Isolation From Three Species of Colletotrichum of Glucan-Containing Polysaccharides That Elicit Browning and Phytoalexin Production in Bean

Anne J. Anderson

Assistant Professor of Plant Pathology, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Approved for publication as Technical Paper No. 4524 of the Oregon Agricultural Experimental Station, Corvallis.
Accepted for publication 14 June 1977.

ABSTRACT

ANDERSON, A. J. 1978. Isolation from three species of Colletotrichum of glucan-containing polysaccharides that elicit browning and phytoalexin production in bean. Phytopathology 68:189-194.

Glucan-containing polysaccharides from three species of *Colletotrichum* initiate symptoms of a defense response (browning and phytoalexin production) in bean, *Phaseolus vulgaris*. Two species, *C. trifoli* and *C. destructivum*, were not pathogenic on bean whereas the third, *C. lindemuthianum*, was a bean pathogen. The polysaccharides were obtained from culture filtrates and were not adsorbed by anion and cation exchange resins. Gel filtration on Agarose 5 M showed that each *Colletotrichum* sp. produced a high-molecular-weight fraction rich in glucan that initiated the symptoms when less than 10^{-7} g of glucose equivalents was applied to bean cotyledons. Lower-molecular-weight fractions from each species were active on bean. These fractions contained glucose, mannose, and galactose; those from *C. trifoli* and *C. destructivum* also contained rhamnose.

Additional key words: elicitor, resistance, hypersensitivity.

Although continuously exposed to many pathogenic microorganisms, plants are generally resistant to invasion owing to mechanisms such as the hypersensitive response (31). In the hypersensitive response, pathogen growth is restricted to the cell or cells that are initially contacted by the pathogen. Events that restrict fungal pathogens may involve low-molecular-weight, antifungal growth inhibitors, termed phytoalexins (7, 28, 29, 31), that accumulate in the hypersensitive plant tissue.

The occurrence of the hypersensitive response as a resistance mechanism in several plant-pathogen interactions suggests the possibility that the response is triggered by specific pathogen components, components termed "elicitors" by Keen et al. (22). Several reports (9, 10, 12, 21, 22, 23, 25, 29, 30) indicate the presence of elicitor activity in impure fungal preparations but only in a few examples (2, 3, 4, 5, 9) have purification and characterization of these active compounds been attempted. In one study, the elicitor activity from various races of the soybean pathogen, *Phytophthora megasperma* var. *sojae*, on soybean has been attributed to specific glucan-rich polysaccharides (3, 4, 5). Another study showed that a glucan-containing fraction extracted from the alpha race of the bean pathogen, *Colletotrichum lindemuthianum*, had elicitor activity on bean (2).

The reports that fungal polysaccharides trigger the hypersensitive response raise the question of the generality of the phenomenon. In an attempt to extend our knowledge of elicitors I have studied three species of *Colletotrichum* for elicitor activity on bean. The species *C. trifoli* (a pathogen of alfalfa) and *C. destructivum* (a pathogen of dodder) caused a hypersensitive response on bean, as did avirulent races of the bean pathogen *C. lindemuthianum* (A. J. Anderson, *unpublished*). The nature of components with elicitor activity that were isolated from *C. lindemuthianum*, *C. trifoli*, and *C. destructivum* are reported in this paper.

00032-949X/78/000029$03.00/0
Copyright © 1978 The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, MN 55121. All rights reserved.

MATERIALS AND METHODS

Culturing of fungi. — The alpha race of *C. lindemuthianum* was maintained and grown in liquid culture as described previously (2). Cultures of *C. destructivum* and *C. trifoli* obtained from Felix Lukezic (Pennsylvania State University), were maintained on potato-dextrose agar at 21 C. Fresh cultures were prepared by transfer of spores at 1 mo intervals. Liquid cultures of *C. destructivum* and *C. trifoli* were grown in the same medium that was used for *C. lindemuthianum* (2).

Isolation of elicitors. — Culture filtrates were obtained from six late-log-phase (10-day-old) 1-liter cultures by filtration through coarse sintered glass funnels. The culture filtrates were centrifuged at 14,000 *g* for 10 min and a clear supernatant fluid was obtained. The combined supernatant fluid was concentrated twentyfold under reduced pressure at 40 C in a rotary vacuum evaporator. Ethanol (three volumes) was added to the concentrated extracts and the precipitates that formed were collected by centrifugation at 37,000 *g* for 10 min. Water (100-120 ml) was added to the precipitated material and the suspensions were dialyzed at 20 C in water for 6 hr. Any insoluble material was removed by centrifugation at 37,000 *g* for 10 min to yield a clear supernatant fluid that was assayed for hexose by the anthrone method (11); for protein by the Lowry procedure (24); and for elicitor activity by the procedures described below. The ethanolic supernatant fluid possessed no elicitor activity.

Assay of elicitor activity. — Elicitor activity in preparations from the *Colletotrichum* spp. was assayed by

measurement of the extent of browning of treated Dark Red Kidney cotyledons and by measuring phytoalexin production in these same tissues. The assay procedure was identical to that described previously (2) except that the antibiotics used in these assays were penicillin G (Sigma) and streptomycin sulphate (Sigma), each at a final concentration of 100 μg/ml. In the assay, one unit of elicitor activity is defined as the amount which, when applied in 100 μliter to the cut surface of a cotyledon, just caused browning and phytoalexin production.

Comparison of the activity of the elicitor preparations from the three *Colletotrichum* spp. involved treatment of the cotyledons with solutions containing equivalent amounts of hexose as measured by the anthrone colormetric procedure (11). A semiquantitative assay of the amounts of the bean phytoalexins produced in the treated cotyledons involved thin-layer silica gel chromatography of a series of dilutions of the bean extracts containing phytoalexins.

Purification of elicitor. — Elicitors from each of the three *Colletotrichum* spp. were purified by passing through a strong cation exchange resin, Dowex 50X2-200 (Sigma Chemical Co., St. Louis, MO 63178), and a strong anion exchange resin, Dowex 1X2-200 (Sigma), followed by molecular sizing on Agarose 5M (Bio Rad). The crude dialyzed preparation (100-120 ml), obtained by ethanol precipitation of the concentrated culture filtrates, was adjusted to pH 2 with 200 mM HCl and applied to a column (10 × 1.5 cm) containing Dowex-50, previously equilibrated with 20 mM HCl. The column was washed with 10 bed volumes of 20 mM HCl and these eluates were combined. The eluate was adjusted to pH 7 by addition of 500 mM phosphate buffer at pH 8 and dialyzed extensively against water prior to assay for hexose, protein, and elicitor activity.

The dialyzed Dowex-50 eluate was adjusted to pH 10 by addition of 1.0 M glycine-NaOH buffer (pH 10) and applied to a column (10 × 1.5 cm) of Dowex 1 equilibrated with 10 mM glycine-NaOH, pH 10. The column was washed with 10 bed volumes of 10 mM glycine-NaOH, pH 10 and the eluates containing nonabsorbed material were combined. The column then was eluted with 10 bed volumes each of 100 mM glycine-NaOH (pH 10) and 10 mM glycine-NaOH (pH 10) containing 0.5 M NaCl. Each eluate was adjusted to pH 7 by addition of 1.0 M sodium acetate buffer pH 4 and dialyzed extensively against water prior to assay for hexose, protein, and elicitor activity. The 10 mM eluate containing elicitor activity was concentrated by evaporation under reduced pressure at 40 C to a final volume of 10 ml.

Gel filtration chromatography of these partially purified elicitor preparations involved application of 1 to 2 ml of the concentrated ion exchange column eluates onto an Agarose 5 M column (43 × 1.0 cm) which was eluted with 0.1 M NaCl. Fifty 3-ml fractions were collected and assayed for hexose. The void volume (fractions 13-18), partially- (fractions 19-31) and fully-included (fractions 32-45) components from each *Colletotrichum* sp. were pooled and assayed for hexose, protein, and elicitor activity. The void- and included volumes of the column were measured by elution of Blue Dextran [molecular weight 2 x 10^6 daltons (Sigma)] and glucose, respectively.

Assay of monosaccharide composition of elicitor preparations by gas chromatographic analysis. — Samples of preparations containing up to the equivalent of 500 μg hexose [as determined by the anthrone colorimetric method (11)] were hydrolyzed and the alditol acetate derivatives were prepared by the method of Jones and Albersheim (19). The acetylation mixtures containing the alditol acetates were treated with 1 ml of water to hydrolyze the remaining acetic anhydride and then 2 ml of chloroform were added. The chloroform extracts of the acetylation mixtures were removed and evaporated to dryness under a stream of purified air at 40 C. These residues containing the alditol acetates were dissolved in 250 μliters of acetone and were analyzed by gas chromatography on a Hewlett Packard Model 5830-A gas chromatograph using flame ionization detectors. Nitrogen, at a flow rate of 17 ml/min, was used as the carrier gas through a 1.83 m x 3.2 mm (o.d.) [6 ft x 1/8 in (o.d.)] copper column containing 3% SP-2340 on 100/120 Supelcoport (Supelco). A 2-μliter sample was injected at a column temperature of 225 C.

Inositol and the standard sugars, mannose, glucose, galactose, and rhamnose, were obtained from Applied Science Laboratories, Inc., State College, PA 16801 and samples of 500 μg of each sugar were used to prepare the derivatives used to standardize the column.

Treatment of elicitor preparations with periodate. — Aliquots of 1.0 mg hexose equivalents of *C. lindemuthianum, C. trifoli* and *C destructivum* elicitor preparations, purified through ion exchange resins, were treated with 0.05 M periodate for 6 hr at 8 C. Then glycerol (250 μliter) was added to remove excess periodate and the extracts were assayed for elicitor activity.

Treatment of elicitor with endoglucanase. — Endoglucanases produced by *Triochoderma viride* were purified from the commercial preparation Cellulysin (Calbiochem, Los Angeles, CA 90063). A solution of 1 g Cellulysin in 100 ml 50 mM sodium acetate pH 4.0 was passed through a column (20 x 5 cm) containing CM Sephadex (C-125-120) equilibrated with 50 mM sodium acetate pH4. The eluate from this column was collected and dialyzed extensively against water and then against 50 mM potassium phosphate pH 8.0. This dialyzed preparation was passed through a column containing DEAE Sephadex (A-125-120) equilibrated with 50 mM potassium phosphate pH 8.0. The column was washed with 10 volumes of 50 mM potassium phosphate pH 7. A wash of 200 mM sodium acetate pH 4 was applied to the column and the eluate was collected in 5-ml fractions. Fractions with endo-β-1, 4-glucanase activity, which was measured by published procedures (20), were combined and the preparation was dialyzed against 50 mM sodium acetate pH 5.2 prior to storage at 4 C. This preparation also contained endo-β-1,3-glucanase as measured by the method of Albersheim and Valent (1) but it was free of any exoglucosidase activity when assayed by the procedure of Jones et al. (20).

Aliquots of 1.0 mg hexose equivalents of *C. lindemuthianum, C. trifoli* and *C. destructivum* elicitor that had been purified through the ion exchange resins were mixed with the purified endoglucanase preparation. After 12 hr incubation at 35 C, these solutions were assayed for elicitor activity.

RESULTS

Detection of elicitor activity in culture filtrates from Colletotrichum lindemuthianum, C. trifoli, and C. destructivum. — The crude extracts prepared by concentration and ethanolic precipitation of the culture filtrates of each *Colletotrichum* sp. caused browning and the production of phytoalexins in the treated Dark Red Kidney bean cotyledons. In each treatment, four phytoalexins (phaseollin, phaseollidin, phaseollinisoflavan, and kievitone) were produced. No browning or phytoalexin accumulation was observed by treating tissues with water or with a preparation of noninculated culture media concentrated twentyfold under reduced pressure at 40 C.

The activity of the crude preparations from the *Colletotrichum* spp. indicated that the culture filtrates contained two to 20 units of elicitor activity/100 μliter. These crude preparations posessed 4- to 6-fold more hexose than protein (Table 1).

Purification of elicitor. — Elicitor activity for each of the three *Colletotrichum* spp. was detected in material that eluted without absorption from both the Dowex 50 and Dowex 1 columns (Table 1). The majority of the applied hexose also was not retained by these column materials (Table 1) and because most of the applied protein was absorbed to the resins, these purification steps enriched for carbohydrate.

The polysaccharides in the eluted preparations from the three *Colletotrichum* spp. were of similar molecular size distribution; a common elution pattern from the Agarose 5 M column was obtained (Fig. 1). For each species about 10% of the applied polysaccharide eluted in the void volume fractions (fractions 13-18), and the remainder was fully (fractions 32-45), or partially included (fractions 19-31), into the column. Comparison of the elicitor activity of the void, intermediate, and included polysaccharides showed that although each was active, the included fractions possessed less units of elicitor per hexose equivalent. Protein was detected only

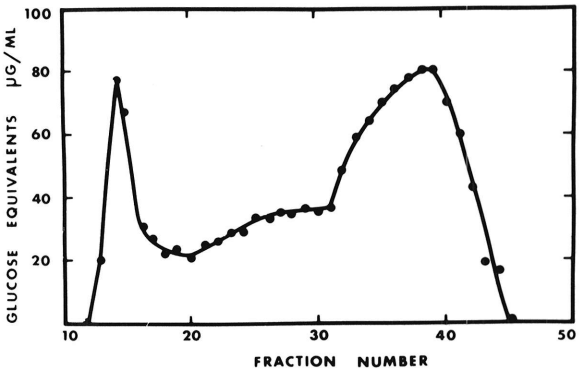

Fig. 1. Chromatography on Agarose 5M of an elicitor preparation from *Colletotrichum trifoli* previously purified from culture filtrates by ethanol precipitation and ion exchange resins.

TABLE 1. Summary of purification from culture filtrates of *Colletotrichum lindemuthianum, C. destructivum,* and *C. trifoli* of elicitors that initiate browning and phytoalexin production in bean.

Elicitor preparation	Total glucose equivalents (mg)	Total protein (mg)	Elicitor activity[a] (total units ±2 x 10^5)
C. lindemuthianum:			
Culture filtrate concentrate	340	75	12 x 10^5
Dowex 50, 20 mM HCl eluate	240	15	24 x 10^4
Dowex 1 eluates,			
10 mM glycine buffer	225	1.5	10 x 10^5
100 mM glycine buffer	13		0
0.5 M NaCl	11		0
C. trifoli:			
Culture filtrate concentrate	375	56	104 x 10^4
Dowex 50, 20 mM HCl eluate	352	12	80 x 10^4
Dowex 1 eluates			
10 mM glycine buffer	250	1.8	50 x 10^4
100 mM glycine buffer	7	0.4	10^3
0.5 M NaCl	2	1.40	0
C. destructivum:			
Culture filtrate concentrate	236	64	20 x 10^4
Dowex 50, 20 mM HCl	182	15	70 x 10^3
Dowex 1 eluates			
10 mM glycine buffer	112	1.0	14 x 10^4
100 mM glycine buffer	2	0.2	0
0.5 M NaCl	1	0.8	0

[a]One unit of elicitor activity is defined as the amount which when applied to the cut surface of a Dark Red Kidney bean cotyledon in 100 μliter of water just initiated the hypersensitive response.

in the included fractions.

Sensitivity of the elicitor to periodate treatment and endoglucanase activity. — Elicitor activity in preparations from *C. lindemuthianum, C. trifoli,* and *C. destructivum* purified through ion exchange chromatography was destroyed by treatment with periodate or by the action of endoglucanases from *T. viride.* These results suggest that the elicitor activity is a function of the polysaccharide moiety.

Comparative activity of the Colletotrichum lindemuthianum, C. trifoli, and C. destructivum elicitor preparations. — The application of 10 μg hexose equivalents from *C. lindemuthianum, C. trifoli,* and *C. destructivum* elicitor preparations purified though the ion exchange resins resulted in the same degree of browning of the Dark Red Kidney bean cotyledons. Essentially identical amounts of the four bean phytoalexins were produced in these assays. The same elicitor preparations from the three *Colletotrichum* spp. also were equally active as measured by browning and phytoalexin production in two other bean cultivars, Bush Blue Lake and Perry Marrow.

Assay of dilutions of the void Agarose 5M preparations on Dark Red Kidney bean cotyledons showed that a minimum of 0.02 (± 0.01) μg hexose equivalents for *C. lindemuthianum,* 0.03 (± 0.01) μg hexose equivalents for *C. trifoli,* and 0.04 (± 0.01) μg hexose equivalents for *C. destructivum* was required to stimulate elicitor activity. Thus, these specific polysaccharide preparations from the three species of *Colletotrichum* possessed almost equivalent elicitor potential on bean.

Monosaccharide composition of elicitor fractions. — Analysis of the alditol acetate derivatives of the elicitor preparations purified through the anion and cation exchange columns indicated that the active fractions from each *Colletotrichum* spp. all contain glucose, mannose, and galactose (Table 2). However, unlike the *C. lindemuthianum* elicitor, the *C. destructivum* and *C. trifoli* preparations also contained rhamnose (Table 2).

Analysis of the fractions separated on the Agarose 5M columns indicated that size difference was accompanied by compositional differences (Table 2). For each of the *Colletotrichum* elicitor preparations, the Agarose 5M void fractions contained mostly glucose, although traces of rhamnose were present in *C. trifoli* and *C. destructivum* extracts (Table 2). The material that included into the column possessed greater amounts of mannose and galactose.

TABLE 2. Monosaccharide composition of preparations from *Colletotrichum lindemuthianum, C. trifoli,* and *C. destructivum* purified for elicitor activity on bean

	Monosaccharide composition			
Elicitor preparation	Rhamnose (%)	Mannose (%)	Galactose (%)	Glucose (%)
C. lindemuthianum, Alpha race:				
Dowex 1 eluates				
10 mM glycine buffer		23	8	69
100 mM glycine buffer		41	51	8
0.5 M NaCl buffer		35	60	5
Agarose 5M eluates				
void fractions			1	99
intermediate fractions		2	2	96
included fractions		44	24	32
C. trifoli:				
Dowex 1 eluates				
10 mM glycine buffer	17	23	21	40
100 mM glycine buffer	26	36	20	18
Agarose 5M eluates				
void fractions	6	4	4	86
intermediate fractions	6	30	16	48
included fractions	16	27	32	28
C. destructivum:				
Dowex 1 eluates				
10 mM glycine buffer	25	20	5	50
100 mM glycine buffer	20	20	29	31
Agarose 5M eluates				
void fractions	4	6	2	88
intermediate fractions	12	19	10	61
included fractions	15	38	19	28

DISCUSSION

Three species of *Colletotrichum* release into the culture filtrates polysaccharides that cause browning and phytoalexin accumulation in bean. These observations compare with studies by Skipp and Deverall (29) and Mercer et al. (25) that *C. lindemuthianum* culture filtrates initiate symptoms in bean that resemble, at the ultrastructural level, a hypersensitive response.

The polysaccharides that elicit phytoalexin production and browning in bean cotyledons were similar in size, composition, and activity, whether they were isolated from *C. trifoli* or *C. destructivum* (species nonpathogenic on bean) or from the bean pathogen *C. lindemuthianum*. A difference was the presence of rhamnose in the elicitor preparations from *C. trifoli* and *C. destructivum;* rhamnose has not been detected in *C. lindemuthianum* (alpha race) elicitor extracts.

The components with elicitor activity were heterogeneous in size. Each of the three *Colletotrichum* spp. produced a high-molecular-weight elicitor fraction, predominantly glucan in nature. The lower-molecular-weight fractions which also possessed elicitor activity all contained glucose, but mannose and galactose were detected along with rhamnose for *C. trifoli* and *C. destructivum*. At present it is not known whether these additional sugars are present in the same or separate polymers. However, it seems likely that a glucan component is essential for elicitor activity. The observation that less than 0.1 μg equivalent of hexose in the active elicitor preparations was sufficient to initiate the resistance response in bean cotyledons indicates the high degree of sensitivity in the plant's reaction.

Although phytoalexin production and plant cell necrosis are characteristics of the hypersensitive response (31), these symptoms also can be induce in plants by many physical (8, 17, 26) and chemical (6, 13, 14, 15, 16, 18, 27) treatments. It is possible that these methods trigger the responses by reactions that are common to those initiated by elicitors. However, most of the chemical treatments are unrelated to the process of pathogenesis. The demonstration of elicitors provides examples of components that could be functional in the normal plant-pathogen interaction.

The elicitor activity of glucan-containing preparations from three *Colletotrichum* spp. augments similar data for glucans from *Phytophthora megasperma* var. *sojae* (3, 4, 5). The isolation from *C. trifoli* and *C. destructivum* of elicitors active in bean demonstrates that resistance in a plant also can be triggered by components from fungal species that are not pathogenic on that plant.

LITERATURE CITED

1. ALBERSHEIM, P., and B. S. VALENT. 1974. Plant pathogens secrete proteins which inhibit enzymes of the host capable of attacking the pathogen. Plant Physiol. 53:684-687.
2. ANDERSON, A. J., and P. ALBERSHEIM. 1975. Host-pathogen interactions. VII. Isolation of a pathogen synthesized fraction rich in glucan, that elicits a defense response in the pathogen's host. Plant Physiol. 56:286-291.
3. AYERS, A., J. EBEL, F. FINELLI, N. BERGER, and P. ALBERSHEIM. 1976. Host-pathogen interactions. IX. Quantitative assays of elicitor activity and characterization of the elicitor present in the extracellular medium of cultures of Phytophthora megasperma var. sojae. Plant Physiol. 57:751-759.
4. AYERS, A. R., J. EBEL, B. VALENT, and P. ALBERSHEIM. 1976. Host-pathogen interactions. X. Fractionation and biological activity of an elicitor isolated from the mycelial walls of Phytophthora megasperma var. sojae. Plant Physiol. 57:760-766.
5. AYERS, A. R., B. VALENT, J. EBEL, and P. ALBERSHEIM. 1976. Host-pathogen interactions. XI. Composition and structure of wall-released elicitor fractions. Plant Physiol. 57:766-774.
6. BAILEY, J. A. 1969. Effects of antimetabolites on production of the phytoalexin pisatin. Phytochemistry 8:1393-1395.
7. BAILEY, J. A. 1974. The relationship between symptom expression and phytoalexin concentration in hypocotyls of Phaseolus vulgaris infected with Collectotrichum lindemuthianum. Physiol. Plant Pathol. 4:477-488.
8. BRIDGE, M. A., and W. L. KLARMAN. 1973. Soybean phytoalexin, hydroxyphaseollin, induced by ultraviolet irradiation. Phytopathology 63:606-609.
9. CRUICKSHANK, I. A. M., and D. R. PERRIN. 1968. The isolation and partial characterization of monilicolin A, a polypeptide with phaseollin-inducing activity from Monilinia fructicola. Life Sci. 7:449-458.
10. DIJKMAN, A. VAN, and A. KAARS-SIJPESTEIJN. 1973. Leakage of pre-absorbed ^{32}P from tomato leaf discs infiltrated with high molecular weight products of incompatible races of Cladosporium fulvum. Physiol. Plant Pathol. 3:57-67.
11. DISCHE, Z. 1962. Color reaction of carbohydrates. Pages 477-512 *in* R. L. Whistler and M. L. Wolfrom, eds. Methods in carbohydrate chemistry. Vol. 1. Academic Press, New York. p.
12. FRANK, J. A. and J. D. PAXTON. 1971. An inducer of soybean phytoalexin and its role in the resistance of soybeans to Phytophthora rot. Phytopathology 61:954-956.
13. HADWIGER, L. A. 1972. Phytoalexin production induced by heavy metals proposed mode of action. Phytopathology 62:761.
14. HADWIGER, L. A., A. JAFRI, S. VON BROEMBSEN, and R. EDDY, JR. 1974. Mode of pisatin induction. Increased template activity and dye-binding capacity of chromatin isolated from polypeptide-treated pea pods. Plant Physiol. 53:52-63.
15. HADWIGER, L. A., and M. E. SCHWOCHAU. 1970. Induction of phenylalanine ammonia lyase and pisatin in pea pods by poly-lysine, spermidine or histone fractions. Biochem. Biophys. Res. Commun. 38:683-691.
16. HADWIGER, L. A., and M. E. SCHWOCHAU. 1971. Specificity of deoxyribonucleic acid intercalating compounds in the control of phenylalanine ammonia lyase and pisatin levels. Plant Physiol. 47:346-351.
17. HADWIGER, L. A., and M. E. SCHWOCHAU. 1971. Ultraviolet light-induced formation of pisatin and phenylalanine ammonia lyase. Plant Physiol. 47:588-590.
18. HESS, S. L., and L. A. HADWIGER. 1971. The induction of phenylalanine ammonia lyase and phaseollin by 9-aminoacridine and other deoxyribonucleic acid intercalating compounds. Plant Physiol. 48:197-202.
19. JONES, T. M., and P. ALBERSHEIM. 1972. A gas chromatographic method for the determination of aldose and uronic acid constitutents of plant cell wall polysaccharides. Plant Physiol. 49:926-936.
20. JONES, T. M., A. J. ANDERSON, and P. ALBERSHEIM. 1972. Studies on the polysaccharide-degrading enzymes secreted by Fusarium oxysporum f. sp. lyco-

persici. Physiol. Plant Pathol. 2:153-166.
21. KEEN, N. T. 1975. Specific elicitors of plant phytoalexin production: determinants of race specificity in pathogens. Science 187:74-75.
22. KEEN, N. T., J. E. PARTRIDGE, and A. I. ZAKI. 1972. Pathogen-produced elicitor of a chemical defense mechansim in soybeans monogenically resistant to Phytophthora megasperma var. sojae. Phytopathology 62:768.
23. KEEN, N. T., M. C. WANG, S. BARTNICKI-GARCIA, G. A. ZENTMYER. 1975. Phytotoxicity of mycolaminarans-β-1, 3-glucans from Phytophthora spp. Physiol. Plant Pathol. 7:91-92.
24. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-275.
25. MERCER, P. C., R. K. S. WOOD, and A. D. GREENWOOD. 1974. Resistance to anthracnose of French bean. Physiol. Plant Pathol. 4:291-306.
26. RAHE, J. E. 1974. Accumulation of phaseollin in hypocotyls of etiolated Phaseolus vulgaris in response to mechanical injury. Proc. Am Phytopathol. Soc. 1:64.
27. SCHWOCHAU, M. E., and L. A. HADWIGER. 1968. Stimulation of pisatin production in Pisum sativum by actinomycin D and other compounds. Arch. Biochem. Biophys. 126:731-733.
28. SKIPP, R. A., and B. J. DEVERALL. 1972. Relationships between fungal growth and host changes visable by light microscopy during infection of bean hypocotyls (Phaseolus vulgaris) susceptible and resistant to physiological races of Colletotrichum lindemuthianum. Physiol. Plant Pathol. 2:357-374.
29. SKIPP, R. A., and B. J. DEVERALL. 1973. Studies on cross-protection in the anthracnose disease of bean. Physiol. Plant Pathol. 3:299-313.
30. VARNS, J. L., W. W. CURRIER, and J. KUĆ. 1971. Specificity of rishitin and phytuberin accumulation by potato. Phytopathology 61:968-971.
31. WOOD, R. K. S. 1974. Diseases in higher plants. Oxford Univ. press, London. 570 p.