Neth. J. Pl. Path. 75 (1969) 58–71

Correlation of phenolic metabolism with histological changes in Phaseolus vulgaris inoculated with fungi[1]

J. E. RAHE, J. KUC', CHIEN-MEI CHUANG and E. B. WILLIAMS

Departments of Biochemistry and Botany and Plant Pathology, Purdue University, Lafayette, Ind., U.S.A.

Accepted 22 August, 1968

Abstract

Acetone powders were prepared at intervals after inoculation of *Phaseolus vulgaris*, variety 'Topcrop', with fungi inciting hypersensitive (*Helminthosporium carbonum*), resistant (*Colletotrichum lindemuthianum*, gamma race), and susceptible (*C. lindemuthianum*, beta race) host responses. Phenylalanine ammonia-lyase activity was determined in extracts of the acetone powders, and quantitative and qualitative measurements were made of phenolic compounds in the acetone filtrates obtained during powder preparation. The developmental morphology of the fungi on host tissue was observed histologically. The data obtained indicate that host-fungus physiology is accompanied by readily detectable and specific alterations of phenolic metabolism during various stages of infection and host response. Phaseollin production in hypocotyls inoculated with *C. lindemuthianum* accompanied the appearance of symptoms in resistant and susceptible reactions; it was produced earlier and in greater amount in resistant reactions.

Introduction

An accumulation of phenolic compounds is frequently observed in plant tissues following inoculation (Farkas and Kiraly, 1962; Kuc', 1966; Kuc', 1967). Fungitoxicity of phenolics is suggested to account, at least in part, for host resistance (Condon et al., 1963; Cruickshank and Perrin, 1963b). Phaseollin has been reported as a key substance in resistance of French bean (*Phaseolus vulgaris*) to certain fungi (Cruickshank and Perrin, 1963a; Pierre, 1966). This study correlates histological observations of host–parasite interactions in bean with changes induced in phenolic compounds and a key enzyme of phenolic metabolism. *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose, and *Helminthosporium carbonum*, race 1, a pathogen of corn which induces a hypersensitive response in bean, were selected because of the different host responses induced by these fungi.

Materials and methods

Growth of etiolated bean seedlings and fungi

Bean seeds were washed in tap water and germinated for 5 days in the dark in vermiculite moistened with Hoagland-Snyder nutrient solution. The seedlings were har-

[1] Journal Paper No. 3470 of the Purdue University Agricultural Experiment Station.

Table 1. Pattern of response of bean varieties to *H. carbonum* and *C. lindemuthianum*

Bean variety	*H. carbonum* (*race 1*)	*C. lindemuthianum*	
		(*beta race*)	(*gamma race*)
'Dark Red Kidney'	hypersensitive	susceptible	susceptible
'Tennessee Green Pod'	hypersensitive	resistant	resistant
'Topcrop'	hypersensitive	susceptible	resistant
'White Kidney'	hypersensitive	resistant	susceptible

Tabel 1. Reactiepatroon van enkele bonevarieteiten ten opzichte van H. carbonum and C. lindemuthianum

vested, washed in tap water after removal of seed coats, and placed in rag dolls. Incubation before and after inoculation was carried out in the dark in a moist chamber maintained at 22 °–24 °C. The host responses to the beta and gamma races of *C. lindemuthianum* and *H. carbonum* are listed in Table 1.

The two races of *C. lindemuthianum* were grown on bean juice agar; *H. carbonum* was grown on V-8 juice agar. The cultures were incubated at 23 °–24 °C in the dark, and inocula were obtained from 8–13 day old cultures. Spore suspensions were prepared by flooding plates with tap water and rubbing the cultures with a glass rod. The resulting suspensions of conidia were filtered through cheese-cloth and the filtrates sprayed onto the bean hypocotyls.

Histology

The bean varieties 'Topcrop', 'Dark Red Kidney', and 'Tennessee Green Pod' were inoculated with *H. carbonum* and the beta and gamma races of *C. lindemuthianum*. Epidermal strips were sectioned freehand from bean hypocotyls at intervals after inoculation and fixed in formalin-acetic acid-alcohol for a minimum of 24 h (Johansen, 1940). The strips were stained in a dilute alcoholic solution of trypan blue or crystal violet, dehydrated through an alcohol series into xylene, and mounted in balsam on glass slides.

Phenylalanine ammonia-lyase (PAL'ase) and assays for phenolics

Acetone powders were prepared from hypocotyls, variety 'Topcrop', and assayed for PAL'ase activity according to the method reported by Biehn (1967). The filtrates obtained during the preparation of acetone powders were concentrated under reduced pressure at 40 °C to a small aqueous volume, adjusted with dilute hydrochloric acid (HCl) to pH 3 and 1 ml/g fresh weight, and extracted 3 times with equal volumes of ethyl acetate. The ethyl acetate fractions were combined and evaporated under reduced pressure to dryness at 40 °C. The residue was redissolved in ethyl acetate (1.5 ml/g fresh wt.). The ethyl acetate portion was then concentrated to dryness, taken up in 90 % ethanol (0.1 ml/g fresh wt.), and used for the assay of phenolic compounds. Quantitative and qualitative comparisons of phenolics obtained from inoculated bean plants by extraction with boiling 85 % ethanol or by cold acetone indicated only minor differences. Acetone extraction makes possible phenolic and enzyme assays from the same plant tissues.

Total phenolic content of the extracts was determined by the method of Swain and

Hillis (1959), using the Folin-Denis reagent prepared according to Horwitz (1965) Samples equivalent to 0.5 g fresh wt. of tissue were applied to plates and component. were separated by thin layer chromatography, using polyamide (Woelm) developed with 70% acetone, 50% ethanol, or 75% ethanol and microcrystalline cellulose (Macherey, Nagel & Co.) developed with 2% acetic acid. Components were detected by ultraviolet radiation at 254 mμ (Mineralite) and diazotized sulfanilic acid (DSA). The relative amounts of components were estimated visually.

Experiments and results

Effect of host age

In this paper host age is defined as the age, in days, of bean seedlings at the time of inoculation. To determine the effect of host age on symptom expression and host–fungus physiology, 'Topcrop', 'Tennessee Green Pod', and 'White Kidney' hypocotyls were inoculated with the beta and gamma races of *C. lindemuthianum*. Symptoms were evaluated visually and PAL'ase activity in extracts of acetone powders of 'Topcrop' hypocotyls of various ages was determined. Varietal resistance to *C. lindemuthianum* is significantly reduced in young etiolated bean hypocotyls. Resistant plants younger than 9 days develop a small number of dark necrotic lesions characteristic of susceptibility, particularly near the cotyledons and on the epicotyls, whereas susceptible plants older than 13 days exhibit areas of relative resistance, characterized by reddish-brown flecking on the lower portions of the hypocotyl. Symptoms are not observed on root tissues of either resistant or susceptible bean plants. The appearance of symptoms characteristic of susceptibility ranges from 72 h after inoculation on 6–10 day

Fig. 1. Phenylalanine ammonia-lyase activity of uninoculated 'Topcrop' hypocotyls of various ages

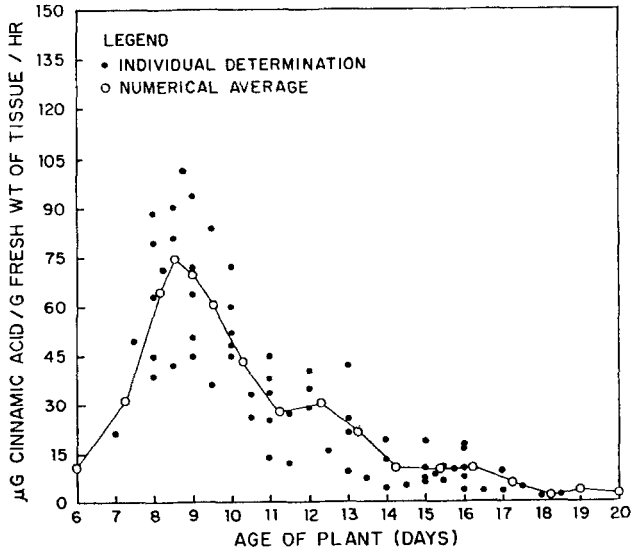

Fig. 1. Fenylalanine ammonia-lyase activiteit van niet geïnoculeerde 'Topcrop' hypocotylen van verschillende leeftijd

old hypocotyls to 96 h on 13–18 day old plants. The level of PAL'ase activity in extracts of acetone powders is markedly influenced by plant age, being highest during the period of rapid hypocotyl growth (Fig. 1).

Histology

Inoculated plants were examined histologically to determine the morphology of penetration and the host cell response. Germ tube development by *H. carbonum* is observed beginning 3–4 h after inoculation. The germ tubes elongate and typically form appressoria within 12–24 h. Host cell response to appressorial stimuli is observed as a granulated condition of the affected cells. These cells rapidly become discolored, giving the inoculated hypocotyl, in the case of very heavy inoculation, a faint reddish-brown color. Using the histological technique described, it has not been possible to detect penetration of host cells from appressoria.

C. lindemuthianum conidia of the beta and gamma races begin germination 4–6 h after inoculation. This germination is observed as a movement of protoplasm out of the conidia into spherical structures typically attached to the conidia directly or by short germ tubes (Fig. 2). The spherical structures, originally hyaline, rapidly become thick walled and pigmented dark brown during the period 8–24 h after inoculation (Fig. 3 and 4). At 36 h after inoculation, germinated conidia and germ tubes are not visible, and the only structures detectable are the pigmented, spherical appressoria (Fig. 5). The disappearance of spore cases and germ tubes is apparently a fixation artifact, as these structures are observed throughout the incubation period on fresh tissue mounted in distilled water. Penetration of host cells from the appressoria occurs 40–48 h after inoculation. Prior to penetration the developmental morphology of *C. lindemuthianum* is the same for both the beta and gamma races on the varieties tested. Bean varieties resistant to a race of *C. lindemuthianum* respond rapidly to penetration from appressoria, and affected cells become granulated and discolored (Fig. 6), similar to those observed in the *H. carbonum*-initiated hypersensitive reactions. This can be seen as visible, reddish-brown flecking on heavily inoculated, resistant hypocotyls beginning at 60–72 h after inoculation. In resistant reactions infection hyphae are not observed beyond the second cell layer, with nearly all hyphae arrested in the outer epidermal cells. In susceptible reactions, primary mycelia (Leach, 1923) penetrate from the appressoria intracellularly to a depth of 3–4 cells with no apparent disturbance of host cell integrity during the period 48–60 h after inoculation (Fig. 7). During the period 60–72 h after inoculation the hyphae in susceptible hosts begin lateral proliferation beneath the epidermal layer (secondary mycelia), causing cellular disorganization (Fig. 8). This lateral growth beneath the epidermal layer appears to be associated with an enzymatic softening of host cell wall tissues.

PAL'ase activity in acetone powders

The hypersensitive response to inoculation (6–24 h after inoculation) with *H. carbonum* is associated with an increase in PAL'ase activity over that in controls in both 8 and 15 day old plants (Fig. 9 and 10). A similar pattern is evident with resistant bean hypocotyls inoculated with *C. lindemuthianum*. The resistant reaction coincides with an increase in PAL'ase activity at the time of penetration (48 h after inoculation) which is not apparent in susceptible bean–fungus combinations. A large increase in PAL'ase activity coincides with symptom development in the case of inoculation with *C. lin-*

demuthianum. This is observed in both young and old plants inoculated with the beta race (susceptible) and young plants inoculated with the gamma race (restricted susceptibility).

Quantitative and qualitative changes in phenolic metabolites associated with host–fungus interaction

The phenolic composition (compounds soluble in organic solvents from acidic aqueous solution, DSA positive, Folin-Denis positive) of host plants is markedly influenced by infection. Changes in total phenolics following inoculation generally reflect changes in PAL'ase activity (Fig. 11 and 12), and are associated with hypersensitive, resistant, and susceptible host cell responses. Table 2–5 give visual estimates of the various phenolic components detected following inoculation. Hypersensitive, resistant and susceptible responses of host cells are distinctly different and readily distinguished by their phenolic patterns. Hypersensitivity in response to *H. carbonum* is associated with the appearance of two components, initially detected 6 h after inoculation and decreasing in amount after reaching maximum concentrations at 12–24 h. The resistant response 48 h after inoculation with the gamma race is accompanied by the appearance of three componentss, of which one or more may be the same as those appearing in response to *H. carbonum* at 12–24 h.

Fig. 2. Germinating conidium 5 h after inoculation. Protoplasm flows from the conidium into the hyaline spherical structure (× 1830).
Fig. 3. Germinated and ungerminated conidia 18 h after inoculation. Ungerminated conidia still contain protoplasm, and appressorium of germinated conidium (center) is pigmented (× 1130).
Fig. 4. Pigmented appressoria 24 h after inoculation. Conidial shells and germ tubes are apparently dissolving (× 1200).
Fig. 5. Pigmented appressoria 36 h after inoculation. Conidial shells and germ tubes are not detectable and penetration of host cells is not apparent (× 670).
Fig. 6. Hypersensitive response of epidermal cells to gamma race 60 h after inoculation. Host cells appear granulated and hyphae are contained (× 670).
Fig. 7. Primary mycelia of beta race 60 h after inoculation. Disorganization and hypersensitivity of host cells are not apparent (× 670).
Fig. 8. Secondary mycelia of beta race 84 h after inoculation (focused 4 cells beneath epidermis). Disintegration of host cell walls at points of mycelial penetration is apparent (× 490).

Fig. 2. Kiemend conidium, 5 uur na inoculatie. Protoplasma stroomt vanuit het conidium in de hyaliene ronde structuur (1830 ×).
Fig. 3. Gekiemde en ongekiemde conidia 18 uur na inoculatie. Ongekiemde conidiën bevatten nog protoplasma, en het appressorium van het gekiemde conidium (centrum) is gepigmenteerd (1130 ×).
Fig. 4. Gepigmenteerde appressoria 24 uur na inoculatie. Conidiumwanden en kiembuizen lossen klaarblijkelijk op (1200 ×).
Fig. 5. Gepigmenteerde appressoria 36 uur na inoculatie. Conidiumwanden en kiembuizen zijn niet waarneembaar en penetratie van gastheercellen kan niet worden waargenomen (670 ×).
Fig. 6. Overgevoeligheidsreactie van epidermiscellen ten opzichte van het gamma fysio, 60 uur na inoculatie. De gastheercellen bieden een korrelige aanblik en de ontwikkeling der hyphen is geremd (670 ×).
Fig. 7. Primair mycelium van het beta fysio, 60 uur na inoculatie. Disorganisatie en overgevoeligheid van de gastheercellen zijn niet waarneembaar (670).
Fig. 8. Secundair mycelium van het beta fysio, 84 uur na inoculatie (ingesteld op 4 cellen beneden epidermis). Disintegratie van de celwanden van de gastheer op punten waar het mycelium penetreert is waarneembaar (490 ×).

Fig. 2–8. Developmental morphology of beta and gamma races of *C. lindemuthianum* on 'Topcrop' hypocotyl tissue

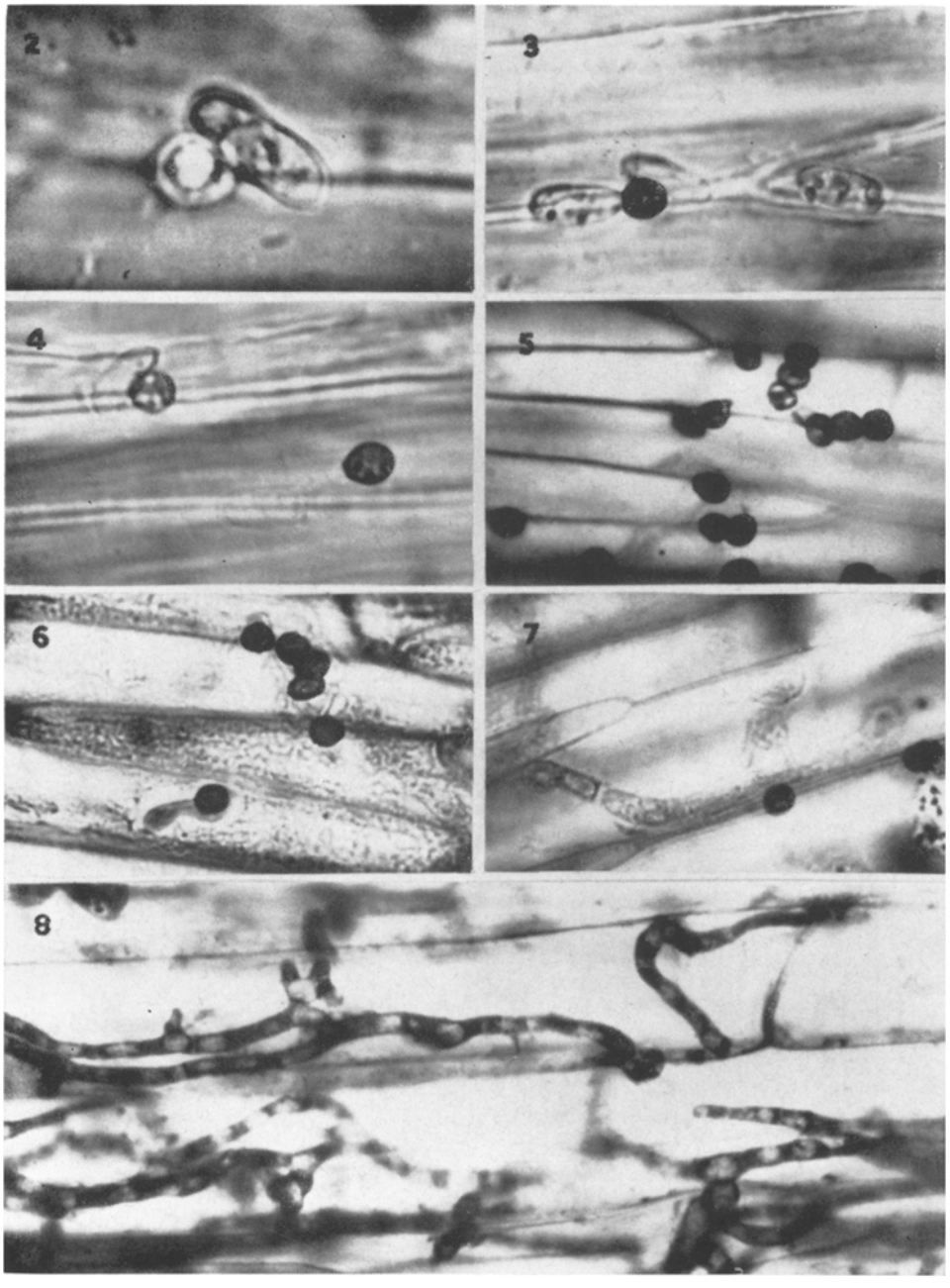

Fig. 2–8. Morfologische ontwikkeling van beta en gamma fysio's van C. lindemuthianum op het weefsel van 'Topcrop' hypocotylen

Fig. 9. Phenylalanine ammonia-lyase activity of 8 day old 'Topcrop' hypocotyls inoculated with *C. lindemuthianum* or *H. carbonum*

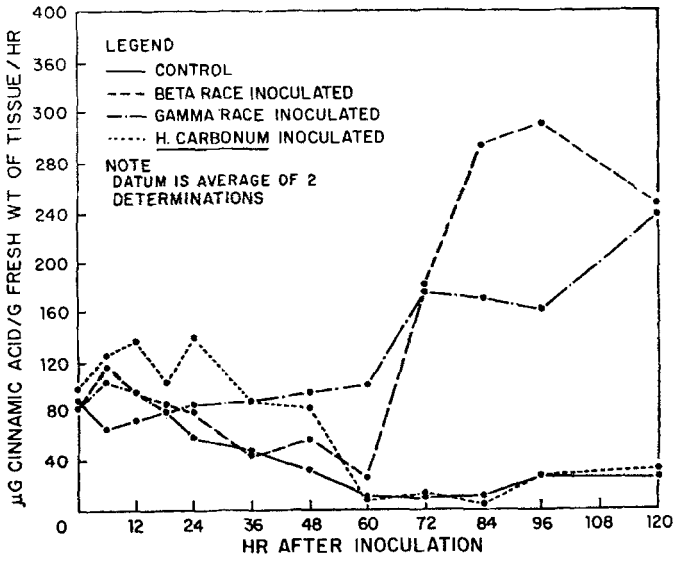

Fig. 9. Fenylalanine ammonia-lyase activiteit van 8 dagen oude 'Topcrop' hypocotylen geïnoculeerd met C. lindemuthianum of H. carbonum

Fig. 10. Phenylalanine ammonia-lyase activity of 15 day old 'Topcrop' hypocotyls inoculated with *C. lindemuthianum* or *H. carbonum*

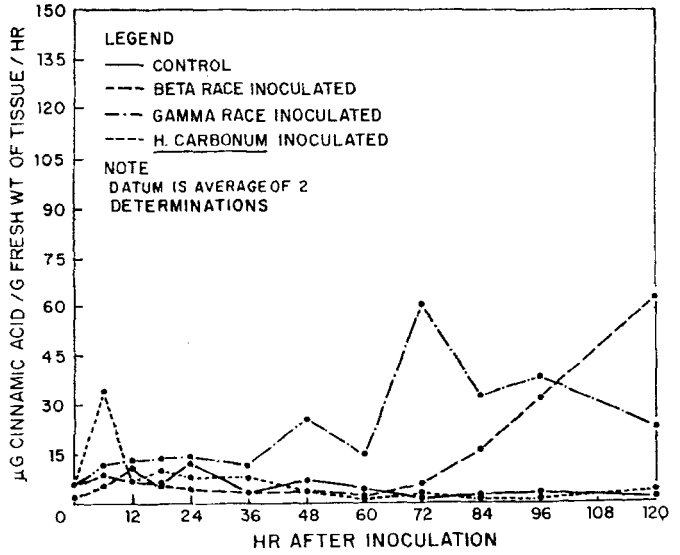

Fig. 10. Fenylalanine ammonia-lyase activiteit van 15 dagen oude 'Topcrop' hypocotylen geïnoculeerd met C. lindemuthianum of H. carbonum

Fig. 11. Total phenolic content of 8 day old 'Topcrop' hypocotyls inoculated with *C. lindemuthianum* or *H. carbonum*

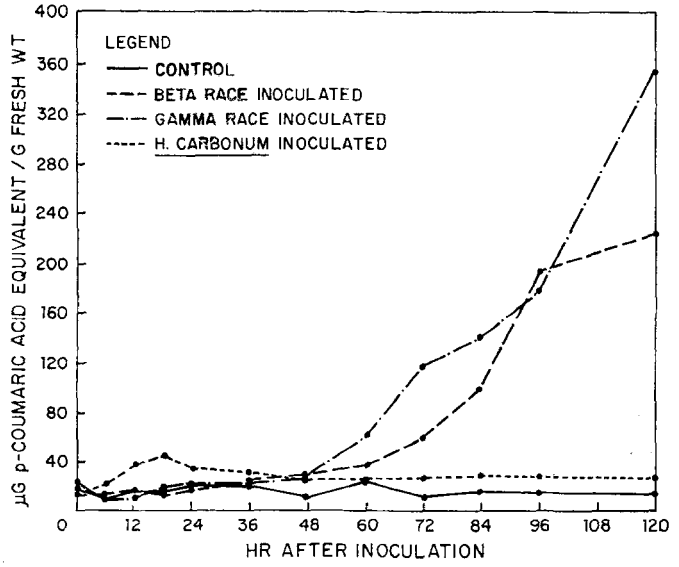

Fig. 11. Totaal fenolgehalte van 8 dagen oude 'Topcrop' hypocotylen, geïnoculeerd met C. lindemuthianum of H. carbonum

Fig. 12. Total phenolic content of 15 day old 'Topcrop' hypocotyls inoculated with *C. lindemuthianum* or *H. carbonum*

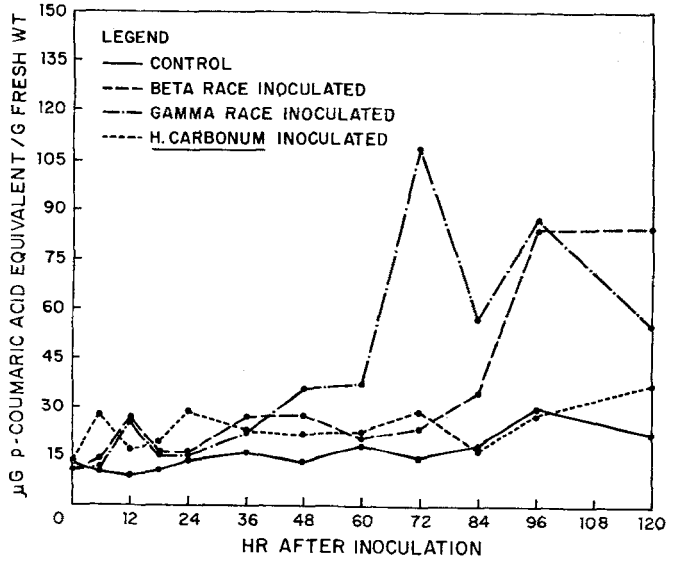

Fig. 12. Totaal fenolgehalte van 15 dagen oude 'Topcrop' hypocotylen, geïnoculeerd met C. lindemuthianum of H. carbonum

Table 2. Key to Table 3–5

R_F	Appearance under UV (254 mμ)	DSA Response
	Cellulose plates – 2% acetic acid	
0.45	absorbs: 0–60 hr blue-purple: 60–120 hr	tan-yellow
0.30	absorbs faintly	tan-yellow
0.19	absorbs	tan-yellow
	Polyamide plates – 70% acetone	
0.85	blue-purple changes to cream-yellow (phaseollin)	tan
0.76	undetected	tan-yellow
0.75	undetected	tan
0.74	tan-brown	pink
0.67	tan-brown	yellow
0.65	undetected	yellow
0.56	absorbs	tan
0.35	absorbs	tan-yellow

Quantitative estimation

– not detectable
+ to + + + + + relative intensity of band
? band obscured by other components

Tabel 2. Sleutel tot Tabel 3–5

The authors are indebted to Dr D. F. Bateman of Cornell University who kindly supplied a sample containing phaseollin. We have isolated a substance from extracts of inoculated hypocotyls whose ultraviolet spectrum, chromatographic characteristics, and DSA response are the same as phaseollin. This substance and authentic phaseollin have R_F values on polyamide of 0.30 (50% ethanol), 0.71 (75% ethanol) and 0.85 (70% acetone). Phaseollin and the corresponding bands from extracts fluoresce blue-purple under radiation of 254 mμ; this fluorescence changes to pale yellow when the plates are kept in air for several days. The ultraviolet spectra of phaseollin, an eluate of phaseollin from thin layer plates, and an eluate of the corresponding band from extracts are the same. Both materials are extracted from aqueous solutions into hexane and both react to give a tan color with DSA and a blue color with ferric chloride–ferricyanide. On the basis of these characteristics we suggest that the isolated material is phaseollin. Phaseollin production is shown in Table 2–5 as the component at R_F 0.85 on polyamide; cellulose chromatography did not give satisfactory separation of phaseollin. Development of plant extracts on polyamide with 50% ethanol also gave a distinct phaseollin band. In all extracts the time of appearance of phaseollin is associated with the appearance of visible symptoms. The amount produced in response to *H. carbonum* was barely detectable and only at 12–24 h with the 50% ethanol solvent. Inoculation with *C. lindemuthianum* induced the production of readily detectable amounts of phaseollin, coinciding with the appearance of visible symptoms. The resistant fleck response at 60–72 h after inoculation with the gamma race was accompanied by the

Table 3. Phenolic components detected in extracts of 'Topcrop' hypocotyls inoculated with the beta race of *C. lindemuthianum*

R_F	*Hours after inoculation*											
	0	*6*	*12*	*18*	*24*	*36*	*48*	*60*	*72*	*84*	*96*	*120*
						Cellulose plates, 8 day old plants						
0.45	–	–	–	–	–	–	–	–	++	+++	?	?
0.30	–	–	–	–	–	–	–	–	++	++	?	–
0.19	–	–	–	–	–	–	–	–	+++	+++	?	?
						Polyamide plates, 8 day old plants						
0.85	–	–	–	–	–	–	–	–	–	+	+	+
0.76	–	–	–	–	–	–	–	–	–	–	–	–
0.75	–	–	–	–	–	–	–	–	–	–	–	–
0.74	–	–	–	–	–	–	–	–	–	–	–	–
0.67	–	–	–	–	–	–	–	–	++	+++	+++	+++
0.65	–	–	–	–	–	–	–	–	–	–	–	–
0.56	–	–	–	–	–	–	–	–	++	+++	+++	?
0.35	–	–	–	–	–	–	–	–	–	–	–	–
						Cellulose plates, 15 day old plants						
0.45	–	–	+	++	+	++	++	++	++	++	++	?
0.30	–	–	–	–	–	–	–	–	–	+	+	–
0.19	–	–	–	–	–	–	–	–	–	++	+	–
						Polyamide plates, 15 day old plants						
0.85	–	–	–	–	–	–	–	–	–	–	+++	+++
0.76	–	–	–	–	–	–	–	–	–	–	–	–
0.75	–	–	–	–	–	–	–	–	–	–	–	–
0.74	–	–	–	–	–	–	+	?	+	++	++++	++++
0.67	–	+	++	++	++	–	+	–	+	++	++++	++++
0.65	–	–	–	–	–	–	–	–	–	–	–	–
0.56	–	–	–	–	–	–	–	–	–	+	+	?
0.35	–	–	+	+	+	–	–	–	–	–	–	–

Tabel 3. Fenolcomponenten in extracten van 'Topcrop' hypocotylen, geïnoculeerd met het beta fysio van C. lindemuthianum

production of much higher levels of phaseolin than were apparent with the appearance of symptoms after inoculation with the beta race at 72–84 h for young plants and 96 h for old plants. A bright blue-fluorescing compound was detected at R_F 0.00–0.05 in extracts developed with 50% ethanol. Its appearance and concentration closely parallelled those of phaseolin. Neither of these substances nor any of the components appearing in Table 2–5 were detected in extracts of control tissues. Blue, green, yellow, and ochre-fluorescing bands were detected following polyamide chromatography, and they are characteristic of necrosis following inoculation with the beta race; these are not included in Table 2–5 except where a distinct DSA color response was produced.

Table 4. Phenolic components detected in extracts of 'Topcrop' hypocotyls inoculated with the gamma race of *C. lindemuthianum*

R_F	*Hours after inoculation*											
	0	*6*	*12*	*18*	*24*	*36*	*48*	*60*	*72*	*84*	*96*	*120*
							Cellulose plates, 8 day old plants					
0.45	–	–	–	–	–	–	++	++	+	–	–	–
0.30	–	–	–	–	–	–	+	+	++	?	?	?
0.19	–	–	–	–	–	–	+++	++	+++	?	?	?
							Polyamide plates, 8 day old plants					
0.85	–	–	–	–	–	–	–	+	+++	++++	++++	++++
0.76	–	–	–	–	–	–	–	–	–	–	–	–
0.75	–	–	–	–	–	–	+	+	+	–	–	–
0.74	–	–	–	–	–	–	–	–	+	++	+++	+++
0.67	–	–	–	–	–	–	++	+++	++++	++++	++++	++++
0.65	–	–	–	–	–	–	–	–	–	–	–	–
0.56	–	–	–	–	–	–	+++	++	?	?	?	?
0.35	–	–	–	–	–	–	–	–	–	–	–	–
							Cellulose plates, 15 day old plants					
0.45	–	–	+	++	++	++	+++	++	++	++	+	–
0.30	–	–	–	–	–	+	++	–	++	+	?	?
0.19	–	–	–	–	–	–	++++	+	++	+	+	+
							Polyamide plates, 15 day old plants					
0.85	–	–	–	–	–	–	–	–	+	++	+++	+++
0.76	–	–	–	–	–	–	–	–	–	–	–	–
0.75	–	–	–	–	–	++	++	+	–	–	–	–
0.74	–	–	–	–	–	–	–	–	–	+	++++	++
0.67	–	–	+	+	+	+	++	+++	++++	++++	+++++	+++++
0.65	–	–	–	–	–	–	–	–	–	–	–	–
0.56	–	–	–	–	–	+	++++	++	+++	–	–	–
0.35	–	+	+	–	+	–	–	–	–	–	–	–

Tabel 4. Fenolcomponenten in extracten van 'Topcrop' hypocotylen, geïnoculeerd met het gamma fysio van C. lindemuthianum

Discussion

Physiologic resistance frequently develops in susceptible hosts as a result of aging; such is the case in bean anthracnose on greenhouse and field grown plants (Griffey and Leach, 1965). In general, the pattern of varietal susceptibility to anthracnose on etiolated bean hypocotyl tissue 13 days old or older is consistent with that observed on pods and young bean seedlings grown in the light, and with published data (Goth and Zaumeyer, 1965). The increased susceptibility of very young etiolated bean plants is associated with rapid growth; for the hypocotyl portion of the plant this occurs 7–11 days after planting. During this period the area of susceptibility on normally resistant hosts progressively moves up the hypocotyl and into epicotyl on plants inoculated at successive intervals after 6 days. The level of PAL'ase is 8–10 fold higher in

Table 5. Phenolic components detected in extracts of 'Topcrop' hypocotyls inoculated with *H. carbonum*

R_F	*Hours after inoculation*											
	0	*6*	*12*	*18*	*24*	*36*	*48*	*60*	*72*	*84*	*96*	*120*
						Cellulose plates, 8 day old plants						
0.45	−	−	+++	++++	++	++	++	+++	+++	+++	++	+
0.30	−	+	++++	++++	+++	+	++	++	+++	+++	++	+
0.19	−	−	−	−	−	−	−	−	−	−	−	−
						Polyamide plates, 8 day old plants						
0.85	−	−	−	−	−	−	−	−	−	−	−	−
0.76	−	−	−	−	−	++	++	++	+	+	+	+
0.75	−	−	−	−	−	−	−	−	−	−	−	−
0.74	−	−	−	−	−	−	−	−	−	−	−	−
0.67	−	−	−	−	−	−	−	−	−	−	−	−
0.65	−	−	+++	+++	+	+++	+++	+++	++	++	++	+
0.56	−	−	−	−	−	−	−	−	−	−	−	−
0.35	−	+	+	+++	+++	−	−	−	−	−	−	−
						Cellulose plates, 15 day old plants						
0.45	−	−	++	+++	++++	+++	+++	++	+++	++	+	+
0.30	−	−	++	+++	++++	+	+	+	+	−	−	−
0.19	−	−	−	−	−	−	−	−	−	−	−	−
						Polyamide plates, 15 day old plants						
0.85	−	−	−	−	−	−	−	−	−	−	−	−
0.76	−	−	−	−	−	++	++	++	+	+	+	+
0.75	−	−	−	−	−	−	−	−	−	−	−	−
0.74	−	−	−	−	−	−	−	−	−	−	−	−
0.67	−	−	−	−	−	−	−	−	−	−	−	−
0.65	−	+	++	+++	+++	+++	+++	+++	++	++	++	+
0.56	−	−	−	−	−	−	−	−	−	−	−	−
0.35	−	++	+	++	+++	−	−	−	−	−	−	−

Tabel 5. Fenolcomponenten in extracten van 'Topcrop' hypocotylen, geïnoculeerd met H. carbonum

8 day old plants than in 15 day old plants. This increased potential for diversion of phenylalanine to phenolic metabolism is not associated with any significant difference in the levels of total phenolics of the two ages of control plants. The authors suggest the possibility that phenolic metabolites are being incorporated into non-extracted polymers, e.g. lignin, during the periods of high PAL'ase activity in actively growing control plants. This is consistent with observations of limited areas of physiologic resistance appearing on the lower portions of susceptible inoculated hypocotyls older than 13 days.

Our observations of the developmental morphology of *C. lindemuthianum* on bean are in general agreement with the germination study of Dey (1919) and the report of Leach (1923) concerning "primary" and "secondary" mycelial development in susceptible

hosts. Pathogen development in etiolated hypocotyls is more rapid than in non-etiolated plants, presumably due to the reduction of cuticle and the succulent condition of the host. This is further reflected in the shortened incubation time required for symptom expression in 6–10 day old plants as compared to 13–18 day old plants.

Histological events following inoculation with various fungi are accompanied by alterations in the phenolic metabolism of the host-fungus complex. These are evident in PAL'ase activity, and in quantitative and qualitative changes in phenolic composition. They are similar in both young and old etiolated hypocotyls. Although the most obvious changes in phenolic metabolism are associated with symptom development in the susceptible host, specific and significant alterations occur during the hypersensitive and resistant host responses.

In interpreting the data, the number of host cells affected at different stages after inoculation must be considered. In susceptible plants, symptom development results in total necrosis of the hypocotyl by 84–96 h in 8 day old plants, and by 108–120 h in 15 day old plants. Partial susceptibility of 8 day old resistant hosts is reflected by partial necrosis of the top 1/4–1/3 of the hypocotyl. In contrast, resistant and hypersensitive flecking was observed on histological sections as granulation of the surface layer of epidermal cells. Of these, only 2–5% reacted to *H. carbonum* inoculation, and 20–30% to *C. lindemuthianum*. Since the phenolic and enzyme data represent assays of whole hypocotyls, the authors suggest that alterations of phenolic metabolism at the site of infection on a cellular level are at least as great in cells exhibiting a hypersensitive or resistant response as in those cells successfully parasitized.

The changes in phenolic metabolism associated with hypersensitivity and resistance in bean hypocotyls are different from those associated with susceptibility. Increased activity of PAL'ase and a corresponding increase in total phenolics occur at the times hypersensitivity to *H. carbonum* (12–24 h) and resistance to *C. lindemuthianum* (48–60 h) are observed histologically. Significant alterations of phenolic metabolism are not detected in the susceptible bean–fungus combination until the time of symptom appearance. Hypersensitivity to *H. carbonum* and varietal resistance to *C. lindemuthianum* appear similar, though not identical. Two of the three components associated with resistance to *C. lindemuthianum* at 48 h appear chromatographically similar to those associated with hypersensitivity to *H. carbonum* at 12–24 h. In both cases the concentration of these compounds decreases with time after expression of hypersensitivity or resistance. In the resistant bean–*C. lindemuthianum* combination, phenolic components appearing after 60 h are similar but generally at higher concentrations than those associated with susceptibility. Considering the fewer number of cells apparently affected in the resistant response, these latter compounds may be an example of the production of "too little, too late" in the case of susceptibility.

Further characterization of the chemical and physiological properties of the phenolic compounds produced in bean–fungus interactions is required before any assessment of their role in resistance can be made. The data presented indicate that phenolic metabolism is profoundly and characteristically influenced by the events following infection of bean hypocotyls with a non-pathogen, and with pathogenic and non-pathogenic races of a bean pathogen.

Samenvatting

Correlatie tussen de stofwisseling van fenolen en histologische veranderingen in Phaseolus vulgaris, na inoculatie met schimmels

Acetonpoeders werden bereid op verschillende tijdstippen na inoculatie van *Phaseolus vulgaris*, cv. 'Topcrop', met schimmels, die een overgevoelige, resistente of vatbare reactie in de gastheer induceren. De activiteit van fenylalanine-ammonia-lyase werd bepaald in extracten van de acetonpoeders, en de fenolen werden kwantitatief en kwalitatief bepaald in de acetonfiltraten, die verkregen werden gedurende de bereiding van het poeder. De morfologische ontwikkeling van de schimmels op het weefsel van de waardplant werd histologisch bestudeerd. De verkregen gegevens wijzen erop dat de fysiologische processen, die in de combinatie van waardplant en schimmel optreden, gedurende verschillende stadia van infectie en reactie van de waardplant, gepaard gaan met duidelijk aantoonbare en specifieke veranderingen in de fenol stofwisseling. Zowel bij resistente als vatbare reacties ging het verschijnen der symptomen gepaard met de vorming van phaseolline in hypocotylen geïnoculeerd met *Colletotrichum lindemuthianum*; bij de resistente reacties werd het eerder en in grotere hoeveelheden gevormd.

References

Biehn, W. L., 1967. Accumulation of phenols in resistant plant–fungus interactions. Ph. D. Thesis, Purdue University, Lafayette, Indiana.

Condon, P., Kuc', J. and Draudt, H. N., 1963. Production of 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin by carrot root tissue. Phytopathology 53: 1244–1251.

Cruickshank, I. A. M. and Perrin, D. R., 1963a. Phytoalexins of the Leguminosae. Phaseolin from Phaseolus vulgaris L. Life Sci. 2: 680–682.

Cruickshank, I. A. M. and Perrin, D. R., 1963b. Studies on phytoalexins. VI. Pisatin: The effect of some factors on its formation in Pisum sativum L. and the significance of pisatin in disease resistance. Aust. J. biol. Sci. 16: 111–128.

Dey, P. K., 1919. Studies in the physiology of parasitism. V. Infection by Colletotrichum lindemuthianum. Ann. Bot. 33: 305–312.

Farkas, G. L. and Kiraly, Z., 1962. Role of phenolic compounds in the physiology of plant disease and disease resistance. Phytopath. Z. 44: 105–150.

Goth, R. W. and Zaumeyer, W. J., 1965. Reactions of bean varieties to four races of anthracnose. Pl. Dis. Reptr 49: 815–818.

Griffey, R. T. and Leach, J. G., 1965. The influence of age of tissue on the development of bean anthracnose lesions. Phytopathology 55: 915–918.

Horwitz, W., 1965. Official methods of analysis. Assoc. Off. Agric. Chem. (A.O.A.C.), 10th ed. Washington, D.C., p. 139.

Johansen, D. A., 1940. Plant microtechnique. McGraw-Hill, New York, p. 41.

Kuc', J., 1966. Resistance of plants to infectious agents. A. Rev. Microbiol. 20: 337–370.

Kuc', J., 1967. Shifts in oxidative metabolism during pathogenesis. In: C. J. Mirocha and I. Uritani (Editors), The dynamic role of molecular constituents in plant–parasite interactions. Association Services Inc., St. Paul, Minn.

Leach, J. G., 1923. The parasitism of Colletotrichum lindemuthianum. Bull. Minn. agric. Exp. Stn 14.

Pierre, R. E., 1966. Histopathology and phytoalexin induction in beans resistant or susceptible to Fusarium and Thielaviopsis. Ph. D. Thesis, Cornell University, New York.

Swain, T. and Hillis, W. E., 1959. The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. J. Sci. Fd Agric. 10: 63–68.