccodes* and *C. atramentarium*	1.00	0.00	16
Between *C. coccodes* and *C. trifolii*	0.47	0.00	192
Between *C. atramentarium* and *C. trifolii*	0.47	0.00	48

[a] Coefficient of similarity values were determined by means of the computer program "Allozyme" (R. Struss, Univ. of Arizona, Tucson). Values theoretically can range from 0.00, indicating no similarity, to 1.00, indicating all loci were identical for all isolates.

In calculating CS values, we decided to ignore MDH in the analyses (Table 4). However, to test the potential effect of MDH, we also calculated the entire isozyme data with MDH interpreted as either one or two loci. The manner in which MDH was interpreted (or omitted) had neglible effect on CS values with the maximum change ±0.02 units. We include MDH in this discussion of variability because it does indicate at least a small degree of variability in *C. acutatum* and also was variable in *C. gloeosporioides*. Lenné and Burdon (16) detected seven isozyme phenotypes among 22 virulent phenotypes of *C. gloeosporioides*. Contrary to our findings, even though isozyme variation was found among populations of the pathogen, there were no differences within individual isolates. The MDH isozyme locus (or loci) was not examined in their studies, however.

Coefficients of similarity have been determined for several fungal species. Micales et al (22), while studying *Cryphonectria cubensis* (Bruner) Hodges isozymes, determined CS intraspecific values to be 0.84–1.00. Bonde et al (3) determined the average intraspecific CS value for *Tilletia indica* to be 0.83 and for *T. barclayana* 0.85. When they compared individuals of *T. indica* with those of *T. barclayana*, a morphologically similar and undoubtedly closely related smut pathogen, the average interspecific CS was very small (0.04) easily differentiating the two species. Bonde et al (2) also determined the intraspecific CS for the "true" *Phakopsora pachyrhizi* Sydow soybean rust pathogen in Asia and Australia to equal 1.00, indicating no detected isozyme variation. Micales et al (21) calculated intraspecific CS values for four species of *Peronosclerospora* downy mildew pathogens of maize to vary from 0.75 to 1.00, depending on the particular species, and Bruckart and Peterson (6) found CS values to be 0.72–0.75 for *Puccinia carduorum* Jacky, a pathogen of *Carduus* thistles. Koch and Kohler (14) found the variation of isozymes and general proteins of formae speciales of *Erysiphe graminis* DC. to be extremely small. The relative frequency at which any divergent band of 176 loci occurred was less than 0.5%. However, the CS values comparing pairs of formae speciales were 0.24–0.28 for five of six comparisons. *E. g. tritici* compared with *E. g. secalis* had a CS of 0.82, indicating a closer relationship.

Strawberry isolates of *Colletotrichum* were polymorphic at 13 of 14 putative isozyme loci, yet variation within *C. acutatum* and *C. fragariae* was small with intraspecies CS values at or approaching 1.00. We conclude that these isolates represent two distinct species. *C. gloeosporioides*, however, was more variable isozymically, with variation at nine of 14 loci and an intraspecific CS of 0.80.

Masel et al (19) detected extensive karyotypic variation involving both chromosome number and length in isolates of *C. gloeosporioides* obtained from *Stylosanthes* spp. The authors

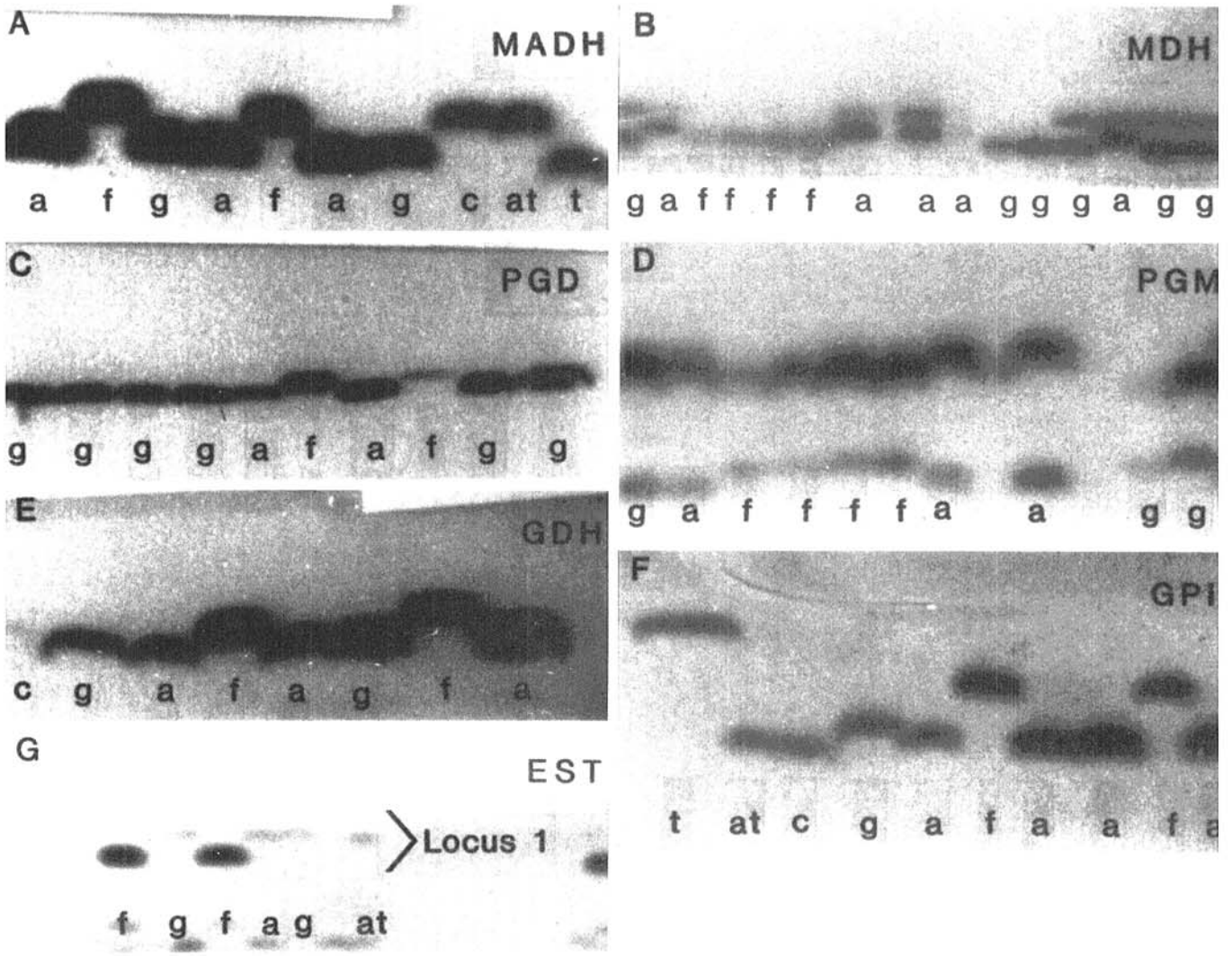

Fig. 1. A–G, isozyme banding patterns (phenotypes) for representative enzymes extracted from *Colletotrichum* spp. a = *C. acutatum*, at = *C. atramentarium*, c = *C. coccodes*, f = *C. fragariae*, g = *C. gloeosporioides*, t = *C. trifolii*. **A,** mannitol dehydrogenase (MADH). **B,** malate dehydrogenase (MDH). One- and two-banded phenotypes are evident for *C. gloeosporioides* and *C. acutatum*. **C,** phosphoglucose dehydrogenase (PGD). **D,** phosphoglucose mutase (PGM). Two putative loci are evident. Locus 1 produces bands in the higher zone and was the more difficult to score. **E,** glutamate dehydrogenase (GDH). Four phenotypes are shown with *C. fragariae* producing the two highest bands and *C. acutatum* and *C. gloeosporioides* the lowest. **F,** glucosephosphate isomerase (GPI). **G,** esterase (EST). Two isozyme loci are evident, however, only locus 1 (upper zone) was consistent throughout the study and was scored. Two of the three phenotypes detected are shown here.

suggest that chromosomal rearrangements may play a role in generating variation in this pathogen and that processes responsible may be active either during somatic growth or parasexual recombination in this presumably haploid organism.

C. fragariae and *C. gloeosporioides* morphologically are very similar to the extent that they are difficult to distinguish without considerable experience. Supposedly, *C. gloeosporioides* has conidia that are rounded at each end, whereas *C. fragariae* has conidia rounded at one end and more pointed at the other. However, many spores (at least 100) have to be examined to make this determination, and not all isolates can be reliably identified using these criteria (M. R. Bonde, G. L. Peterson, and J. L. Maas, *unpublished data*). An isolate of *Colletotrichum* that produces the *Glomerella* sexual stage is presumed to be *C. gloeosporioides*, however, not all isolates of the latter produce the sexual stage (J. L. Maas, *unpublished data*). Whereas *C. fragariae* and *C. gloeosporioides* are morphologically hard to distinguish, they easily are distinguishable using isozymes (as evidenced by the CS of 0.42).

C. acutatum can be distinguished from *C. gloeosporioides* and *C. fragariae* based on growth rate in culture and the shape of conidia. Isozyme results suggest that *C. acutatum* and *C. gloeosporioides* are more closely related than are *C. fragariae* and *C. gloeosporioides*. Indeed, one isolate of *C. gloeosporioides* (phenotype III in Table 3) differed only at one isozyme locus from *C. acutatum* phenotype II and apparently is very closely related to that organism. *C. atramentarium* and *C. coccodes* had an interspecific CS value of 1.00, indicating little genetic variability. This is consistent with the current interpretation that they are the same species (7).

Of the 13 enzymes that we scored, five always distinguished *C. fragariae* and *C. gloeosporioides*. Cytochrome oxidase was the single enzyme (with one putative locus) that separated all three pathogens in every instance. Our results indicate that isozymes can be used as a means of identifying species of *Colletotrichum*. The data with *C. coccodes* and *C. trifolii* suggest that the technique may be applicable to a wide range of species from different hosts.

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