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Studies on the toxins of *Pyricularia* spp.—the phytotoxicity of culture filtrates*

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Abstract

Filtrates from cultures of 5 *Pyricularia* spp. from different graminaceous hosts and, isolates of *P. oryzae* from rice belonging to 10 races were analysed for phytotoxic components by thin-layer chromatography and bioassay techniques. A number of phytotoxic components including pyriculol was detected. A component which was apparently the reduced form of pyriculol was also detected in some of the toxic eluates of the chromatograms. The components could be distinguished by their characteristic UV absorption spectra. Most of the components appeared to be unsaturated and phenolic in nature. The number and nature of the components varied with the isolate, medium and cultural condition.

Introduction

In an earlier communication we reported that although the toxins α-picolinic acid and piricularin[1] could not be detected in culture filtrates of *Pyricularia* spp. from various gramineous hosts, the phytotoxic compound pyriculol[2] was frequently detectable in cultures of *Pyricularia* spp. including races of *P. oryzae*[3]. However, ethyl acetate extracts of culture filtrates, where none of the three toxic components could be detected, were found to be still toxic to rice plants implying the presence of other phytotoxic components in the filtrates. Evidence is presented in the present paper for the occurrence of many phytotoxic components in culture filtrates of *Pyricularia* as revealed by thin-layer chromatography (TLC), bioassay techniques and UV spectrophotometry.

Materials and Methods

Pyricularia spp. from different hosts, *viz.*, *Eleusine coracana* (*P. setariae*[4]), *Setaria italica* (*P. setariae* Nisikado), *Panicum repens*

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[*P. oryzae*[4] = *P. grisea* (Cke) f. sp. *panicae* f. sp. nov.[5]], *Brachiaria mutica* [*P. brachiariae-mutica*[4] = *P. grisea* (Cke) Sacc. f. sp. *brachiariae* f. sp. comb. nov.[5]], *Leersia hexandra* [*P. leersiae — hexandrae*[4] = *P. leersiae* (Sawada) Ito[5]] and 10 races of *P. oryzae* Cav. from rice (*Oryza sativa*) were used. For convenience, these isolates are referred to as *Pyricularia* spp. from the respective hosts. The isolates were grown in different media as shake or stationary cultures. Shake cultures were grown for 5–10 days whereas stationary cultures were incubated for 40 days in line with the procedure of Tamari *et al.* (1965)[6] and Umetsu *et al.* (1972)[7]. The media, cultural conditions, extraction of the filtrates with ethyl acetate, thin-layer chromatography and recording of UV spectra of the eluates have been previously described[3]. After chromatography in benzene: ethyl acetate (7 : 3), the thin-layer plates were viewed under UV (254 and 356 nm) and the UV absorbing/fluorescing areas marked and their R_f determined. Corresponding areas in the control chromatograms were also marked. Extracts from the same volumes of un-inoculated media were used as controls. The marked areas were scrapped off the plates and extracted twice with twice distilled ethanol and centrifuged at 4,000 r.p.m. for 5 min. The supernatants were dried at room temperature in 100 ml beakers. The residues were taken in 2 ml distilled water and bioassayed as follows:

(*a*) *Seedling growth inhibition.*—Five germinated seeds of *O. sativa* (CO 13) with uniform radicles and plumules were placed in the eluates The beakers were covered with 'Alfoil' and left at room temperature. Growth measurements were made at the end of 5 days on shoots and the longest roots. Distilled water was used for the controls.

(*b*) *Leaf punch method.*—The upper surface of intact first leaves of the respective host plants of *Pyricularia* spp. was gently punched with a capillary tube (1 mm θ). Drops of eluates (0·01 ml) were placed on the punched areas. Five to 6 drops were kept on each leaf. Distilled water drops served as controls. The plants were left in a growth room maintained at $30 \pm 1°$ C during the light period (12 h) and $20 \pm 1°$ C during the dark period (12 h).

UV spectra of toxic eluates.—Samples which were phytotoxic in both bioassays were scanned for their UV spectra before and after multiple development on silica gel G thin-layers in benzene, and benzene: ethyl acetate (9 : 1, 8 : 2 and 7 : 3) solvent systems. The components thus obtained moved as single spots in the last mentioned solvent system and their UV spectra are presented.

Colour reactions to spray reagents [8, 9, 10, 11].—Some of the frequently occurring toxic components were tested with the following reagents. Folin-Ciocalteu reagent (1), diazotized *p*-nitraniline (2), tetrazotized

benzidine (3) and neutral ferric chloride (4) were used to test the phenolic nature of the components. Bromophenol blue (5) revealed acids and 2, 4-dinitrophenyl-hydrazine (2, 4-DNPH) (6) free aldehyde and keto groups. Fluorescein-bromine (7), Bayer's test (8) and Iodine vapours (9) were used to test unsaturation.

Experimental and Results

Initially the 6 isolates of *Pyricularia* were grown as shake cultures in sucrose-nitrate and Iwasaki media for 10 and 5 days respectively. One litre each of the filtrates was extracted with ethyl acetate and the concentrated samples were streaked individually on Whatman No. 3 chromatographic paper, 38 × 18 cm (previously washed in the developing solvent and dried) and co-chromatographed with authentic pyriculol in benzen : ethyl acetate (7 : 3) solvent system. Ethyl acetate extracts of the two un-inoculated media (1 l each) were also similarly examined (control). When the chromatograms were viewed under UV, the marker pyriculol was located as a reddish brown spot at an R_f of 0·98. The control chromatograms showed no distinct areas of absorption or fluorescence. The other chromatograms showed a pale blue fluorescent band opposite the marker in the case of all culture filtrate samples except that of the isolate from *P. repens* grown in Iwasaki medium. In this case, a UV absorbing band was located opposite to the marker. The bands, when eluted with ethanol, dried and bioassayed with rice seeds, inhibited root growth to an extent of 41–100% and shoot growth to an extent of 6–100%. The corresponding regions from the control chromatograms stimulated root and shoot growth.

The pale blue fluorescent bands gave a UV spectrum as shown in figure 1, *a* which on rechromatography on paper in benzene : ethyl acetate (7 : 3) or butanol : acetic acid : water (4 : 1 : 1) gave a UV spectrum as shown in figure 1, A. This component (A) inhibited root growth by about 18% and shoot growth by about 4% at a concentration of 400 μg/ml. At lower concentrations, root growth was stimulated but shoot growth was inhibited by about 3% even at a concentration of 1 μg/ml.

In the case of the isolate from *P. repens*, however, the UV absorbing band gave a different spectrum with absorption peaks at 227, 275 and 350 nm suggestive of pyriculol. However, when this band was rechromatographed on paper in the 2 solvent systems mentioned before, the absorption peaks suggestive of pyriculol disappeared and the resultant spectrum resembled that of 'A' (figure 1, A). On the contrary, when the band was rechromatographed on silica gel thin-layers in benzene : ethyl acetate (7 : 3), 6 bands resulted, one of which was identical to pyriculol in its R_f (0·35) and UV spectral characteristics. Similarly, the blue fluorescent bands from the

other isolates were also found to resolve into a number of bands by TLC. Since these preliminary experiments indicated the poor resolution of the paper chromatographic technique and the possible degradation of some of the components including pyriculol to the toxic component 'A', further analyses of the ethyl acetate extracts of the culture filtrates were done by TLC (*vide* Materials and Methods).

Phytotoxic components in culture filtrates of Pyricularia *grown in sucrose-nitrate medium as shake cultures.*—In a preliminary experiment, ethyl acetate extracts of 400 ml each of the un-inoculated medium and filtrate from 10 day old culture of *P. oryzae* were bioassayed for phytotoxicity with rice seeds. The extract from the un-inoculated medium stimulated root growth (38%) and inhibited shoot growth slightly (13%) as compared to the corresponding water controls. On the other hand, the extract from the culture filtrate inhibited root growth markedly (83%) and shoot growth to a certain exent (20%).

The ethyl acetate extractable crude toxic fraction could be resolved into a number of components by TLC. Although the crude fraction from 400 ml of the filtrate was toxic, none of its components was toxic even when they were obtained from 1 l each of the filtrates of the 6 isolates, implying that the components were not present in toxic concentrations and the toxicity of the crude fraction was probably cumulative. However, when the extracts from 2 l volumes of the filtrates were chromatographed, certain UV absorbing/fluorescing areas of the chromatograms caused typical growth inhibitions in rice seedlings and necrotic lesions on host leaves (Plate I).

The response of rice seedlings to the components of the crude toxic fractions, their R_f values and the type of their UV spectra are given in table 1.

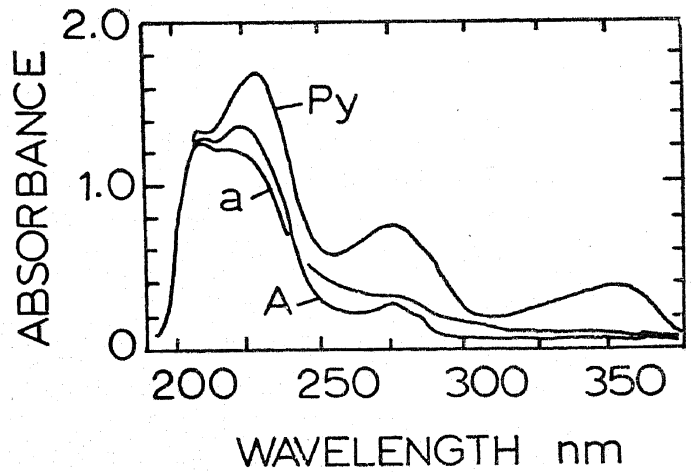

Figure 1. UV spectra in ethanol of eluate from paper chromatograms (R_f 0·98) before (*a*) and after rechromatography (A). Py = authentic pyriculol.

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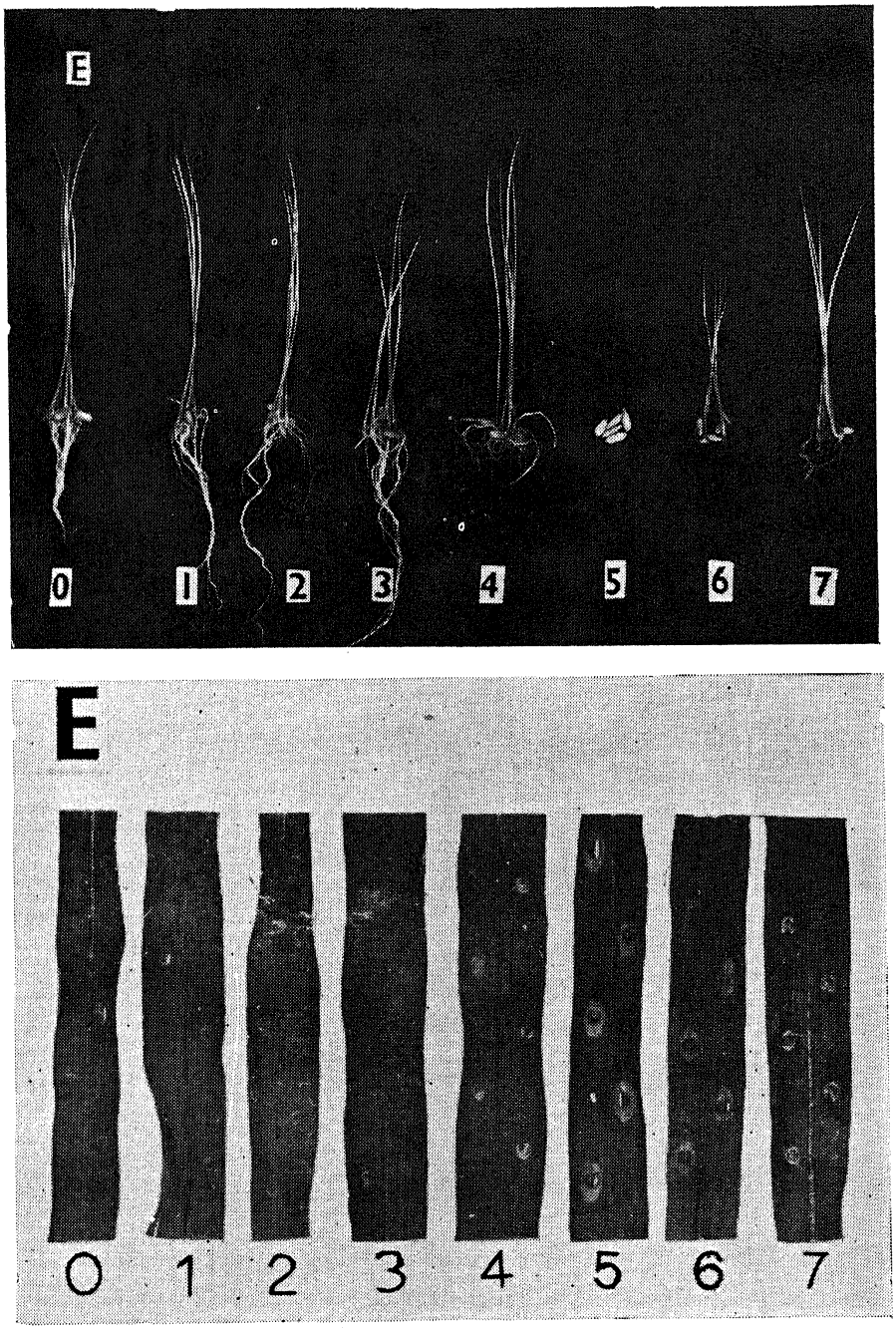

Plate 1. The typical response of rice seedlings to TLC eluates, in this case, for the isolate from *B. mutica* (*vide* table 1). 0 = water control. 1–7: R_f 0·98, 0·91, 0·75, 0·56, 0·35, 0·19 and 0·05. Note the toxicity of eluates 4–7 to rice seedlings and the typical necrotic lesions on the host leaves (*B. mutica*) produced by the same eluates.

(*facing page* 80)

Table 1. Growth response of rice seedlings to components of ethyl acetate extracts of culture filtrates of *Pyricularia* spp. grown in sucrose-nitrate medium as shake cultures.

Eluates from R_f		Un-inoculated medium	Isolates from O.S.	E.C.	S.I.	P.R.	B.M.	L.H.
	R	+ 48	+ 64	+ 49	+100	+ 84	+ 60	+ 57
0·98	S	+ 7	− 1	+ 2	0	− 5	+ 6	+ 1
	ST	—	A	A	A	A	A	A
	R	+ 36	+ 50	+ 45	+ 89	+ 94	+ 71	+ 41
0·91	S	+ 2	− 2	+ 1	+ 4	− 3	− 1	− 3
	ST	—	—	—	—	—	—	—
	R	+ 77	+ 50	+125	+156	+ 84	+ 97	+ 64
0·75	S	+ 7	0	− 6	− 3	− 12	− 13	0
	ST	—	B	B	B	B	B	B
	R	+ 44	− 33	+ 81	− 36	− 30	− 46	+ 84
0·56	S	− 2	− 22	+ 11	− 3	− 4	− 5	+ 4
	ST	—	C	C	C	C	C	C
	R	*	−100	*	*	*	*	*
0·45	S		− 13					
	ST		D					
	R	+ 61	−100	*	*	*	−100	*
0·35	S	+ 1	− 36				−100	
	ST	—	E				F	
	R	*	*	− 28	*	*	*	*
0·29	S			− 9				
	ST			G				
	R	+ 44	−100	− 97	−100	−100	−100	−100
0·19	S	+ 1	−100	+ 2	−100	− 17	− 36	− 32
	ST	—	H	H	H	H	H	H
	R	+ 85	+ 15	−100	+ 46	− 36	− 42	+ 40
0·05	S	+ 1	+ 11	− 76	− 5	− 8	− 7	+ 10
	ST	—	—	I	—	J	J	—

O.S. : *O. sativa*
E.C. : *E. coracana*
S.I. : *S. italica*
P.R. : *P. repens*
B.M. : *B. mutica*
L.H. ; *L. hexandra*

R : Root } % inhibition (−) or stimulation (+)
S : Shoot } calculated over water controls
ST : Spectral type in ethanol (*vide* figure 2)
* : No UV absorbing/fluorescent band
— : No UV spectrum
F : Pyriculol

It will be seen from the table that in the case of the un-inoculated medium, the eluates from the chromatograms were not toxic. These eluates did not give any UV spectra. However, some of the eluates of the culture filtrate samples inhibited root and shoot growth, the former to a greater extent than the latter. Although the eluates from R_f 0·98 in all the 6 isolates were not toxic, the UV spectra of these samples were identical with the spectrum of the toxic component 'A' (figure 1, A) obtained from paper chromatograms. The eluates from R_f 0·75 stimulated root growth but slightly inhibited shoot growth. Figure 2 B shows the UV spectrum of

Table 2. Growth response of rice seedlings to components of ethyl acetate extracts of culture filtrates of *Pyricularia* spp. grown in sucrose-nitrate medium as stationary cultures.

Eluates from R_f		Isolates from O.S.	E.C.	S.I.	P.R.	B.M.	L.H.
0·96	R	+ 40	+ 89	+ 76	+ 56	+ 69	+ 87
	S	+ 8	+ 7	+ 2	− 6	0	− 2
	ST	A	A	A	A	A	A
0·74	R	+ 96	+120	+ 69	+ 64	+ 80	+120
	S	+ 2	+ 9	− 8	− 4	− 4	− 4
	ST	B	B	B	B	B	B
0·57	R	*	*	+ 44	+ 40	+ 80	+ 89
	S			− 2	− 2	0	− 2
	ST			C	C	C	C
0·35	R	*	0	− 68	− 78	−100	+ 69
	S		− 12	− 20	− 10	− 94	− 2
	ST		F	F	F	F	F
0·21	R	+ 44	−100	− 62	+ 78	− 71	+ 11
	S	− 4	− 48	− 12	+ 11	− 6	− 1
	ST	H	H	H	H	H	H
0·05	R	+107	−100	+ 64	− 61	+ 71	+ 86
	S	− 4	− 37	− 2	− 8	− 4	− 2
	ST	—	I	I	I	I	—

O.S. : *O. sativa*
E.C. : *E. coracana*
S.I. : *S. italica*
P.R. : *P. repens*
B.M. : *B. mutica*
L.H. : *L. hexandra*

R : root } % inhibition (−) or stimulation (+)
S : Soot } calculated over water controls
ST : Spectral type in ethanol (*vide* figure 2)
— : No UV spectrum
* : No UV absorbing/fluorescent band
F : Pyriculol

this component. The eluates from R_f 0·56 were toxic in the case of 4 of the 6 isolates. Although no toxicity was evident at this R_f with the isolate

Table 3. Growth response of rice seedlings to components of ethyl acetate extracts of culture filtrates of *Pyricularia* spp. grown in Iwasaki medium as shake cultures.

Eluates from R_f		Un-inoculated medium	Isolates from O.S.	E.C.	S.I.	P.R.	B.M.	L.H.
	R	+ 5	+ 79	+ 38	+ 31	+ 76	+ 57	+ 31
0·98	S	+ 2	0	+ 5	+ 3	+ 2	+ 1	+ 5
	ST	—	A	A	A	A	A	A
	R	+ 40	+ 74	+ 63	+ 51	+ 76	+ 34	+ 39
0·90	S	+ 3	+ 6	+ 3	+ 3	0	+ 10	+ 3
	ST	—	—	—	—	—	—	—
	R	+ 50	+ 83	+ 73	+ 75	+113	+ 71	+ 51
0·77	S	+ 2	+ 2	− 8	− 3	− 8	− 7	+ 1
	ST	—	B	B	B	B	B	B
	R	+ 41	+ 56	*	− 64	− 63	*	*
0·56	S	+ 15	+ 5		− 13	− 8		
	ST	—	C		C	C		
	R	+ 36	+101	− 33	+ 46	− 74	− 55	− 42
0·35	S	+ 8	+ 5	− 16	+ 1	− 16	− 18	− 38
	ST	—	K	K	K	F	K	K
	R	+ 91	− 88	*	−100	−100	*	− 80
0·21	S	+ 10	− 34		− 60	− 29		− 36
	ST	—	L		L	L		M
	R	+ 31	− 81	*	*	*	*	*
0·10	S	− 12	+ 1					
	ST	—	N					
	R	+ 68	− 23	− 80	+ 77	− 16	− 51	− 58
0·05	S	− 4	− 13	− 18	0	− 19	− 20	− 27
	ST	—	0	I	—	0	I	0

O.S. : *O. sativa*
E.C. : *E. coracana*
S.I. : *S. italica*
P.R. : *P. repens*
B.M. : *B. mutica*
L.H. : *L. hexandra*

R : Root } % inhibition (−) or stimulation (+)
S : Shoot } calculated over water controls
ST : Spectral type in ethanol (*vide* figure 2)
* : No UV absorbing/fluorescent band
— : No UV spectrum
F : Pyriculol

from *E. coracana* and *L. hexandra*, these two eluates also gave the same UV spectra as the other samples (figure 2, C) indicating that the component was not present in toxic concentrations. The isolate from *O. sativa* showed toxicity at R_f 0·45 and the UV spectrum of the eluate is given in figure 2, D. Eluates from R_f 0·35 were toxic in the case of the isolates from *O. sativa* and *B. mutica*. Although toxicity of the latter could be ascribed to pyriculol (figure 2, F), the former gave a different UV spectrum (figure 2, E). The isolate from *E. coracana* showed toxicity at R_f 0·29 and the UV spectrum of the eluate is shown in figure 2, G. With all isolates, eluates from R_f 0·19 were toxic and the spectra of these samples were identical (figure 2, H). Toxicity was also evident at R_f 0·05 in the case of

Table 4. Growth response of rice seedlings to components of ethyl acetate extracts of culture filtrates of *Pyricularia* spp. grown in Iwasaki medium as stationary cultures.

Eluates from R_f		Isolates from O.S.	E.C.	S.I.	P.R.	B.M.	L.H.
0·98	R	+ 71	0	+ 21	+ 52	+ 67	+40
	S	+7	+ 6	+ 16	+ 12	+ 6	+ 7
	ST	@	@	@	@	@	@
0·55	R	−100	−100	−100	− 52	*	+ 64
	S	−100	−100	−100	− 7		+ 10
	ST	P	P	P	P		—
0·35	R	*	*	*	−100	− 74	+ 38
	S				− 52	− 14	0
	ST				K	K	K
0·25	R	−100	−100	−100	*	− 21	*
	S	−100	−100	−100		− 7	
	ST	Q	Q	Q		R	
0·06	R	−100	−100	−100	− 60	−100	+ 38
	S	−100	−100	− 53	− 15	−100	+ 2
	ST	S	S	S	I	I	—

O.S. : *O. sativa*
E.C. : *E. coracana*
S.I. : *S. italica*
P.R. : *P. repens*
B.M. : *B. mutica*
L.H. : *L. hexandra*

R : Root } %inhibition (−) or stimulation (+)
S : Shoot } calculated over water controls
ST : Spectral type in ethanol (*vide*, figure 2)
— : No UV spectrum
* : No UV absorbing/fluorescent band
@ : Not studied.

the isolates from *E. coracana*, *P. repens* and *B. mutica* but the UV spectrum of the eluate of the *E. corocana* isolate (figure 2, I) differed from those of the other two isolates (figure 2, J),

Phytotoxic components in culture filtrates of Pyricularia *grown in sucrose-nitrate medium as stationary cultures.*—The 6 isolates of *Pyricularia* were grown for 40 days and the ethyl acetate extracts from 1 l filtrates were analysed as before and the results are presented in table 2.

It is evident from the results that components 'A' and 'B' detected in shake cultures were also present in stationary cultures at about the same R_f. Similarly, component 'C' was also located at about the same R_f as before in the case of 4 of the 6 isolates. However, the eluates were not toxic indicating that the component was not present in toxic concentrations. Again, although pyriculol was detectable at R_f 0·35 with all isolates except that from *O. sativa*, this component did not appear to be present in toxic concentrations in the case of the isolates from *E. coracana* and *L. hexandra*.

Table 5. Phytotoxic components detected in culture filtrates of races of *P. oryzae*

Races	MEDIUM			
	Sucrose-nitrate		Iwasaki	
	Cultural condition			
	Shake	Stationary	Shake	Stationary
Indian				
IC1	A C H J	A F H I	A C F U I	A C K L I
IC17	A C H J	A F H J	A C K L U N	A K L N
ID1	A C H J	A C F H J	A F U I	A C K I
IE1	A C H J	A H J	A C F U N	A C K Q R
IF1	A C H U J	A F H I	A C F U N	A C K L I
Philippine				
P16	A C H J	A H J	A C F N	A C K L I
P26	A C H J	A F H I	A C F I	A C F L I
P92	A C H J	A F H J	A C F L O	A C F L N
P149	A C H J	A C H J	A C F N	A C F I
P150	A C H J	A C H J	A C K L N	A C K L N

F : Pyriculol

The components 'H' and 'I' were also detectable at about the same R_f values as before. However, it may be seen that these were present in toxic concentrations only in filtrates of some isolates but not in others. Thus, 6 of the 10 components, recorded in shake cultures, were also detected in stationary cultures.

Table 6. Physico-chemical properties of toxic components detected in culture filtrates of *Pyricularia* spp.

R_f	Toxic component	Colours under UV	Reactions to spray reagents @ 1, 2, 3, 4	5	6	7, 8, 9	λ_{max} nm in ethanol
0·97	A	Blue	+	—	+	+	225 (1·32) 274 (0·24) 282 (I)
0·75	B	Absorption	+	*	*	+	257·5
0·56	C	Yellow	+	—	+	+	216 (1·60) 259 (1·1) 333 (0·42)
0·56	P	Absorption	+	+	—	+	237·5 (0·76) 278 (1·18)
0·45	D	Pale blue	*	*	*	*	214 (1·84) 255 (BP) (1·12)
0·35	E	Absorption	*	*	*	*	215 (1·98) 278 (1·68)
0·35	F	Reddish brown	+	—	+	+	232 (1·62) 278 (0·7) 352 (0·36)
0·35	K	Absorption	+	—	+	+	223 (1·38) 277 (0·36) 285 (I)
0·29	G	Blue streak	*	*	*	*	227 (0·94) 260 (BP) (0·22)
0·25	Q	Absorption	+	—	+	+	232 (BP) (0·82) 278 (1·18)
0·25	R	Yellow	*	*	*	*	275 (BP)
0·20	H	Bright yellow	+	—	+	+	216·5 (1·80) 240 (I) 261 (1·04) 327 (0·56)
0·20	L	Yellow	+	—	+	+	216 (1·66) 282·5 (0·98) 325 (I)
6·20	M	Pale yellow	*	*	*	*	225 (1·74) 276 (0·64) 286 (I)
0·10	N	Pale blue	*	*	*	*	275
0·10	U	Grey	*	*	*	*	222 (1·42)·276·5 (0-62) 317·5 (1·13)
0·06	I	Blue	+	—	+	+	219·5 (1·74) 254 (0·84) 293 (BP) (0·26)
0·06	J	Pale blue	*	*	*	*	221·5 (1·54) 265 (BP) (0·70)
0·06	O	Yellow	*	*	*	*	276 (I)
0·06	S	Blue	+	—	+	+	225 (I) 277 (0·48)
0·06	T	Grey	+	—	+	+	218 (1·41) 251 (0·90) 354 (1·16)

F: Pyriculol; @: *vide* Materials and Methods; BP: Broad peak; I: inflection +: positive; —: negative; *not tested; (Figures in paranthesis refer to absorption values).

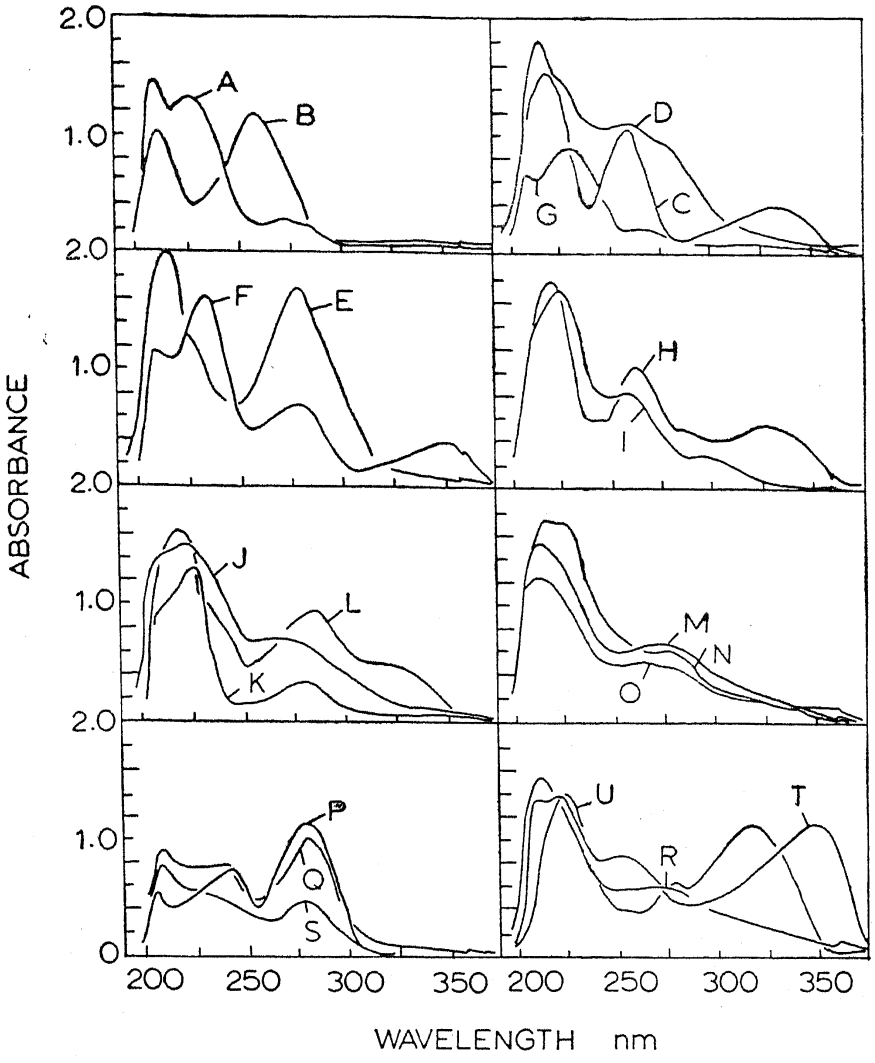

Figure 2. UV spectra in ethanol of phytotoxic components of *Pyricularia* spp. F = Pyriculol.

Phytotoxic components in culture filtrates of Pyricularia *grown in Iwasaki medium as shake cultures.*—The 6 isolates were grown for 6 days and the ethyl acetate extracts were analysed for phytotoxicity as before. The results are presented in table 3.

It is evident from the data that the toxic components 'A', 'B', 'C' and 'I', detected in shake and stationary cultures grown in sucrose-nitrate medium, were also detectable in cultures grown in Iwasaki medium. It may also be noted that the eluates from R_f 0·35 were toxic in the case of the isolates from *E. coracana*, *P. repens*, *B. mutica* and *L. hexandra*. The eluate of the isolate from *P. repens* gave a typical spectrum of pyriculol (figure 2, F) but the other eluates gave a different spectrum (figure 2, K). The same component was also detected in the case of the isolates from *O. sativa* and *S. italica* though not present in toxic concentrations. Toxi-

city was again evident at R_f 0·21 but the UV spectrum of the eluates of the isolates from *O. sativa*, *S. italica* and *P. repens* (figure 2, L) was different from that of *L. hexandra* (figure 2, M). The isolate from *O. sativa* showed toxicity at R_f 0·10 and the UV spectrum of the eluate is shown in figure 2, N. The toxic eluates from R_f 0·05 gave two types of UV spectra. With the isolates from *E. coracana* and *B. mutica*, the spectra corresponded to that shown in figure 2, I. However, in the case of the isolates from *O. sativa*, *P. repens* and *L. hexandra*, the spectra of the eluates were different (figure 2, 0). Thus, the UV spectra of certain toxic eluates (K, L, M, N, O) appeared to be different from those recorded in the previous experiments.

Phytotoxic components in culture filtrates of Pyricularia *grown in Iwasaki medium as stationary cultures.*—The phytotoxic components from cultures of the 6 isolates grown for 40 days are shown in table 4.

Eluates from R_f 0·55 were toxic in 4 of the 6 isolates and the UV spectrum of the eluates (figure 2, P) appeared to be different from those recorded in the earlier experiments. Eluates from R_f 0·35 were toxic in the case of the isolates from *P. repens* and *B. mutica*. The UV spectra of these samples were identical with those recorded for some of the eluates from the same R_f (figure 2, K) in the earlier experiment (table 3) where the isolates were grown as shake cultures in the same medium. The same component was also detected in the filtrate of the isolate from *L. hexandra* though not in toxic concentration. Toxicity was also evident at R_f 0·25 but the eluates gave 2 types of UV spectra which appeared to be different from all other spectra recorded in the previous experiments. The spectrum (figure 2, Q) was registered by the eluates of the isolates from *O. sativa*, *E. coracana* and *S. italica*. On the other hand, with the isolate from *B. mutica*, the eluate showed a different spectrum (figure 2, R). Again, while the toxic eluates from R_f 0·06 gave a UV spectrum as shown in figure 2, I in the case of the isolates from *P. repens* and *B. mutica*, the eluates of the isolates from *O. sativa*, *E. coracana* and *S. italica* gave a different UV spectrum (figure 2, S) which appeared to be different from those described so far.

Besides the above, another component (figure 2, T) was also detected at R_f 0·06 in the case of the isolate from *B. mutica* grown in sucrose-nitrate medium as shake culture. This component extracted from 2 l of the filtrate casued 89% root inhibition and 16% shoot inhibition in rice seedlings.

Toxic components detected in filtrates of races of P. oryzae *grown in sucrose-nitrate and Iwasaki media as shake and stationary cultures.*—The following races, *viz.*, IC1, IC17, ID1, IE1, IF1 (Indian), P16, P26, P92, P149 and P150 (Philippines) were examnied. Ethyl acetate extracts from

200 ml each of the filtrates were analysed by TLC and the spectra of the UV absorbing/fluorescing components were compared with those mentioned earlier and the results are presented in table 5.

It will be seen from the table that many of the components already described were detectable in filtrates of the races of *P. oryzae*. Further, another component (figure 2, U) was also detected in filtrates of the race IF1 grown in sucrose-nitrate medium and in the Indian races grown in Iwasaki medium as shake cultures. This component when extracted from 1 l of Iwasaki medium caused 67% root and 25% shoot inhibition in rice seedlings.

Phytotoxic components from shake cultures grown in Tamari's medium.— The 6 isolates were grown for 10 days and the toxic components from 200 ml each of the filtrates were studied by TLC and spectrophotometry. The components A, C, I, K and L were identified in the filtrates.

The physico-chemical properties of the toxic components detected in culture filtrates of the isolates of *Pyricularia* are shown in table 6.

It will be seen from the table that the components generally differed in their R_f values and, when they did not, differed spectrally. The colour reactions of the components tested indicate that most of them are unsaturated and phenolic in nature. In addition, component 'P' appeared to be an acid.

Discussion

It is evident from the results presented that ethyl acetate extracts of culture filtrates of *Pyricularia* could be resolved into a number of phytotoxic components, including pyriculol, by TLC and bioassay techniques (tables 1–4). It is also obvious that though pyriculol was not detectable in certain filtrates, they contained other phytotoxic components.

Regardless of the isolate, medium or cultural condition, eluates of the chromatograms from R_f 0·55–0·57, 0·35, 0·25, 0·19–0·21, 0·10–0·11 and 0·05–0·07 were generally phytotoxic. However, phytotoxicity at a particular R_f could not be ascribed to the same component. For instance, while some of the toxic eluates from R_f 0·35 showed pyriculol (figure 2, F), others contained components whose UV spectra (figure 2, E and K) were different from that of pyriculol. A similar situation could also be seen with reference to eluates from other R_f values (table 6). A further point of interest is that although some of the eluates were not toxic, the UV spectra of their components corresponded to those detected in some of the phytotoxic eluates suggesting that the components were not present in toxic concentrations in such eluates. It is also evident that not all the compo-

nents were produced by the same isolate or in the same medium or under the same cultural condition. Such differences were also found in the case of *P. oryzae* races (table 5).

Reference to table 6 will show that most of the components detected in the toxic eluates are phenolic in nature and also unsaturated like pyriculol but could be distinguished by their characteristic UV absorption spectra. Of the many components of the culture filtrates described here, we have been able to detect pyriculol in blast diseased leaves of *B. mutica*[12]. We have also detected component 'A' in blast lesions of the 6 gramineous hosts and component 'B' in lesions from *B. mutica* and *P. repens* (unpublished).

Since this work was done, Iwasaki *et al.*[13] reported the isolation of many phytotoxic substances from the culture broth of *P. oryzae* of which three compounds were considered to be related to pyriculol. Of these, one was established as a reduction product of pyriculol (UV absorption maxima in ethanol at 219, 254 and 293 nm) by $NaBH_4$ reduction and microbial transformation of pyriculol with *P. oryzae*. In the present work, the UV spectrum of component 'I' detected in some of the toxic eluates from R_f 0·05–0·06 (tables 1–4), closely corresponds to that of reduced pyriculol (table 6). Further work is in progress regarding the identity of this component as well as others and their phytotoxicity.

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