Screening for Resistance to and Pathogenic Specialization of Fusarium oxysporum f. sp. phaseoli, the Causal Agent of Bean Yellows

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ABSTRACT

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Bean cultivars were root-dip inoculated with suspensions of microconidia of *Fusarium oxysporum* f. sp. *phaseoli* and placed in chambers at 20, 24, and 28 C. Although optimum temperature for growth of the fungus in vitro was about 28 C, a variable proportion of plants of a susceptible cultivar (Bush Blue Lake 274) did not develop symptoms of wilt when incubated at 28 C after inoculation. All plants developed symptoms at 20 C even with the lowest inoculum level. The cultivar Preto Uberabinha behaved as resistant regardless of the test conditions employed, whereas the cultivar Tenderette was resistant to inoculum containing 10^4 conidia/ml, but completely susceptible to that containing 10^6 conidia/ml. The differential response of bean cultivars and lines inoculated with strains from Brazil, the USA, and The Netherlands allowed the characterization of two pathogenic races of *F. oxysporum* f. sp. *phaseoli*. The European and North American strains appeared to represent a single race, and the Brazilian strains a different race. The strains from the USA and Europe were highly virulent on *Phaseolus coccineus*, whereas the Brazilian strains failed to induce wilting symptoms on this species.

Additional key words: *Phaseolus vulgaris*, Fusarium wilt, host reaction.

A vascular disease of beans (*Phaseolus vulgaris* L.), first described in the USA in 1929 (9) and later called bean yellows, is caused by *Fusarium oxysporum* (Schlecht) f. sp. *phaseoli* Kendrick and Snyder (12). In Brazil, bean yellows was reported in 1966 (4) and has become an important disease, particularly for snap bean crops in certain southeastern areas (5,6,8). There is evidence for more than one race of the pathogen in Brazil (3). Limited tests in the USA failed to detect differences in pathogenicity of strains from California and South Carolina (1). In England (7) and The Netherlands (N. Hubbeling, *personal communication*), a similar disease occurs on *Phaseolus coccineus*, but apparently it never has been reported on local cultivars of *P. vulgaris*. It was suggested that the disease of the runner bean described in England might be incited by a race of *F. oxysporum* f. sp. *phaseoli* different from the one occurring in the USA (1).

In a Rhode Island experimental field plot, differences in susceptibility to bean yellows were noticed among bean cultivars under conditions of natural infection (11). Cruz et al (6) found resistance in local dry bean and exposed snap bean cultivars to strains of the fungus isolated in São Paulo, Brazil.

The importance of inoculation procedures and environmental conditions when screening for resistance to Fusarium wilts has been demonstrated in several instances (10,17,18). In a recent study on the Fusarium wilts of cucurbits, incubating temperature and inoculum level were included among factors that affect the interpretation of results of pathogenicity tests (2).

The purposes of this paper are: to (i) establish a suitable and reproducible technique for testing the reaction of beans to *F. oxysporum* f. sp. *phaseoli*, and (ii) to compare the virulence of strains of the pathogen from different geographical origins towards bean cultivars and accessions. Preliminary reports have been published (14,15).

MATERIALS AND METHODS

The following strains of *F. oxysporum* f. sp. *phaseoli* were used: three strains (2107-A, 2107-B, and 2116) from diseased specimens of dry and snap beans collected in 1973-74 from Rio de Janeiro, Brazil; ATCC 18131 isolated originally by G. M. Armstrong and J. K. Armstrong in South Carolina; and WAG-76 from runner beans in The Netherlands and obtained from N. Hubbeling (Institute of Phytopathological Research, Wageningen, The Netherlands).

Seedlings were grown in vermiculite until the primary leaves were one-third expanded (usually 7 days old); then they were pulled and their root system was washed, uniformly clipped, and inoculated. On the day seeds were planted, the fungus (monoconidial cultures) was transferred from storage in soil tubes to plates of potato-dextrose agar and incubated at 24 C. On day 4, mycelial disks taken from these plates were added to flasks of potato-dextrose broth. The flasks were placed on a rotary shaker for 3 days at room temperature (about 22 C). For the preparation of conidial suspensions, contents of the flasks were filtered through a double layer of cheesecloth and the filtrates were centrifuged (10 min at 8,000 rpm), followed by resuspension of the conidial pellets in sterile distilled water. Finally, the concentration of conidia was adjusted with a haemacytometer or by optical transmittance (600 nm) in a Bausch & Lomb Spectronic-20 colorimeter. Roots were dipped momentarily in such suspensions (3) and the inoculated seedlings were planted individually in 10-cm diameter pots containing a mixture of compost, muck soil, and sand (3:3:1, v/v). Uninoculated, susceptible controls were included. Results were recorded after 30 days and the reaction of individual plants was scored according to a disease severity rating (DSR) that ranged from 1.0 (plants showing vascular discoloration, but no external symptoms) to 5.0 (plants dead). Plants with DSR = 1.0 to 2.0 (mild external symptoms followed by recovery) were rated resistant. Plants with DSR = 4.0 (severe leaf dropping, no recovery, plants dying) to 5.0 were rated susceptible. Plants with DSR = 3.0 (stunting and chlorosis, limited leaf dropping, plants surviving inoculation) were considered intermediate in reaction.

To study the effect of incubation temperatures and inoculum dosages upon the reaction of beans to the yellows organism, seedlings were kept in controlled environment chambers (12-hr day and 10,000 lux) at 20, 24, and 28 C. Plants were subjected to approximately 10^4, 10^5, and 10^6 conidia/ml. To compare the virulence of strains of the fungus, a collection of more than 50 bean cultivars and accessions (including 41 Brazilian dry bean intro-

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TABLE 1. Effects of incubation temperature and inoculum dosage on the reaction of two bean cultivars to strain 2107-A of *Fusarium oxysporum* f. sp. *phaseoli* from Brazil

Incubation temperature	Inoculum dosage	Cultivar Tenderette			Cultivar Bush Blue Lake 274		
		Disease severity[a]		Plants with external symptoms	Disease severity		Plants with external symptoms
(C)	(conidia/ml)	Average	Range	(%)	Average	Range	(%)
28	10^6	1.0[b]	...	0	2.4	1.0–4.0	70
	10^5	1.0	...	0	1.4	1.0–3.0	30
	10^4	1.0	...	0	1.0	...	0
24	10^6	1.0	...	0	3.8	3.0–5.0	100
	10^5	1.0	...	0	2.7	1.0–4.0	75
	10^4	1.0	...	0	1.8	1.0–3.0	70
20	10^6	1.4	1.0–2.0	40	4.7	4.0–5.0	100
	10^5	1.1	1.0–2.0	15	4.6	4.0–5.0	100
	10^4	1.0	...	0	2.6	2.0–3.0	100

[a]Disease severity rating (1.0 = no external symptoms to 5.0 = plants dead).
[b]Average values of 20 plants per cultivar in two separate tests, 30 days after inoculation.

ductions from the Instituto Agronomico do Estado de São Paulo, Campinas, Brazil) was inoculated (about 10^6 conidia/ml) in the greenhouse with the air temperature of 18-23 C.

RESULTS

The reaction of two bean cultivars to a Brazilian strain of *F. oxysporum* f. sp. *phaseoli* was greatly influenced by temperature of incubation and concentration of spores in the inoculum; disease severity was inversely proportional to the temperature of incubation and directly proportional to the inoculum dosage (Table 1). At 20 C, all plants of the susceptible Bush Blue Lake 274 showed external wilting symptoms (DSR = 2.6–4.7), regardless of the inoculum concentration. Furthermore, all seedlings of this cultivar inoculated with the two highest dosages and kept at 20 C were dying or already dead within 30 days. Conversely, at 28 C, 30 and 70% of the plants of Bush Blue Lake 274 showed no external symptoms after inoculation with 10^6 and 10^5 conidia/ml, respectively. The lowest inoculum dosage (10^4 conidia/ml) in the 28

TABLE 2. Effect of the incubation temperature on the in vitro radial growth of three strains of *Fusarium oxysporum* f. sp. *phaseoli*[a]

Incubation temperature	Diameter of 4-day-old colonies (mm)		
(C)	2107-A	ATCC 18131	WAG-76
16	20.3[b]	24.3	23.0
20	30.3	32.0	36.4
24	36.6	44.0	42.6
28	39.3	47.3	45.6
32	18.8	23.5	21.7

[a]Origin of strains: 2107-A = Brazil, ATCC 18131 = USA, and WAG-76 = The Netherlands.
[b]Average values of four separate tests.

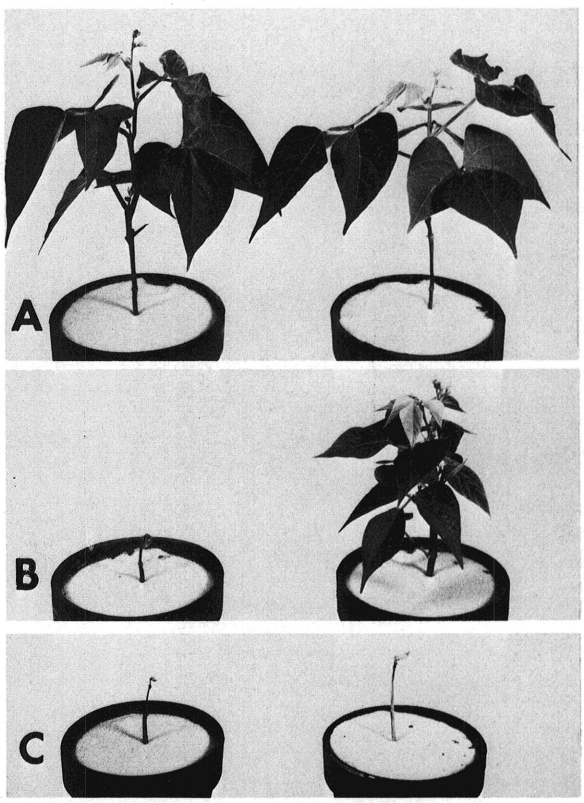

Fig. 1. Characteristic reactions of the bean cultivars **A)** Preto Uberabinha, **B)** Tenderette, and **C)** Bush Blue Lake 274 incubated at 12-hr day, 10,000 lux, and 20 C after inoculation with strain ATCC 18131 of *Fusarium oxysporum* f. sp. *phaseoli* 10^6 conidia/ml (left) and 10^5 conidia/ml (right).

TABLE 3. Effect of the inoculum dosage on the reaction of three bean cultivars to strain ATCC 18131 of *Fusarium oxysporum* f. sp. *phaseoli* under controlled environment[a]

Cultivar	Disease severity[b] per inoculum dosage (conidia/ml)					
	10^6		10^5		10^4	
	Avg.	Range	Avg.	Range	Avg.	Range
Preto Uberabinha	1.5[c]	1.0–2.0	1.0	...	1.0	...
Tenderette	4.9	4.0–5.0	3.2	2.0–4.0	1.1	1.0–2.0
Bush Blue Lake 274	5.0	...	4.9	4.0–5.0	3.1	2.0–4.0

[a]Constant incubating conditions (12-hr day, 10,000 lux, and 20 C).
[b]Disease severity rating (1.0 = no external symptoms to 5.0 = plants dead).
[c]Average values of 20 plants per cultivar in two separate tests, 30 days after inoculation.

C chamber proved insufficient to detect the susceptibility of this cultivar. At 24 C, disease severity was intermediate (DSR = 1.8–3.8 for Bush Blue Lake 274), the highest concentration of spores being the only one that completely prevented escapes in the susceptible cultivar.

The cultivar Tenderette displayed a resistant reaction to the Brazilian strain of the pathogen under all the conditions studied (DSR = 1.0–1.4). Even when submitted to the most severe test, this cultivar remained resistant, although some of the inoculated plants developed incipient to mild external symptoms of the disease.

Incubation of inoculated seedlings at 20 C also resulted in severe disease caused by all other stains of the bean yellows organism presently studied. Thus, these results indicated that the incubating temperature most conducive to increased disease severity did not correlate with temperatures favorable to rapid growth of the fungus in culture (Table 2); this is also the case with other wilt *Fusaria* (2,13).

By incubating at 20 C and varying the spore concentration in the

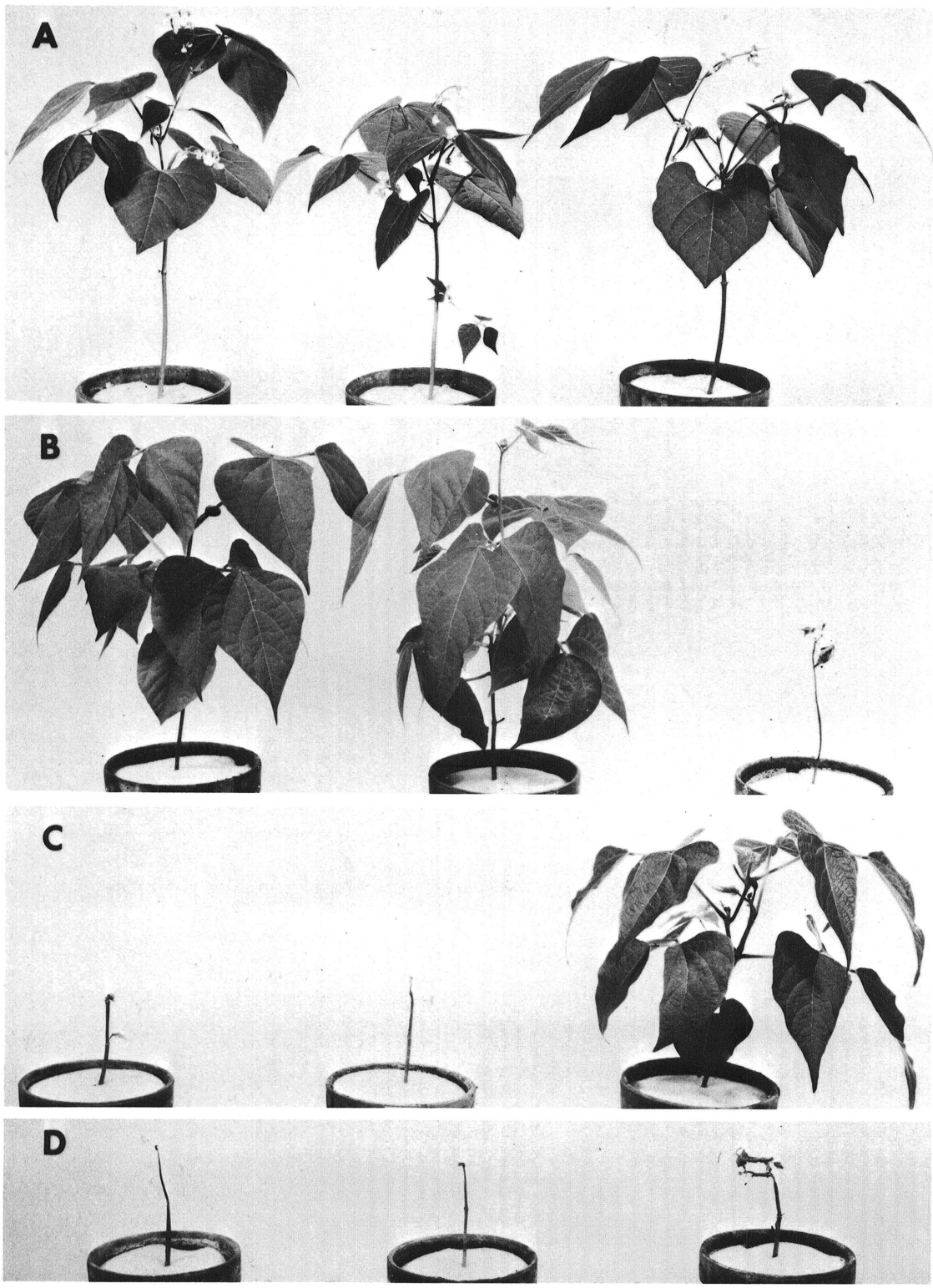

Fig. 2. Characteristic reactions of the bean cultivars **A)** Wis. (BBSR) 130, **B)** Preto Uberabinha, **C)** Pintado, and **D)** Bush Blue Lake 274 inoculated with strains WAG-76 (left), ATCC 18131 (center), and 2107-A (right) of *Fusarium oxysporum* f. sp. *phaseoli*.

inoculum, two types of resistance to the USA strain of the pathogen (ATCC 18131) could be distinguished (Table 3, Fig. 1). The cultivar Preto Uberabinha (a dry bean grown commercially in Brazil) maintained its high resistance when the inoculum dosage was increased from 10^4 to 10^6 conidia/ml (DSR = 1.0–1.5); however, the response of the cultivar Tenderette to this particular strain ranged from resistance (DSR = 1.1) to intermediate (DSR = 3.2) to complete susceptibility (DSR = 4.9) with the increasing inoculum dosages.

Inoculation of the collection of bean cultivars and accessions in the greenhouse led to a clear distinction between two pathogenic races of *F. oxysporum* f. sp. *phaseoli*. Virulence of the strains from the USA and Europe was identical. The Brazilian strains were identical to each other, but differed from the North American and European strains with respect to virulence towards selected bean cultivars (Table 4, Fig. 2). Some cultivars (eg, Slimgreen, Wis. [BBSR] 130, and Rosinha-Sem-Cipo) were resistant to both races; some (eg, Topcrop, Pintado, Branco Uberlandia, and Floresta-5) were resistant to the Brazilian race only; others (eg, Preto Uberabinha and Rosinha G-1) were resistant to the North American-European race only; and, finally, others (eg, Bush Blue Lake 274 and Roxoti) were susceptible to both races. Two accessions of *P. coccineus* (PI 321088 and PI 319449) were killed by the European and USA strains, but when inoculated with the Brazilian strains these plants developed a restricted vascular discoloration only (Fig. 3).

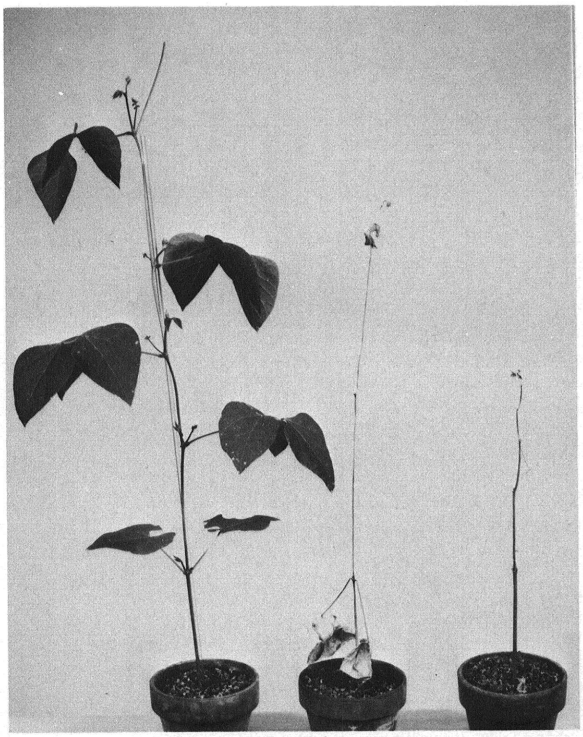

Fig. 3. Characteristic reactions of a *Phaseolus coccineus* accession (PI 319449) inoculated with strains 2107-A (left), ATCC 18131 (center), and WAG-76 (right) of *Fusarium oxysporum* f. sp. *phaseoli*.

DISCUSSION

The studies performed under controlled environment enabled us to define a set of testing conditions suitable to screening beans for resistance to *F. oxysporum* f. sp. *phaseoli*. With these conditions (approximately 20 C and 10^5–10^6 conidia/ml), it was possible to differentiate races among the available strains and also to obtain reproducible data when investigating the inheritance of resistance to the pathogen through segregating generations from selected bean crosses (16).

The results of the greenhouse inoculations indicated that the strains from the USA and The Netherlands belong to a same race, and that the strains from Brazil belong to a distinct race. Some of the cultivars listed were highly resistant to the USA-European race and highly susceptible to the Brazilian race; others had an opposite reaction. Therefore, these cultivars can be used as a set of differentials for the identification of races in *F. oxysporum* f. sp. *phaseoli*.

The fact that relatively high temperatures (above 24 C) tended to suppress full symptom expression might have some epidemiological significance. For example, under warm conditions, even "susceptible" cultivars such as Bush Blue Lake 274 possibly could behave as "resistant" with the lower inoculum densities expected in the field. This could explain, at least in part, why the disease has never become limiting in certain regions of the USA where the causal organism long has been present. A second possibility might be that some degree of resistance to the USA race might be widespread among commercial bean genotypes and would be

TABLE 4. Reaction of selected bean cultivars and accessions inoculated with two strains[a] of *Fusarium oxysporum* f. sp. *phaseoli*

Species and bean cultivar or accession	Disease severity[b] and strain			
	2107-A		ATCC 18131	
	DSR	Class[c]	DSR	Class
Phaseolus vulgaris				
Slimgreen	1.2[d]	R	1.6	R
Rosinha-Sem-Cipo (*)[e]	1.8	R	1.6	R
Wis. (BBSR) 130	1.0	R	1.2	R
Preto Uberabinha (*)	4.2	S	1.0	R
Rosinha G-1 (*)	5.0	S	1.4	R
Topcrop	1.0	R	4.5	S
Pintado (*)	1.0	R	5.0	S
Branco Uberlandia (*)	1.0	R	5.0	S
Floresta-5 (*)	1.0	R	5.0	S
Bush Blue Lake 274	5.0	S	5.0	S
Roxoti (*)	5.0	S	5.0	S
Phaseolus coccineus				
PI 321088	1.0	R	5.0	S
PI 319449	1.0	R	4.8	S

[a]Origin of strains: 2107-A = Brazil, ATCC 18131 = USA.
[b]Disease severity rating (1.0 = no external symptoms to 5.0 = plants dead).
[c]Symbols: R = resistant (DSR = 1.0–2.0), and S = susceptible (DSR = 4.0–5.0).
[d]Average values of 20 plants per cultivar, 30 days after inoculation.
[e](*) Brazilian dry bean cultivars obtained from the I.A.C. (Campinas, São Paulo, Brazil).

sufficient to protect the plants subjected to natural infection. In support of this hypothesis, Howard and Anderson (11) stated that only two of 11 cultivars showed symptoms of the disease when planted in a randomized, four-replicate design at Rhode Island under natural soil infestation. In our greenhouse tests, a degree of resistance to the USA strain, comparable to that of Tenderette, was detected in other snap bean cultivars such as Bountiful, Early Gallatin, and Bush Blue Lake 109 (author's *unpublished*). In addition, plants of cultivar Slimgreen were highly resistant to that strain.

Distinct types of resistance in a single host species to other *formae speciales* of *F. oxysporum* that incite wilts exist and are genetically controlled (10,18). A comparable situation may occur in the case of bean yellows in which type *B* resistance (cultivar Tenderette × strain ATCC 18131) is overcome under certain conditions that favor disease development, whereas type *A* resistance (cultivar Preto Uberabinha × strain ATCC 18131) is fully expressed under the same conditions.

The race of the fungus from Europe caused severe wilt not only on *P. coccineus*, but also on cultivars of *P. vulgaris*. From these results, one can speculate that some degree of the resistance must be present in cultivars of the latter species grown in The Netherlands where the disease apparently is restricted to the runner bean (N. Hubbeling, *personal communication*).

The reactions of certain cultivars (eg, Pintado and Rosinha-Sem-Cipo) to the Brazilian strains indicated that these strains may belong to the same race of the pathogen isolated in São Paulo by Cruz et al (6).

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