Resistance

Race Characterization of Brazilian Isolates of *Colletotrichum lindemuthianum* and Detection of Resistance to Anthracnose in *Phaseolus vulgaris*

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ABSTRACT

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Nine races (alpha, delta, epsilon, zeta, eta, teta, kappa, lambda, and mu) of *Colletotrichum lindemuthianum* were identified from 201 bean isolates collected from 16 states in Brazil. Of the nine races, zeta, eta, teta, and mu were new races. The identification was based on a set of differential cultivars: Michelite, Aiguille Vert, Dark Red Kidney, Widusa, Imuna, BO 22, Sanilac, Cornell 49-242, Kaboon, TO, PI 207262, and Mexico 222. Seventy-two cultivars and breeding lines were tested for resistance to all of the races. The lines A 321, A 373, and A 387 and the cultivars AB 136, Evolutie, GO 2338, GO 3367, and TU were resistant to all races tested. Forty-nine sources of resistance to race alpha, 44 to delta, 52 to epsilon, 53 to zeta, 56 to eta, 38 to theta, 32 to kappa, 42 to lambda, and 36 to mu were identified among the components of the germ plasm studied.

Pathogenic variability of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. was first observed by Barrus (5). Later he used 69 cultivars of bean to characterize 15 isolates of the fungus and found two distinct races, alpha and beta (6). Based on identical criteria and nomenclature, races gamma (8), delta (2), epsilon (7), alpha-Brasil (14), lambda (18), kappa (20)—or ebnet (32), jota (18), and lambda (15) were detected from 1942 to 1979. Other nomenclature systems used to designate races of *C. lindemuthianum* are not widely accepted because their differentiation involves reactions of local cultivars (36). Races such as "Mexicano" I, II, and III (37), "Brasileiro" I and II (26), races Ba-1 to Ba-10 (29), and ebnet race (32) are examples of racial designations of limited use. Furthermore, isolates exist that do not fit in any of the described races (4,24,25).

Because of the diversity of race nomenclature, the use of local sets of differential cultivars, and different rating scales (6,12,13,17,20,21,26), it is difficult to compare races of *C. lindemuthianum* (10,37) at the international level.

In Brazil, past investigations of the pathogenic variability of this fungus were limited to the states of Rio Grande do Sul, Santa Catarina (27), Paraná (3,24), São Paulo (19,28), Minas Gerais, Rio de Janeiro, and Espírito Santo (26,29). Between 19 and 32 isolates of the fungus were tested and characterized based on sets containing a maximum of three internationally accepted cultivars. Thus it is difficult to compare these results with those obtained in Europe and North America.

Therefore, it is important to test all available Brazilian isolates against a set of internationally used differentials. This paper also reports the identification of new races of *C. lindemuthianum* and new resistant sources to Brazilian races of *C. lindemuthianum*.

MATERIALS AND METHODS

Monoconidial isolates of *C. lindemuthianum* were obtained by plating spore suspensions of 201 isolates from 384 seed and pod

samples collected from 16 states in Brazil (Tables 1 and 2). Diseased materials were incubated individually in wet plastic chambers (10.8 × 10.8 × 0.3 cm) on wet filter paper at 20 C until spore masses were formed. Spores were transferred to culture medium, and the isolates were identified according to descriptions of the Commonwealth Mycological Institute (11). All cultures were maintained on modified Mathur's medium (19). Also, the method developed by Takatsu (34) was adopted to preserve conidial masses of *C. lindemuthianum*.

Races were identified based on the results of the inoculations of 12 differential cultivars (Table 1). One isolate of each of the nine races was deposited in the Herbarium of the Commonwealth Mycological Institute, Kew, England.

Clean seeds of the differentials were produced in pots in greenhouses. Seeds of all cultivars and breeding lines tested were permanently stored at the Banco de Germoplasma of Centro Nacional de Recursos Genéticos (CENARGEN/EMBRAPA), Brasília.

To produce inoculum, spore masses of 2-wk-old colonies were transferred to the surface of four petri dishes containing 30 ml of modified Mathur's medium (19). Plates were incubated in the dark at 20 C for 10 to 20 days. Spore suspensions were prepared using sterile distilled water and calibrated using a hemacytometer.

All 201 isolates (Table 2) were tested twice by inoculating to differential cultivars, following the method of Champion et al (9). Seeds of differential cultivars were treated with 1% sodium hypochlorite for 1 min before washing with sterile distilled water. Seeds were germinated on wet filter paper at 20 C under fluorescent lamps with a 12-hr photoperiod for 5 to 7 days. Four healthy seedlings of each cultivar with a main root at least 10 cm long were inoculated by immersion in 10^6 spores per milliliter suspensions for 1 hr. Inoculated seedlings were laid with their tips aligned on double sheets of 19 × 28 cm Cel 065 Germtest paper (Delo/Elos, Rua General Jardim 645, 8° Andar/582, 01000 São Paulo, SP) with 4-cm gaps between seedlings. The paper was wet with sterile water before seedlings were wrapped. Each package consisted of a set of four seedlings of each differential cultivar inoculated with one isolate of the fungus. The inoculated seedlings were incubated in a dark Mangeldorf germinator (Biomatic Aparelhos Científicos Ltda., Rua Cel. Massut 124, 90000 Porto Alegre, RS) at 20 C and 100% relative humidity for 7 days. Seedling reaction was scored using a scale of 0 to 5 (Fig. 1). Plants with ratings of 1, 2, or 3 were considered resistant, and higher scores indicated susceptible materials. To confirm the results obtained by the method of Champion et al (9), a group of 48 isolates, which included representatives of all races detected in each state, was tested by a seedling inoculation method developed by Giessen and Steenbergen (16). The bottom of the Styrofoam boxes (40 × 24 × 21 cm) was covered with Kimpack paper (Seedburo Equipment Co., Chicago, IL) and a perforated aluminum plate measuring 36 × 20 × 1 cm covered with Germtest paper. Five-mm holes were made at 4 × 5 cm intervals on the Germtest paper, for a total of 36 holes per box.

Each box received a set of four seedlings of each of the 12 differential cultivars. Individual seedlings were placed in holes with

TABLE 1. Characterization of Brazilian isolates of races of *Colletotrichum lindemuthianum* by inoculating seedlings of 12 differential cultivars[a]

Number of isolates	Differential cultivars												Race[d]
	a	b	c	d	e	f	g	h	i	j	k	l[b,c]	
121	+	+		+									alpha (60)
26	+	+	+	+	+		+						delta (13)
22	+	+											epsilon (11)
8	+	+		+	+		+			+	+		zeta (4)
10	+	+		+								+	eta (5)
3	+	+	+		+	+	+		+			+	theta (1.5)
3	+	+	+	+	+		+	+					kappa (1.5)
1	+	+	+	+	+	+	+		+				lambda (0.5)
7	+	+	+	+	+		+					+	mu (3.5)

[a] Seedlings were inoculated in moist dark chambers at 20 C.
[b] a = Michelite; b = Aiguille Vert; c = Dark Red Kidney; d = Widusa; e = Imuna; f = BO 22; g = Sanilac; h = Cornell 49-242; i = Kaboon; j = TO; k = PI 207262; and l = Mexico 222.
[c] + indicates susceptible reaction.

TABLE 2. Races of *Colletotrichum lindemuthianum* collected from *Phaseolus vulgaris* in a nationwide survey in Brazil from 1984 to 1985

State	Cultivar	Races	Number of isolates
Rio Grande do Sul	Carioca	delta	2
	Carioca Preto	alpha	3
	Maquiné	alpha	1
	Paulista	alpha	1
	Preto	alpha, delta	9
	Rico 23	alpha	2
	Tahyu	mu	3
Santa Catarina	Carioca	alpha	4
	Preto	alpha, delta, epsilon, kappa, mu	20
	Vermelho	delta	1
Paraná	Bico de ouro	alpha	1
	Bolinha amarela	delta	2
	Brilhante	kappa	1
	Carioca	alpha, delta	12
	Chumbinho	alpha, epsilon, zeta	3
	Jolis	alpha	2
	Lustroso	epsilon	1
	Mexico	eta	3
	Mouro	delta	1
	Preto	zeta	3
	Rancheiro	delta	2
	Rio Ivaí	alpha, epsilon	2
	Rio Tabaí	alpha, epsilon	5
	Rosinha	delta, epsilon	4
	TO	zeta	1
São Paulo	Aroana	alpha	3
	Bico de ouro	mu	1
	Carioca	alpha, epsilon, kappa	14
	Jalo	delta	2
Rio de Janeiro	Preto	alpha, epsilon	3
Espírito Santo	Aroana	alpha	3
	Carioca	alpha, epsilon	3
	Costa Rica	alpha	2
Espírito Santo	Mata mulher	zeta	3
	Preto	alpha	3
	Sintético	delta	1
Minas Gerais	Jalo	delta, theta	2
	Macarrão(p)[a]	theta	2
	Manteigão	alpha, delta	4
	Preto	delta	4
	Rico 23(p)	alpha	1
	Rosinha	alpha, mu	3
	Roxinho	alpha, epsilon	3
	Vermelho	alpha	8
Mato Grosso do Sul	Carioca	alpha	2
	Roxinho	alpha, delta	3
Mato Grosso	Carioca	alpha	2
	Rosinha	alpha	1
Goiás	Carioca	delta	1
	Jalo	delta	1
	Roxinho	alpha, eta	8
Distrito Federal	Carioca	alpha	4
	Jalo	delta	1
	Preto	alpha, eta	3
	Rico 23(p)	alpha, eta	3
	Rosinha	alpha	2
	Vagem(p)	alpha, eta	8
Bahia	Carioca	alpha, epsilon	4
	Mulatinho	delta	1
Sergipe	Carioca	alpha	1
Alagoas	Mulatinho	alpha	1
Paraíba	Mulatinho(p)	alpha	1
Rondônia	Carioca	alpha	1

[a] p indicates isolation from pods. The other isolates were obtained from seeds.

the root system crossing the aluminum support plate and remaining in touch with the wet Kimpack paper. The box was covered with transparent plastic and placed in a growth chamber (Percival Manufacturing Co., Boone, IA) at 20 C, with 14 hr light (Phillips Daylight, TL 20/54 RS) and 10 hr dark, for 3 days to allow for root and hypocotyl growth. The plastic cover was then removed, and the seedlings received a uniform spray with 100-ml spore suspension (10^6 spores per milliliter) per box. The box was again sealed with the plastic sheet and incubated in the dark for 24 hr at 20 C; it then received 14 hr light and 10 hr darkness for 6 days. The seedlings were also evaluated using the 0 to 5 scale (Fig. 1).

The index of virulence of the races of *C. lindemuthianum* was calculated as the percentage of bean cultivars or breeding lines susceptible to each race.

The index of resistance was calculated as the percentage of the races that infected a cultivar. Whenever a cultivar or line had even one plant developing disease, the cultivar or line was considered susceptible in calculating the index of virulence or the index of resistance.

RESULTS

The reactions of the 12 differential cultivars to the 201 isolates of *C. lindemuthianum* confirmed the presence of races alpha, delta, epsilon, kappa, and lambda in Brazil (Tables 1 and 2). In addition, four new races were identified as zeta, eta, teta, and mu (Fig. 2). Among the isolates tested, 121, 26, 22, 8, 10, 3, 3, 1, and 7 were races alpha, delta, epsilon, zeta, eta, teta, kappa, lambda, and mu respectively (Table 1). Forty-eight isolates comprising all races were also inoculated by the method of Giessen and Steenbergen and the same results were obtained. The distribution of these races in Brazil is summarized in Table 3.

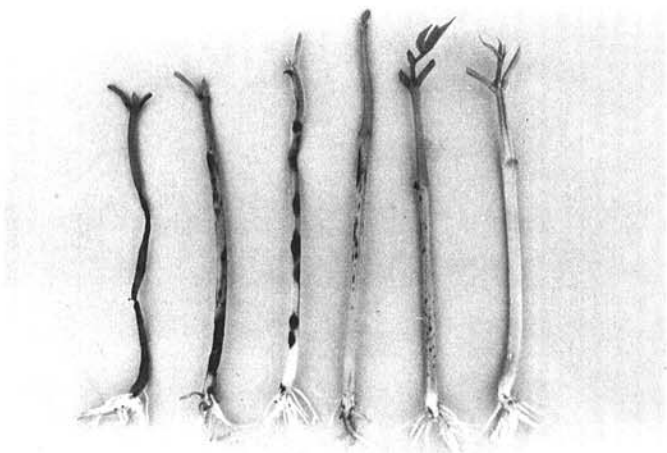

Fig. 1. Bean seedlings with scores 5 to 0 (left to right) used to evaluate resistance to *Colletotrichum lindemuthianum*. Seedlings with scores of 0, 1, and 2 were considered resistant and those with scores of 3, 4, and 5 susceptible.

None of the races of *C. lindemuthianum* was pathogenic on all 72 sources of resistance tested. Most of the lines and cultivars showed an index of resistance above 50 (Table 4). The lines A 321, A 373, A 381 and cultivars AB 136, Evolutie, GO 2338, GO 3367, and TU were shown to be resistant to all races detected. The remaining components of the germ plasm studied were susceptible to at least one race. The number of sources of resistance to each of the races varied from 36 in the case of races zeta and lambda to 59 in eta (Table 4).

DISCUSSION

Although the two methods of inoculation showed similar results, seedlings seemed more responsive to the method of Champion (9) than to the method of Giessen and Steenbergen (16). Only the cultivar Cornell 49-242 showed a differential response with a score of 2 and 3 when inoculated with races alpha, eta, teta, and mu by the first method and no symptoms when inoculated by the method of Giessen and Steenbergen (16).

The best-known race nomenclature for *C. lindemuthianum* is based on letters of the Greek alphabet (3,6,8,17–19,24,33,35). Therefore, the new races eta, theta, zeta, and mu were named according to this system. The number of races detected shows a great variability of the fungus in Brazil, reflecting the diversity of this species in South America.

The selection and use of differentials must only include cultivars with potential use as a resistance source. They also must be available for national or international breeding programs. The newly adopted differentials TO, PI 207262, and Mexico 222 comply with these basic requirements. These three cultivars, although considered by Fouilloux (14), Kruger et al (20), and Schwartz et al (33) as important sources of resistance, were shown to be susceptible to the newly reported races. Race zeta was pathogenic to varieties TO and PI 207262; races eta and mu attacked Mexico 222; and teta was pathogenic to Mexico 222 and Kaboon.

Among the cultivars employed, only Michelite, Dark Red Kidney, Perry Marrow, and Cornell 49-242 were previously used for identification of races of *C. lindemuthianum* in Latin America (19,26,29,37).

The ARE gene present in Cornell 49-242 (22) is the main source of resistance to anthracnose present on Brazilian cultivars of recent development (1). The detection of race kappa on commercial seeds produced in the states of Santa Catarina, Paraná, and São Paulo represents a threat to this extensive use of ARE-gene resistance in Brazil (30,31).

The race alpha was always found in previous surveys in Brazil (3,19,23,26,29). The present data confirmed the widespread presence of this race in the country; approximately 60% of the isolates were alpha.

Anthracnose is present in all bean-producing areas of Brazil, but

TABLE 3. Distribution of physiological races of *Colletotrichum lindemuthianum* in Brazil

State[a]	Number of isolates of each race									Total per state
	Alpha	Delta	Epsilon	Zeta	Eta	Theta	Kappa	Lambda	Mu	
Rio Grande do Sul	14	3							3	20
Santa Catarina	16	3	3				1		2	25
Paraná	18	7	9	5	3		1			43
São Paulo	12	2	4				1		1	20
Rio de Janeiro	2		1							3
Espírito Santo	9	1	2	3						15
Minas Gerais	15	5	2			3		1	1	27
Mato Grosso do Sul	4	1								5
Mato Grosso	3									3
Goiás	7	2			1					10
Distrito Federal	14	1			6					21
Bahia	3	1	1							5

[a]One isolate of race alpha was obtained from Alagoas, Paraíba, Rondônia, and Sergipe.

TABLE 4. Response of 72 sources of resistance to bean anthracnose when inoculated with nine races of *Colletotrichum lindemuthianum*

Line or cultivar[a]	Races[b]									Index of resistance
	Alpha	Delta	Epsilon	Zeta	Eta	Theta	Kappa	Lambda	Mu	
A 321	R	R	R	R	R	R	R	R	R	100
A 381	R	R	R	R	R	R	R	R	R	100
AB 136	R	R	R	R	R	R	R	R	R	100
Evolutie	R	R	R	R	R	R	R	R	R	100
GO 2338	R	R	R	R	R	R	R	R	R	100
GO 3367	R	R	R	R	R	R	R	R	R	100
TU	R	R	R	R	R	R	R	R	R	100
A 255	R	R	R	R	R	R		R	R	89
A 259	R	R	R	R	R	R	R	R/S	R	89
A 262	R	R	R	R	R	R/S	R	R	R	89
A 263	R	R	R	R	R		R	R	R	89
A 323	R	R/S	R	R	R	R	R	R	R	89
Aroana 80	R	R	R	R	R	R		R	R	89
Aysó	R	R	R	R	R	R		R	R	89
Carioca 80	R	R	R	R	R	R		R	R	89
Cornell 49242	R	R	R	R	R	R		R	R	89
GO 2641	R	R	R	R/S	R	R	R	R	R	89
GO 2696	R	R	R	R/S	R	R	R	R	R	89
GO 5653	R	R	R		R	R		R	R	89
GO 8160	R	R/S	R	R	R	R	R	R	R	89
GO 10921	R	R	R		R	R	R	R	R	89
Iguaçu	R	R	R	R	R	R		R	R	89
Moruna 80	R	R	R	R	R	R		R	R	89
PI 207262	R	R	R		R	R	R	R	R	89
Rio Piquirí	R	R	R	R	R	R		R	R	89
Rio Vermelho	R	R	R	R	R	R		R	R	89
TO	R	R	R		R	R	R	R	R	89
A 254	R	R	R	R/S	R	R/S	R	R	R	78
A 317	R/S	R	R	R/S	R	R	R	R	R	78
A 322	R	R	R	R/S	R	R	R	R		78
BAT 841	R	R	R		R	R	R	R/S	R	78
BO 22	R	R	R	R	R		R		R	78
Coco la creme	R	R	R	R	R		R		R	78
GO 3991	R	R	R	R/S	R	R/S	R	R	R	78
GO 6220	R/S	R	R	R	R	R	R	R/S	R	78
GO 7310	R	R	R	R/S	R	R	R	R	R/S	78
Kaboon	R	R	R	R	R		R		R	78
PI 173022	R		R	R	R	R		R	R	78
Vermelhão	R	R	R	R	R		R	R/S	R	78
A 140	R	R	R	R/S	R	R			R	67
A 296	R	R	R	R/S	R	R			R	67
A 329	R/S	R	R	R	R		R/S	R	R	67
Mexico	R	R	R	R			R	R		67
Mulatão	R/S	R	R	R	R	R	R	R/S	R/S	67
Olho de Pombo	R	R/S	R	R	R		R	R		67
Renka	R	R	R		R	R/S	R		R	67
A 319	R	R/S	R	R	R	R				56
A 374	R	R	R		R		R			56
GO 4360	R		R	R/S			R	R	R	56
GO 6975	R	R	R		R		R/S			44
PI 165422	R	R/S		R			R/S	R/S	R	44
A 154	R	R/S		R	R	R/S				33
A 252	R		R		R					33
A 331	R/S	R/S	R/S	R	R	R/S	R			33
Rio Tibagí		R					R		R	33
A 248	R/S		R		R					22
A 250			R/S	R		R/S			R	22
A 264	R			R/S	R		R/S	R/S	R/S	22
A 305		R/S			R	R	R/S			22
GO 6071			R/S		R	R			R/S	22
A 153					R					11
A 242			R/S		R					11
A 243			R/S		R					11
A 257		R/S	R/S	R/S			R/S	R/S	R	11
A 320	R									11
Index of virulence	28	26	24	50	18	46	43	50	39	

[a] Five breeding lines tested (A 253, A 256, A 279, A 286, GO 8519) did not show resistance to any of the nine races.
[b] R indicates resistance and R/S indicates that some of the plants tested were susceptible and others were resistant.

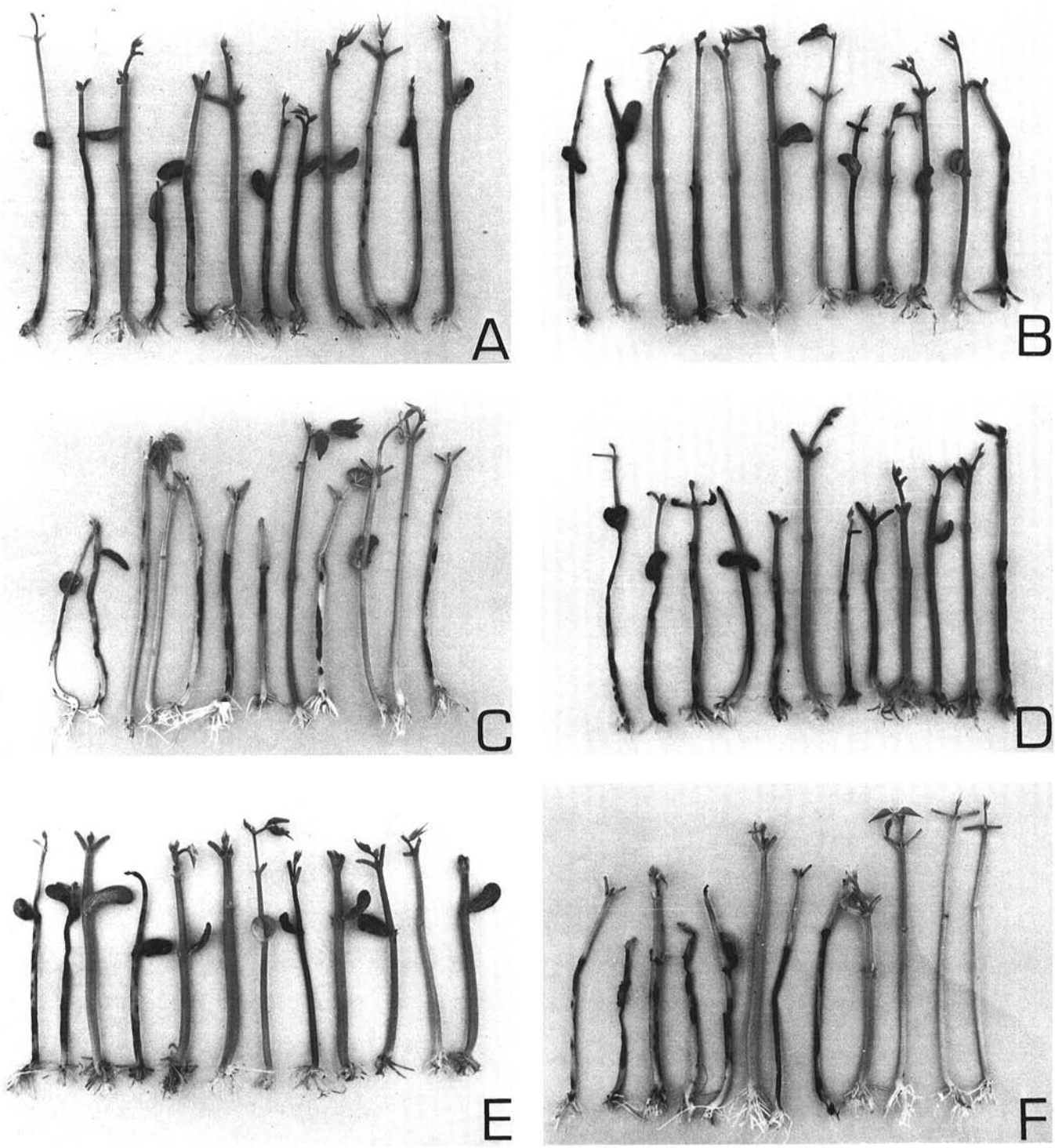

Fig. 2. The new races zeta (A), eta (B), teta (C), and mu (D) of *Colletotrichum lindemuthianum*, the common race alpha (E), and kappa (F), based on the reactions of the differential varieties (left to right): Michelite, Aiguille Vert, Dark Red Kidney, Widusa, Imuna, BO 22, Sanilac, Cornell 49-242, Kaboon, TO, PI 207262, and Mexico 222.

some races of *C. lindemuthianum* have limited spread, probably because most of the growers use locally grown seeds (23). The development of the seed industry could accelerate the spread of those races if seed health is not seriously considered.

Race alpha-Brazil described by Fouilloux (14) was not found in this survey. Recently Schwartz et al (33) could not infect varieties Cornell 49-262 and Mexico 222 with an alpha-Brazil isolate as previously reported (14,15).

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