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Relationship between preformed/post-infectional antifungal substances in leaves of *Panicum repens* L. and compatibility with *Pyricularia* spp.*

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ABSTRACT

Preformed and post-infectional antifungal substances appear to be involved in the compatibility of *Pyricularia* spp. with *Panicum repens* L. Diffusates and extracts of 'uninoculated' or leaves inoculated with the compatible or incompatible isolates showed antifungal activity. However, the antifungal activity was found to be localized. Antifungal activity was also evident in mechanically injured leaves or leaves treated to cell free germination fluid of an incompatible isolate. The presence of preformed and post-infectional protectants was confirmed by thin-layer chromatography. Post-infectional protectants appeared earlier in leaves inoculated with the incompatible isolate than in leaves inoculated with the compatible isolate. The incompatible isolate was more sensitive than the compatible isolate to preformed and post-infectional protectants.

1. INTRODUCTION

IN an earlier paper[1] we reported that pre-inoculation of leaf tissues of *P.repens* L. with an incompatible isolate of *Pyricularia oryzae* Cav. (P_1) or a UV mutant derived from it (M_1) protected the leaves from subsequent infection by a compatible isolate (PR). The protection, though localized, appeared to be the result of an interaction between the host and the pre-inoculant. In the present communication we describe experiments which indicate the involvement of preformed and post-infectional antifungal substances in the compatibility of *Pyricularia* spp. with *P. repens*.

2. MATERIALS AND METHODS

BIOASSAY OF DIFFUSATES: Detached *P. repens* leaves (16 for each treatment) were floated on benzimidazole and inoculated with either the incompatible P_1/M_1 or the compatible PR isolate (4×10^5 conidia/ml) as

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B 7—April 77

described previously.[1] About 4 ml of conidial suspension was used for each treatment. The control leaves received distilled water droplets. The leaves were incubated under diffuse light for 5 days in a growth room. At the end of this period, infection droplets and the droplets from control leaves were collected by means of Pasteur pipettes. The amount of diffusates thus collected varied between 1·0–1·25 ml. The diffusates were centrifuged at 4000 r.p.m. for 15 min and the cell free supernatants dried over $CaCl_2$ in a vacuum desiccator at room temperature. The dry residues were taken in 1·0 ml portions of water and distributed equally (0·25 ml) on 4 microscope slides. The material was allowed to air dry at room temperature. Drops (*ca* 0·01 ml) of conidial suspension (10×10^4/ml) of P_1, M_1, or PR were placed on the dry residues and the slides incubated in Petri dish moist chambers for 24 hr in the growth room. Duplicate slides were kept for each treatment. At the end of incubation, percentage germination, germ tube length and appressorial formation were assesed as before.[1]

BIOASSAY OF LEAF EXTRACTS: Since cross-protection appeared to be highly localized,[1] leaf samples for assessment of fungitoxic materials within the tissue were prepared as follows. The leaves, after removal of the diffusates, were washed free of any benzimidazole adhering to the abaxial surface. Leaves inoculated with the incompatible isolates were cut to exclude much of the uninoculated regions. In the case of leaves inoculated with the compatible isolate, portions not showing symptoms were excluded. Leaf tissue below the water droplets was used in the case of uninoculated controls.

A weighed amount of leaf tissue (500 mg fresh weight) was extracted thrice with 10 ml of 80% ethanol in a pestle and mortar, centrifuged at 6000 r.p.m. for 15 min and the supernatant dried under vacuum at 45–47° C in a rotary evaporator. The dried material was taken in 1·5 ml water when a turbid solution resulted. The extract was again centrifuged at 6000 r.p.m. for 15 min when a clear supernatant was obtained with a little residue. Aliquots (0·12 ml) of the supernatant were air dried on microscope slides. The residue was dispersed in 0·5 ml water and 0·12 ml aliquots were dried on slides as before. Conidial suspension (0·01 ml) of P_1, M_1 or PR was placed on the dry residues. The slides were incubated in moist chambers and percentage germination, germ tube length and appressorial formation were assessed as before.[1] Distilled water controls were also kept for all bioassays.

THIN-LAYER CHROMATOGRAPHY OF LEAF EXTRACTS: *P. repens* leaves (50–60 for each treatment) were inoculated with a conidial suspension (12 ml) of the compatible PR or incompatible P_1. The healthy controls

received distilled water drops. Leaf tissue samples (1·2 to 1·5 g fresh weight) were prepared as before at 24, 48, 72 and 120 hr incubation and extracted thrice with 30 ml of 80% cold methanol (AR) in a precooled pestle and mortar, centrifuged at 6000 r.p.m. for 15 min and the supernatants collected. Cold methanol was preferred to ethanol since it reduced the interference by pigments during subsequent chromatography. Preliminary experiments indicated no difference between the two alcohols in the extraction of fungitoxic materials. The methanol in the supernatant was driven off at 50° C under vacuum in a rotary film evaporator and the aqueous residue (pH 5·5) was extracted 6 times with ethyl acetate (AR). The pooled ethyl acetate extracts were taken to dryness *in vacuo* in a rotary evaporator at 45° C. These samples are referred to as Fraction 1 samples.

The mother liquor was acidified to pH 2·0 with H_2SO_4 (AR), extracted three times with ethyl acetate and the pooled extracts dried as before. The samples are termed here as Fraction II samples.

Healthy leaves (1·5 g fresh weight) were similarly processed immediately after detachment (0 time samples).

Eastman silica gel chromatogram sheets (No. 6061 without fluorescent indicator) were activated at 120° C for 30 min. Samples representing 750 mg fresh weight of leaves in ethyl acetate were streaked 2 cm from one edge of the sheets. The chromatograms were developed in benzene: ethyl acetate (7: 3) to a distance of 13 cm and dried at room temperature. Antifungal activity on the chromatograms was detected by a direct bioassay technique.

DETECTION OF ANTIFUNGAL ACTIVITY ON THIN-LAYER CHROMATOGRAMS: Antifungal materials on the chromatograms were bioassayed according to the method recommended by Keen *et al.*[2] However, *Cladosporium herbarum* spores were used. Conidia from one week old cultures growing on potato-dextrose agar were harvested and washed by centrifugation. A highly concentrated conidial suspension was sprayed on the chromatograms which were then oversprayed with half strength potato-dextrose agar at *ca* 45° C. The sprayed chromatograms were incubated in moist chambers at room temperature for 48 hr. The chromatograms were briefly air dried and sprayed again with half strength potato-dextrose agar and incubated further in a moist chamber for 24 hr. At the end of this period, fungitoxic zones appeared as white bands on a dark background and their Rf values were measured.

3. EXPERIMENTAL AND RESULTS

ANTIFUNGAL ACTIVITY OF DIFFUSATES AND EXTRACTS OF HEALTHY AND INOCULATED LEAVES: The effect of diffusates and extracts of healthy/P_1, M_1 or PR inoculated leaves on the germination response of P_1, M_1 and PR conidia is presented in tables 1–3.

It is evident that diffusates from healthy or inoculated leaves did not appreciably affect conidial germination in the three isolates. However, diffusates from healthy leaves inhibited germ tube growth in the incompatible P_1 and M_1 as against a slight stimulation in the compatible PR isolate. Diffusates from P_1 and M_1 inoculated leaves inhibited germ tube growth in all the three isolates but this inhibition was greater in the incompatible P_1 and M_1 than in the compatible PR (tables 1 and 2). Diffusates from leaves inoculated with the compatible PR, also inhibited germ tube growth in P_1, but stimulated it in PR (table 3).

Appressorial formation, in general, was suppressed in diffusates from healthy leaves, but this effect was greater in the incompatible P_1 and M_1 than in the compatible PR. Diffusates from leaves inoculated with P_1 or PR did not further affect appressorial formation in the PR isolate (tables 1 and 3). The diffusates from M_1 inoculated leaves, however, suppressed appressorial formation in PR to a greater extent than the diffusates from healthy leaves (table 2). Similarly, the percentage of P_1 appressoria was decreased to a greater extent in the diffusate from P_1 inoculated leaves than in the 'healthy control.'

Table 1. Germination response of PR and P_1 conidia in diffusates and leaf extracts of *P. repens* inoculated with the P_1 isolate

Treatment	% germination		Increase (+) or decrease (−) in germ tube length (% of control)		% appressoria	
	PR	P_1	PR	P_1	PR	P_1
Water (control)	97·8	96·8	..	..	16·0	50·9
Diffusate						
Healthy	98·2	98·2	+ 4·3	−11·8	8·7	15·8
Inoculated	96·9	96·7	− 3·6	−27·8	8·2	5·8
Leaf extract						
Healthy (S)	98·8	97·5	+44·2	−26·4	4·8	7·9
Inoculated (S)	96·7	88·0	+25·7	−68·8	1·5	4·0
Healthy (R)	99·2	96·1	+57·6	−19·0	6·1	8·7
Inoculated (R)	93·4	87·1	+35·7	−67·1	1·2	2·3

S = supernatant, R = residue.

Table 2. Germination response of PR and M_1 conidia in diffusates and leaf extract of *P. repens* inoculated with the M_1 isolate:

Treatment	% germination		Increase (+) or decrease (−) in germ tube length (% of control)		% appressoria	
	PR	M_1	PR	M_1	PR	M_1
Water (control)	97·7	86·6	..	..	27·2	25·0
Diffusate						
Healthy	97·9	84·2	+ 4·4	−19·1	12·3	0·0
Inoculated	98·6	80·3	− 6·7	−27·3	0·2	0·0
Leaf extract						
Healthy (S)	99·9	78·3	+77·3	+14·6	1·0	0·0
Inoculated (S)	98·9	75·0	+59·3	−21·8	1·0	0·0
Healthy (R)	99·8	74·4	+74·8	+ 2·5	1·0	0·0
Inoculated (R)	99·3	70·2	+43·1	− 0·9	0·7	0·2

S = supernatant, R = residue.

Table 3. Germination response of PR and P_1 conidia in diffusates and leaf extracts of *P. repens* inoculated with the PR isolate

Treatment	% germination		Increase (+) or decrease (−) in germ tube length (% of control)		% appressoria	
	PR	P_1	PR	P_1	PR	P_1
Water (control)	95·8	96·3	..	..	28·5	54·3
Diffusate						
Healthy	96·7	95·9	+ 3·5	− 3·8	18·0	0·0
Inoculated	97·8	94·4	+27·1	−16·5	18·7	0·7
Leaf extract						
Healthy (S)	96·8	95·9	+40·7	−12·0	0·7	0·2
Inoculated (S)	96·2	85·8	+22·0	−59·3	0·0	0·1
Healthy (R)	97·8	96·0	+54·2	−22·0	0·7	0·4
Inoculated (R)	96·3	89·7	+42·4	−57·1	0·0	0·0

S = supernatant, R = residue.

Extracts from healthy leaves did not appreciably affect germination of P_1 and PR conidia (tables 1 and 3) but reduced the germination of M_1 conidia by 8–12% (table 2). Extracts from M_1 inoculated leaves further reduced germination in the same isolate but did not affect germination of PR conidia. Extracts from leaves inoculated with the incompatible P_1 or compatible PR did not also appreciably affect germination of PR conidia but reduced the germination of P_1 conidia by 7–10% as compared to 'healthy' (tables 1 and 3).

Extracts of healthy or inoculated leaves stimulated germ tube growth of the compatible PR but the stimulation was less in the 'inoculated' than in the healthy (tables 1–3). On the contrary, extracts of leaves inoculated with P_1 or PR inhibited germ tube growth in P_1 and this inhibition was greater in the 'inoculated' than in the 'healthy' (tables 1 and 3). Extracts of healthy leaves stimulated germ tube growth in M_1 but extracts of M_1 inoculated leaves inhibited the germ tube growth in the same isolate (table 2).

In leaf extracts appressorial formation was further reduced or even negligible as compared to diffusates. However, the reduction was greater in the 'inoculated' than in the 'healthy'.

To sum up, the results clearly indicate that both healthy and inoculated leaf tissues contained fungitoxic material that was more inhibitory to the incompatible P_1 and M_1 than the compatible PR.

ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS PREPARED FROM REGIONS IN BETWEEN P_1 PROTECTED SITES: In an earlier paper[1] we reported that cross-protection was not evident in leaves of *P. repens* when the compatible PR was inoculated in regions in-between previously inoculated P_1 sites. The object of the present experiment was to find out if antifungal activity was also localized. Regions in-between P_1 inoculated sites were excised and leaf extracts prepared (*vide*, Materials and Methods). The germination response of the compatible PR and incompatible P_1 in the leaf extracts is shown in table 4.

It is evident from the results that the extracts did not appreciably affect germination of conidia of both isolates or inhibit their germ tube growth. Appressorial formation was negligible in the treatments. Thus, no antifungal activity could be demonstrated in areas of leaf tissue in-between P_1 protected sites.

Table 4. Germination response of PR and P_1 conidia in *P. repens* leaf extracts obtained from regions in-between P_1 inoculated sites.

Treatment	% germination		Increase (+) or decrease (−) in germ tube length (% of control)		% appressoria	
	PR	P_1	PR	P_1	PR	P_1
Water (control)	97·5	96·3	..	..	29·8	53·4
Leaf extracts						
Healthy (S)	97·7	94·3	+32·5	+ 2·3	0·6	0·3
Inoculated (S)	95·4	94·7	+35·4	+ 4·6	0·3	0·0
Healthy (R)	96·9	96·1	+30·9	+ 4·7	0·2	0·1
Inoculated (R)	97·2	95·3	+34·0	− 0·5	0·3	0·0

S = supernatant, R = residue.

ANTIFUNGAL ACTIVITY OF DIFFUSATES AND EXTRACTS OF LEAVES TREATED TO P_1 GERMINATION FLUID: The antifungal response of *P. repens* leaf tissue treated to cell-free germination fluid of P_1 is reported here. Conidia of P_1 (4×10^5/ml) were germinated for 24 hr in distilled water and germination fluid obtained by centrifugation.[1] Drops of distilled water or germination fluid were placed on detached leaves and treated further as mentioned under Materials and Methods.

The germination response of PR and P_1 conidia to the treatments is shown in table 5. Germination of conidia of both isolates, was reduced in diffusates from leaves treated to the germination fluid as compared to the 'healthy' diffusate or controls. Diffusates from 'healthy' stimulated germ tube growth in PR but tended to inhibit it in P_1. The diffusate from the 'treated' inhibited germ tube growth of P_1 to a greater extent than that of PR. Germ tube growth of PR and P_1 was stimulated in the 'healthy' leaf extracts. This stimulation tended to be less in the 'treated' extracts in the case of PR but in P_1, slight inhibition was observed.

Appressorial formation in both isolates was reduced in the 'healthy' diffusates but the extent of this reduction was greater in the incompatible P_1 than in the compatible PR. As compared to the diffusate from 'healthy', the 'treated' diffusate tended to further reduce appressorial formation in PR but stimulated it in P_1.

Table 5. Germination response of PR and P_1 conidia in diffusates and extracts of *P. repens* leaves treated to P_1 germination fluid

Treatment	% germination		Increase (+) or decrease (−) in germ tube length (% of control)		% appressoria	
	PR	P_1	PR	P_1	PR	P_1
Control						
Water	95·3	95·5	...	...	30·7	53·5
Germination fluid	82·8	93·9	−28·0	− 6·6	32·9	59·1
Diffusate						
Healthy	96·6	95·5	+17·5	− 4·7	19·2	9·7
Treated	73·6	77·9	− 3·6	−20·6	13·5	22·9
Leaf extracts						
Healthy (S)	98·5	96·7	+43·8	+ 8·8	0·8	0·0
Treated (S)	97·9	95·8	+38·2	− 1·8	0·6	0·0
Healthy (R)	97·4	96·3	+45·7	+ 8·9	0·2	0·0
Treated (R)	96·3	96·1	+40·8	− 0·4	0·6	0·0

S = supernatant, R = residue.

ANTIFUNGAL ACTIVITY OF DIFFUSATES AND EXTRACTS OF PHYSICALLY INJURED LEAVES: The adaxial surface of detached leaves was physically injured with a stainless steel needle. Drops of distilled water were placed on the injured sites. Uninjured control leaves were also similarly treated. The leaves were incubated for 5 days and processed as before.

The results are presented in table 6. Germination of conidia of both PR and P_1 isolates was not appreciably affected in any of the treatments. Germ tube growth in the compatible PR was stimulated in all treatments except in the diffusates from 'injured' where a slight inhibiton was noted. On the contiary, germ tube growth in the incompatible P_1 was inhibited in all treatments and the extent of this inhibition tended to be greater in the 'injured' than in the 'uninjured' treatments. The diffusates reduced appressorial formation in both isolates as compared to the control but there was no appreciable difference between 'uninjured' and 'injured' treatments. However, appressorial formation in the diffusates was inhibited to a greater extent in P_1 than in PR.

THIN-LAYER CHROMATOGRAPHIC STUDY OF ANTIFUNGAL SUBSTANCES IN HEALTHY AND INOCULATED LEAVES: Results of earlier experiments suggested that the healthy leaves of *P. repens* and leaves inoculated with the compatible or incompatible isolates of *Pyricularia* contained fungitoxic material. Attempts were, therefore, made to separate the fungitoxic substances by TLC (*vide*, Materials and Methods). Healthy and inoculated tissues were

Table 6. Germination response of PR and P_1 conidia in diffusates and extracts of physically injured *P. repens* leaves

Treatment	% germination		Increase (+) or decrease (−) in germ tube length (% of control		% appressoria	
	PR	P_1	PR	P_1	PR	P_1
Water (control)	95·1	96·5	..	..	31·1	54·2
Diffusate						
Uninjured	97·3	96·2	+ 5·2	−14·1	12·2	2·2
Injured	96·2	95·4	− 2·8	−20·7	14·7	1·3
Leaf extract						
Uninjured (S)	97·6	95·7	+48·9	−16·3	0·4	0·2
Injured (S)	96·1	94·6	+40·0	−23·3	0·3	0·0
Uninjured (R)	98·7	97·3	+48·4	−11·9	0·9	0·5
Injured (R)	97·4	96·8	+37·2	−21·6	1·3	0·7

S = supernatant, R = residue.

examined for antifungal substances at 24 hr intervals over a period of 120 hr from the time of inoculation. The Rf values of antifungal bands detected on thin-layer chromatograms are shown in table 7. Plate I shows a typical chromatogram bioassayed directly for antifungal substances.

Ethyl acetate extracts of uninoculated and inoculated leaves showed antifungal bands on the chromatograms indicating the presence of fungitoxic substances both in uninoculated and inoculated tissues. Fraction I samples of uninoculated and inoculated leaves showed an antifungal band between Rf 0·59 and 0·73 at all incubation periods. Extracts of uninoculated and leaves inoculated with the compatible PR showed an additional band with Rf 0·11 and 0·07 respectively at 72 hr incubation. However, extracts of leaves inoculated with the incompatible P_1 showed an additional band even from 24 hr after inoculation. The Rf of this band varied from 0·13 at 24 hr to 0·26 at 120 hr incubation. Antifungal activity at these Rf values appeared to be 'post-infectional' since extracts of uninoculated or leaves inoculated with the compatible PR did not show antifungal bands at the same or similar Rf values.

Fraction II samples of uninoculated leaves also showed an antifungal band. Extracts of inoculated leaves showed strong antifungal activity between Rf 0·64 and 0·73. While this antifungal component was detectable 24 hr after inoculation in leaves inoculated with the incompatible P_1, this was detected only from 48 hr after inoculation in leaves inoculated with the compatible PR. In addition, P_1 inoculated leaves showed slight antifungal activity between Rf 0·14 and 0·19 at all incubation periods.

GERMINATION RESPONSE OF PR AND P_1 CONIDIA TO PREFORMED AND POST-INFECTIONAL ANTIFUNGAL COMPONENTS: Some of the preformed and post-infectional antifungal components detected in the previous experiment were subjected to further study. Antifungal material from corresponding halves of bioassayed chromatograms was eluted thrice with ethyl acetate (AR) and the samples taken to dryness at room temperature. The residues were taken in ethyl acetate (1·0 ml) and aliquots (0·12 ml) were dried on microscope slides. Suspensions (0·01 ml) of P_1 or PR conidia (10×10^4/ml) were placed on the dry residues and the slides incubated in a moist chamber upto 48 hr. Water controls were also kept. Duplicates were maintained for each treatment.

The Rf values of the antifungal components tested and germination response of the two isolates to the components are shown in table 8.

Table 7. Rf values of antifungal bands shown by extracts of healthy and inoculated leaf tissues of *P. repens*.

Treatment	Incubation time (h)									
	Fraction I					Fraction II				
	0	24	48	72	120	0	24	48	72	120
Uninoculated	0·68	0·59	0·59	0·62	0·68+	0·90	..	..	..	..
	..	..	..	0·11	..	..	0·15*	0·15*	0·15*	0·15*
Inoculated (PR)		0·62	0·65	0·59	0·73		..	0·64	0·69	0·73
		..	..	0·07	..		0·17	..	..	0·17*
Inoculated (P_1)		0·62	0·61	0·65	0·73+		0·69	0·73	0·72	0·70+
		0·13*	0·20*	0·24*	0·26+		0·17*	0·14*	0·18*	0·19*

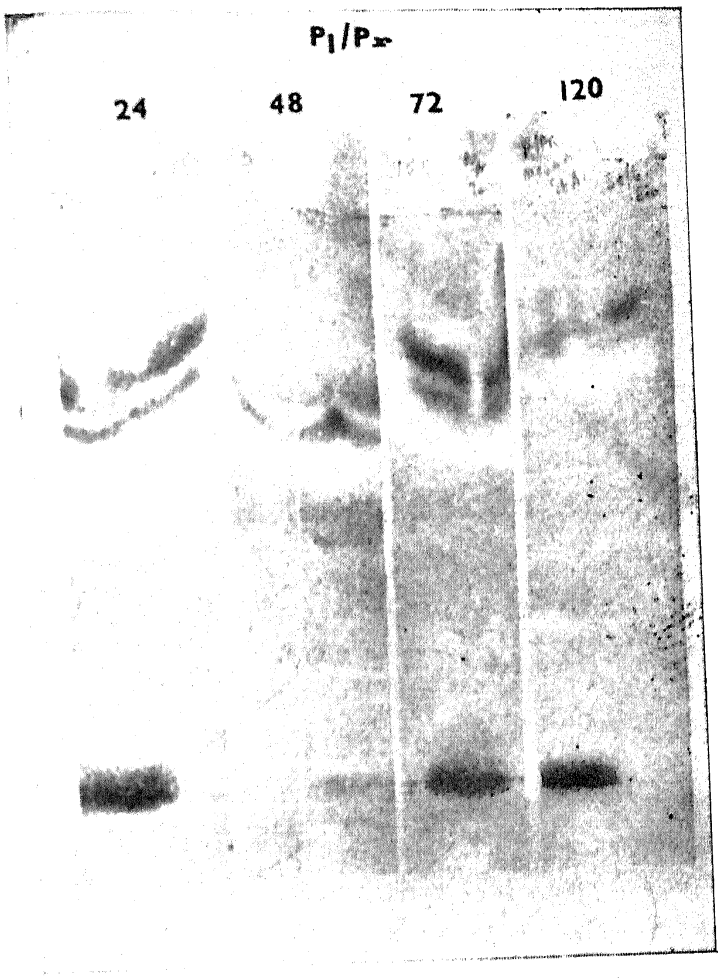
* : faint, + : *vide*, table 8.

Table 8. Germination response of PR and P_1 conidia to preformed and post-infectional antifungal substances from *P. repens* leaves

Treatment	Incubation time (h)	% germination		Increase (+) or decrease (−) in germ tube length (% of control) with appressorium		Increase (+) or decrease (−) in germ tube length (% of control) without appressorium		% appressoria	
		PR	P_1	PR	P_1	PR	P_1	PR	P_1
Water (control)	24	97·2	96·9	..	..	..	..	37·1	50·9
	48	97·0	97·1	..	..	..	..	35·2	51·4
Preformed (Rf 0·68)[a]	24	15·2	5·7	−42·4	nil	−70·8	−87·4	18·5	0·0
	48	15·9	6·2	−47·9	nil	−76·3	−91·5	19·1	0·0
Post-infectional (Rf 0·73)[b]	24	32·2	0·0	nil	nil	−87·6	nil	0·0	0·0
	48	37·3	0·0	nil	nil	−87·5	nil	0·0	0·0
(Rf 0·26)[c]	24	51·2	0·0	nil	nil	−86·7	nil	0·0	0·0
	48	37·3	0·0	+20·2	nil	−33·3	nil	51·9	0·0
(Rf 0·70)[d]	24	60·2	0·0	−38·7	nil	−8·9	nil	15·2	0·0
	48	74·9	0·0	+36·6	nil	−18·7	nil	60·8	0·0

a : from uninoculated, *b*, *c* and *d* : from P_1 inoculated.

Proc. Indian Acad Sci.,
Vol. 85 B, No. 4, 1977, pp. 257–268



A typical chromatogram showing the occurrence of antifungal substances in extracts of *P. repens* leaves.

(*facing page* 266)

It is evident from the results that the antifungal components from uninoculated or inoculated leaf tissues were inhibitory to germination and germ tube differentiation in both isolates. However, the incompatible P_1 appeared to be more sensitive to the antifungal components than the compatible PR.

4. Discussion

Uninoculated leaves of *P. repens* as well as leaves inoculated with the incompatible P_1, M_1 or the compatible PR showed antifungal activity when the diffusates and leaf extracts were bioassayed with P_1, M_1 or PR conidia (tables 1, 2, 3). However, it is evident from the results that the incompatible P_1 or M_1 was more sensitive than the compatible PR to the fungitoxic materials. It may also be seen that these materials affected appressorial formation to a greater extent in the incompatible than in the compatible isolate suggesting that differentiation of infection structures may be affected by the fungitoxic materials.

Bioassay of diffusates and extracts of mechanically injured leaves also yielded similar results indicating cellular damage could evoke antifungal responses (table 6). That such responses could also be elicited in the host by metabolites of the fungus, in this case, P_1, is also evident from the results presented in table 5. It may be seen again that germ tube growth of the incompatible P_1 was affected to a greater extent than that of the compatible PR by the fungitoxic materials. These results thus support the earlier conclusion[1] that cross-protection against 'blast' disease in *P. repens* leaves is the result of an interaction between the host and the preinoculant and not due to a mechanical barrier imposed by appressoria of the protectant isolate. Results presented in table 4 further indicate the localization of the antifungal response in protected leaves. It is, therefore, not surprising that cross-protection was also localized.

Chromatographic studies of healthy and inoculated leaves of *P. repens* lent further evidence for the presence of preformed and post-infectional antifungal substances. The points of interest here are that post-inoculation antifungal material appeared earlier in leaves inoculated with the incompatible P_1 than in leaves inoculated with the compatible PR (table 7) and that the incompatible isolate was more sensitive than the 'compatible' to preformed and post-infectional antifungal components as judged by their germination response (table 8).

In conclusion it, therefore, appears that both preformed and post-infectional antifungal substances are involved in the compatibility of *Pyricularia* spp. with *P. repens*. Since the phytoalexin concept was proposed,[3]

numerous reports have appeared on the production of antifungal substances in plants following inoculation with fungi.[4] The evidence presented here indicates that preformed protectants may also be of considerable importance in host-parasite relations as pointed out by Matrin[5] and Brian.[6]

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