Induction of Pisatin Formation in the Pea Foot Region by Pathogenic and Nonpathogenic Clones of Fusarium solani

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ABSTRACT

The pisatin-inducing potential and the relative pisatin sensitivity of two pathogenic and two nonpathogenic clones of *Fusarium solani* were compared to evaluate the role of pisatin as a resistance component in the foot region of pea seedlings grown in infested soil. High quantities of pisatin accumulated more quickly in the foot region (basal stem, upper taproot and hypocotyl) of peas grown in nonsterile soil infested with clones of *Fusarium solani* f. sp. *phaseoli* than with clones of *F. solani* f. sp. *pisi*. However, after a slight delay, pathogenic clones were eventually responsible for the highest accumulation of pisatin/gram of tissue. The pattern of pisatin accumulation described above was partially obscured when *F. solani* clones were added to soil with a higher percent organic matter, since such soil promoted substantial increases in the pisatin in seedlings in the absence of the *F. solani* inoculum.

Percentage spore germination, rate of germ-tube growth, linear growth on agar and sporulation of all *F. solani* clones were retarded when grown in vitro at pisatin concentrations of 30 to 200 μg/ml and the pathogenic clones did not consistently excel as the most pisatin insensitive. However, pathogenic clones did consistently excel in degrading pisatin. The pisatin sensitivity of both pathogenic and nonpathogenic clones was associated with the potential of pisatin to inhibit protein synthesis in the fungus.

On the basis of dramatic increases in protein synthesis observed only in pea tissue inoculated with a nonpathogenic clone of *F. solani*, we suggest that pisatin production in pea seedlings may be coupled with additional host responses which cumulatively render the plant resistant.

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The phytoalexin, pisatin, may be the source of resistance in peas to clones of *Fusarium solani* (Mart.) Appel & Wr. other than those of *F. solani* f. sp. *pisi* (F. R. Jones) Snyd. & Hans. (6, 11). However, there are no reports of pisatin production in that portion of the plant normally attacked by this fungus, i.e., the foot region, defined by Cook and Flentje (1) as the "lower 1-cm of epicotyl, upper 1-cm of taproot, and hypocotyl". We studied the ability of the foot region of pea plants to produce and accumulate pisatin in the presence of clones of *F. solani* pathogenic and nonpathogenic to peas to more closely define the role of pisatin in root disease resistance. Each clone of the fungus was also used in correlated studies including in vitro effects of pisatin on fungal growth and biodegradation of pisatin.

MATERIALS AND METHODS.—*Induction of pisatin formation and pisatin assay*.—Pea seeds of the variety 'Alaska' were germinated 1-inch deep in either of two soil types collected from areas of no known pea cultivation. Soil No. 1 was a Palouse silt loam soil from the Washington State University campus, and soil No. 2 was a Ritzville silt loam soil from a long-established clean-tilled cherry orchard at the Washington State Dry Land Research Unit, Lind, Washington. Prior to planting, the potted, nonsterilized soils were infested with chlamydospores (about 3,000 spores/g air-dry soil) of one of four clones of *F. solani*. Two of the clones designated P-A and P-NZ were pathogenic to peas and were subcultures of SA-1 and NZ-1 of Cook et al. (2). Nonpathogenic clone NP-W was obtained from infected bean plants from Prosser, Washington. Nonpathogenic clone NP-I was isolated from an infected bean stem from central Idaho. Plants grown in noninfested, nonsterile soil served as controls. Plants were removed from the soil at intervals of 8 (just emerging), 9, 10, 12, 16, 21, and 28 days after planting. The foot region was cut into three equal sections each 6-mm long referred to as the upper, middle and lower sections of the foot region. The middle section included the region of cotyledon attachment. Analyses were based on 2 g (fresh wt) of tissue from each of the sections which were immediately weighed, frozen with liquid nitrogen and analyzed for pisatin content as described previously (8).

Effect of pisatin on germination of macroconidia, germ tube growth and subsequent sporulation.—Pisatin was dissolved in small (μl) volumes of 95% ethanol then stirred into warm 2% water agar (40 C) such that the concentration of phytoalexin in the agar was 100 or 200 μg/ml. Control plates had ethanol only added. A dilute suspension of freshly-formed or 2-month-old macroconidia of the four clones was atomized onto the agar surfaces. Plates were incubated in the dark at room temperature. Ten

randomly-selected spores from each plate were observed at 1 hr intervals for spore germination, germ tube growth and sporulation.

Effect of pisatin on fungal growth—linear growth and dry weight determinations.—Linear growth rate was determined on Vogel's medium (10) containing 0, 30, 50, 100, or 200 μg pisatin/ml on glass slides. Inoculum plugs 3-mm in diameter cut from the leading edge of rapidly growing cultures of the fungi were used to start the cultures. The glass slide cultures were placed on glass rod supports in plates lined with moist filter paper and incubated at room temperature in the dark. Radial measurements of growth were made daily with the aid of a dissecting microscope. In addition shake cultures of each of the four clones of *F. solani* were grown at room temperature in the dark in Vogel's medium containing 0, 10, or 30 μg pisatin/ml of medium. Ten macroconidia were added to 50 ml of the medium in 250-ml Erlenmeyer flasks to initiate growth. Culture mats were collected on filter paper at 12-hr intervals over 10 days then dried for 24 hr at 90 C and weighed.

In vitro degradation of pisatin.—Cultures of *Ascochyta pisi* Lib., *Monilinia fructicola* (Wint.) Honey and clones P-A and NP-W of *F. solani* were grown in 50 ml of Vogel's medium for 5 days. The contents were transferred to sterile 250-ml Erlenmeyer flasks containing 250 μg crystalline pisatin -^{14}C (∿sp act = 0.027 mc/mM). The cultures were shaken continuously in the dark at room temperature. The mycelium in each flask was recovered after 24 hr by filtration, frozen with liquid nitrogen, and then homogenized with glass beads in a mortar in cold 95% ethanol. Fungal remnants were removed by filtration, spread thinly in planchets, and counted for radioactivity. Both the ethanol extract of the fungal tissue and a petroleum ether extract of the medium were dried in vacuo and the pisatin content of each quantified (8). To evaluate the effects of staling products and pH on degradation of pisatin, rapidly growing cultures (dry weight 40-50 mg) of clones P-A and NP-W of *F. solani* were recovered by filtration, washed with sterile distilled water, scraped into 50 ml of fresh Vogel's medium containing 500 μg pisatin, and replaced on a rotary shaker in the dark at 22 C. The pH was maintained between 6.5 and 6.9 by addition of NaOH when necessary. At intervals through 36 hr the pisatin remaining in the medium was extracted, purified and quantified.

Rate of protein synthesis in pea pods inoculated with F. solani.—Immature pea pods (3 g per treatment) were split, placed with the endocarp up in petri plates, and evenly sprayed with a suspension of macroconidia (10^4 spores/ml) of clone P-A or NP-W of *F. solani*, or water only, and then kept in the dark at room temperature. After 9 hr they were rinsed to remove the fungi, blotted to remove any excess water and then pulse-treated with L-leucine-^{14}C for 1 hr (8). The noninoculated pods (water-sprayed) were simultaneously treated with L-leucine-^{3}H. At the end of the pulse label period, each inoculated treatment (^{14}C-labeled) and its noninoculated control (^{3}H-labeled) were combined. Buffer-soluble proteins were extracted and separated on a G-200 silica-gel column as described previously (8). The soluble proteins were eluted (2-ml fractions) with .01M Tris (pH 7.6) buffer. The elution of proteins from the column was followed by measuring the optical density at 280 nm. Aliquots of 0.7 ml were removed from each fraction, combined with 10 ml of scintillation fluid – composed of Triton-X; toluene: liquifluor (New England Nuclear), 5.0:9.25:0.75, and the radioactivity measured by scintillation spectrometry. To assess any isotope effect or counting artifacts in this dual label technique two additional lots of noninoculated pods were dual-labeled, and processed as described above.

Effect of pisatin on the rate of protein synthesis in Fusarium solani.—The rate of total protein synthesis in pisatin-treated and control cultures of *F. solani* was estimated on the basis of the incorporation of L-leucine-U-^{14}C (sp act = 254 mc/mmole) into the trichloroacetic acid insoluble components of mycelia and spores. Flasks containing 5 ml of Vogel's medium and 30 μg pisatin/ml were inoculated with 10^6 macroconidia/ml and incubated in shake culture in the dark at 24 C. Control flasks contained no pisatin. Cultures from each treatment were pulse-labeled with 0.5 μc L-leucine-^{14}C for 15 min at 2-or 4-hr intervals through 72 hr. Unlabeled L-leucine and 1 ml of 50% trichloroacetic acid were added to terminate the pulse-label period. The mycelium was washed extensively in 10% trichloroacetic acid

TABLE 1. Pisatin concentration in upper (U) and middle (M) sections of the foot region of pea plants grown in Lind silt loam soil[a] containing 3,000 macroconidia of *Fusarium solani* per g air-dry soil

Plant age (days)[b]	μg pisatin/g fresh weight of pea tissue					
	Control[c]		Clone P-A[c]		Clone NP-W[c]	
	U	M	U	M	U	M
8	Tr	Tr	Tr	Tr-10	Tr	Tr
9	Tr	Tr	Tr	12-16	Tr	Tr
10	Tr	Tr	Tr	18-27	Tr	45-53
12	Tr	40-45	13-24	47-53	Tr	160-183
14	Tr	16-29	192-246	204-273	Tr	22-35
16	6-13	Tr	105-222	117-150	12-15	40-63
28	...	...	166-286	327-411[d]	...	...

[a]Silt loam soil collected from an orchard at Lind, Washington.

[b]Plants were harvested at intervals and pisatin was then extracted from the foot region sections.

[c]'Control'–sampled plants were grown in nonsterile, noninoculated soil; 'Clone P-A' – sampled plants were grown in 'Control' soil infested with subculture SA-1 of *Fusarium solanum* f. *pisi* from R. J. Cook (2) which is pathogenic on pea; 'Clone NP-W'–sampled plants grown were grown in 'Control' soil infested with a *Fusarium* sp. isolated from bean plants from Prosser, Washington (nonpathogenic on pea).

[d]These 28-day-old plants were severely rotted in the foot region.

TABLE 2. Pisatin concentration in upper (U), middle (M), and lower (L) sections of the foot region of pea plants grown in Pullman silt loam containing 3,000 macroconidia of *Fusarium solani* per g air-dry soil

Plant age (days)[a]	μg pisatin/g fresh weight of pea tissue														
	Control[b]			Clone P-A[b]			Clone P-NZ[b]			Clone NP-W[b]			Clone NP-I[b]		
	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L
8	Tr	91-97	Tr	Tr-17	69-132	0	0-20	50-139	0	34-96	112-206	0	0-70	103-196	0
9	Tr-44	54-147	Tr	Tr-14	28-114	Tr	20-48	50-139	0	43-60	170-175	Tr	Tr-27	82-185	Tr
10	24-79	123-149	Tr-35	52-66	146-162	Tr-24	36-52	128-158	Tr	Tr-15	103-110	0	Tr-25	95-203	Tr
12	Tr-90	122-253	16-41	30-61	102-160	Tr	Tr-20	97-104	Tr	Tr-24	88-100	Tr	Tr-50	92-93	Tr
14	Tr-54	113-222	Tr	28-65	110-113	Tr	Tr-22	81-152	0	Tr-43	63-120	Tr	18-100	122-148	Tr-36
16	17-42	66-105	Tr	10-20	109-141	Tr	31-40	56-86	Tr	28-30	62-64	Tr	13-120	67-102	Tr
21	47-95	92-109	Tr	25-33	40-59	Tr	Tr-37	63-66	0	Tr-41	36-109	0	Tr-97	93-102	Tr
28	50-59	46-64	0	23-71	44-54	0	16-64	11-95	0	Tr-65	32-76	0	Tr-121	23-244	Tr-36

[a]Plants were harvested at intervals and pisatin was then extracted from the foot region sections.

[b]'Control' – sampled plants were grown in nonsterile soil; 'Clone P-A' – sampled plants were grown in 'Control' soil infested with subculture SA-1 of *Fusarium solani* f. *pisi* from R. J. Cook et al. (2) which is pathogenic on pea; 'Clone NP-Z' – sampled plants were grown in 'Control' soil infested with subculture NZ-1 of *Fusarium solani* f. *pisi* from R. J. Cook et al. (2) which is pathogenic on pea; 'Clone NP-W' – sampled plants grown in 'Control' soil infested with a *Fusarium* sp. isolated from bean plants from Prosser, Washington (nonpathogenic on pea); 'Clone NP-I' – sampled plants grown in 'Control' soil infested with a *Fusarium* sp. isolated from an infected bean stem in central Idaho (nonpathogenic on pea).

followed by washes (30-sec rinse) in chloroform: methanol (2:1, v/v) and 95% ethanol. The dry mycelial residue was combusted to $^{14}CO_2$ (8) which was trapped in ethanolamine and counted in a liquid scintillation spectrometer. The disk-gel electrophoretic techniques of Ornstein and Davis (7) were utilized to separate the soluble proteins of germinated spore of clone NP-W grown 6 hr on Vogel's medium with or without 30 μg/ml pisatin.

RESULTS.–*Induction of pisatin formation.*–The quantity of pisatin produced (Table 1) in the foot region of the pea was meager when seedlings were grown in the non-infested silt loam soil obtained from Lind, Washington. However, when the pea pathogen P-A was present in the soil, pisatin production had increased significantly by seedling emergence and continued to increase over the 4-week test period by which time severe decay had occurred. Tissues of the severely diseased foot region contained up to 411 μg pisatin/g fresh weight of tissue at the end of the test. High concentrations of pisatin were reached somewhat earlier in seedlings grown in the soil with the nonpathogenic clone NP-W, but the level dropped off after the initial sharp increase. The contrast between pisatin production in inoculated and that in noninoculated treatments diminished in the silt loam soil from Pullman which was higher in organic matter content (Table 2). Pisatin production by plants grown in the silt loam from Pullman resembled that in the silt loam soil from Lind; the nonpathogenic clone, NP-W, consistently induced earlier formation of high pisatin concentrations than did pathogenic clones. Also, there was a marked tendency with all clones for an initial increase in phytoalexin about two weeks after planting followed by a decrease in pisatin concentrations except when damage to the foot region was severe. Pisatin accumulation was highest in the immediate area of cotyledonary attachment. Generally, only trace amounts of the compound were produced in lower sections of the foot region (∿ 1 cm below cotyledonary attachment).

The relative pisatin toxicity to Fusarium solani clones.–Spore germination, rate of germ tube growth, time required for sporulation, and percent inhibition of linear growth were chosen as criteria which might relate to the pathogenic potentials of the *F. solani* clones. The effects of two or more concentrations of pisatin on clones P-A, P-NZ, NP-I and NP-W are compared in Fig. 1 and Tables 3, 4, and 5. Clones P-A (pathogenic) and NP-W (nonpathogenic) differ markedly in their response to pisatin. Although the germination, linear growth, and sporulation time of P-A is significantly retarded in the presence of pisatin, this clone is clearly less pisatin-sensitive than clone NP-W. Two other clones P-NZ (pathogenic) and NP-I (nonpathogenic), which are clearly distinguished by pathogenicity tests, are not clearly distinguished by these pisatin sensitivity criteria.

Pisatin did not completely prevent spore germination (Table 3) and after an initial retardation of germ tube elongation (Fig. 1) all clones (except NP-W) eventually attained a rate of growth comparable to clones on control media. There were some detectable morphological differences between clones grown in pisatin and those grown in the absence of pisatin. For example the germ tubes of clone NP-W grown at high (200 μg pisatin/ml) concentrations were more extensively branched. All clones sporulated freely except NP-W on agar containing 200 μg pisatin/ml.

Effect of pisatin on synthesis of the soluble proteins by F. solani.–Since protein synthesis is a sensitive measure of the cellular well-being of an organism, the effect of pisatin on the rate of protein synthesis was evaluated. The rate of protein synthesis of P-A macroconidia grown in 30 μg pisatin/ml of

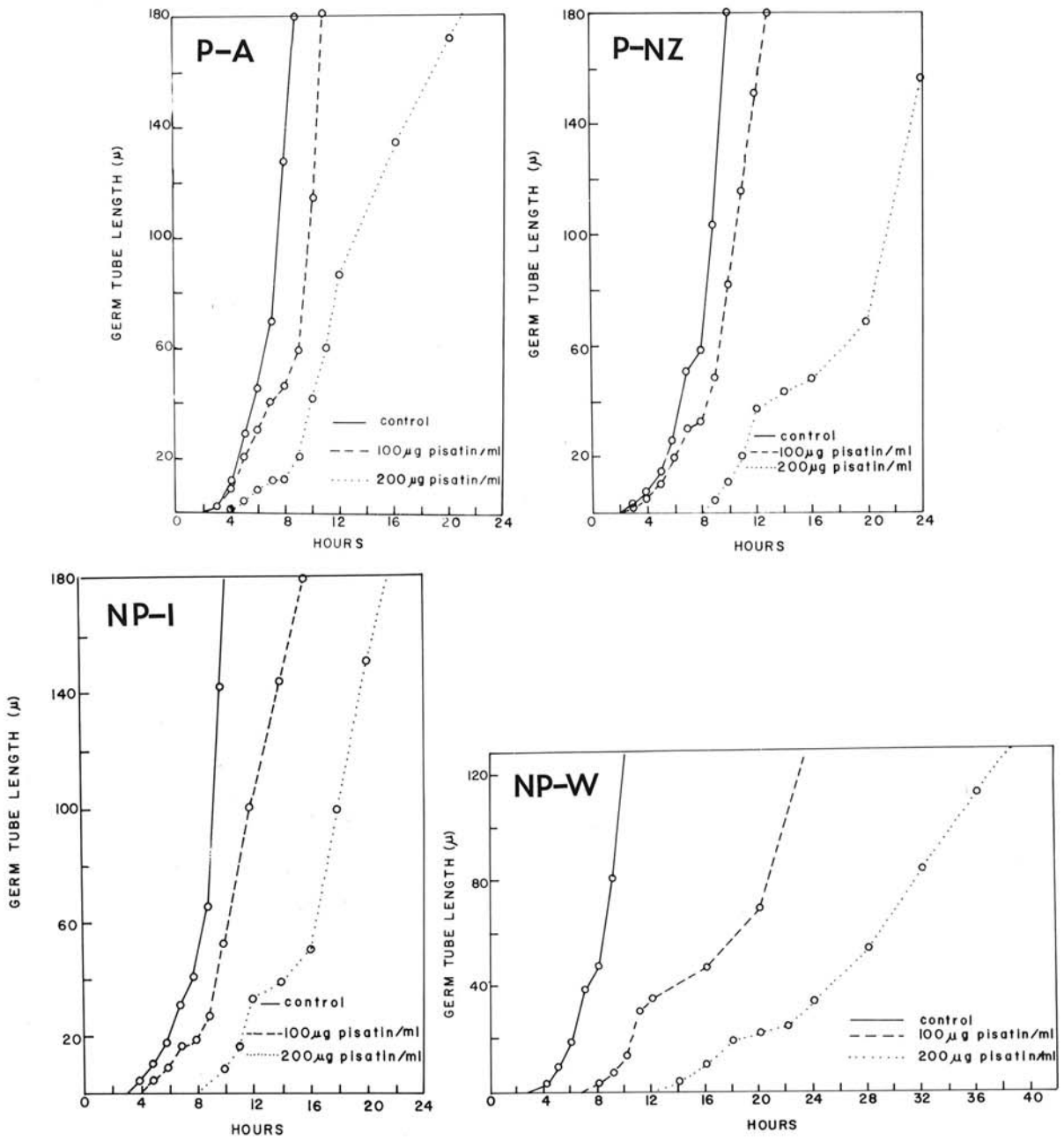

Fig. 1. Effect of pisatin on germ tube elongation of the four clones of *Fusarium solani* (P-A, P-NZ, NP-I, and NP-W) grown on water agar. Extrapolations to the edges of the plotting areas shown were based upon actual data.

medium was only 58% that of the control (without pisatin) 4 hr after the start of the incubation period. At 16 hr the rate of protein synthesis of P-A had linearly increased to 140% that of the control. The rate of protein synthesis of NP-W macroconidia was less than 50% of the control rate 8 hr after the start of the incubation period and linearly increased to 120% within 20 hr. After 28 hr, the rate of protein synthesis in both P-A and NP-W was apparently no longer affected by the pisatin in the media. Some clear differences were also detected after 6 hr in the protein bands of NP-W separated by disk-gel electrophoresis. At least three band changes (probably due to changes in location on the gel) appeared in pisatin-treated cultures. These band changes may reflect gel pattern changes due to cross linking (9) or other changes which are not related to protein synthesis.

In vitro degradation of pisatin.—The sensitivity of pathogenic or nonpathogenic organisms to pisatin

TABLE 3. Effect of pisatin on the germination of macronidia of four clones of *Fusarium solani* on water agar

Clone[a]	Pisatin concentration (μg/ml)	Percent germination after 5 hr	10 hr	20 hr	40 hr	80 hr	120 hr
P-A	0	100					
	100	100					
	200	48	100				
P-NZ	0	100					
	100	100					
	200	0	24	80	80	80	80
NP-I	0	100					
	100	89	100				
	200	0	64	90	97	97	97
NP-W	0	100					
	100	0	100				
	200	0	0	3	15	15	15

[a]P-A and P-NZ are clones of *Fusarium solani* f. sp. *pisi*. NP-I and NP-W are clones of *Fusarium* sp. nonpathogenic on peas (see Materials & Methods).

TABLE 4. Time required for initiation of spore production by four clones of *Fusarium solani* on water containing pisatin

Clone[a]	Pisatin concentration (μg/ml) 0 hr	100 hr	200 hr
P-A	15	15	32
P-NZ	22	30	42
NP-I	22	33	44
NP-W	28	60[a]	80[b]

[a]P-A and P-NZ are clones of *Fusarium solani* f. sp. *pisi*. NP-I and NP-W are clones of *Fusarium* sp. nonpathogenic on peas (see Materials & Methods).

[b]Very few spores produced.

TABLE 5. Percent inhibition of linear growth of four clones of *Fusarium solani* by pisatin

Clone[b]	percent inhibition[a] Pisatin concentration 30 μg/ml	50 μg/ml	100 μg/ml	200 μg/ml
P-A	5	7	11	49
P-NZ	2	7	14	32
NP-I	11	13	39	56
NP-W	41	59	63	93

[a]Percent inhibition is based on colony radius compared to that of control cultures after 7 days of growth on Vogel's medium solidified with agar.

[b]P-A and P-NZ are clones of *Fusarium solani* f. sp. *pisi*. NP-I and NP-W are clones of *Fusarium* sp. nonpathogenic on peas (see Materials & Methods).

may be tempered by their inherent ability to detoxify or eliminate this compound. *A. pisi* or clone P-A of *F. solani* were able to degrade 250 μg pisatin in 250 ml liquid culture within 24 hr. By contrast, 33-53% and 40-45% of the radioactive pisatin remained in cultures of clone NP-W of *F. solani* and *M. fructicola*, respectively (Table 6). Much of the label from the degraded pisatin-^{14}C was recovered from the medium of all cultures except that of *M. fructicola*. In the latter case almost all radioactivity from the degraded pisatin remained in the mycelium. A small amount of nonpisatin radioactivity was extracted from cultures of *F. solani* f. sp. *pisi*, and was almost totally comprised of a water soluble and alcohol-insoluble component. The breakdown of pisatin by two clones of *F. solani* yielded no single product.

Except for the buffering action of the medium no control of pH was attempted in the experiments described above. In later experiments the effect of pH and staling on the ability of fungal cultures to degrade pisatin was considered since pisatin is converted to anhydropisatin in the presence of acid. In these experiments, washed mycelium was added to flasks containing 500 μg pisatin and 50 ml of fresh Vogel's medium maintained at pH 6.5-6.9. Pisatin was completely degraded by clone P-A of *F. solani* within 20-24 hr. Clone NP-W required 48-72 hr for complete degradation. When cultures of either clone were autoclaved before addition of pisatin, 80% of the pisatin was recovered. Pisatin degradation also occurred in the presence of culture filtrates of *F. solani* clones; however, the rate of degradation occurred at a slower rate.

Effect of pathogenic and nonpathogenic conidia on the synthesis of proteins in pea tissue.—Phytoalexin production in peas is only one response dependent on protein synthesis (8). Therefore, by determining the relative rate at which all soluble proteins are synthesized in response to

pathogen and nonpathogen, a clearer view of the total host response is possible. Within 9 hr after a conidial suspension of the nonpathogenic clone, NP-W, was inoculated onto the endocarp surface of excised pea pods, there was a marked increase in protein synthesis over the normal synthesis of water-treated tissue (Fig. 2). The increased rate of synthesis appeared to be a general increase in the synthesis of all sizes of soluble proteins. There was only a slight change in protein synthesis when the pods were inoculated with the pathogenic clone P-A.

DISCUSSION.—The phytoalexin pisatin has been implicated as the primary constituent which renders pea tissue resistant to certain plant pathogenic organisms which are normally nonpathogenic to peas (4, 11). This study was directed towards estimating the potential effectiveness of pisatin in the specific pea tissues parasitized by pea root rotting organisms. In general, our results support the view that pisatin can, at certain concentrations, retard but not prevent germination and growth of root rotting organisms.

It is technically difficult (because of the limits of pisatin solubility) to supplement healthy tissues with pure pisatin in levels comparable to those induced in the tissue by pathogenic organisms. Therefore, the estimated concentrations of pisatin needed for disease resistance must be based on both the content of pisatin in the pea tissue and in vitro growth of pathogenic organisms at various pisatin concentrations. The highest concentrations (up to 411 μg/g) of pisatin in the foot region were detected, not in response to a nonpathogen of pea, but rather in pea tissue most severely rotted by a pea pathogen, *F. solani* f. sp. *pisi*. The initial accumulations in the foot region were highest in response to *F. solani* f. sp. *phaseoli* but these values were gradually surpassed by treatments involving the pathogens. This result supports Muller's (6) basic postulate that rate of phytoalexin accumulation may be a paramount factor in the effective action of a phytoalexin, but does not support the alternative concept that pathogens succeed by failing to stimulate high quantities of the phytoalexin.

Phytoalexin production is greatly influenced by the soil microflora associated with the foot region of the pea. Aseptically grown pea seedlings (up to 20 cm) do not produce detectable levels of pisatin. However, pea seedlings in comparable stages of development in potted soil produce substantial levels

TABLE 6. Recovery of radioactive pisatin[a] and nonpisatin compounds from culture of pathogenic and nonpathogenic fungi

	Percent of pisatin extracted from noninoculated control[b]			
	Pathogens		Nonpathogens	
	Ascochyta pisi	Clone[c] P-A	Clone[c] NP-W	*Monilinia fructicola*
Pisatin-^{14}C	0	0	33-53	40-45
Nonpisatin compounds:				
Medium	13	31-60	41-53	1
Mycelium	13	3-5	3-13	40-42

[a]250 μg of pisatin-^{14}C were added to 3-day-old cultures (about mg dry weight) of the fungi growing in 50 ml of Vogel's medium. After 24 hr the pisatin which remained was extracted with petroleum ether from the fungal tissue and from the medium. Radioactivity was determined for the purified pisatin and for the water-soluble compounds in the medium and mycelium as a result of pisatin degradation by the fungi.

[b]The control consisted of incubation of radioactive pisatin in noninoculated medium for 24 hr after which the pisatin was extracted with petroleum ether and quantitated.

[c]P-A is a clone of *Fusarium solani* f. sp. *pisi*. NP-W is a clone of *Fusarium* sp. nonpathogenic on peas (see materials and methods).

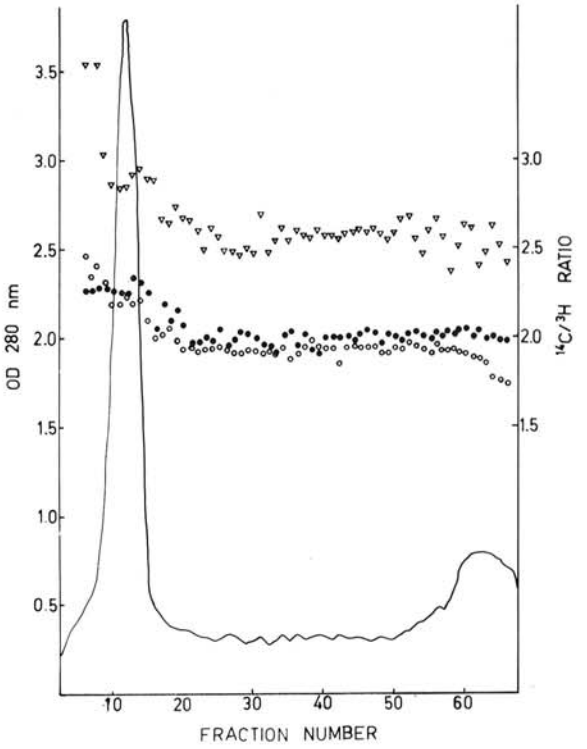

Fig. 2. Rate of protein synthesis (measured as the incorporation of ^{3}H and ^{14}C-labeled L-leucine in a 1-hr pulse period) 9 hr after the endocarp of the pea pod was inoculated with *Fusarium solani* clones P-A or NP-W or treated with H_2O. The *F. solani* inoculated tissue was always pulse-labeled with L-luecine-^{14}C; therefore, the higher the $^{14}C/^{3}H$ ratio, the greater the protein synthesis in the inoculated treatment. All ratios are greater than 1.0 since the data represent relative rates of protein synthesis and have been calculated as described previously (5) to correct for the d.p.m. of isotope administered but not for the molecular uptake rates which differs presumably because of differences in the sp. act. of the L-leucine precursor. ▽ = $^{14}C/^{3}H$ ratio of protein synthesis in NP-W inoculated tissue/H_2O treated tissue; ● = $^{14}C/^{3}H$ ratio of protein synthesis in P-A-treated tissue/H_2O-treated tissue; ○ = $^{14}C/^{3}H$ ratio of protein synthesis in H_2O-treated tissue/H_2O-treated tissue.

of pisatin. None of the soils used contained *F. solani* f. sp. *pisi* clones or any other known pea pathogen prior to inoculation. The accumulation of pisatin in the foot region in soils inoculated with clones of *F. solani* was compared with the background level of induction by the normal microflora of the soil. This background level of induction was low in seedlings grown in sand or the silt loam soil from Lind, Washington and was high in the Pullman soil. The Lind soil is low in organic matter content and presumably had a low microflora level. The pisatin concentration accumulations in seedlings grown in the non-infested and *F. solani* f. sp. *pisi*-infested silt loam soil from Pullman were similar. The addition of *Fusarium* propagules did not effectively augment the action of the inherent microflora. The generally high pisatin accumulation in all treatments and the varied accumulation in the presence of different *F. solani* clones suggests that the action (or interaction) of many microorganisms may be involved in pisatin accumulation. In each soil the middle section of the foot region accumulated the most pisatin. This higher level may be due to increased microbial activity in this vicinity nurtured by seed exudation (3).

The role of pisatin as a resistance factor must include a consideration of its relative toxicity to potential pathogens. Both pathogenic and nonpathogenic clones express a sensitivity to pisatin. This sensitivity results, in part, if not totally, from an alteration in the rate of protein synthesis.

If only clones P-A and NP-W of *F. solani* are considered in in vitro toxicity tests the results suggest that pisatin accounts for a major portion of the pea's resistance response. That is (i) clone NP-W stimulates high accumulations of pisatin in the pea tissue, (ii) these levels are sufficiently high to retard development of NP-W in vitro, (iii) NP-W is more pisatin-sensitive than P-A and (iv) NP-W is slower than P-A in degrading (and presumable detoxifying) pisatin.

Optimism for the role of this phytoalexin as the sole resistance factor against soil-borne fungi is somewhat dampened when clone NP-I of *F. solani* is included in the comparisons. For example, spores of clone NP-I (nonpathogenic) germinated slightly better than those of the pathogenic clone P-NZ at 100 or 200 μg pisatin/ml, and subsequent germ tube growth at the highest concentration was also somewhat less inhibited. Nonpathogenic NP-I sporulated as well as P-NZ at all pisatin levels tested. Linear growth of NP-I at 200 μg/ml was more rapid than that of pathogenic P-A, whereas growth as measured by dry weight increases in liquid culture was less rapid than for clone P-A. Thus, on the basis of pisatin sensitivity, the nonpathogenic clone NP-I was not clearly distinguished from the two pathogenic clones. Thus, there is doubt in this case that pisatin alone restrains the nonpathogenic clone NP-I of *F. solani* from successfully colonizing pea roots, especially since this clone appears to be no more sensitive to pisatin than pathogenic clone P-NZ. Neither of these fungi appear to promote pisatin accumulations to levels which are strongly inhibitory to their growth.

The resistance in peas to Fusarium root rot may well be comprised of multiple factors, one of which is pisatin. It is possible to establish that pisatin formation is an antagonistic reaction of the pea plant to various soil organisms and that *F. solani* f. sp. *pisi* and presumably other pea pathogens must tolerate this reaction during the infection process. However, the full magnitude of the host tissue response should not be overlooked. The dynamic response of the pea tissue inoculated with *F. solani* f. sp. *phaseoli* detected as gross changes in protein synthesis (Fig. 2) suggests that the resistance response effects the synthesis of many soluble enzymes in addition to those utilized in pisatin production. To conclude that pisatin singly accounts for the success or failure of all *F. solani* clones to invade pea tissue seems unwarranted at this time.

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