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# Sensitivity to, and metabolism of, phaseollin in relation to the pathogenicity of different isolates of Botrytis cinerea to bean (Phaseolus vulgaris)

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**Abstract**

The pathogenicity of five isolates of *Botrytis cinerea* to bean *(Phaseolus vulgaris)* was compared with their sensitivity to the phytoalexin phaseollin and their ability to metabolize phaseollin. Three of the five isolates were pathogenic to bean, as they formed spreading lesions on bean leaves; the other two isolates were considered nonpathogenic to bean, since they produced lesions limited in size or no lesions at all.

The isolates were about equally sensitive to phaseollin, except for one nonpathogenic strain which was more sensitive than the other ones to higher concentrations of phaseollin. The three pathogenic isolates metabolized phaseollin to 6a-hydroxyphaseollin readily in shake cultures containing 9 $\mu$g phaseollin/ml, while nonpathogenic isolates were not able, or less able, to do so.

## Introduction

Several studies have shown a correlation between pathogenicity of different fungal species and tolerance of growing mycelium to phytoalexins produced by their host plants (Cruickshank, 1962; Cruickshank and Perrin, 1971; Jones et al., 1975; Mansfield and Deverall, 1974; Perrin et al., 1974; VanEtten, 1973). Some notable exceptions, however, have been reported by Pueppke and VanEtten (1974) and Ward et al. (1974). In a number of cases pathogenic species were also better able to metabolize their host phytoalexins than nonpathogenic species (Higgins and Millar, 1970; Mansfield and Widdowson, 1973; Nonaka, 1967; De Wit-Elshove, 1969), but such a correlation was not found by Van den Heuvel and Glazener (1975) and Sakuma and Millar (1972).

Apparently, there is no general rule that pathogenicity is associated with a relative insensitivity to the host phytoalexins or the ability to metabolize them. These mechanisms may, however, contribute to susceptibility in certain host-pathogen interactions.

Differences in pathogenicity between clones, formae speciales, strains or physiological races of a fungal pathogen were found to be associated with a differential sensitivity of mycelium to the host phytoalexins or with a differential metabolism of these substances (Christenson and Hadwiger, 1973; Harrower, 1973; Van 't Land et al., 1975; Uritani and Kojima, 1975; Van Etten and Smith, 1975), with the exception of *Colletotrichum lindemuthianum* and phaseollin (Bailey, 1974; Van den Heuvel and Vollaard, 1976).

Van den Heuvel and Glazener (1975) have shown that an isolate of *Botrytis cinerea*, obtained from diseased *Phaseolus vulgaris* tissue, was able to metabolize the bean phytoalexin phaseollin to the less toxic product, 6a-hydroxyphaseollin. In the present paper the sensitivity of five isolates of *B. cinerea*, obtained from different sources, to phaseollin and their ability to metabolize phaseollin have been investigated in relation to their pathogenicity to bean.

## Materials and methods

*Fungal isolates and inoculation of bean leaves.* The five isolates of *Botrytis cinerea* Pers. ex Fr. used in this study were isolated from different host plants; viz. isolate BC-1 from bean *(P. vulgaris)*; isolate BC-3 from raspberry; isolate BC-4 from tomato; isolate BC-5 from grape (CBS strain 121.39); and isolate BC-6 from flax *(B. cinerea* f. *lini* van Beyma, CBS strain 131.28). The latter two isolates were obtained from Centraalbureau voor Schimmelcultures (CBS), Baarn.

Bean plants (*Phaseolus vulgaris* L. 'Dubbele Witte z. dr.') were grown in the greenhouse at 22° to 27°C. Inoculum plugs (5 mm diameter) taken from the edge of 3-day-old colonies of *B. cinerea* isolates grown on PDA plates at 23°C were placed upside down on the adaxial side of primary leaves on 11- to 13-day-old plants. Ten leaves (two leaves/plant) were used for each isolate and one mycelium plug was placed on each leaf. The inoculated plants were kept at 20° to 25°C in transparant humidity chambers lined with moist filter paper to maintain a high relative humidity.

*Sensitivity to phaseollin.* The bioassays employed for comparing the sensitivity of growing mycelium of the *B. cinerea* isolates to phaseollin were essentially the same as described previously (Van den Heuvel and VanEtten, 1973).

*Preparation of shake cultures.* The abilities of different *B. cinerea* isolates to metabolize phaseollin were compared using shake cultures. Three-hundred-ml Erlenmeyer flasks containing 50 ml of a modified Richard's solution, with 2% glucose as the only carbon source, were inoculated with small pieces of mycelium and incubated at 24°C on a reciprocal shaker (ca 130 strokes/min). After 4 days of incubation the cultures were ground slightly (Sorvall Omnimixer) and diluted with fresh Richard's solution to give 0.6 mg (dry weight) mycelium/ml. After an additional 24 h period of shaking the actively growing cultures were again diluted with fresh Richard's solution to give 1.5 mg (dry weight) mycelium/ml. Eight aliquots of 6 ml were taken from these cultures and transferred to 25-ml Erlenmeyer flasks. Purified phaseollin, dissolved in dimethylsulfoxide (DMSO), was added to these cultures to give a final phaseollin concentration of 9 μg/ml, a concentration slightly inhibitory to fungal growth. The final concentration of DMSO in the cultures was 0.5%. Control cultures received DMSO without phaseollin.

The methods used for determining mycelial dry weights and preparing phaseollin solutions were as described by Van den Heuvel and Glazener (1975).

*Extraction and quantitative analysis of phaseollin and 6a-hydroxyphaseollin.* After the cultures had been shaken with phaseollin for 3, 24 or 48 h, or without phaseollin for 24 h, 6 ml 96% ethanol was added to each flask to halt metabolic activity and to

extract phaseollin and 6a-hydroxyphaseollin from the mycelium. Further steps in the extraction procedure were similar to those described previously (Van den Heuvel and Glazener, 1975). Quantitative analysis of the two compounds by thin-layer chromatography (TLC) and in situ densitometry was also carried out as outlined earlier (Van den Heuvel and Glazener, 1975), except that TLC plates were developed in an unsaturated tank with benzene: methanol (9:1) as the solvent system. Samples of phaseollin and 6a-hydroxyphaseollin were spotted as reference compounds on TLC plates.

All values were obtained from duplicate samples, and each experiment was repeated at least once.

In control experiments the recovery of phaseollin from flasks containing medium with phaseollin, but without fungus, was examined after shaking for 3, 24 of 48 h. An average of 65% of added phaseollin was recovered, together with two unidentified compounds which were probably nonbiological breakdown products of phaseollin. The combined average proportion of these products was 18%. Had they not been formed, the recovery of phaseollin would have been about 83%. Extracts from fungal cultures contained small, variable quantities of such substances. In view of these results, only actually-recovered percentages of phaseollin and 6a-hydroxyphaseollin are presented.

Fig. 1. Pathogenicity of five isolates of *Botrytis cinerea* to leaves of bean *(Phaseolus vulgaris)* as measured by lesion diameter at daily intervals. The isolates used were: BC-1 (○), BC-3 (●), BC-4 (▽), BC-5 (□) and BC-6 (■).

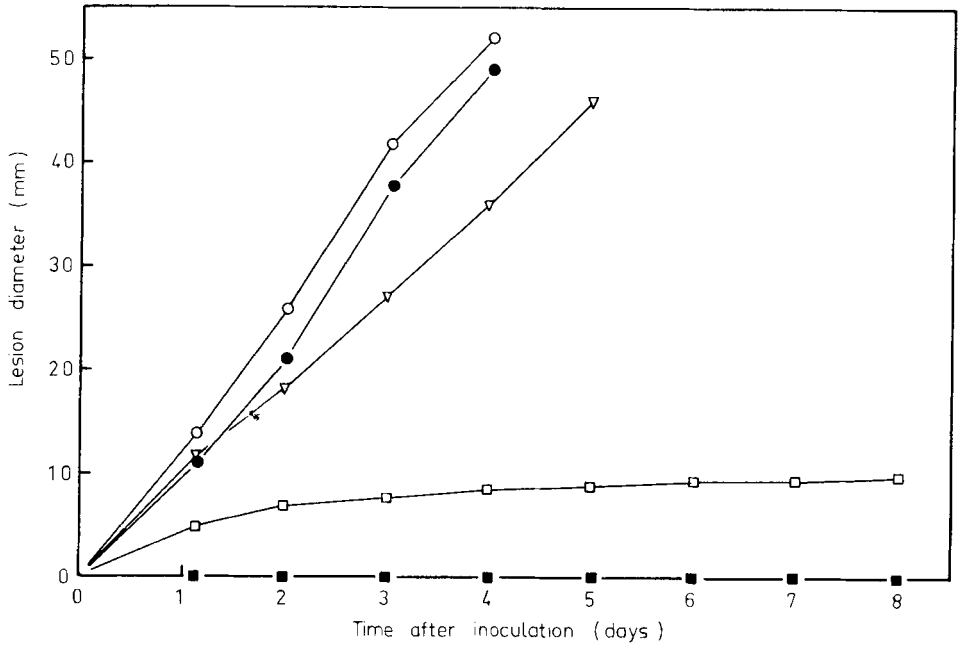


*Fig. 1. Pathogeniteit van vijf isolaten van Botrytis cinerea voor bladeren van boon (Phaseolus vulgaris), bepaald door dagelijkse meting van de lesiediameter. De vijf isolaten waren: BC-1* (○), *BC-3* (●), *BC-4* (▽), *BC-5* (□) *en BC-6* (■).

## Results

*Pathogenicity to bean leaves.* Three isolates of *B. cinerea* (BC-1, BC-3 and BC-4) produced rapidly spreading lesions in bean leaves (Fig. 1). After such lesions had reached a diameter of about 50 mm, the infected leaves started to wilt and drop.

On leaves inoculated with isolate BC-5, lesions limited in size (ca 10 mm diameter) were formed. Infection with isolate BC-6 did not lead to any visible discolouration or necrosis, apart from a rare pin-point lesion. Isolates BC-5 and BC-6 where therefore classified as nonpathogenic, whereas the three former strains were regarded as pathogenic.

*Sensitivity to phaseollin.* The three pathogenic isolates of *B. cinerea* and isolate BC-5 were about equally sensitive to phaseollin (Fig. 2). Isolate BC-6 was more sensitive than the other isolates to concentrations of 0.1 and 0.2 mM phaseollin (32.2 and 64.4 µg/ml, respectively).

Fig. 2. Inhibition of radial mycelial growth of five isolates of *Botrytis cinerea* by various concentrations of phaseollin. Same symbols as in Fig. 1.

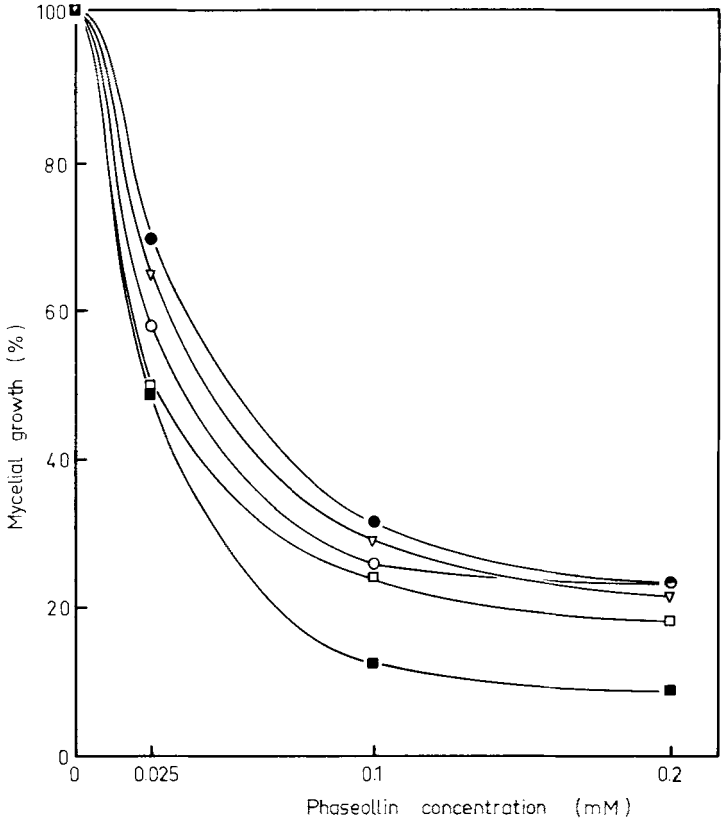


*Fig. 2. Remming van de radiale myceliumgroei van vijf isolaten van Botrytis cinerea door verschillende concentraties phaseolline. Zelfde symbolen als in Fig. 1.*

Fig. 3. Recovery of phaseollin (●) and its metabolite, 6a-hydroxyphaseollin (○), from chromatographed extracts of shake cultures of five isolates of *Botrytis cinerea*, incubated with 9 μg phaseollin/ml for 3, 24 or 48 h.

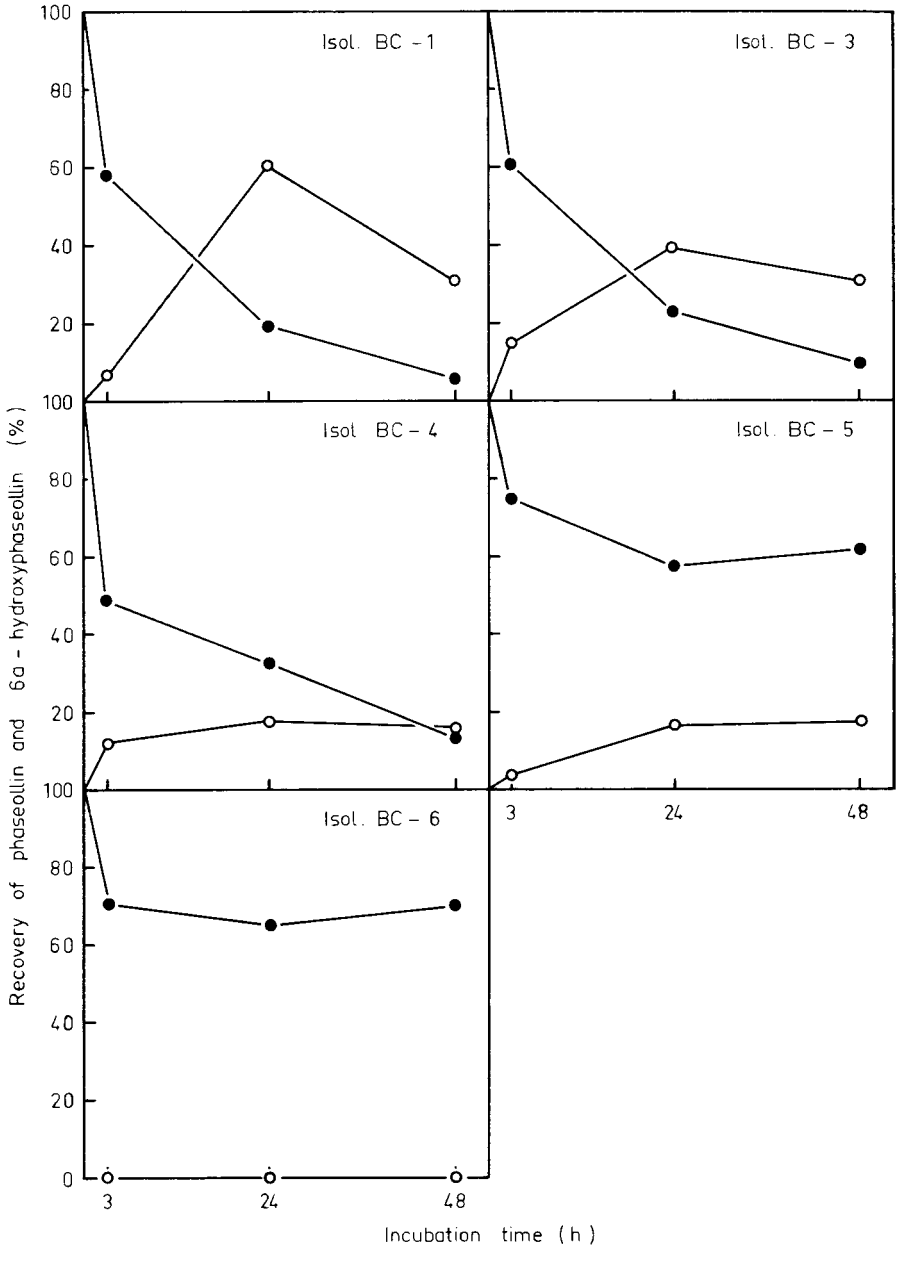


*Fig. 3. Hoeveelheid teruggevonden phaseolline (●) en zijn omzettingsprodukt, 6a-hydroxyphaseolline (○), in gechromatografeerde extracten van schudculturen van vijf isolaten van Botrytis cinerea,na incubatie met 9μg phaseolline/ml gedurende 3, 24 of 48 h.*

*Metabolism of phaseollin.* Phaseollin disappeared from shake cultures of the three pathogenic isolates at similar rates (Fig. 3). Less than 15% of the original amount of phaseollin was left in these cultures after incubation for 48 h. Concurrent with the disappearance of phaseollin ($R_f$ 0.68), a metabolic product appeared which was identified as 6a-hydroxyphaseollin, by $R_f$ value (0.41), reaction with diazotized *p*-nitroaniline spray reagent, and UV spectrometry (Van den Heuvel and Glazener, 1975). This product also tended to disappear from the cultures, but no other metabolite(s) was (were) detected.

Little phaseollin disappeared from the cultures of both nonpathogenic *B. cinerea* isolates. Some 6a-hydroxyphaseollin was produced in cultures of isolate BC-5, however, whilst no metabolite was detected in cultures of isolate BC-6. When cultures of the latter isolate were exposed to even lower concentrations of phaseollin, viz. 3 and 6 μg/ml, there was still no apparent metabolism of phaseollin. Therefore, the inability of this isolate to metabolize phaseollin could not be ascribed to its relatively higher sensitivity to phaseollin.

## Discussion

Evidence has been obtained that pathogenicity of *B. cinerea* isolates to bean is associated with their ability to metabolize phaseollin in vitro readily, whereas nonpathogenicity is associated with a limited ability or an inability to do so. No clear correlation was found between sensitivity to phaseollin and (non)pathogenicity to bean, when comparing the nonpathogenic isolates BC-5 and BC-6, however, both a higher sensitivity to phaseollin and a greater inability to metabolize phaseollin were found to be correlated with the more pronounced nonpathogenicity of the latter isolate to bean.

Metabolism of phaseollin by *B. cinerea* apparently always takes place via the intermediate 6a-hydroxyphaseollin, which is less inhibitory to *B. cinerea* than phaseollin (Van den Heuvel and Glazener, 1975). Hence, this conversion may be regarded as part of a detoxification process. Further steps of this detoxification process, such as the metabolism of 6a-hydroxyphaseollin, are still unclear. In no case was 6a,7-dihydroxyphaseollin, as produced by *Colletotrichum lindemuthianum* (Van den Heuvel and Vollaard, 1976), or any other metabolite of phaseollin detected.

If the correlation between ability to metabolize phaseollin and pathogenicity to bean is not merely coincidental, the differences in ability to detoxify phaseollin may be responsible for the differential reactions in infected bean plants. It seems reasonable that an isolate with a marked ability to metabolize phaseollin, may keep the level, after its induction in the infected tissue low. This might therefore contribute to the fungus being able to cause spreading lesions. On the other hand, in bean leaves infected with a nonpathogenic isolate, toxic levels of the phytoalexin may accumulate, which strongly inhibit the fungus, so that only lesions limited in size are formed. Research on the accumulation of phaseollin and its metabolite, 6a-hydroxyphaseollin, and other phytoalexins in bean tissues infected with different *B. cinerea* isolates is in progress.

## Samenvatting

*Gevoeligheid voor en omzetting van phaseolline in relatie tot de pathogeniteit van verschillende isolaten van Botrytis cinerea voor boon (Phaseolus vulgaris)*

De pathogeniteit van vijf verschillende isolaten van *Botrytis cinerea* voor boon *(Phaseolus vulgaris)* werd vergeleken met hun gevoeligheid voor het fytoalexine phaseolline en hun vermogen om phaseolline om te zetten.

Drie van de vijf isolaten bleken pathogeen voor boon te zijn, omdat ze op bonebladeren zich uitbreidende lesies vormden. De twee andere isolaten werden als niet-pathogeen voor boon beschouwd, aangezien ze op bonebladeren alleen lesies van beperkte omvang of in het geheel geen lesies vormden (Fig. 1). De isolaten waren alle ongeveer even gevoelig voor phaseolline, behalve een niet-pathogeen isolaat, dat relatief sterker geremd werd door hogere concentraties phaseolline (Fig. 2). In schudculturen, waaraan 9 $\mu$g phaseolline/ml was toegevoegd, werd phaseolline door de drie pathogene isolaten vrij vlot omgezet tot 6a-hydroxyphaseolline, maar door de twee niet-pathogene isolaten niet of slechts in geringe mate (Fig. 3).

Geconcludeerd wordt dat er bij de onderzochte isolaten van *B. cinerea* geen duidelijke correlatie bestaat tussen gevoeligheid voor phaseolline en pathogeniteit voor boon, maar wel tussen vermogen tot omzetting van phaseolline en pathogeniteit voor boon.



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## References


Bailey, J. A., 1974. The relationship between symptom expression and phytoalexin concentration in hypocotyls of *Phaseolus vulgaris* infected with *Colletotrichum lindemuthianum*. Physiol. Pl. Path. 4: 477–488.

Christenson, J. A. & Hadwiger, L. A., 1973. Induction of pisatin in the pea foot region by pathogenic and nonpathogenic clones of *Fusarium solani*. Phytopathology 63: 784–790.

Cruickshank, I. A. M., 1962. Studies on phytoalexins. IV. The antimicrobial spectrum of pisatin. Aust. J. biol. Sci. 15: 147–159.

Cruickshank, I. A. M. & Perrin, Dawn R., 1971. Studies on phytoalexins. XI. The induction, antimicrobial spectrum and chemical assay of phaseollin. Phytopath. Z. 70: 209–229.

Harrower, K. M., 1973. Differential effects of phytoalexin from *Pisum sativum* on two races of *Ascochyta pisi*. Trans. Br. mycol. Soc. 61: 383–386.

Heuvel, J. van den & Glazener, Judy A., 1975. Comparative abilities of fungi pathogenic and non-pathogenic to bean *(Phaseolus vulgaris)* to metabolize phaseollin. Neth. J. Pl. Path. 81: 125–137.

Heuvel, J. van den & VanEtten, H. D., 1973. Detoxification of phaseollin by *Fusarium solani* f. sp. *phaseoli*. Physiol. Pl. Path. 3: 327–339.

Heuvel, J. van den & Vollaard, P. J., 1976. Metabolism of phaseollin by different races of *Colletotrichum lindemuthianum*. Neth. J. Pl. Path. 82: 103–108.

Higgins, Verna J. & Millar, R. L., 1970. Degradation of alfalfa phytoalexin by *Stemphylium loti* and *Colletotrichum phomoides*. Phytopathology 60: 269–271.

Jones, D. R., Unwin, C. H. & Ward, E. W. B., 1975. The significance of capsidiol induction in pepper fruit during an incompatible interaction with *Phytophthora infestans*. Phytopathology 65: 1286–1288.

Land, B. G. van 't, Wiersma-van Duin, E. D. & Fuchs, A., 1975. In vitro and in vivo conversion of pisatin by *Ascochyta pisi*. Acta bot. neerl. 24: 251 (Abstr.).
Mansfield, J. W. & Deverall, B. J., 1974. Changes in wyerone acid concentrations in leaves of *Vicia faba* after infection by *Botrytis cinerea* or *B. fabae*. Ann. appl. Biol. 77: 227–235.
Mansfield, J. W. & Widdowson, D. A. 1973. The metabolism of wyerone acid (a phytoalexin from *Vicia faba* L.) by *Botrytis fabae* and *B. cinerea*. Physiol. Pl. Path. 3: 393–404.
Nonaka, F., 1967. Inactivation of pisatin by pathogenic fungi. Agric. Bull. Saga Univ. 24: 109–121.
Perrin, Dawn R., Biggs, D. R. & Cruickshank, I. A. M., 1974. Phaseollidin, a phytoalexin from *Phaseolus vulgaris:* isolation, physicochemical properties and antifungal activity. Aust. J. Chem. 27: 1607–1611.
Pueppke, S. G. & VanEtten, H. D., 1974. Pisatin accumulation and lesion development in peas infected with *Aphanomyces euteiches*, *Fusarium solani* f. sp. *pisi*, or *Rhizoctonia solani*. Phytopathology 64: 1433–1440.
Sakuma, T. & Millar, R. L., 1972. Relative abilities of pathogens and nonpathogens of alfalfa to induce production of and degrade medicarpin. Phytopathology 62: 499 (Abstr.).
Uritani, I. & Kojima, M., 1975. Difference in sensitivity to sweet potato phytoalexins between pathogenic and nonpathogenic strains of *Ceratocystis fimbriata*. Abstr. XIIth int. bot. Congr., Leningrad: 495.
Van Etten, H. D., 1973. Differential sensitivity of fungi to pisatin and phaseollin. Phytopathology 63: 1477–1482.
VanEtten, H. D. & Smith, D. A., 1975. Accumulation of antifungal isoflavonoids and 1a-hydroxyphaseollone, a phaseollin metabolite, in bean tissue infected with *Fusarium solani* f. sp. *phaseoli*. Physiol. Pl. Path. 5: 225–237.
Ward, E. W. B., Unwin, C. H. & Stoessl, A., 1974. Postinfectional inhibitors from plants. XIII. Fungitoxicity of the phytoalexin, capsidiol, and related sesquiterpenes. Can. J. Bot. 52: 2481–2488.
Wit-Elshove, Annemarie de, 1969. The role of pisatin in the resistance of pea plants – some further experiments on the breakdown of pisatin. Neth. J. Pl. Path. 75: 164–168.

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