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Comparative abilities of fungi pathogenic and nonpathogenic to bean (Phaseolus vulgaris) to metabolize phaseollin

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Abstract

The abilities of fungi pathogenic and nonpathogenic to bean (*Phaseolus vulgaris*) to metabolize the phytoalexin phaseollin were compared when grown in shake cultures containing 12 to 15 µg phaseollin/ml. Under these conditions phaseollin was metabolized by five out of seven pathogens and by three out of five nonpathogens.

Disappearance of phaseollin was accompanied by the appearance of metabolic products in cultures of *Fusarium solani* f. sp. *phaseoli*, *Colletotrichum lindemuthianum*, *Botrytis cinerea* and *Cladosporium herbarum*. The nonpathogen *C. herbarum* detoxified phaseollin to 1a-hydroxyphaseollone as rapidly as the pathogen *F. solani* f.sp. *phaseoli*. Phaseollin was converted to 6a-hydroxyphaseollin by the pathogens *B. cinerea* and *C. lindemuthianum*, and this product was further metabolized by the latter fungus. 6a-Hydroxyphaseollin was less fungitoxic to *B. cinerea*. *C. lindemuthianum* was equally sensitive to both compounds.

Phaseollin was not metabolized by the pathogens *Fusarium oxysporum* f. sp. *phaseoli* and *Thielaviopsis basicola*.

Introduction

In a number of cases smaller amounts of phytoalexins have been found in plant tissues showing susceptible reactions when infected with pathogenic fungi as compared with tissues reacting hypersensitively following infection with nonpathogens, at least at earlier stages of infection (Christenson and Hadwiger, 1973; Hess et al., 1971; De Wit-Elshove and Fuchs, 1971). Such reduced accumulations of phytoalexins might result from the induction of less phytoalexin production by pathogens or from partial conversion of phytoalexin by pathogens. Several studies have demonstrated that pathogenic fungi were able in vitro to metabolize phytoalexins produced by their respective host plants (Bailey, 1974; Christenson and Hadwiger, 1973; Cruickshank et al., 1974; Van den Heuvel and VanEtten, 1973; Higgins and Millar, 1970; Mansfield and Widdowson, 1973; Nonaka, 1967; Sakuma and Millar, 1972; Stoessl et al., 1973; De Wit-Elshove, 1969), whereas nonpathogens were unable or less able to do so (Christenson and Hadwiger, 1973; Higgins and Millar, 1970; Nonaka, 1967; De Wit-Elshove, 1969). However, a few cases have been reported of nonpathogens readily metabolizing such phytoalexins (Heath and Higgins, 1973; Sakuma and Millar, 1972; Stoessl et al., 1973). Some pathogens metabolized the host phytoalexin within infected plant tissues, as their metabolites were found in tissue extracts (Mansfield and Widdowson, 1973; VanEtten and Smith, 1975).

Information on the metabolism of phaseollin, the principal phytoalexin of bean, by fungal species is scarce. Phaseollin was readily metabolized in vitro by the bean pathogens *Fusarium solani* f. sp. *phaseoli* and *Colletotrichum lindemuthianum* (Bailey, 1974; Cruickshank et al., 1974; Van den Heuvel and VanEtten, 1973) and by *Stemphylium botryosum*, a pathogen of alfalfa, being nonpathogenic to bean (Heath and Higgins, 1973). VanEtten and Smith (1975) showed that *F. solani* f. sp. *phaseoli* was also able to metabolize phaseollin in infected bean hypocotyls, since its metabolic product, 1a-hydroxyphaseollone, was found in tissue extracts.

In general, mycelial growth of bean pathogens was found to be less sensitive to inhibition by phaseollin than was growth of nonpathogens (Cruickshank and Perrin, 1971; VanEtten, 1973). This might be correlated with a greater ability of pathogens to metabolize or detoxify phaseollin. The purpose of this investigation was to determine whether pathogenicity to bean is related to such an ability in broth culture.

Materials and methods

Fungal isolates. Seven pathogens and five nonpathogens of bean (*Phaseolus vulgaris* L.) were compared for their abilities to metabolize phaseollin in vitro.

The pathogens used in this study were orginally isolated from diseased *P. vulgaris* tissues, unless otherwise stated, and were: *Botrytis cinerea* Pers. ex Fr., *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. race α 2, *Fusarium oxysporum* Schlecht. ex Fr. f. sp. *phaseoli* Kend. & Snyd. (CBS strain 935.73), *Fusarium solani* (Mart.) Appel & Wollenw. f. sp. *phaseoli* (Burkh.) Snyd. & Hans., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Thielaviopsis basicola* (Berk. & Br.) Ferraris (CBS strain 150.67, from tobacco).

Nonpathogens used were: *Alternaria zinniae* Pape, *Ascochyta pisi* Lib. race D2, *Aureobasidium pullulans* (de Bary) Arnaud isolate A-36 (Van den Heuvel, 1970b), *Cladosporium herbarum* (Pers.) Link ex Fr. isolate A-133 (Van den Heuvel, 1970b) and *Fusarium solani* (Mart.) Appel & Wollenw. f. sp. *cucurbitae* Snyd. & Hans. Although *A. zinniae* can cause large necrotic spots on bean leaves in darkness (Van den Heuvel, 1970a), it forms only small limited lesions under natural light conditions and is, therefore, regarded here as a nonpathogen of bean.

The isolates of *C. lindemuthianum* and *A. pisi* were kindly provided by N. Hubbeling, IPO, Wageningen. Isolates of *F. oxysporum* f. sp. *phaseoli* and *T. basicola* were obtained from Centraalbureau voor Schimmelcultures (CBS), Baarn.

Stock cultures of most fungi were maintained on slants of PDA at 2° or 15 °C.

Preparation of shake cultures. The methods used for growing the fungi and preparing shake cultures were essentially the same as described by Van den Heuvel and VanEtten (1973).

Spore suspensions, prepared from 1- to 2-week-old cultures of sporulating fungi grown on PDA, oatmeal agar or malt agar at 21° to 23 °C under dark or light conditions, were used as starting material. The spore suspensions were filtered through one to four layers of cheesecloth, loosely woven cotton gauze or glass wool to remove larger mycelial fragments. The filtrates were centifuged at 3,000 *g* for 10 min at 4 °C, or at 12,000 *g* for 20 min at 4 °C (*A. zinniae* only). The pellets were resuspended in glucose-asparagin (GA) liquid medium (Van den Heuvel and VanEtten, 1973) or

carrot root extract (Mathre and Ravenscroft, 1966; *T. basicola* only) to give a spore concentration of 7×10^5 spores/ml. One to three 250-ml portions were added to 1000-ml Erlenmeyer flasks and incubated at 23 °C on a reciprocal shaker (c. 130 strokes/min) for 24 h.

Nonsporulating fungi (*R. solani* and *S. sclerotiorum*) were grown as still cultures on liquid GA-medium at 23 °C for 5 to 10 days. About five mycelial mats of each fungus were rinsed with sterile distilled water and slightly homogenized in c. 250 ml fresh GA-medium (Sorvall Omnimixer, setting 2 or 3, two or three times 15 sec). The homogenate was incubated at 23 °C on a reciprocal shaker for 24 h as above. Further steps in the procedure were the same for sporulating and nonsporulating fungi.

After the 24-h period of shaking, three 5-ml samples of the actively growing mycelium of each fungus were collected on tared Whatman No. 50 filter paper using a vacuum filtration apparatus and weighed after drying at 80 °C for 1 h. Portions equivalent to 6 mg (dry weight) of the mycelium remaining in the shake cultures were then collected on Schleicher & Schüll No. 595 filter paper and resuspended in 4 ml of fresh GA-medium in 25-ml Erlenmeyer flasks. These cultures were incubated on a shaker as above, allowing a 30-min adaptation period before treatment with phaseollin.

Purified crystalline phaseollin was prepared by the prodecure of VanEtten and Bateman (1970). Concentrations of this compound were determined spectrophotometrically from the extinction (E) at 280 nm using the conversion factor $E^{1\,cm}_{280\,nm}$ 1.00 = 32.9 µg/ml (Cruickshank and Perrin, 1971). Phaseollin was added to the cultures in dimethylsulfoxide (DMSO) to give a final DMSO concentration of 0.5%. The DMSO was required to keep phaseollin in solution in the media. The final phaseollin concentration in the media was, unless otherwise stated, between 12 and 15 µg/ml. Most fungi grew well in shake cultures containing such relatively low concentrations of phaseollin, as was measured by determining fungal dry weights after incubation for 24 h. Control cultures received DMSO without phaseollin. In most experiments eight cultures were used for extraction of phaseollin and its metabolic products.

Extraction of phaseollin and its metabolic products. After the cultures had been shaken with phaseollin for 3, 24 or 48 h, or without phaseollin for 24 h, 4 ml 96% ethanol was added to each flask to halt metabolic activity and to extract phaseollin and similar compounds from the mycelium. The mycelium was removed by filtration through Schleicher & Schüll No. 595 filter paper and rinsed twice with 1 ml of 50% ethanol. Ethanol was removed from the filtrates by evaporation under reduced pressure. The aqueous fraction remaining was partitioned twice with 10 ml of chloroform. The chloroform fractions were combined and evaporated to dryness. The residue was dissolved in 1.00 ml of 1-propanol; 0.85 ml of this solution was transferred to a small vial and dried under a stream of nitrogen.

Qualitative and quantitative analysis of phaseollin and its metabolic products. Thin-layer chromatography (TLC) and in situ densitometry were used for qualitative and quantitative analysis of phaseollin and its metabolic products. The residues dried under nitrogen were redissolved in 35 µl of 1-propanol; 25-µl volumes of these solutions were spotted on precoated silica gel TLC plates (Merck Silica gel 60 F_{254},

layer thickness 0.25 mm), previously scored into 18 mm wide lanes. Samples of phaseollin and 1a-hydroxyphaseollone were spotted as reference compounds. The chromatograms were developed in a tank saturated with benzene: ethyl acetate: methanol (25:8:4) as solvent system.

Qualitative analysis of phaseollin and its metabolic products was accomplished by comparing R_f values of components of chromatographed extracts with those of reference compounds, reactions with spray reagents and UV and mass spectrometry.

Quantitative measurement of phaseollin and its metabolic products was accomplished by in situ densitometry of a developed and dried TLC plate using a Vitatron TLD 100 flying spot densitometer equipped with a recorder. A mercury lamp supplied with an UVB liquid filter irradiated the plate with ultraviolet light in the 254 nm region to induce fluorescence of the plate. Quenching of fluorescence by substances present on the plate was measured by a readout unit containing a 0.25 mm diaphragm, a 525 nm filter and a photomultiplier. The plate was scanned in the direction of development at a speed of 3 cm/min. At the same time the plate was rapidly moving to and fro perpendicularly to the scan axis, which resulted in a two-dimensional scanning of each lane between two scored lines. Each sample was scanned twice and relative peak areas were averaged. Phaseollin was quantitated using a standard curve prepared from three duplicate samples of different known quantities of phaseollin applied to each assay plate. Metabolic products of phaseollin were quantitated using the same standard curve, on the assumption that such products would cause a similar specific fluorescence quenching.

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 a fungus, was investigated after shaking for 3, 24 or 48 h. In these experiments 74 to 80% (average 77%) of added phaseollin was recovered, together with two unidentified products, one of them probably being identical with compound C (Van den Heuvel and VanEtten, 1973). The average relative abundances of these compounds were 11 and 9%. Had these products not been formed, the recovery of phaseollin would have been about 97%. It is not known whether and at what time these products of abiotic degradation of phaseollin were formed during shaking or extraction. After different shaking periods similar amounts of these compounds were found. Extracts from fungal cultures contained no detectable or only small varying quantities of such substances. In view of these results only actually recovered percentages of phaseollin and its metabolites are presented.

Results

Metabolism of phaseollin by pathogens. Phaseollin disappeared for the greater part from shake cultures of four out of seven pathogens within a period of 48 h (Fig. 1 and 2). At the same time metabolic products of phaseollin were detected in cultures of *F. solani* f. sp. *phaseoli*, *B. cinerea* and *C. lindemuthianum*. In cultures of *R. solani* a substance (R_f 0.55) accumulated slowly to a relative abundance of 16% after 48 h of incubation; it is uncertain whether this substance was a metabolic product of phaseollin.

In cultures of *S. sclerotiorum* phaseollin disappeared slowly to 67% of the original

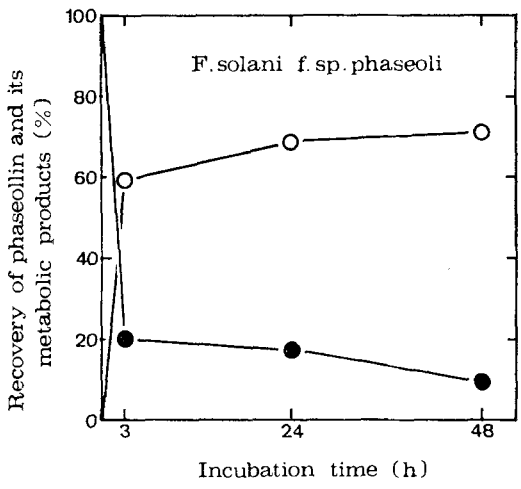

Fig. 1. Recovery of phaseollin (●) and its metabolic product (○) from chromatographed extracts of shake cultures of *F. solani* f. sp. *phaseoli*, a fungus pathogenic to bean, incubated with 12 to 15 μg phaseollin /ml for 3, 24 or 48 h.

Fig. 1. Hoeveelheid teruggevonden phaseolline (●) en zijn omzettingsprodukt (○) in gechromatografeerde extracten van schudculturen van F. solani f. sp. phaseoli, een schimmel pathogeen voor boon, na incubatie met 12 tot 15 μg phaseolline/ml gedurende 3, 24 of 48 uur.

amount added. Since in this experiment mycelial growth was only 28% compared with growth in cultures without phaseollin, the experiment was repeated with a lower concentration of phaseollin (6.5 μg/ml) added to the cultures. Although in the latter experiment phaseollin did not inhibit mycelial growth, a similar proportion (70%) of the phytoalexin remained in the cultures after 48 h of incubation. In this case, however, a substance with a low R_f value (0.09) accumulated in the cultures to a concentration equivalent to 27% of added phaseollin after 48 h of incubation, whereas only a trace (approximately 6%) of this compound was detected in the experiment previously mentioned. Again, it is uncertain whether this product was a metabolite of phaseollin. In view of these results, it is assumed that *S. sclerotiorum* has the ability to metabolize phaseollin, though slowly.

Two bean pathogens, viz. *F. oxysporum* f. sp. *phaseoli* and *T. basicola*, apparently did not metabolize phaseollin. Since the strains of both pathogens used were not recent isolates, their pathogenicity to bean was checked by inoculation experiments. Eleven-day-old seedlings of the bean cultivars Red Kidney and Dubbele Witte z. dr. were placed in suspensions containing spores of one of both fungi for 1 h, and were then planted in pots with steamed soil and kept at 17° to 25°C in the greenhouse. Two weeks after inoculation, all plants inoculated with *T. basicola* showed numerous dark-brown lesions on the roots. Three weeks after inoculation, a small percentage of the plants inoculated with *F. oxysporum* f. sp. *phaseoli* showed yellowing of leaves or discolouration of vascular bundles of the stem or both. These results confirm the pathogenicity of both fungal strains to bean.

Metabolism of phaseollin by nonpathogens. Two out of five nonpathogenic fungi, viz. *A. pullulans* and *F. solani* f. sp. *cucurbitae*, were not able to metabolize phaseollin (Fig. 3). Although only 72% of added phaseollin remained in shake cultures of *A. pullulans* after 48 h of incubation, in another experiment where 7.1 μg of phaseollin/ml was added to such cultures, 84% of the phaseollin was still detected after 48 h.

Fig. 2. Recovery of phaseollin (●) and its metabolic products (○, □) from chromatographed extracts of shake cultures of six fungi pathogenic to bean, incubated with 12 to 15 μg phaseollin/ml for 3, 24 or 48 h.

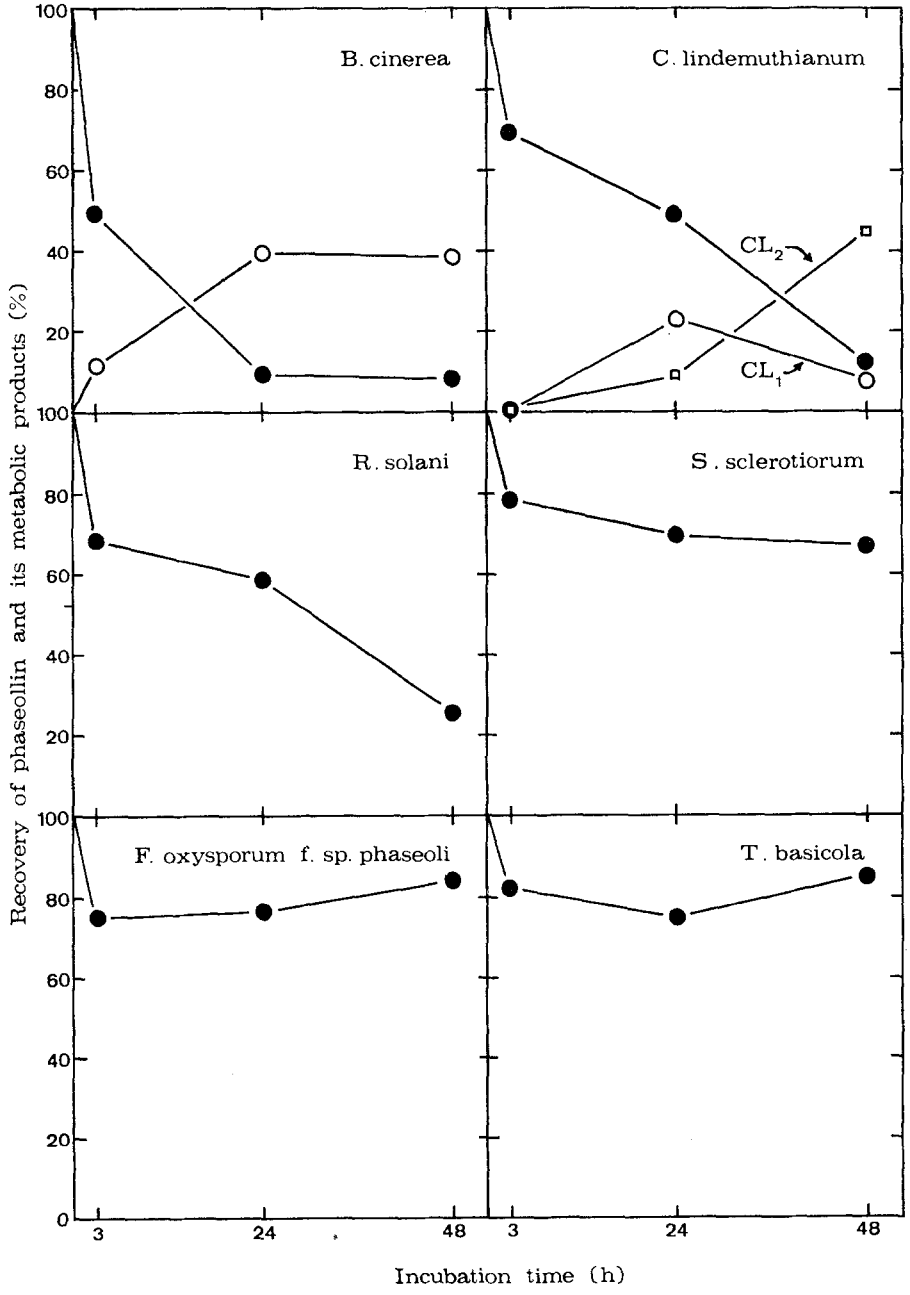

Fig. 2. Hoeveelheid teruggevonden phaseolline (●) en zijn omzettingsprodukten (○, □) in gechromatografeerde extracten van schudculturen van zes schimmels, pathogeen voor boon, na incubatie met12 tot 15 μg phaseolline/ml gedurende 3, 24 of 48 uur.

Fig. 3. Recovery of phaseollin (●) and its metabolic products (○) from chromatographed extracts of shake cultures of five fungi nonpathogenic to bean, incubated with 12 to 15 μg phaseollin/ml for 3, 24 or 48 h.

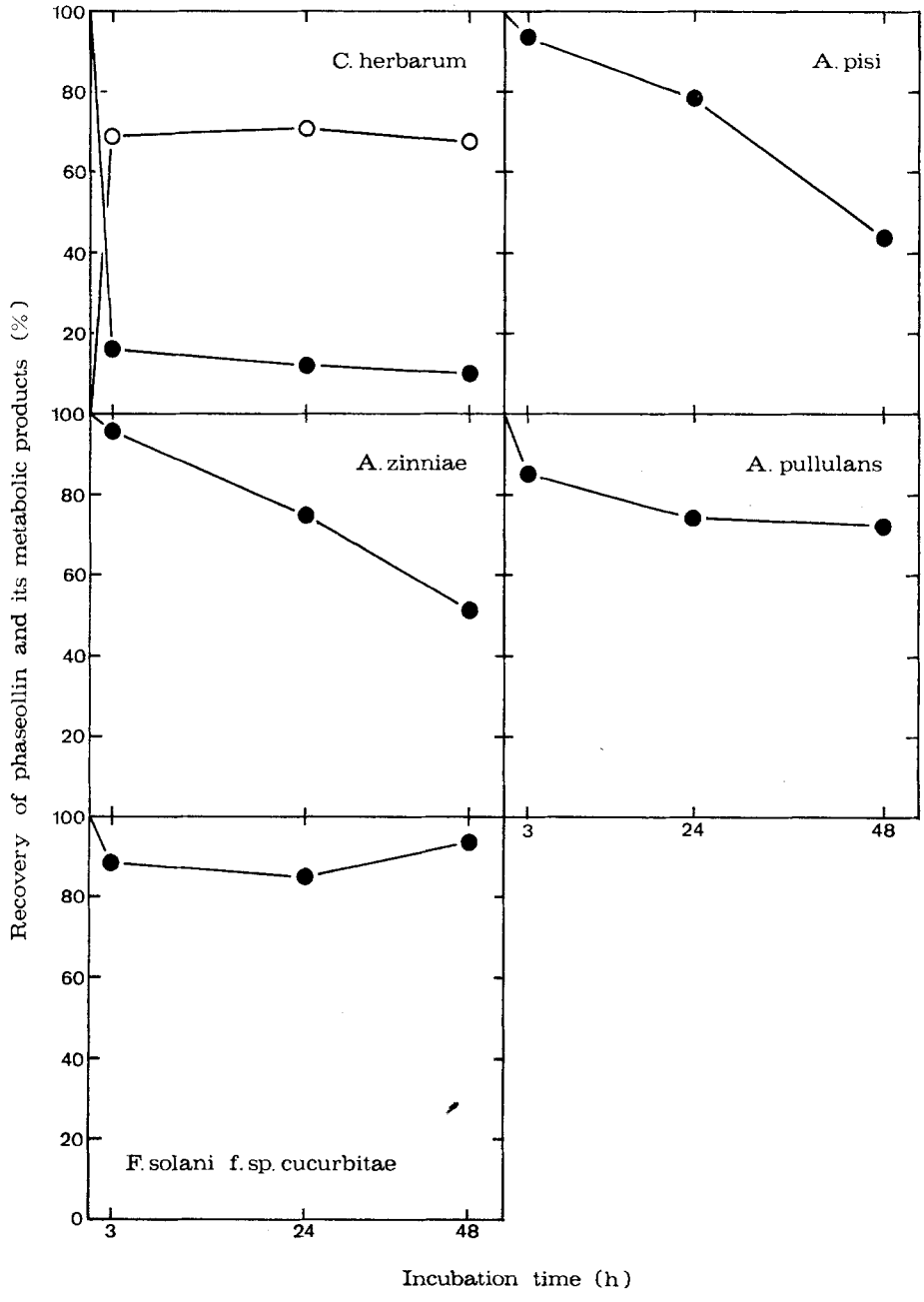

Fig. 3. Hoeveelheid teruggevonden phaseolline (●) en zijn omzettingsprodukten (○) in gechromatografeerde extracten van schudculturen van vijf schimmels, niet-pathogeen voor boon, na incubatie met 12 tot 15 μg phaseolline/ml gedurende 3, 24 of 48 uur.

Three other nonpathogens assayed showed an ability to metabolize phaseollin. In cultures of *A. pisi* and *A. zinniae* phaseollin disappeared rather slowly to 44 and 52%, respectively, after 48 h of incubation. In cultures of *C. herbarum*, however, phaseollin was rapidly converted to a metabolic product (R_f 0.45) that accumulated to 69% during the first hours of incubation.

Identification of the metabolic products

F. solani f. sp. phaseoli and C. herbarum. The compound produced by these fungi proved to be 1a-hydroxyphaseollone, which, in earlier experiments (Van den Heuvel and VanEtten, 1973; Van den Heuvel et al., 1974), was found to be a detoxification product of phaseollin formed by *F. solani* f. sp. *phaseoli*. The product was identical with an authentic sample of 1a-hydroxyphaseollone, as compared by R_f value (0.41–0.47) following co-chromatography on thin-layer silica gel plates, reaction with spray reagents, and UV spectrum. A mass spectrum of the metabolite formed by *C. herbarum* was identical with that of an authentic sample of la-hydroxyphaseollone.

B. cinerea. The phaseollin metabolite produced by *B. cinerea* is a white solid that is stable to basic but sensitive to acid conditions. It reacts positively with a number of spray reagents for phenolic compounds (diazotized *p*-nitroaniline, diazotized benzidine, vanillin in H_2SO_4, Fast Blue Salt B). It exhibits UV absorption in ethanol at λ_{max} (log ε) 207 (4.50), 229 (4.50), 280 (4.00), 286 (3.97) and 313 (3.44) nm, the latter peak almost appearing as a broad shoulder at 306–315 nm. Its UV absorption is shifted in base (2×10^{-3} M Na-ethoxide in ethanol) to give λ_{max} (log ε) 229 (4.50), 254 (4.18), 284 (4.01) and 290 (4.01) nm, with a shoulder around 320 (3.48) nm. The optical rotation of this compound is $[\alpha]_{578}^{20} = -167°$ (MeOH), such a high negative rotation being characteristic for pterocarpans.

IR, mass and NMR spectra of the metabolite were in good agreement with those reported by Burden et al. (1974) for 6a-hydroxyphaseollin, this being the structure proposed for their metabolite 1, a phaseollin metabolite produced by *C. lindemuthianum*. Direct comparison of our metabolite with a sample of metabolite 1, kindly provided by J. A. Bailey, ARC Unit of Plant Growth Substances and Systemic Fungicides, Wye College, Wye, showed that both compounds were identical in R_f value following co-chromatography on silica gel thin layers plates, reaction with spray reagents, and UV spectrum. Treatment of our metabolite with hydrochloric acid yielded a product with characteristics identical with those obtained by Burden et al. (1974) after treatment of their metabolite 1 with formic acid, confirming the presence of a tertiary hydroxyl group at C_{6a}. Methylation of our compound, $C_{20}H_{18}O_5$ (M^+ 338), with diazomethane yielded a monomethylether with m/e 352 ($C_{21}H_{20}O_5$, 46%), 337 (100%), 334 (7%), 319 (13%) and 309 (37%); as would be expected, the hydroxyl group at C_{6a} did not react.

6a-Hydroxyphaseollin has been proposed by Sims et al. (1972) as the structure of a phytoalexin from soybean. Our phaseollin metabolite of B. *cinerea* differed slightly from a sample of the soybean phytoalexin, kindly provided by N.T. Keen, Department of Plant Pathology, University of California, Riverside, in R_f value following co-chromatography on silica gel thin layer plates, reaction with vanillin/H_2SO_4 spray reagent, and UV spectrum.

However, the results presented here and by Burden et al. (1974) justify the conclusion that the phaseollin metabolite of *B. cinerea* is identical with 6a-hydroxyphaseollin.

C. lindemuthianum. Two metabolic products were found, tentatively called compounds CL_1 and CL_2. After 24 h of incubation more of compound CL_1 than of compound CL_2 had accumulated; after 48 h compound CL_1 had decreased, whereas compound CL_2 had increased strongly. This indicates that phaseollin was first converted to compound CL_1 and that the latter was further converted to compound CL_2. These products might well be identical with the phaseollin metabolites 1 and 2 produced by *C. lindemuthianum* race γ as described by Bailey (1974) and Burden et al. (1974).

Compound CL_1 appeared to be identical with 6a-hydroxyphaseollin, when comparisons were made utilizing R_f values following co-chromatography on thin-layer silica gel plates, reaction with diazotized *p*-nitroaniline spray reagent, and UV and mass spectral analysis.

Compound CL_2 seemed at first to be identical with metabolite 2 of *C. lindemuthianum* race γ, since its R_f values following thin-layer chromatography, its reaction with diazotized *p*-nitroaniline reagent, and its UV and mass spectrum were in agreement with those reported for metabolite 2 (Burden et al., 1974). However, the positive reaction of compound CL_2 with Gibbs' reagent does not agree with the proposed structure of metabolite 2, since Gibbs' reagent gives a positive reaction only with phenols having an unsubstituted position *para* to the hydroxyl group (King et al., 1957). This indicates that compound CL_2 contains a hydroxyl group at either C_1 or C_4. A negative reaction with molybdate reagent (Pridham, 1959) indicated the absence of an *ortho*-dihydroxyl group, in this case at C_3 and C_4. These results are in favour of 1,6a-dihydroxyphaseollin as being the structure for compound CL_2, rather than 6a, 7-dihydroxyphaseollin as proposed for metabolite 2 by Burden et al. (1974). The possibility should not be excluded that different races of *C. lindemuthianum* may metabolize 6a-hydroxyphaseollin in different ways.

Antifungal activity of phaseollin and 6a-hydroxyphaseollin

B. cinerea (isolate from bean), *B. cinerea* (CBS strain 121.39, from grape, *F. solani* f. sp. *cucurbitae* and *C. lindemuthianum* race α 2 were used for determining the inhibitory activities of phaseollin and its derivative, 6a-hydroxyphaseollin. The bioassays carried out on 1.0 ml of medium in small (35 mm diameter) Petri plates were essen-

Table 1. Inhibition of radial mycelial growth of fungi by phaseollin and 6a-hydroxyphaseollin

Fungi	Percent inhibition	
	phaseollin (0.05 mM = 16.1 μg/ml)	6a-hydroxyphaseollin (0.05 mM = 16.9 μg/ml)
B. cinerea (isol. bean)	46	27
B. cinerea (isol. grape)	63	20
F. solani f. sp. *cucurbitae*	53	38
C. lindemuthianum race α 2	21	21

Tabel 1. Remming van de radiale myceliumgroei van schimmels door phaseolline en 6a-hydroxyphaseolline.

tially as described previously (Van den Heuvel and VanEtten, 1973). Net radial mycelial growth was determined by measuring two diameters of each of two duplicate colonies and subtracting the diameter of the inoculum plug. Growth of the fungi was measured after 2 to 4 days of incubation, when growth in the controls had almost reached the edge of the plate, except in the case of *C. lindemuthianum*, which grew very slowly.

The metabolite 6a-hydroxyphaseollin is less fungitoxic than phaseollin to three out of the four fungi, but is as toxic as phaseollin to *C. lindemuthianum* (Table 1). Similar results were obtained when the effects of a higher concentration (0.1 mM) of phaseollin and 6a-hydroxyphaseollin were compared.

Discussion

Most fungi grew well in shake cultures containing relatively low concentrations of phaseollin (12 to 15 μg/ml). The mycelial growth of four out of thirteen fungi (viz. *C. lindemuthianum*, *R. solani*, *S. sclerotiorum* and *A. pisi*), however, was less than 50% compared to growth in cultures without phaseollin, yet, these fungi were able to metabolize phaseollin. Addition of smaller amounts of phaseollin to cultures of *S. sclerotiorum* reduced inhibition of mycelial growth, but did not increase phaseollin metabolism. In preliminary experiments (not reported here) with cultures of *C. lindemuthianum* containing lower concentrations of phaseollin an accelerated conversion of compound CL_1 to CL_2 was observed.

Five out of seven bean pathogens were able to metabolize phaseollin, most of them following a different pathway. *F. solani* f. sp. *phaseoli*, which was included mainly for comparison with other fungi, formed 1a-hydroxyphaseollone, this metabolite being less fungitoxic than phaseollin (Van den Heuvel and VanEtten, 1973). *C. lindemuthianum* formed two metabolites, CL_1 and CL_2, the former, identified as 6a-hydroxyphaseollin, appearing to be as toxic to *C. lindemuthianum* as phaseollin. The fungitoxicity and final characterization of compound CL_2 requires further study. It is as yet uncertain whether these two fungi would be able to convert their metabolites to less toxic or nontoxic products. Bailey (1974) showed that, in his system, *C. lindemuthianum* was able to degrade phaseollin via the antifungal intermediates 1 and 2, and he did not detect any further fungitoxic metabolites.

B. cinerea converted phaseollin to 6a-hydroxyphaseollin, which was less fungitoxic than phaseollin to each of two isolates of this fungus of different pathogenicity. Research is being carried out to determine whether or not differences in pathogenicity to bean between these and other isolates of *B. cinerea* are correlated with differences in their sensitivity to phaseollin or in their ability to metabolize this phytoalexin. Preliminary experiments with shake cultures containing 4 mg (dry weight) of *B. cinerea* mycelium/ml and lower concentrations of phaseollin indicated that 6a-hydroxyphaseollin was further metabolized without formation of detectable products. In no case was any metabolite similar to compound CL_2 detected.

Phaseollin or its metabolic products may finally be degraded to CO_2 and H_2O. This possibly occurred in cultures where phaseollin disappeared without formation of detectable products. The phytoalexin pisatin was reported to be broken down to CO_2 by some pea pathogens (De Wit-Elshove, 1969; De Wit-Elshove and Fuchs, 1971).

The ability of a fungus to metabolize phaseollin is not unique to bean pathogens, as was already shown for the alfalfa pathogen *Stemphylium botryosum* (Heath and Higgins, 1973; Higgins et al., 1974). This fungus was able to convert phaseollin to phaseollinisoflavan, which was not less fungitoxic than phaseollin. Phaseollin is metabolized by *A. pisi* and *A. zinniae*, pathogens of pea and zinnia, respectively, without formation of detectable products, and is converted by *C. herbarum*, an epiphytic organism isolated from bean leaves, to 1a-hydroxyphaseollone. This product was shown to be less fungitoxic to a wide variety of fungi (Van den Heuvel and VanEtten, 1973; VanEtten and Smith, 1975). It was suggested by the latter authors that many saprophytes may degrade plant tissue and, therefore, should have the ability to metabolize compounds such as phaseollin. On the other hand, *A. pullulans*, which was also isolated from bean leaves, did not possess this ability.

The results indicate that a fungus pathogenic to bean does not necessarily have the ability to metabolize phaseollin, as was shown for *F. oxysporum* f. sp. *phaseoli* and *T. basicola*. In addition, a fungus having the ability to metabolize phaseollin is not necessarily pathogenic to bean. It may be concluded that the pathogenicity to bean is not primarily determined by the ability of a fungus to metabolize or detoxify phaseollin and possibly also other phytoalexins in vitro. This ability may be of some help to a number of pathogens in colonizing bean tissues, and may, therefore, partly explain susceptibility of bean to these pathogens. Other factors may also determine pathogenicity to bean, such as a relative insensitivity of some pathogens to the actual amounts of phytoalexins accumulating in infected bean tissues.

Samenvatting

Vergelijkend onderzoek naar het vermogen van schimmels, pathogeen en niet-pathogeen voor boon (Phaseolus vulgaris) om phaseolline om te zetten

Een vergelijkend onderzoek werd verricht naar het vermogen van schimmels, al dan niet pathogeen voor boon (*Phaseolus vulgaris*), om het fytoalexine phaseolline om te zetten.

In schudculturen waaraan 12 tot 15 µg phaseolline/ml was toegevoegd, kon phaseolline worden omgezet door vijf van de zeven onderzochte pathogenen en door drie van de vijf getoetste niet-pathogenen (Fig. 1, 2 en 3).

Het verdwijnen van phaseolline ging gepaard met het verschijnen van omzettingsprodukten in culturen van *Fusarium solani* f. sp. *phaseoli*, *Colletotrichum lindemuthianum*, *Botrytis cinerea* en *Cladosporium herbarum*. De niet-pathogene schimmel *C. herbarum* detoxificeerde phaseolline tot 1a-hydroxyphaseollon even snel als het pathogeen *F. solani* f. sp. *phaseoli*. Phaseolline werd omgezet tot 6a-hydroxyphaseolline door de pathogenen *B. cinerea* en *C. lindemuthianum* en dit produkt werd door *C. lindemuthianum* weer verder omgezet tot een verwante verbinding. 6a-Hydroxyphaseolline was minder fungitoxisch dan phaseolline voor *B. cinerea*, maar even fungitoxisch voor *C. lindemuthianum* (Tabel 1).

Phaseolline werd niet omgezet door de bonepathogenen *Fusarium oxysporum* f. sp. *phaseoli* en *Thielaviopsis basicola*.

Geconcludeerd wordt dat er geen direkt verband bestaat tussen het vermogen van een schimmel tot omzetting of detoxificatie van phaseolline in vitro en zijn pathogeniteit voor boon.

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Book review

G. F. Weber: Bacterial and fungal diseases of plants in the tropics. University of Florida Press, 1973. 673 p., 210 ill. Price $ 22.50.

The number of general books on phytopathology in the tropics is still small, but slowly increasing. Weber's book is the first encyclopedia compiling all important bacterial and fungal diseases of as many crops as possible in one volume of reasonable size. The diseases are arranged alphabetically according to common names of host plants. The purpose of the book is to help diagnose the diseases from symptoms and characteristics of the pathogen.

About 1300 diseases of 94 host plants are described. Many food crops of importance for the local population are included. 46 diseases of maize are dealt with and another twenty names of pathogens 'associated with corn' are listed. Some selected literature references are given for each plant; 31 titles are included for maize.

Illustration is kept simple and printed on the same paper as the book; the 210 photographs show symptoms and details of the pathogens.

Some readers may expect more information on control measures. This subject is dealt with deliberately in general on three pages only and no details are given for each disease separately. The authors' aim was solely to make diagnosis easier. Moreover control measures and products are changing rapidly.

This book will be of use for students, advisers, lecturers and also farmers in tropical agriculture, because the author has succeeded in composing an attractive and easily readable volume.

D. Mulder