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Influence of chemical profiles of host plants on the infestation diversity of *Retithrips syriacus*

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Abstract. The onset of biotic stress in the host plants as a result of increased insect population size leads to enhanced levels of secondary metabolites and associated phenolic enzyme activity· Of the three host plants examined, viz. *Ricinus communis* (castor), *Eucalyptus globulus* (eucalyptus) and *Manihot utilissima* (tapioca), castor was the host most preferred by *Retithrips syriacus*· Despite the fact that tapioca had the highest levels of secondary compounds, thrips infestation persisted· However, fecundity and growth were reduced because of the relatively high levels of primary metabolites. Gallic acid was found to be the most toxic of the phenolic acids, followed by pyrogallol, resorcinol, phloroglucinol and vanillic acid· The less toxic phenolic acids and flavanoids were detected in leaves that harboured thrips, while the preponderance of gallic acid was found in uninfested hosts. Thus the interaction of *Retithrips syriacus* with the hosts is governed essentially by the biochemical profiles of its hosts, which tend to be altered subsequent to infestation, thus manifesting induced resistance through enhanced production of phenolics

Keywords. *Retithrips syriacus;* castor; eucalyptus; tapioca; primary and secondary metabolites.

1. Introduction

The mechanisms of host plant resistance in response to insect infestation consists of a series of biochemical events, including increased production of phenolics, mediated by the activity of such enzymes as phenylalanine ammonia-lyase, tyrosine ammonia-lyase, peroxidase and polyphenoloxidase. The primary metabolites include carbohydrates and proteins, which are exploited by the herbivores for their growth and development (Rockstein 1978; Ananthakrishnan 1990; Jayaraj and Uthamasamy 1990). These primary metabolites also function as precursors of secondary substances, which are major elements of resistance in plants (Whittaker and Feeny 1971; Haslam 1985). The secondary substances determine the suitability of the substrate for colonization and exploitation by the herbivores and thus govern host preferences and acceptability. Age-correlated biochemical profiles of host tissues also significantly influence infestation patterns (Ananthakrishnan 1956; Suresh Kumar and Ananthakrishnan 1988). The present investigation involved analysis of the relative levels of primary and secondary metabolites in host tissues and the impact of changes in the constitution of phenolic acids on infestation of *Retithrips syriacus* (Mayet) on three hosts, viz. *Ricinus communis* (castor); *Eucalyptus globulus* (eucalyptus) and *Manihot utilissima* (tapioca).

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2. Materials and methods

2.1 *Primary, secondary metabolites*

Host plant leaves of the same age, which were free of thrips and which were heavily infested with thrips, were analysed for their biochemical profiles. Estimation of total proteins (Lowry *et al* 1951), total carbohydrates (Dubois *et al* 1956) and total amino acids (Moore and Stein 1948) were carried out for the status of primary metabolites. Total phenols (Bray and Thorpe 1954) and total flavanoids (Bate-Smith 1962) were analysed quantitatively for the levels of secondary substances. The activity of phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL) (Higuchi 1966), polyphenol oxidase (PPO) (Palmer 1963) and peroxidase (PO) (Loebenstein and Linsey 1961) were assayed using the enzyme extract taken from host leaves. Thin-layer chromatography and UV spectrophotometric analyses were used to identify the individual phenols and flavanoids (Harborne 1984). Individual sugars were identified using paper-chromatographic separation techniques (Harborne 1984). Fatty acids were analysed through HPLC with a Hewlett-Packard (HP-1090) system at 230nm using a Hypersil ODS 5 μm column, water and acetonitrile as solvents, and. a flow rate of 0·45 ml/min according to Schuster's (1985) gradient programme. The retention time and percentage of methylated fatty esters were recorded. The methylene chloride extracts of host leaves and thrips anal exudates were injected into a Hewlett–Packard 5971A GCMS (electron impact to 70 eV) interfaced with 5890A second-series GCMS computer systems with an NBS mass spectral library containing the spectra of over 38,000 compounds. The column was a fused silica capillary column (10 m×0·2 mm) with a cross-linked methylsilicone phase. Helium was used as the carrier gas. The temperature programme was 40°C to 250°C at 10° C per minute hold; injector transfer line and ion sources were set at 230° C, 200° C and 220° C respectively for detecting the mass spectrum of the volatiles specific to castor, eucalyptus and tapioca.

2.2 *Thrips infestation*

Thrips population density was determined as an average of the number of individuals occurring on 50 leaves from each of the hosts sampled. Observations on adult longevity, fecundity (Wasilla and Venkataraman 1967), larval growth index (Ananthakrishnan 1990) and Dobie's index of susceptibility (Dobie 1977) were based on the same sample size. Leaf disc experiments utilizing a four-armed glass chamber measuring 15 cm long and 7 cm in diameter were used to determine differential attraction of thrips. Attraction was defined as the number of individuals moving towards the leaf discs which were quantified as an average of 10 observations. The mobility index was defined as the ratio of distances travelled (10 cm) to time (min). Based on differential attraction, castor was found to be the most preferred host. The influence of individual phenols such as vanillic acid, gallic acid, resorcinol, phloroglucinol and pyrogallol were determined by spraying aqueous solutions of 0·1 to 1 ppm concentration of each of the phenols onto the surface of castor leaves maintained individually in 18-cm-diameter petri dishes and introducing thrips (three replicates). The deterrent effect was calculated on the basis

of feeding index. The total area considered for the feeding index was 5 cm^2 and for the infestation ratio the number of individuals was 20. All data obtained for population density, longevity, fecundity, larval growth index and index of susceptibility were analysed statistically for correlations against levels of primary and secondary metabolites, based on five pairs of observations considering the cumulative levels of primary and secondary metabolites on the one hand and thrips data on the other. Analysis of variance was used to determine the differential influence of the different phenolic acids with respect to the feeding index and infestation ratio of the thrips.

3. Results

Levels of primary metabolites in the three hosts were significantly higher in infested leaves. While carbohydrates, proteins and amino acids ranged from 185·4 to 231·4 mg/g, 28·6 to 41·6 mg/g and 0·08 to 0·15 mg/g tissue respectively in uninfested castor, infested leaves had 20% higher carbohydrate and 64·2% higher protein levels. Carbohydrates in uninfested eucalyptus ranged from 106·4 to 153·7 mg/g with infested leaves having 49% higher levels. Levels of total proteins and amino acids in eucalyptus were significantly higher than in castor, ranging from 41·2 to 83·8 mg/g up to a maximum of 115 mg/g in infested leaves and from 0·180 to 0·230 mg/g with the infested leaves bearing 35% higher levels, respectively. Of the three hosts, the highest levels of primary metabolites were recorded in tapioca (carbohydrates 340 mg/g, proteins 155 mg/g, amino acids 0·272 mg/g in uninfested leaves) and infested leaves also had significantly higher levels (16·8% carbohydrates, 31·1% proteins, 23·1% amino acids).

The level of total phenols varied in direct relation to the quantity of primary metabolites and was highest in tapioca (18·2 to 40·14 mg/g) followed by castor (15·6 to 35 mg/g) and eucalyptus (9·2 to 22 mg/g). Total flavanoids were significantly higher by 68% in castor followed by 70% increase in eucalyptus and 62% in tapioca in the infested hosts compared to the uninfested state. The activity of phenolic enzymes also increased with the onset of infestation, indicating induced resistance in the hosts. While PAL activity ranged from 0·021 to 0·02750 (μ cinnamic acid/h/100mg protein) in castor, corresponding to the degree of infestation, sharp increases (up to 89%) were recorded in eucalyptus and tapioca. TAL ranged from 1·22 to 7·61 (μ coumaric acid/h/l00 mg protein) in castor and increased in activity by 45% and 40% in eucalyptus and tapioca respectively. Accordingly, resistance to infestation, in terms of increased production of phenolics, was highest in tapioca followed by castor and eucalyptus. However, because of the significantly lower levels of readily utilizable primary metabolites in eucalyptus, the most suitable host was castor.

Highest population sizes (271 ± 24 individuals/leaf) were recorded in castor with average adult longevity being 16·7 ± 0·15 days. Average fecundity was 75 ± 1·1 eggs/female. Populations on eucalyptus were 43% less than those on castor and were lowest on tapioca (44 ± 2·4 to 114 ± 3·4). Adult longevity of thrips was reduced by 18% and 30% in the latter two hosts respectively. That eucalyptus and tapioca are less preferred hosts of thrips was also evidenced by the much reduced fecundity (62·2 ± 5 and 44 ± 8·3 respectively) in these hosts. The larval growth index (LGI) and the index of susceptibility (IS) also revealed castor to be the most favoured host

(LGI 18·2 ± 0·9, IS 1·94 ± 0·36) followed by eucalyptus (LGI 12·5 ± 0·78, IS 1·64 ± 0·11). Tapioca was the least suited of the hosts (LGI 8·7 ± 1·1, IS 1·46 ± 0·24). Analysis of variance for population sizes showed distinct variations ($P < 0·05$, Cd 30·93) indicating host-specific interactions. Abiotic factors such as high temperatures (approximately 38·2 ± 2·l°C) or heavy rainfall (above 90mm) were found to be detrimental to thrips populations. The optimal temperature for thrips was found to be approximately 26 ± 1·1° C.

Population densities correlated with levels of primary and secondary metabolites (table 1). The differential attraction and the mobility index of thrips were maximum towards castor (29·7, 0·5319) less towards eucalyptus (15·6, 0·31) and least towards tapioca (5·7, 0·18). The sugar constitutions of the three hosts were distinctly different. While galactose and galacturonic acids were detected in castor, rhamnose was identified in eucalyptus as the predominant sugar, apiose and rhamnose were identified in tapioca. Gallic acid was the most widely distributed phenolic and occurred in all three hosts, either individually in uninfested castor, in combination with syringic acid in eucalyptus or with salicylic acid and phloroglucinol in tapioca. Subsequent to infestation, vanillic acid, phloroglucinol, syringic acid and pyrogallol occurred in castor; while protocatechuic acid occurred in infested eucalyptus and tapioca, *p*-hydroxybenzoic acid was detected in all the three infested hosts. Such flavanoids as apigenin, hesperitin, chrysoeriol, naringenin and quercetin were common to all the three hosts prior to infestation. Kaempferol and hesperidin were detected in eucalyptus and castor respectively. With the onset of infestation the composition of flavanoids like the phenolic acids was altered, with dihydroquercetin appearing in castor, naringin in eucalyptus and luteolin in castor and tapioca. Linolenic (0·072%), linoleic (1·699%), palmitic (0·441%), stearic (0·363%) margaric (0·381%) and lauric acids (0·230%) were seen in uninfested castor; only the former three acids were detected subsequent to infestation. Lauric acid was the predominant constituent in uninfested eucalyptus (22·748%) followed by oleic (8·837%), linolenic (0·612%), linoleic (2%) and arachidic acids (0·353%). Lauric acid was much reduced in infested eucalyptus, which also contained traces of margaric acid. Tapioca had the same fatty acids profile as castor but in much higher levels and unlike in castor stearic, margaric and arachidic acids were detected when tapioca was infested. Lauric acid was the predominant fatty acid (32·351%) in uninfested leaves. Bioassays for the toxicity of the individual phenolic acids reveal

Table 1. Coefficient of correlation relating levels of metabolites and population sizes and growth parameters of *Retithrips syriacus*

	Ricinus communis		*Eucalyptus globulus*		*Manihot utilissima*	
	Primary metabolites	Secondary metabolites	Primary metabolites	Secondary metabolites	Primary metabolites	Secondary metabolites
Population size	0·88	−0·83*	0·96**	−0·92	0·97**	−0·75
Fecundity	0·97**	−0·99***	0·95	−0·99***	0·97**	−0·92*
Longevity	0·98**	−0·97**	0·96**	−0·97**	0·98**	−0·92*
LGI	0·91**	−0·86**	0·96**	−0·99***	0·92	−0·97**
IS	0·95***	−0·91**	0·96**	−0·98**	0·92**	−0·94*

*$P < 0·05$. **$P < 0·01$. ***$P < 0·0001$

gallic acid to be most toxic followed by pyrogallol, resorcinol, phloroglucinol, and finally vanillic acid (table 2).

Table 2. Analysis of variance for the toxicity bioassay of the phenolic acids.

Phenolic acid	Concentration ranges (ppm)	CD at 5% level of significance	
		Feeding index	Infestation ratio
Vanillic acid	0·1 to 0·8	0·1652*	1·18*
Resorcinol	0·1 to 0·7	0·290*	2·60*
Phloroglucinol	0·1 to 0·7	0·147*	0·980*
Pyrogallol	0·1 to 0·3	0·213*	0·954*
Gallic acid	0·1, 0·2	0·2434*	0·121*

*$P < 0.05$

A comparison of volatiles in host leaves and anal exudates of thrips revealed the mobilization of naphthalene from castor, undecane from eucalyptus and other related compounds from the substrates were also detected in tapioca (table 3). This suggests adaptive mechanisms in colonization.

Table 3. GC-MS analysis of the host leaves and the anal exudates of *Retithrips syriacus* on the respective hosts.

	Compound	Molecular wt.	Retention time	% quality
Ricinus communis	9,12-Octadecadienal dimethyl acetal	255	10·927	59
	Naphthalene	184	18·892	68
Anal exudates of *R. syriacus* on *R. communis*	1-Naphthelene methyl palmitate	396	5·896	52
	1, 1, 2-Benzene dicarboxylic acid	236	9.168	64
	2-Thiophene carboxylic acid	254	10·576	53
	Naphthalene	394	16·417	78
	Cyclopentane butanoic acid	458	21·067	50
Eucalyptus globulus	Phytol	296	10·935	58
	Cyclohexane	204	4·689	52
	Undecane	282	15·199	55
Anal exudates of *R. syriacus* on *E. globulus*	Cyclohexanone	183	12·880	60
	Phosphothioic acid	236	24·731	59
	Undecane	282	18·892	50
	Naphthalene	184	18·892	52
	2-Furan carboxaldehyde	236	24·731	59
Manihot utilissima	Cyclohexanone	183	14·576	53
Anal exudates of *R. syriacus* on *M. utilissima*	Dodecanoic acid	229	11·788	56
	Naphthalinone	310	10·66	53

4. Discussion

The results show that infestation patterns and changes in the levels of the primary and secondary metabolites are related events. Uninfested host leaves had high levels of primary metabolites and importantly, relatively lower levels of secondary substances. This might be factor contributing to the initial establishment of thrips populations. Subsequently the onset of heavy infestations was observed in conjunction with higher levels of carbohydrates, proteins and amino acids and particularly total phenols with associated phenolic enzyme activity and the flavanoids. Such increases in total phenols are considered elements of induced resistance in hosts against herbivory. However, despite high levels of phenols in tapioca, thrips infestations were observed to an appreciable extent. This could possibly be attributed to the proportionately high levels of readily utilizable primary substrates. Increases in total phenols and flavanoids were found to significantly reduce adult longevity and fecundity, and retard larval growth specially so in eucalyptus and tapioca. The negative impact of total phenols in castor was only to a lesser degree, directly proportional to the levels of phenols observed and the relative levels of primary metabolites.

The profiles of individual phenolic acids and flavanoids differed in the uninfested and infested hosts indicating transformations induced by infestation. However the prevalence of gallic acid prior to infestation in most instances may be considered a factor of inherent host resistance. Assays for toxicity of the individual phenolic acids shows the prevalence of less toxic ones in infested hosts. It is also possible that the prevalence of less toxic phenolic acids may contribute to the ability of thrips to infest the host corresponding to high levels of primary metabolites. The acute toxicity of gallic acid was reported in the case of *Schizaphis graminum* by Todd (1971). Such fatty acids as linoleic and linolenic acids derived from the host leaves play significant roles in the development of larvae, adult emergence, growth and reproduction (Dadd 1983). The occurrence of lauric acid in significantly higher quantities in tapioca could contribute to its being less suitable as a host. The individual sugars are known to synergize with fatty and amino acids (Chippendale 1978) determining substrate acceptability as either feeding attractants or deterrents with insect responses being species specific. Plant volatiles too are important components of host substrates and influence host selection (Visser and Ave 1978).

Certain of the volatiles such as naphthalene, cyclohexanone and their derivatives, originally detected in the host were also seen in the excreta of the thrips (table 3), indicative of their possible toxic nature and hence their ready excretion by thrips (Howard *et al* 1983, 1987). The implications of such compounds contributing specifically to host resistance needs to be further investigated. That the derivatives in the anal droplets indicate their origin in the hosts suggests the prevalence of dynamic adaptational abilities of the thrips which contribute to successful establishment of populations that tend to exploit the readily utilizable substrates. Thus host responses to infestations including increased production of phenolics and alterations in the composition of phenolic acids and flavanoids can be considered manifestations of induced resistance, which significantly alters parameters such as adult longevity and fecundity of the thrips.

However, persistant colonization by thrips can be correlated to the levels of primary and secondary metabolites, the relative abundance of which determines host selection and infestation.

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