

# Physiological Specialization and Effects of Inoculum Concentration of *Fusarium oxysporum* f. sp. *phaseoli* on Common Beans

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## ABSTRACT

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Physiological specialization of a strain of *Fusarium oxysporum* f. sp. *phaseoli* from Colorado was compared to that of other strains from South Carolina (American Type Culture Collection strains) and Colombia on the International Center of Tropical Agriculture (CIAT) bean *Fusarium* wilt differential cultivars of *Phaseolus vulgaris*. The Colorado and Colombian strains produced differential reactions and are considered to be distinct races. The Colorado race exhibited the broadest virulence spectrum. Cultivar HF 465-63-1 was highly resistant to all races. Varying inoculum concentrations of the Colorado race changed the expected disease reaction order of the differential cultivars at inoculum densities lower than  $10^6$  conidia per milliliter. The variation of cultivar disease reactions to different inoculum densities can lead to a false classification of races.

*Fusarium* wilt of beans, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *phaseoli* J.B. Kendrick & W.C. Snyder (FOP), is an important disease of common bean (*Phaseolus vulgaris* L.) (1). Little is known about the pathogenic variability of FOP. Two strains from the Netherlands and ATCC 18131 from the American Type Culture Collection were characterized as the same physiological race, and a strain from Brazil was identified as a different physiological race with a set of 11 *P. vulgaris* and two *P. coccineus* L. cultivars (24). Aloj et al (2) described a strain of FOP from Italy as a different physiological race from ATCC 18131 and the Brazilian FOP strains used by Ribeiro and Hagedorn (24). Recently it was reported that strains of FOP from Brazil and Colombia belong to two different races (6).

It is virtually impossible to genetically characterize the pathogenicity in an asexual fungus such as *F. oxysporum*; thus pathogenic variability is identified by the specific parasitic interaction of the fungus with a set of differential cultivars (18). Windels (30) stated that this classical method of identifying races in *F. oxysporum* can be strongly influenced by environmental conditions, cultivar selection and number, strain virulence, host age, inoculum type and density, and cri-

teria of race classification (4,10,12,16, 24,27,29).

Ribeiro and Hagedorn (24) reported that *Fusarium* wilt severity in common beans increased proportionally to inoculum concentration and was influenced by cultivar and temperature interactions. In muskmelon, watermelon, and chickpea, *Fusarium* wilt severity also increased with an increase in inoculum concentration; but in general, severity depended on the level of resistance of cultivars tested and the incubation temperature (7,13,20).

The objectives of this study were to characterize the pathogenic variability of three strains of FOP by inoculating differential cultivars obtained from the CIAT Bean *Fusarium* Wilt Differential Nursery, and to measure the effects of varying inoculum concentrations of a strain from Colorado on common beans. We use the terms virulence and aggressiveness as oligogenic and polygenic traits, respectively, to differentiate pathogenicity, which is the ability to cause disease (15). The term strain is used for those cultures with unknown race identity (31).

## MATERIALS AND METHODS

**Race experiments.** The ATCC 18131 strain of FOP (FOP-SC) was obtained from M. Silbernagel, USDA/ARS IAREC, Prosser, Washington. This strain was originally isolated by G. M. Armstrong and J. K. Armstrong in South Carolina (3). The Colorado strain of FOP was recovered from one of several infected pinto beans, cultivar U.I. 114, collected by H. F. Schwartz in northeast Colorado in 1990. Specific characterization of this strain as *F. oxysporum* was conducted following identification criteria proposed by Nelson et al (21) and E. Ruppel (USDA/ARS, Fort Collins,

CO, *personal communication*). To determine forma specialis, a pathogenicity test (23) was performed with different common bean cultivars, and the strain used in this study was selected because of its aggressiveness. A voucher specimen has been submitted to the American Type Culture Collection as FOP-CO1. The FOP strain from Colombia (FOP-CL25) was obtained from M. Pastor Corrales, International Center of Tropical Agriculture (CIAT), Colombia. Inocula of FOP-SC and FOP-CO1 were derived from single-spore (macroconidia) stock cultures that were grown at room temperature in culture tubes containing autoclaved finely sieved sandy soil mixed with 2% powdered oatmeal and 15% distilled water (w/w), and stored at 4 C until use. Inoculum of FOP-CL25 was derived from a single-spore (macroconidia) stock culture grown in culture tubes containing potato-dextrose agar (PDA), and stored at 4 C until use.

Seed of the CIAT Bean *Fusarium* Wilt Differential Nursery entries was obtained from M. Pastor Corrales. The nursery contained the following common bean lines or cultivars: HF-465-63-1 (cream-beige, small seed), BAT 477 (cream-beige, medium seed), Mortino (purple mottled, large seed), Ecuador 605 (pink, medium seed), TIB 3042 (purple, large seed), Diacol Calima (red mottled, large seed), RIZ 30 (red spotted, small seed), IPA 1 (cream-beige, small seed), A 211 (black, small seed), and ICA 032 (brown, large seed). Only original CIAT seed was used in the experiments. In the first experiment, only cultivars A 211, BAT 477, Diacol Calima, ICA 032, and HF 465-63-1 were used because of limited seed stocks. Pinto U.I. 114 was not included as a differential but had been used as a susceptible host to verify pathogenicity of both strains. In the second experiment, cultivars Mortino, Ecuador 605, TIB 3042, Diacol Calima, IPA 1, RIZ 30, HF 465-63-1, BAT 477, and pinto U.I. 114 (presumed universal susceptible check by Salgado and Schwartz, *unpublished*) were inoculated.

Two seeds were planted in 13-cm-diameter plastic pots (approximately 1 L in volume) containing potting soil and placed in the greenhouse for germination. Inoculation procedures were adapted from those described by Pastor Corrales and Abawi (23).

For the first experiment, conidial inocula of FOP-SC and FOP-CO1 were

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prepared by sprinkling a few milligrams of soil stock culture onto petri dishes containing PDA (DIFCO Laboratories, Detroit, MI) at a pH of  $5.6 \pm 0.2$  and incubated in the laboratory for 28 days at room temperature and a 12-hr dark-fluorescent-light cycle. Conidia (mainly microconidia) and a few chlamydospores were suspended in sterile distilled water, filtered through a double layer of cheesecloth, and washed twice at 2,700 rpm at 4 C for 10 min. The inoculum concentration was adjusted to  $10^6$  conidia per milliliter of distilled water with a hemacytometer. Four hundred milliliters of the conidial suspension was added to a 1,000-ml beaker and stirred continuously during inoculation. In the second experiment, a similar procedure was followed except that the inoculum of FOP-CO1 was obtained from a 10-day-old culture, and FOP-CL25 was obtained from a culture-tube slant containing PDA. The conidial suspensions consisted mainly of microconidia.

Plants were inoculated 12 days after planting when unifoliolate leaves were three-fourths to fully expanded. To facilitate soil separation from roots and to speed the regaining of turgidity after transplanting, plants were not watered the day before inoculation. A maximum of 12–14 uniform seedlings per cultivar per inoculation were carefully removed from the potting soil, and the root systems were immediately washed with tap water. Cleaned plants were maintained for less than 5 min in a plastic tank containing tap water. Subsequently, one-third of the distal root system was clipped, the remaining root system was placed in the conidial suspension for 5 min, and two plants were transplanted to a 13-cm-diameter plastic pot containing potting soil. Noninoculated, clipped, water-dipped checks were also included in each experiment.

During inoculation, the greenhouse temperature was maintained at 21–25 C. A cool postinoculation temperature was

maintained to reduce possible effects of high temperature on disease reactions and to favor plant recovery after transplanting, because it was observed that plants inoculated on sunny days suffered severe unifoliolate leaf scalding. Inoculated plants were incubated in the greenhouse on a 16–21 C/32 C night/day cycle. The relative humidity ranged from 50 to 100%. Supplemental halide lighting was provided for 5 hr, from 3:00 to 8:00 pm, to provide plants with a minimum daily light cycle of approximately 12 hr. Photosynthetically active radiation measured at the bench level inside the greenhouse with the artificial light was approximately  $350\text{--}400 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

Plants were fertilized every 3 days starting 4 days after inoculation with a 15-30-15 NPK (Stern's Miracle-GRO) liquid fertilizer (6 g/20 L water). Thirteen days after inoculation, plants received the same fertilizer at the rate of 12 g/20 L water every 3 days until the end of the experiment. The solution was applied at the rate of approximately 100 ml per pot. Four to 7 days after inoculation, dead plants or those that did not recover from transplanting were removed. Plants were watered every day, and after inoculation rotated every 3 days on the bench to more uniformly expose plants to temperature and light variations within the greenhouse.

External symptoms were rated 14 and 21 days after inoculation according to the CIAT severity scale of 1 (no visible symptoms) to 9 (plant foliage 100% wilted, chlorotic, or dead). Ratings of 3 corresponded to 10% wilted and/or chlorotic, 5 to 25%, and 7 to 50%. An average disease severity of 1–3 indicated a resistant reaction class, 3.1–6 an intermediate, and 6.1–9 a susceptible (23). Internal ratings were recorded 21 days after inoculation by cutting the main stem at the primary node and noting internal discoloration. Values for external symptoms obtained at 21 instead of 14 days after inoculation were statistically

analyzed, because most resistant and some susceptible cultivars exhibited some degree of symptom remission from chlorosis, stunting, or wilting of primary leaves by 21 days after inoculation, presumably due to transplant recovery and/or host response to infection. Most susceptible plants were dead by 14 days. The experimental design was completely randomized and repeated once. Data were pooled because homogeneity tests of equal error variance for the two tests showed that the error-variance terms were equal ( $P \leq 0.05$ ) (SAS Institute, Cary, NC). Because of a significant cultivar-by-strain interaction ( $P \leq 0.05$ ) in both experiments, differences between strains within each cultivar were analyzed (19) and are shown in Table 1.

**Inoculum-density experiment.** In this experiment only FOP-CO1 was used. The inoculation procedure was the same as described above, except that four inoculum concentrations of  $1 \times 10^3$ ,  $1 \times 10^4$ ,  $1 \times 10^5$ , and  $1 \times 10^6$  conidia per milliliter were used. The cultivar Mortino was included only in the first test because of germination problems in the second. The experimental design was completely randomized and repeated once with 20 seedlings inoculated for each cultivar and test. The homogeneity test of equal error variance for the two tests indicated that the error-variance terms were unequal ( $P \leq 0.05$ ). Because of a significant cultivar-by-inoculum density interaction ( $P \leq 0.05$ ), differences among cultivars at each inoculum level were analyzed separately as shown in Table 2. Means of each treatment for both tests were combined and  $\log_{10}$  transformed only for the linear-regression analysis of disease severity and inoculum density. The linear regression for each cultivar was tested for the significance of the model.

## RESULTS

**Race experiments.** In the first race experiment, there were no differences in host susceptibility to FOP strains for

**Table 1.** Characterization of variability in virulence of South Carolina (FOP-SC), Colorado (FOP-CO1), and Colombian (FOP-CL25) strains of *Fusarium oxysporum* f. sp. *phaseoli* by inoculation of sets of bean (*Phaseolus vulgaris*) cultivars from CIAT and pinto U.I. 114

| Cultivar    | Cultivar set A      |        |                      |        | Cultivar set B       |        |                       |        |
|-------------|---------------------|--------|----------------------|--------|----------------------|--------|-----------------------|--------|
|             | FOP-SC <sup>y</sup> |        | FOP-CO1 <sup>y</sup> |        | FOP-CO1 <sup>y</sup> |        | FOP-CL25 <sup>y</sup> |        |
|             | Mean                | Rating | Mean                 | Rating | Mean                 | Rating | Mean                  | Rating |
| A 211       | 7.8                 | S      | 8.4                  | S      | ...                  |        | ...                   |        |
| BAT 477     | 8.0                 | S      | 8.5                  | S      | 8.9 <sup>z</sup>     | S      | 1.2 <sup>z</sup>      | R      |
| D. Calima   | 5.9 <sup>z</sup>    | I      | 7.3 <sup>z</sup>     | S      | 8.4 <sup>z</sup>     | S      | 9.0 <sup>z</sup>      | S      |
| ICA 032     | 7.6                 | S      | 8.3                  | S      | ...                  |        | ...                   |        |
| HF 465-63-1 | 2.1                 | R      | 2.9                  | R      | 2.5 <sup>z</sup>     | R      | 1.2 <sup>z</sup>      | R      |
| Mortino     | ...                 |        | ...                  |        | 8.8                  | S      | 9.0                   | S      |
| Ecuador 605 | ...                 |        | ...                  |        | 8.7 <sup>z</sup>     | S      | 9.0 <sup>z</sup>      | S      |
| TIB 3042    | ...                 |        | ...                  |        | 8.9                  | S      | 9.0                   | S      |
| RIZ 30      | ...                 |        | ...                  |        | 8.1 <sup>z</sup>     | S      | 1.2 <sup>z</sup>      | R      |
| IPA 1       | ...                 |        | ...                  |        | 8.4 <sup>z</sup>     | S      | 1.2 <sup>z</sup>      | R      |
| U.I. 114    | ...                 |        | ...                  |        | 9.0 <sup>z</sup>     | S      | 1.3 <sup>z</sup>      | R      |

<sup>y</sup> Mean disease severity recorded 21 days after inoculation. CIAT severity scale: 1 (no external symptoms) to 9 (plant 100% diseased). A rating of R = resistant (1–3); I = intermediate (3.1–6); and S = susceptible (6.1–9). A total of 26–28 plants per cultivar were inoculated with each strain in set A, and 24–48 in set B; ... indicates cultivar not inoculated.

<sup>z</sup> Means between strains within the cultivar set were significantly different at  $P \leq 0.05$  (Duncan's multiple range test) for each cultivar comparison.

**Table 2.** Effect of inoculum concentration of a strain of *Fusarium oxysporum* f. sp. *phaseoli* (FOP-CO1) after inoculation on a set of bean (*Phaseolus vulgaris*) cultivars from CIAT and pinto U.I. 114

| Cultivar    | Test 1 concentration (conidia/ml)     |                 |                 |                 |                 | Test 2 concentration (conidia/ml) |                 |                 |                 |                 |
|-------------|---------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------------------------|-----------------|-----------------|-----------------|-----------------|
|             | 0                                     | 10 <sup>3</sup> | 10 <sup>4</sup> | 10 <sup>5</sup> | 10 <sup>6</sup> | 0                                 | 10 <sup>3</sup> | 10 <sup>4</sup> | 10 <sup>5</sup> | 10 <sup>6</sup> |
| Mortino     | 1.0 a <sup>xy</sup><br>C <sup>z</sup> | 1.2 b<br>C      | 2.1 bc<br>C     | 4.6 c<br>B      | 9.0 a<br>A      | ...                               | ...             | ...             | ...             | ...             |
| BAT 477     | 1.1 a<br>D                            | 1.4 b<br>D      | 3.7 b<br>C      | 7.1 b<br>B      | 8.9 a<br>A      | 1.2 a<br>D                        | 1.4 b<br>D      | 4.6 b<br>C      | 9.0 a<br>B      | 9.0 a<br>B      |
| HF 465-63-1 | 1.0 a<br>B                            | 1.0 b<br>B      | 1.4 c<br>AB     | 1.1 d<br>B      | 2.1 c<br>A      | 1.0 a<br>B                        | 1.0 b<br>B      | 2.1 b<br>B      | 2.8 c<br>B      | 3.1 b<br>B      |
| D. Calima   | 1.0 a<br>C                            | 1.1 b<br>C      | 1.6 c<br>C      | 3.2 c<br>B      | 8.6 a<br>A      | 1.1 a<br>D                        | 1.7 b<br>D      | 2.3 b<br>D      | 6.7 b<br>C      | 8.9 a<br>B      |
| TIB 3042    | 1.0 a<br>C                            | 1.0 b<br>C      | 3.8 b<br>B      | 8.7 ab<br>A     | 9.0 a<br>A      | 1.0 a<br>D                        | 2.5 b<br>D      | 3.9 b<br>C      | 8.9 a<br>B      | 9.0 a<br>B      |
| IPA 1       | 1.0 a<br>C                            | 1.7 b<br>C      | 2.1 bc<br>C     | 4.7 c<br>B      | 8.2 a<br>A      | 1.0 a<br>D                        | 2.2 b<br>D      | 7.3 a<br>C      | 7.0 b<br>C      | 8.8 a<br>B      |
| RIZ 30      | 1.0 a<br>C                            | 1.0 b<br>C      | 1.0 c<br>C      | 2.8 cd<br>B     | 6.6 b<br>A      | 1.0 a<br>D                        | 1.2 b<br>C      | 8.7 a<br>B      | 8.8 a<br>B      | 9.0 a<br>B      |
| Ecuador 605 | 1.0 a<br>C                            | 1.1 b<br>C      | 2.3 bc<br>BC    | 3.3 c<br>B      | 8.9 a<br>A      | 1.0 a<br>D                        | 1.2 b<br>D      | 3.1 b<br>C      | 8.7 a<br>B      | 8.9 a<br>B      |
| U. I. 114   | 1.0 a<br>C                            | 3.0 a<br>B      | 8.3 a<br>A      | 9.0 a<br>A      | 9.0 a<br>A      | 1.0 a<br>D                        | 5.6 a<br>C      | 9.0 a<br>B      | 9.0 a<br>B      | 9.0 a<br>B      |

<sup>x</sup> Mean severity of rating recorded 21 days after inoculation. CIAT severity scale: 1 (no external symptoms) to 9 (plant 100% diseased). A total of 20 plants per cultivar were inoculated with each inoculum concentration during each test; ... indicates cultivar not inoculated.

<sup>y</sup> Means with the same lowercase letter (a-d) within each column of each test are not significantly different at  $P \leq 0.05$  (Duncan's multiple range test).

<sup>z</sup> Means with the same uppercase letter (A-D) within each row of each test are not significantly different at  $P \leq 0.05$  (Duncan's multiple range test).

**Table 3.** Regression equations and coefficients for *Fusarium* wilt severity of eight cultivars of *Phaseolus vulgaris* 21 days after inoculation with five levels of conidial densities of a strain of *Fusarium oxysporum* f. sp. *phaseoli* (FOP-CO1) from Colorado

| Cultivar       | Regression equation                     | Regression coefficient |
|----------------|---|------------------------|
| BAT 447        | Y1 = 1 + 1.1239 × log <sub>10</sub> (x) | 0.8855                 |
| HF 465-63-1    | Y2 = 1 + 0.2030 × log <sub>10</sub> (x) | 0.8696                 |
| Diacol Calima  | Y3 = 1 + 0.8245 × log <sub>10</sub> (x) | 0.7684                 |
| Pinto U.I. 114 | Y4 = 1 + 1.4940 × log <sub>10</sub> (x) | 0.9720                 |
| TIB 3042       | Y5 = 1 + 1.1669 × log <sub>10</sub> (x) | 0.8818                 |
| IPA 1          | Y6 = 1 + 1.0095 × log <sub>10</sub> (x) | 0.9302                 |
| RIZ 30         | Y7 = 1 + 0.9320 × log <sub>10</sub> (x) | 0.8964                 |
| Ecuador 605    | Y8 = 1 + 0.9251 × log <sub>10</sub> (x) | 0.8175                 |

cultivars A 211, BAT 477, and ICA 032. The cultivar HF 465-63-1 was resistant, and pinto U.I. 114 was susceptible to both strains. Diacol Calima had an intermediate and susceptible disease reaction to FOP-SC and FOP-CO1, respectively. In the second race experiment, cultivars Mortino, Ecuador 605, TIB 3042, and Diacol Calima had susceptible reactions to both FOP strains. However, there was an interaction for mean severity between cultivars Ecuador 605 and Diacol Calima with both strains. HF 465-63-1 was resistant to both strains, and clear qualitative disease reactions were observed in the remaining cultivars. Cultivars BAT 477, RIZ 30, IPA 1, and pinto U.I. 114 were resistant to FOP-CL25 and susceptible to FOP-CO1 (Table 1).

Infected vascular tissue of susceptible entries had a characteristic dark brown discoloration. Resistant cultivars U.I. 114, BAT 477, RIZ 30, and IPA 1 had a disease-severity rating of 1 to 2 to FOP-CL25, with no internal discoloration. Internal discoloration of the stem was observed with HF 465-63-1 inoculated with each strain even in plants expressing

no external wilt symptoms. FOP-CL25 produced an external pink mass of conidia on stems of dead plants.

**Inoculum-density experiment.** This experiment was conducted to determine if disease reactions in the CIAT and U.I. 114 cultivars were affected by inoculum densities. The expected disease-rating classes were not changed at the level of 10<sup>6</sup> conidia per milliliter for either test (Table 2). All cultivars except HF 465-63-1 were susceptible to FOP-CO1 at this high concentration. In the first test, at 10<sup>5</sup> conidia per milliliter, a change ( $P \leq 0.05$ ) in ranking order from expected susceptible-to-intermediate reactions was observed for cultivars Diacol Calima, IPA 1, Mortino, and Ecuador 605; a change from susceptible to resistant was observed for cultivar RIZ 30. Differences observed between both tests at 10<sup>5</sup> conidia per milliliter are assumed to be caused by a strong environment-by-cultivar-by-inoculum interaction.

Swanson and Van Gundy (27) reported that under conducive disease-testing conditions, cowpea lines with polygenes for resistance to *Fusarium* wilt

reacted as susceptible, and only lines with major genes for resistance were detected. At 10<sup>4</sup> conidia per milliliter, a highly variable response was observed in all cultivars except HF 465-63-1 and U.I. 114, which were highly resistant and susceptible, respectively (Table 2). Mean severity differences ( $P \leq 0.05$ ) among inoculum densities within cultivars were observed in both tests and for all cultivars analyzed, which confirms the hypothesis mentioned above of environment-by-inoculum-by-cultivar interactions.

There was a linear increase in disease severity with increased inoculum concentration for all cultivars by logarithmic model [ $y = a + b \times \log_{10}(x)$ ]. Wilt severity increased linearly ( $P \leq 0.05$ ) with increased inoculum concentration for all cultivars (Table 3). The coefficients of regression ( $R^2$ ) among cultivars ranged from 0.76 to 0.97, with the lowest value for Diacol Calima and the highest for U.I. 114. The slope (b) of the linear regression of resistant cultivar HF 465-63-1 differed ( $P \leq 0.05$ ) from the other cultivars; however, no differences ( $P \leq 0.05$ ) were observed among the slopes of the other cultivars (Wilk Lambda statistical test).

## DISCUSSION

Characterization of strains of *F. oxysporum* as formae speciales and races has been based on the host-pathogen interaction (9). Parlevliet and Zadoks (22) defined a race as a pathogen characterized by specific virulence genes identified by a specific set of host cultivars. However, this subdivision has not been recognized by the International Code of Botanical Nomenclature (30). Based on host-pathogen interaction, VanderPlank

(28) proposed a definition for virulent races as strains that interact differentially with host cultivars, and for aggressive races as strains that vary in aggressiveness but act independently of host cultivars, or in other words, when a non-significant strain-by-cultivar interaction is observed. The term aggressive race was questioned by Caten (8) as meaningless because it suggests that a host-pathogen interaction is under polygenic control with a continuous phenotypic variation and concluded that "the existence of discrete classes is central to any concept of race."

We followed the criteria of using discrete qualitative classes such as susceptible and resistant (plus an intermediate class) for race classification as suggested by Caten (8) and reported for Fusarium wilt in beans (24) and in other leguminosae crops such as peas (5) and chickpeas (17). This criterion of classification is used to rank genes with major effects, and cultivar ranking is dependent on the strain used (22,28). The VanderPlank criteria of cultivar-by-race interactions for definition of virulent races can be questioned, at least with the bean cultivars and FOP strains used in this study.

In the first race experiment (Table 1), differential interactions ( $P \leq 0.05$ ) between the cultivar Diacol Calima and both FOP strains from the United States suggested that these strains qualified as different physiological (virulent) races sensu VanderPlank. Although there was a significant cultivar-by-strain interaction, the lack of a clear resistant/susceptible reaction suggests that there is polygenic control for reaction in the cultivar Diacol Calima controlling this difference (aggressiveness) between both strains. In the second race experiment, the significant cultivar-by-strain interaction within susceptible cultivars for both FOP strains clearly showed the limitations of race analysis by differential interaction. Analysis of simple effects revealed that both U.S. and Colombian FOP strains interacted significantly, not only within cultivars such as BAT 477, which showed a clear differential resistant/susceptible response to both strains, but also within cultivars such as Diacol Calima, which was susceptible to both FOP strains. Demmink et al (12) reported for Fusarium wilt in carnation that a significant interaction can "result from independent variation of two or more distinct resistance/virulence components in the host/parasite interaction." This cultivar-by-strain interaction has been related in other pathosystems with nonspecific resistance (horizontal, sensu VanderPlank) controlled by polygenes (22).

Because of the small number of host differentials used, we do not have conclusive evidence of FOP virulence differences between FOP-SC and FOP-CO1. However, the cultivars U.I. 114,

BAT 477, RIZ 30, and IPA 1 clearly differentiate FOP-CL25 and FOP-CO1 as distinct physiological races by disease reactions. FOP-CO1 has a broader virulence spectrum than FOP-CL25 on this set of differential cultivars selected at CIAT with Latin American collections of FOP. Of nine cultivars tested, all but HF 465-63-1 were susceptible to FOP-CO1, compared with only four cultivars susceptible to FOP-CL25. Differences among cultivars to FOP-CL25 revealed two virulence pathotypes. This clear phenotypic response was not observed with FOP-CO1, where three groups were detected. One group contained the resistant cultivar HF 465-63-1, and another group included the cultivar RIZ 30, which was susceptible but different from a third group that included U.I. 114, Mortino, BAT 477, and TIB 3042.

We selected the CIAT Bean Fusarium Wilt Nursery to evaluate the diversity of FOP races. The number of resistance genes in the differential cultivars BAT 477, U.I. 114, IPA 1, and RIZ 30 are unknown. However, evidence of different genes for resistance (assuming each cultivar has a major gene) was observed for the cultivars Mortino, Ecuador 605, and Diacol Calima, which showed a resistant response to a Brazilian race of FOP (23) and susceptible responses to FOP-CL25 and FOP-CO1 races. On the contrary, FOP-CL25 can be differentiated from FOP-CO1 and Brazilian races by resistant reactions of RIZ 30 and IPA 1. Further research is required to characterize the number and identity of resistance genes in these cultivars, and to develop a universally acceptable set of differential cultivars for race identification and nomenclature. The experiment can be conducted through a series of partial diallelic crosses (resistant by resistant and resistant by susceptible) (14), and separate inoculations of  $F_2$  populations with the different races described above and others that could be obtained from cooperators. To monitor local FOP pathogenic variability (11), we also recommend greenhouse and/or field evaluations with a set of commercial cultivars (CO 33142, CO 59196, and Jamapa) with known major genes for resistance to the Colorado race (25,26).

Ribeiro and Hagedorn (24) reported the effect of temperature on bean cultivar by FOP inoculum density on modifying disease reaction classes. The bean cultivar Tenderette developed susceptible wilt symptoms with  $10^6$  conidia per milliliter, but a resistant response was observed at  $10^4$  conidia per milliliter at 20°C. No wilt symptoms were observed at any inoculum level under incubation temperatures of 24 and 28°C. In Fusarium wilt of chickpea, it was reported that at incubation temperatures of 25 and 30°C and different inoculum densities, temperature accounted for most of the wilt variability (7). The importance of a standard

inoculum concentration was illustrated by the variable disease reactions of CIAT bean cultivars exposed to different levels of inoculum of the Colorado race. The variation of disease reaction of cultivars to different inoculum densities can lead to a false classification of race. The variability in disease rating classes observed with inoculum densities lower than  $10^6$  conidia per milliliter may be related to disease escape and/or a polygenic complex for resistance. The disease reaction at the highest inoculum concentration ( $10^6$  conidia per milliliter) masks this interaction. Failure to reproduce similar wilt severity in the repeated test also suggested that environmental effects contributed to a cultivar-by-inoculum concentration interaction.

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