# Differential Sensitivity of Fungi to Pisatin and to Phaseollin

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ABSTRACT

Forty-six fungal isolates representing 13 species were tested for their tolerance to pisatin, and 33 *Fusarium* isolates representing three species were tested for their tolerance to phaseollin. In general, pea pathogens were more tolerant of pisatin than were nonpathogens of pea and bean pathogens were more tolerant of phaseollin than nonpathogens of bean. There were, however, several notable exceptions. *Aphanomyces euteiches*, a pathogen of pea, was markedly inhibited by pisatin, whereas several nonpathogens of pea were highly tolerant of pisatin. In addition, several nonpathogens of bean were highly tolerant of phaseollin. The antifungal properties of both pisatin and phaseollin also depended on the bioassay conditions.

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The antifungal properties of the phytoalexins pisatin (from pea) and phaseollin (from bean) have been investigated for a large number of fungi either pathogenic or nonpathogenic to pea and bean. Of 50 fungal isolates assayed against pisatin, 45 were strongly inhibited; 44 of these were nonpathogens of pea and one was a pea pathogen (2). The five fungi that were tolerant of pisatin were all pathogens of pea. Of 27 fungal isolates assayed against phaseollin, all but five were markedly inhibited; these latter five were bean pathogens (7). No nonpathogen of pea or bean was tolerant of pisatin or phaseollin. These results implied that the sensitivity of fungi to these phytoalexins was related to their pathogenicity.

The striking differences in sensitivity to pisatin and phaseollin associated with so many fungi suggested that these compounds might be useful in the development of selective media for the isolation and/or enumeration of fungal pathogens of pea and bean. I found, however, that pisatin and phaseollin lacked sufficient antifungal activity to be used as the bases for selective media, that their antifungal activities were markedly affected by the bioassay conditions, and that there are additional exceptions to the previously observed patterns of sensitivity of pathogens and nonpathogens to these compounds. Although these findings precluded the use of pisatin and phaseollin for the selective isolation or enumeration of fungal pathogens of pea or bean, some of the results obtained are pertinent to the type of evidence that has been used to imply that phytoalexins are involved in disease resistance. This paper reports information from our selective medium studies which bears on this point.

MATERIALS AND METHODS.—*Cultures.*—*Ascochyta pisi* Lib., *A. pinodella* L. K. Jones, and *Mycosphaerella pinodes* (Berk. & Blox.) Vest. were provided by V. R. Wallen, Cell Biology Research Institute, Central Experimental Farm, Ottawa, Ontario, Canada. *Aphanomyces euteiches* Drechs. was obtained from R. D. Lumsden, ARS-USDA, Plant Industry Station, Beltsville, Maryland. *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyder & Hansen isolates no. 84, 85, and O.I., and *F. oxysporum* Schl. f. sp. *pisi* (van Hall) were supplied by W. T. Schroeder, N.Y. State Agricultural Experiment Station, Geneva, N.Y. *F. solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyder & Hansen isolates SRF, R-10, FB, and O.I.; *F. oxysporum* Schl. f. sp. *cepae* (Hanz.) Snyder & Hansen, *F. roseum* (Lk.) Snyder & Hansen 'Avenaceum'; *Neurospora crassa* Shear & Dodge; *Helminthosporium turcicum* Pass.; *Phytophthora infestans* (Mont.) de Bary; *Rhizopus stolonifer* (Ehrenberg) Lind.; and *Thielaviopsis basicola* (Berk. & Br.) Ferr. were obtained from various investigators at Cornell University, Ithaca, N.Y. *F. solani* isolates no. 1 to 10 were obtained from W. C. Snyder, University of California, Berkeley, whereas the F series isolates of *F. solani*, the *Penicillium* sp. and the *Trichoderma* sp. were isolated on Kerr's selective medium (11) from soil with a history of pea root-rot problems.

*P. infestans* and *H. turcicum* were maintained on V-8 agar medium [200 ml V-8 juice (Campbell Soup Co.), 3 g $CaCO_3$, 20 g agar, 800 ml $H_2O$]; all other isolates were grown on potato-dextrose agar (PDA).

*Bioassays.*—Petri plates (9.0-cm diam) that contained PDA or V-8 agar were seeded with either a small amount of mycelium or a uniformly distributed spore suspension of a given fungus. The cultures were incubated for 2-3 days at 24 ± 2 C in the dark and then 4.0-mm diam mycelial plugs, cut with a cork borer either from the advancing margins of the fungal colonies or from plates containing germinated spores, were removed from the plates. The plugs were placed on the surface of Martin's peptone-glucose agar (PGA) medium (12) [10 g glucose, 5 g peptone, 1 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 22 g agar and 1,000 ml $H_2O$] contained in 35 × 10 mm plastic petri plates (no. 1008, Falcon Plastics, Oxnard, Calif.). Dimethylsulfoxide (DMSO) solutions of phaseollin and pisatin were added to the plates prior to the addition of 1.0 ml of molten PGA medium. Plates that did not receive phytoalexins served as controls. Both test and control plates contained DMSO at a final concentration of 2%. Pisatin was tested at 100 μg/ml and phaseollin at 50 μg/ml because these concentrations approached, but did not exceed, the limits of solubility of the compounds in 2% DMSO.

Plates were kept in the dark at 24 ± 2 C until net radial growth was 25 ± 4 mm. Radial growth was determined by measuring two diameters for each of two replicate colonies and substracting the diameter of the inoculum plug. All bioassays were repeated at least once. Variation rarely exceeded 15% between experiments and usually was ca. 5%.

*Pathogenicity.*—*Fusarium* isolates (F series) were tested for pathogenicity by growing peas, *Pisum sativum* L. 'Progress No. 9' and beans, *Phaseolus vulgaris* L. 'Red Kidney' in steamed soil contained in 102-mm (4-inch) diam clay pots in a 28 ± 5 C greenhouse. Approximately 15 seeds were planted in each pot and one week later the seedlings were inoculated with spore suspensions prepared from 1-week-old cultures of *Fusarium* which had been grown at 26 ± 2 C in diffuse daylight on PDA contained in petri plates (9.0-cm diam). Spores from one petri plate culture were used to inoculate one pot of seedlings. Both noninoculated plants, and plants inoculated with an authentic isolate of *F. solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli* were included as controls. Disease symptoms were evaluated 4 weeks after inoculation.

*Phytoalexins.*—Phaseollin was prepared as previously described (15). Pisatin was isolated from pea stems infected with *Rhizoctonia solani* Kuehn by a modification of the procedures employed by Cruickshank & Perrin (4) and Hadwiger et al. (9). Infected peas were obtained by the same procedure used to obtain *R. solani*-infected bean hypocotyls (16). Pea stems bearing 1- to 2-week-old *R. solani* lesions were ground in 95% ethanol (1:4, w:v) for 1-2 min in a Waring Blendor. The suspension was filtered through several layers of cheesecloth and Whatman No. 1 filter paper. Sodium phosphate buffer (0.01 M, pH 7.0) was added to the ethanol extract (1:4, v:v) and the ethanol removed by evaporation at ca. 40 C under reduced pressure. The buffered solution was partitioned first with four volumes and then with one volume of petroleum ether (85% hexane). The petroleum ether fractions were combinde and evaporated to dryness under reduced pressure at 40 C. The residue was dissolved in a small amount of ethanol and chromatographed on preparative silica gel plates (2.0-mm thick and with fluorescence indicator; Brinkmann Instruments, Westbury, N.Y.) in hexane-ethyl acetate-methanol (60:40:1, v:v:v) (10). Material with $R_f$ of approximately 0.33 (dark-quenching area under short wave ultraviolet light) was eluted in ethanol or ethyl acetate and filtered through glass fiber paper (Reeve Angel, Clifton, N.J.) to remove the silica gel. The compound isolated had an ultraviolet absorption spectrum identical to that reported for pisatin (λ max at 309, 286, and 280 nm) (13), and had the same $R_f$ value (0.11) as an authentic sample of pisatin on analytical silica gel plates (9) when irrigated in chloroform. The identity of pisatin was confirmed by mass spectrometric analysis.

Phaseollin and pisatin were quantified by using their reported molar extinction coefficients in ethanol [log ε = 3.96 at 279 nm for phaseollin (5), and log ε = 3.86 at 309 nm for pisatin (4)].

RESULTS.—*Effect of pisatin or phaseollin on radial mycelial growth of fungi.*—The sensitivity of 13 fungal species to pisatin were evaluated using PGA medium supplemented with 100 μg of pisatin/ml. In general, pathogens of pea were more tolerant to pisatin than were nonpathogens of pea (Table 1). The four fungal species that were very tolerant of pisatin

TABLE 1. Response of fungal pathogens and nonpathogens of peas to pisatin[a]

| Fungus | Culture[b] period (days) | Growth (mm)[c] Control | Growth (mm)[c] Pisatin (100 μg/ml) | Inhibition (%) |
|---|---|---|---|---|
| *Fusarium solani* f. sp. *pisi** | 4 | 23.8 | 21.7 | 9 |
| *Ascochyta pisi** | 11 | 24.3 | 23.6 | 3 |
| *Ascochyta pinodella** | 3 | 23.9 | 23.0 | 4 |
| *Mycosphaerella pinodes** | 5 | 22.0 | 20.4 | 7 |
| *Aphanomyces euteiches** | 2 | 23.5 | 1.8 | 92 |
| *Fusarium solani* f. sp. *phaseoli* | 6 | 24.4 | 8.0 | 67 |
| *Penicillium* sp. | 5 | 27.0 | 15.5 | 43 |
| *Trichoderma* sp. | 2 | 24.8 | 9.3 | 63 |
| *Fusarium solani* f. sp. *cucurbitae* | 4 | 24.0 | 0 | 100 |
| *Neurospora crassa* | 1 | 25.3 | 3.8 | 85 |
| *Helminthosporium turcicum* | 4 | 25.3 | 13.0 | 49 |
| *Phytophthora infestans*[d] | 4 | 22.3 | 1.5 | 93 |
| *Rhizopus stolonifer* | 1 | 28.0 | 12.9 | 54 |

[a] Fungi were bioassayed on 1.0 ml of media in 35-mm diam plastic petri plates. Unless stated otherwise, the medium contained 10 g glucose, 5 g peptone, 22 g agar, 1 g $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 20 g agar, and 1,000 ml $H_2O$. Pisatin was dissolved in dimethylsulfoxide (DMSO) and added to the medium to give final concentrations of 100 μg pisatin/ml and 2% DMSO. DMSO (2%) without pisatin was added to the control medium.

[b] Measurements were taken when net radial growth in controls was 25 ± 4 mm.

[c] Growth was calculated by measuring two diameters for each of two replicate colonies and subtracting the diameter of the mycelial plug (4 mm) used to inoculate the plates.

[d] Grown on V-8 agar medium.

* Pea pathogens.

TABLE 2. Response of *Fusarium* isolates to phaseollin and pisatin[a]

| *Fusarium* isolate | Culture period (days) | Growth (mm) Control | Growth (mm) Pisatin (100 μg/ml) | Growth (mm) Phaseollin (50 μg/ml) | (%) Inhibition Pisatin | (%) Inhibition Phaseollin |
|---|---|---|---|---|---|---|
| *F. solani* f. sp. *pisi* no. 84 | 4 | 25.5 | 26.0 | 22.0 | −2 | 14 |
| *F. solani* f. sp. *pisi* no. 85 | 4 | 28.5 | 27.0 | 24.0 | 5 | 16 |
| *F. solani* f. sp. *pisi* O.I. | 4 | 26.5 | 23.8 | 20.0 | 10 | 25 |
| *F. solani* f. sp. *phaseoli* SRF | 5 | 22.0 | 9.0 | 19.5 | 59 | 11 |
| *F. solani* f. sp. *phaseoli* R-10 | 5 | 21.5 | 8.5 | 17.0 | 60 | 21 |
| *F. solani* f. sp. *phaseoli* FB | 5 | 22.5 | 10.5 | 21.0 | 53 | 7 |
| *F. solani* f. sp. *phaseoli* O.I. | 6 | 24.0 | 12.0 | 23.8 | 50 | 1 |
| *F. oxysporum* f. sp. *cepae* | 3 | 26.0 | 12.0 | 7.0 | 54 | 73 |
| *F. oxysporum* f. sp. *pisi* no. 1061 | 3 | 24.5 | 12.0 | 9.0 | 51 | 63 |
| *F. roseum* 'Avenaceum' | 3 | 21.5 | 15.0 | 14.0 | 30 | 35 |
| Summary | | | | | | |
| *F. solani* f. sp. *pisi* | | | | | ⩽10 | ⩽25 |
| *F. solani* f. sp. *phaseoli* | | | | | ⩾50 | ⩽21 |

[a] Fungi were bioassayed as described in Table 1 except that a phaseollin treatment was added. Phaseollin was dissolved in dimethylsulfoxide (DMSO) and added to the medium to give final concentration of 50 μg phaseollin/ml and 2% DMSO.

were pea pathogens. However, one pea pathogen, *Aphanomyces euteiches*, was strongly inhibited.

Various *Fusarium* species were tested for their sensitivity to pisatin and to phaseollin (Table 2). *F. solani* f. sp. *phaseoli*, a bean pathogen, was only slightly inhibited by phaseollin, as was expected. On the other hand, of two pea pathogens tested, *F. solani* f. sp. *pisi* was tolerant of both pisatin and phaseollin whereas *F. oxysporium* f. sp. *pisi* was as sensitive to pisatin as many of the nonpathogens of pea. Despite the lack of an absolute correlation between the pathogenicity of the isolates and their response to pisatin and phaseollin, a characteristic pattern of response of the *F. solani* f. sp. *pisi* and *F. solani* f. sp. *phaseoli* isolates to phaseollin and pisatin was apparent. *F. solani* f. sp. *pisi* isolates were inhibited ⩽ 10% by pisatin and ⩽25% by phaseollin. *F. solani* f. sp. *phaseoli* isolates were inhibited ⩾ 50% by pisatin and ⩽ 21% by phaseollin. None of the other *Fusarium* isolates showed this pattern (Table 2).

*F. solani* f. sp. *pisi* and *F. solani* f. sp. *phaseoli* cannot be distinguished from each other, or from other *F. solani* isolates, on the basis of their conidial morphology. Rather, identification is established on

the basis of their pathogenicity to bean or to pea. It was of interest, therefore, to determine whether the characteristic pattern of response of *F. solani* isolates to pisatin and phaseollin could be utilized to distinguish formae speciales *pisi* and *phaseoli*. Ten coded isolates of *F. solani* were obtained from W. C. Snyder, University of Calif., Berkeley, and tested in the standard bioassay. To allow for some variation among isolates, the inhibition range was arbitrarily increased by 5% (the normal variation in our assay). Thus, any isolate that sustained $\leqslant$ 15% inhibition by pisatin and $\leqslant$ 30% inhibition by phaseollin was designated as *F. solani* f. sp. *pisi*; any isolate that was inhibited $\geqslant$ 45% on pisatin and $\leqslant$ 26% on phaseollin was designated as *F. solani* f. sp. *phaseoli*. On the basis of inhibition patterns, isolates 2 and 5 were identified as *F. solani* f. sp. *pisi*, and isolates 1 and 7 as *F. solani* f. sp. *phaseoli* (Table 3). Isolates 3, 4, 6, 8, 9, and 10 could not be assigned to either group. W. C. Snyder (*personal communication*) confirmed these findings and revealed that isolates 4, 8, and 10 were *F. solani* f. sp. *cucurbitae* and isolates 3, 6, and 9 were soil saprophytes.

Since identification of authentic *F. solani* f. sp. *pisi* and *F. solani* f. sp. *phaseoli*, was accomplished, the possibility of utilizing the procedure to identify soil-borne isolates of *F. solani* was tested. Over 50 *Fusarium solani* isolates were collected from soil which had a history of pea root-rot problems and 13 were selected and bioassayed against phaseollin and pisatin (Table 4). All 13 were tested for their pathogenicity on pea and five were tested for their pathogenicity on bean. The isolates were not classified as pathogenic unless they produced large lesions ($\geqslant$ 1 cm in diam) similar to those produced by the authentic isolate of *F. solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli*. Two of the 13 isolates (F-1, F-28) were pathogenic on pea and were highly to

TABLE 3. Response of coded *Fusarium solani* isolates to phaseollin and to pisatin[a]

| Isolate code no. | Culture period (days) | Growth (mm) | | | (%) Inhibition | |
|---|---|---|---|---|---|---|
| | | Control | Pisatin (100 μg/ml) | Phaseollin (50 μg/ml) | Pisatin | Phaseollin |
| 1 | 14 | 21.5 | 11.5 | 18.5 | 47 | 14 |
| 2 | 3 | 25.0 | 22.0 | 18.0 | 12 | 28 |
| 3 | 14 | 22.0 | 14.0 | 15.0 | 36 | 32 |
| 4 | 3 | 25.0 | 0.0 | 2.0 | 100 | 92 |
| 5 | 3 | 25.0 | 22.5 | 21.0 | 10 | 16 |
| 6 | 3 | 23.5 | 14.0 | 5.0 | 40 | 79 |
| 7 | 14 | 24.0 | 10.5 | 19.5 | 56 | 19 |
| 8 | 3 | 26.0 | 0.0 | 0.0 | 100 | 100 |
| 9 | 4 | 25.0 | 20.0 | 8.5 | 20 | 66 |
| 10 | 3 | 22.5 | 3.0 | 1.5 | 87 | 93 |

[a] Fungi were bioassayed as described in Table 2.

TABLE 4. Response of unknown *Fusarium* isolates to phaseollin and to pisatin and their pathogenicity to pea and bean

| *Fusarium* isolate | Culture period (days) | Growth (mm)[a] | | | Inhibition (%) | | Pathogenicity[b] | |
|---|---|---|---|---|---|---|---|---|
| | | Control | Pisatin (100 μg/ml) | Phaseollin (50 μg/ml) | Pisatin | Phaseollin | On pea | On bean |
| F-1 | 3 | 26.0 | 25.0 | 20.3 | 4 | 22 | + | – |
| F-2 | 2 | 26.8 | 25.8 | 21.5 | 4 | 20 | F | M.F. |
| F-3 | 4 | 23.5 | 16.8 | 15.0 | 29 | 36 | – | N.T. |
| F-4 | 4 | 24.5 | 17.0 | 14.8 | 31 | 40 | – | N.T. |
| F-6 | 4 | 24.0 | 17.8 | 14.5 | 26 | 40 | – | N.T. |
| F-7 | 4 | 23.0 | 19.0 | 14.8 | 17 | 36 | – | N.T. |
| F-13 | 3 | 24.5 | 21.5 | 23.5 | 12 | 4 | – | M.F. |
| F-24 | 2 | 25.0 | 18.7 | 17.0 | 25 | 32 | – | N.T. |
| F-26 | 3 | 24.5 | 21.0 | 21.5 | 14 | 12 | – | – |
| F-28 | 3 | 24.0 | 22.5 | 21.0 | 6 | 13 | + | M.F. |
| F-29 | 3 | 27.0 | 21.0 | 20.0 | 22 | 26 | – | N.T. |
| F-34 | 4 | 27.8 | 21.0 | 18.0 | 24 | 35 | – | N.T. |
| F-45 | 3 | 25.5 | 10.8 | 12.0 | 58 | 53 | – | N.T. |

[a] Fungi were bioassayed as described in Table 2.

[b] (–) No symptoms on pea stems or bean hypocotyls, (+) large lesions on pea stem or bean hypocotyls (stem or hypocotyls girded by a lesion 1 to 2 cm in length) like those produced by an authentic isolate of *F. solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli*, (F) small flecks (ca. 1 mm) on pea stems, (M.F.) minute flecks (<1 mm) on bean hypocotyls, (N.T.) isolates were not tested on bean. Isolates rated –, F, or M.F. were not considered to be pathogenic. None of these isolates produced symptoms on peas characteristic of those produced by *F. oxysporum* f. sp. *pisi*.

TABLE 5. Response of fungi on peptone-glucose medium supplemented with pisatin and rose bengal[a]

| Fungus | Culture period (days) | Growth (mm) | | Inhibition (%) |
|---|---|---|---|---|
| | | Control | Pisatin (100 μg/ml) | |
| *Helminthosporium turcicum* | 6 | 23.0 | 21.8 | 5 |
| *Trichoderma* sp. | 3 | 22.8 | 22.5 | 1 |
| *Penicillium* sp. | 6 | 24.3 | 24.0 | 1 |
| *Fusarium solani* f. sp. *pisi* | 7 | 21.8 | 20.2 | 7 |

[a] Fungi were bioassayed as in Table 1 except pisatin and control medium contained in addition 33 μg of rose bengal/ml.

moderately tolerant of pisatin and phaseollin (Table 4) and agreed with the pre-established response pattern for *F. solani* f. sp. *pisi* (i.e., they were inhibited $\leqslant$ 15% by pisatin and $\leqslant$30% by phaseollin). However, since several nonpathogenic isolates (F-2, F-13, F-26) exhibited the same response, the inhibition pattern did not permit specific identification of *F. solani* f. sp. *pisi*. Of particular interest was the finding that one isolate (F-26) was very tolerant of phaseollin and pisatin yet produced no symptoms on either bean or pea.

*Effect of bioassay conditions on response of fungi to pisatin and to phaseollin.*—The above assays involved measurements of radial growth on a medium (PGA) which supported rapid growth of fungi. If rose bengal, a compound which reduces the growth rate of fungi (12), was added to the PGA medium (33 mg rose bengal/liter) the pattern of response of fungi to pisatin was changed (Table 5). On PGA-rose bengal medium, *F. solani* f. sp. *pisi* appeared no more tolerant of pisatin than were several nonpathogens of pea (compare Table 1 & 5).

It was also observed that spores of *F. solani* f. sp. *phaseoli* bioassayed under different conditions varied in their response to phaseollin. Spores incorporated into PGA or PDA supplemented with 50 μg/ml of phaseollin produced few, if any, colonies. In a typical experiment where about 25 spores were incorporated into 1.5 ml of PGA medium, 27 colonies developed in the controls while only one developed in the phaseollin-supplemented medium. The number of spores that produced colonies was low regardless of whether new (1- to 2-week-old) or old (1- to 2-month-old) spores were used, or whether the relative number of macro- and microconidia added to the medium were varied. When liquid medium (PGA medium without agar) containing phaseollin (50 μg/ml) was used, the rate of spore germination was slowed, but about 90-100% of the spores did germinate and produced visible colonies. Low recovery of *F. solani* f. sp. *phaseoli* when spores are incorporated into solid medium containing phaseollin apparently reflects some intrinsic property of this type of bioassay for this pathogen, since there was essentially no repression of recovery of either the bean pathogen *Thielaviopsis basicola*, when assayed in the same manner, or of *F. solani* f. sp. *pisi* assayed similarly against 100 μg of pisatin/ml.

It has been reported (4) that some of the pterocarpanoid phytoalexins are adsorbed by plastic. However, when phaseollin or pisatin was added to liquid medium in plastic petri plates and the liquid medium assayed 3 days later, no loss of phytoalexin from the medium could be detected.

DISCUSSION.—Assays of 46 fungal isolates, representing 13 species, for their tolerance to pisatin and 33 *Fusarium* isolates for their tolerance to phaseollin indicated, as had been previously reported (2, 7), a general pattern of greater tolerance of pathogens than nonpathogens to the plants' phytoalexins; yet, there were notable exceptions. The pea pathogen, *A. euteiches*, was markedly inhibited by 100 μg of pisatin/ml and *F. oxysporum* f. sp. *pisi* was inhibited by pisatin as much as were most nonpathogens of pea. Furthermore, several of the *Fusarium* spp. isolated from the soil and determined to be not pathogenic to pea or bean were very tolerant of pisatin and/or phaseollin. It has previously been shown that the bean pathogen *Rhizoctonia solani*, is very sensitive to phaseollin (14). There exist, therefore, pathogens of bean and pea that are sensitive to phaseollin and pisatin, respectively, and there exist nonpathogens of both pea and bean that are insensitive to these phytoalexins. Obviously, the relative sensitivity to a phytoalexin as determined by mycelial growth in an in vitro assay cannot be the sole criterion used to evaluate an organism's pathogenic potential.

Cruickshank (2) found that the pea pathogen, *Septoria pisi* Westenel, was (like *A. euteiches*) very sensitive to 100 μg of pisatin/ml. In subsequent studies (6) he determined the ability of *S. pisi* and of 17 other fungi to induce an accumulation of pisatin in drops of spore suspensions added to seed cavities of detached pea pods. The concentration of pisatin that accumulated ranged from 10 μg/ml to 116 μg/ml of "diffusate" solution. The lowest concentrations occurred when *S. pisi* was used as the inoculum. He concluded that when a pathogen that is sensitive to pisatin causes a susceptible reaction, the fungus presumably does not stimulate significant phytoalexin accumulation (3, 6). Since *A. euteiches* is very sensitive to pisatin, it would appear necessary for *A. eutiches* to repress the accumulation of pisatin in situ if the concept of pisatin as a resistant factor in peas is to be upheld.

The results obtained using different assay systems brings into question, however, the meaning of in vitro assays. The response of fungi to pisatin was quite

different depending on whether PGA medium or PGA medium supplemented with rose bengal was used. In addition, *F. solani* f. sp. *phaseoli* spores responded differently depending on whether they were bioassayed in liquid medium or in solid medium. It has previously been demonstrated that hyphal growth and spore germination are affected differently by phaseollin (1, 7). It also has been reported recently that fungi respond differently to wyerone acid (a phytoalexin from *Vicia faba* L.) in different media (8). Since these conditions are not the same as those in the host tissue, it is not possible to say what assay would best reflect the situation in situ. It is clear that some means other than comparison of in situ concentrations of phytoalexins and in vitro measurements of antifungal activity are needed to permit a more accurate assessment of the role of these compounds in disease resistance.

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