Journal of Tropical Forest Science 12(2):221–226 (2000)

ROLE OF FOLIAR PROTEIN AND POLYPHENOL AND THEIR RELATIONSHIP TO CLONAL RESISTANCE IN TEAK AGAINST THE LEAF SKELETONISER, *PALIGA MACHOERALIS* WALKER (LEPIDOPTERA: PYRALIDAE)

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Received June 1997

JAIN, A., ROYCHOUDHURY, N. & BHARGAVA, A. 2000. Role of foliar protein and polyphenol and their relationship to clonal resistance in teak against the leaf skeletoniser, *Paliga machoeralis* Walker (Lepidoptera: Pyralidae). Leaf protein and polyphenol contents were estimated for selected resistant (ORANR-2, ORANR-3, ORANR-4, ORANP-7 and MHSC-A3) and susceptible (APT-8, APT-14, TNT-2 and ORPB-15) clones of teak to the leaf skeletoniser, *Paliga machoeralis*. The results were correlated with the degree of resistance/susceptibility. The protein and polyphenol contents of clonal leaves were directly and inversely proportional respectively, to the amount of leaf damage. The ratio of protein to polyphenol played a decisive role in determining the degree of resistance. A low ratio indicates higher resistance and vice versa.

Key words: Teak - clonal resistance - skeletoniser - leaf - protein - polyphenol

JAIN, A., ROYCHOUDHURY, N. & BHARGAVA, A. 2000. Peranan protein dan polifenol daun serta kaitannya dengan kerintangan klon dalam pokok jati terhadap ulat perangka daun, *Paliga machoeralis* Walker (Lepidoptera: Pyralidae). Kandungan protein dan polifenol dianggarkan bagi klon rintang yang terpilih (ORANR-2, ORANR-3, ORANR-4, ORANP-7 dan MHSC - A3) dan klon rentan (APT-8, APT-14, TNT-2 dan ORPB-15) bagi jati terhadap perangka daun, *Paliga machoeralis*. Keputusan adalah seimbang masing-masing secara langsung dan secara songsang dengan jumlah kerosakan daun. Nisbah protein kepada polifenol memainkan peranan yang penting dalam menentukan darjah kerintangan. Nisbah yang rendah menunjukkan kerintangan yang tinggi, dan nisbah yang tinggi menunjukkan kerintangan yang rendah.

Introduction

Host resistance has provided a highly practical approach to solving insect problems (Ortman & Peters 1980, Smith 1989, Roychoudhury *et al.* 1995a). Clonal resistance in teak (*Tectona grandis* L.f.) (family Verbenaceae) against its prime defoliators has been well established (Tewari 1992) but information still remains limited regarding the phytochemicals influencing the dynamics of insect–plant interactions (Hanover 1975, 1980, Norris & Kogan 1980, Matson & Scriber 1987) and which is decisive in imparting resistance against insects (Saxena 1986). Foliar

constituents have a significant role in population dynamics, insect behaviour and the physiology of defoliating insects in a number of ways (Jain *et al.* 1995, Roychoudhury *et al.* 1995b). The present study is an attempt to examine the role of leaf protein and polyphenol in clonal resistance of teak against the leaf skeletoniser, *Paliga machoeralis* Walker (Lepidoptera:Pyralidae), a devastating defoliator of teak.

Preliminary screenings were conducted on 167 clones of teak from ten Indian states, to identify teak clones resistant to *P. machoeralis*, at the National Teak Germ Plasm Bank, Chandrapur (Maharashtra), by Roychoudhury and Joshi (1996). Further studies were carried out on selected teak clones through field observations and feeding bioassays in the laboratory (Roychoudhury *et al.* 1995c). All these studies suggest that teak clones exhibit resistance (antixenosis/non-preference) to the leaf skeletoniser.

Materials and methods

To find out the role of protein and polyphenol in clonal resistance of teak against *P. machoeralis*, nine clones of teak, viz. ORANR-3, MHSC-A3, ORANR-2, ORANP-7, ORANR-4, APT-8, TNT-2, ORPB-15 and APT-14, were ranked as most resistant, highly resistant, resistant, moderately resistant, susceptible, highly susceptible and most susceptible respectively based on our earlier work (Roychoudhury & Joshi 1996, Roychoudhury *et al.* 1995c). Leaves of intermediate maturity (3 months old) (Roychoudhury *et al.* 1995b) were collected from one-year-old plants during late August (coinciding with the outbreak of the teak leaf skeletoniser) and dried at 40 °C, pulverised and subjected to analyses. The protein content was estimated at 660 nm on a PC-based UV/VIS spectrophotometer by the extraction technique described by Lowry *et al.* (1951). The polyphenol content was determined using the method given by Folin and Ciocalteu (1927) with some modifications (Singleton 1974). The readings were taken at 700 nm again with the PC-based UV/VIS spectrophotometer. The data were based on three trials, each containing three replications for each clone. These were subjected to statistical analyses by ANOVA using CRD design by SPSS statistical package and the results correlated with degree of resistance. Data were angular transformed to conform to normal distribution.

Results and discussion

Leaf protein

The protein content of contributory leaves of selected teak clones exhibited significant ($F=7.632$, $p<0.01$; d.f. 8,18) variation in their mean values (Table 1). Clonal leaves revealed gradual decrease in protein content with increased degree of resistance. The most resistant and susceptible clones, ORANR-3 and APT-14, showed the lowest and highest amounts of protein ($p<0.01$) respectively compared to other clones in this work. The protein content of clones MHSC-A3, ORANR-2,

Table 1. Data on protein and polyphenol contents of contributory leaves of selected teak clones and their ratio

Clone	Origin	Resistance/ susceptibility	Protein (%)		Polyphenol (%)		Protein: Polyphenol Ratio
			Mean ± SE	Angular transformed	Mean±SE	Angular transformed	
ORANR-3	Orissa	MR1	5.92 ± 1.02 (4.50 - 6.88)	13.99	4.51 ± 0.36 (4.06 - 4.93)	12.27	1.31
MHSC-A3	Maharashtra	HR	10.03 ± 0.98 (8.79 - 11.18)	18.44	4.40 ± 0.36 (3.92 - 4.80)	12.10	2.28
ORANR-2	Orissa	R	10.07 ± 1.17 (8.94 - 11.69)	18.45	3.53 ± 0.26 (3.21 - 3.85)	10.84	2.85
ORANP-7	Orissa	MR2	10.24 ± 0.95 (8.94 - 11.19)	18.65	3.48 ± 0.25 (3.21 - 3.81)	10.74	2.94
ORANR-4	Orissa	MR2	10.40 ± 1.10 (8.94 - 11.61)	18.78	3.10 ± 0.34 (2.64 - 3.46)	10.13	3.35
APT-8	Andhra Pradesh	MS1	10.52 ± 0.50 (10.00 - 11.19)	18.93	2.87 ± 0.18 (2.68 - 3.12)	9.74	3.67
TNT-2	Tamilnadu	S	11.94 ± 0.59 (11.18 - 12.63)	20.20	2.46 ± 0.32 (2.08 - 2.86)	9.00	4.85
ORPB-15	Orissa	HS	14.10 ± 3.40 (9.67 - 17.93)	21.90	1.97 ± 0.21 (1.68 - 2.16)	8.09	7.16
APT-14	Andhra Pradesh	MS2	15.50 ± 1.28 (13.88 - 17.00)	23.16	1.72 ± 0.28 (1.45 - 2.10)	7.55	9.01
SEm				1.315		0.483	1.310
$CD_{0.05}$				2.763		1.015	2.752
$CD_{0.01}$				3.785		1.390	3.770
p value				*0.000*		*0.000*	*0.000*

- Data based on pooled mean of three trials.
- Range values are inside parentheses.
- MR1=most resistant, HR=highly resistant, R=resistant, MR2=moderately resistant, MS1=moderately susceptible, S=susceptible, HS=highly susceptible, MS2= most susceptible.
- SEm=standard error of mean.
- CD=critical difference.

ORANP-7, ORANR-4, APT-8 and TNT-2 failed to exhibit conclusive results ($p > 0.05$). Further, clones TNT-2 and ORPB-15 showed no significant ($p > 0.05$) difference among themselves. However, clone APT-14 revealed significant ($p < 0.05$) difference when compared with others except clone ORPB-15, where no conclusive difference existed.

Leaf polyphenol

The polyphenol content of selected clonal leaves of teak revealed significant ($F = 23.091$, $p < 0.01$; d.f. 8,18) differences in their mean values (Table 1). Leaves of teak clones showed gradual increase in polyphenol content in relation to increase in degree of resistance. The most resistant clone, ORANR-2, showed a significantly higher concentration ($p < 0.01$) compared to others, except clone MHSC-A3 where no difference ($p > 0.05$) was recorded. The polyphenol content of clones ORANR-2, ORANP-7 and ORANR-4 showed no significant difference among themselves ($p>0.05$). Further, clones APT-8 and TNT-3 exhibited no conclusive difference ($p>0.05$). The most susceptible clone, APT-14, revealed significantly ($p< 0.05$) the lowest content of polyphenol compared to the others except clone ORPB-15, where no significant difference was observed ($p > 0.05$).

Ratio of leaf protein to polyphenol

The ratio of foliar protein to polyphenol of tested teak clones showed significant ($F=13.860$, $p<0.01$; d.f. 8,18) variation in their mean values (Table 1) and exhibited correlation with regard to the degree of resistance. It was found to be inversely proportional to resistance. A lower ratio was recorded for the most resistant clone, ORANR-3, whereas the most susceptible clone, APT-14, exhibited a higher ratio.

The present work demonstrates that the foliar protein content of selected teak clones is inversely proportional to the degree of resistance. These findings are in agreement with the observations of Jayaraj (1967) and Uthamasamy *et al.* (1971), who have also recorded increased protein content in varieties of castor and bhendi susceptible to leaf hoppers *Empoasca flavescens* and *Amrasca devastans* (Jassidae) respectively. The polyphenol content of clonal leaves exhibits an increasing trend in relation to resistance. Although, contradictory opinions exist regarding the role of polyphenols in the dynamics of insect–plant interactions (Porter & Hemingway 1989, Roychoudhury *et al.* 1995d), the present study clearly suggests the allomonic effects of foliar polyphenol in teak clones against the leaf skeletoniser. A similar notion has also been envisaged for some other insect–host interactions (Reese 1979). Apart from these, in the present work, a good correlation, ($r = -0.779$, $p < 0.01$), was observed between proteins and polyphenols, the ratio of which strongly influences the degree of resistance in teak clones. The ratio between these two is more important than their absolute concentrations. A lower ratio indicates higher resistance whereas a higher ratio is an indicator of susceptibility. The ratio of protein to polyphenol is considered to be one parameter

of the nutritional quality of a leaf (McManus *et al.* 1983); a lower ratio in a host plant has been found to affect all aspects of performance including feeding guilds on *Diacrisia casignetum* (Banerjee & Haque 1985). As a whole, the present study suggests that the contents of leaf protein, polyphenol and their ratio can be considered as determinants of clonal resistance in teak against *P. machoeralis.*

Acknowledgements

The authors are grateful to B. N. Gupta, Director General, Indian Council of Forestry Research and Education, Dehradun, and R. B. Lal, Director, Tropical Forest Research Institute, Jabalpur (MP), for their encouragement and providing research facilities.

References

BANERJEE, T. C. & HAQUE, N. 1985. Influence of host plants on development, fecundity and egg hatchability of the arctiid moth, *Diacrisia casignetum. Entomologia Experimentalis Applicata* 37:193–198.

FOLIN, O. & CIOCALTEU, V. 1927. On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry* 73:627.

HANOVER, J. W. 1975. Physiology of tree resistance to insects. *Annual Review of Entomology* 20:75–95.

HANOVER, J. W. 1980. Breeding forest trees resistant to insects. Pp. 487–511 in Maxwell, F. G. & Jennings, P. R. (Eds.) *Breeding Plants Resistant to Insects.* John Wiley & Sons, New York.

JAIN, A., ROYCHOUDHURY, N. & JOSHI, K. C. 1995. Population outbreak of defoliator, *Hyblaea puera* Cramer and skeletonizer, *Eutectona machaeralis* Walker on teak, in relation to leaf chemical status. *Myforest* 31:49–54.

JAYARAJ, S. 1967. Effect of leafhopper infestation of carbohydrate and nitrogen in castor varieties in relation to their resistance to *Empoasca flavescens* (F.) (Homoptera: Jassidae). *Indian Journal of Experimental Biology* 5:156–162.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193:265–275.

MATSON, W. J. & SCRIBER, J. M. 1987. Nutritional ecology of insect folivores of woody plants: water, nitrogen, fibre and mineral considerations. Pp. 105–146 in Slansky, F. & Rodriguez, J. (Eds.) *Nutritional Ecology of Insects, Mites & Spiders.* John Wiley & Sons, New York.

MCMANUS, J., LILLEY, T. H. & HASLAM, E. 1983. Plant polyphenols and their associations with proteins. Pp. 123–137 in Hedin, P. A. (Ed.) *Plant Resistance to Insects.* American Chemical Society, Washington.

NORRIS, D. M. & KOGAN, M. 1980. Biochemical and morphological bases of resistance. Pp. 23–62 in Maxwell, F. G. & Jennings, P. R. (Eds.) *Breeding Plants Resistant to Insects.* John Wiley & Sons, New York.

ORTMAN, E. F. & PETERS, D.C. 1980. Introduction. Pp. 3–13 in Maxwel, F. G. & Jennings, P. R. (Eds.) *Breeding Plants Resistant to Insects.* John Wiley & Sons, New York.

PORTER, L. J. & HEMINGWAY, R.W. 1989. Significance of the condensed tannins. Pp. 988–1027 in Rowe, J. W. (Ed.) *Natural Products of Woody Plants II.* Springer-Verlag, Germany. 1243 pp.

REESE, J. C. 1979. Interactions of allelochemicals with nutrients in herbivore food. Pp. 309–330 in Rosenthal, G. A. & Janzen, D. H. (Eds.) *Herbivores : Their Interactions with Secondary Plant Metabolites.* Academic Press, New York.

ROYCHOUDHURY, N., JAIN, A., JOSHI, K. C. & GUPTA, B. N. 1995a. Concept of natural resistance of trees to insects. *Advances in Forestry Research in India* 13:210–220.

ROYCHOUDHURY, N., JAIN, A. & JOSHI, K. C. 1995b. Alteration of growth and development in leaf skeletonizer, *Eutectona machaeralis* Walker, due to variations in teak leaves of different maturity. *Indian Journal of Experimental Biology* 33:227–229.

ROYCHOUDHURY, N., JAIN, A. & JOSHI, K. C. 1995c. Resistance in teak clones against leaf skeletonizer, *Eutectona machaeralis* Walker (Lepidoptera:Pyralidae). *Advances in Forestry Research in India* 13:140–157.

ROYCHOUDHURY, N., JAIN, A., JOSHI, K. C. & BHARGAVA, A. 1995d. Insect–host interactions: role of polyphenols. *Tropical Sylvics* 3:1–2.

ROYCHOUDHURY, N., JAIN, A. & JOSHI, K. C. 1996. Search for natural resistance in teak clones against *Eutectona machaeralis* Walker (Lepidoptera : Pyralidae). *Indian Journal of Forestry* 19:205-213.

SAXENA, R. C. 1986. Biochemical bases of insect resistance in rice varieties. Pp. 142–159 in Green, M. B. & Hedin, P. A.(Eds.) *Natural Resistance of Plants to Pests : Role of Allelochemicals.* American Chemical Society, Washington.

SINGLETON, V. L. 1974. *Analytical Fractionation of the Phenolic Substances of Grapes and Wine and Some Practical Uses of Such Analysis.* Advances in Chemistry Series 137. American Chemical Society: 184.

SMITH, C. M. 1989. *Plant Resistance to Insects : A Fundamental Approach.* John Wiley & Sons, New York. 286 pp.

TEWARI, D. N. 1992. *A Monograph on Teak (Tectona grandis Linn.f.).* International Book Distributors, Dehradun. 479 pp.

UTHAMASAMY, S., JAYARAJ, S. & SUBRAMANIAN, T. R. 1971. Studies on the varietal resistance of bhendi, *Abelmoschus esculentus* (L.), to the leafhopper, *Amrasca devastans* (Dist.) (Homoptera: Jassidae). *South Indian Horticulture* 19:53–59.