

Chemical Ecology of *Probergrothius sanguinolens* (Hemiptera : Pyrrhocoridae) in Relation to Herbivory and Carnivory

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(Received 22 March 1995; after revision 30 April 1996; Accepted on 30 April 1996)

Qualitative and quantitative fluctuations of primary metabolites and allelochemicals such as phenols, flavonoids and volatile profiles of diets exert a strong influence on the performance and fitness of *Probergrothius sanguinolens* in terms of post embryonic development, adult longevity, egg output, egg hatchability, total growth index, adult emergence, sex ratio, number of ovipositions and fecundity.

Key Words : *Probergrothius sanguinolens*, *Sterculia foetida*, Fecundity, Facultative carnivory

Introduction

Nutrient imbalance in plant tissue tend to reduce their nutritional value and many plant allelochemicals have been known to interfere with the utilization of nutrients (Mattson & Scriber 1987, Broadway & Duffey 1988, Slansky 1992). Phytophagous insects tend to exhibit numerous adaptations that allow them to compensate for the nutritional deficiencies in their plant foods including choosing nutrient-rich plant tissue (Ananthkrishnan 1992, Slansky 1993), increasing the feeding rate (Ananthkrishnan et al. 1990, Simpson & Simpson 1990), synchronizing phenology with the food plant (Slansky & Rodriguez 1987) manipulating plant tissues to improve their nutrient content (Ananthkrishnan &

Raman, 1988) and relying on microorganisms to synthesize required nutrient (Houk & Griffiths 1980, Kukor & Martin 1987).

"Seed predation" is widespread among Hemipteran bugs and largely occur in families Pyrrhocoridae, Lygaeidae, Coreidae, Cydnidae and occasionally in Pentatomidae, Scutelleridae, and Miridae (Slater 1972, Janzen 1978, Malipatil 1979, Mc Pherson 1982, Schaefer & Mitchel 1983). The present investigation was undertaken to study the influence of biochemical variations of host tissues on the biology, growth and reproduction of *Probergrothius sanguinolens*. In addition the role of facultative carnivory exerted by *P. sanguinolens* were explained.

Materials and Methods

Insect Culture

Adults of *P. sanguinolens* were collected from the fields and reared in the laboratory under room temperature ($29^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 75 - 90% RH) in glass troughs (25 × 10 cm).

A sand bed of 2.5 cm thick was provided as oviposition substratum. Eggs laid in the soil were tagged and as soon as the neonate nymphs emerged, they were separated and reared on separate plastic containers having the respective food materials.

Nutritional Influence of Different Hosts on Growth and Reproduction

In order to analyse the influence of different diets, on the development and reproduction of *P. sanguinolens*, plant diets (containing seeds of *Sterculia foetida*, *Azadirachta indica*, *Adansonia digitata*), animal diets (freshly killed termites, snails, ants and beetles) and mixed diet containing all these materials in equal proportions were provided separately to the neonate nymphs till the next generation. Observations were made on life cycle parameters such as larval duration, adult emergence, sex ratio, total growth index (TGI), pre oviposition period, adult longevity, fecundity, number of ovipositions and egg hatchability. Due to nymphal mortality of *P. sanguinolens* on *Azadirachta indica* and *Adansonia digitata*, freshly emerged adults were introduced to study the reproductive parameters.

Volatile Analysis

Hexane extracts of the seeds were injected on to a coupled Hewlett packard 5890 GC/MSD interfaced with GC/MSD chemstation with an NBS 49K mass spectral library. Helium was used as the carrier gas.

Mass spectral data obtained during the assay were compared with the mass spectra of compounds available in chemstation NBS 49K library. The quality (%) and the retention time (min.) were also noted.

Biochemical Analyses

Biochemical profiles of the host seeds were analysed following the methods of Lowry et al. (1951) for total proteins, Dubois et al. (1956) for total carbohydrates, Folch et al. (1957) for lipids, Moore and Stein (1948) for amino acids, Bray and Thorpe (1954) for phenols, Johnson and Schall (1952) for OD phenols and Harborne (1988) for individual phenols and flavonoids.

The biochemical profiles of the ovary, fat body and haemolymph were estimated using the 5th day old adults of *P. sanguinolens* reared separately on different hosts. The antennae of the adults were severed with fine scissors and 10 μl of haemolymph was collected and the same adults were dissected out and 100 mg of fat body and ovary were collected for biochemical analyses. In all the experiments five replicates were maintained.

Results

Studies on the life cycle parameters of *P. sanguinolens* on different host seeds revealed that the nymphs fed on *S. foetida* showed faster developmental rate (28.5 days), while incomplete development resulted when the nymphs were reared on the other two seeds. A significant increase in the fecundity (137.0/female), adult longevity (63.1 days) and egg hatchability (94%) was noticeable when adults were fed on *S. foetida* seeds (table 1). A four fold increase in the number of ovipositions (5.6 per female) was evident when fed on *S. foetida* seeds as compared to other host seeds.

Table 1 Growth and life cycle parameters of *Probergrothius sanguinolens* on different alternate hosts

Hosts	Developmental stages (days)					Adult emergence (%)	Sex ratio (female: male)	TGI	Pre-oviposition period (days)	Adult female longevity (days)	Egg output/ Female	Egg hatchability (%)	No of ovipositions/ Female
	I	II	III	IV	V								
<i>Stereocilia foetida</i>	2.2 ±0.1	3.0 ±0.2	6.3 ±2.4	7.0 ±0.5	10.0 ±0.25	98	1:1.2	0.89	10.0±1.2	63.1±2.1	137.0±8.9	94	5.6±0.73
<i>Azadirachta indica</i>	6.3 ±0.8	8.0 ±0.7	9.0 ±0.7	-	-	-	-	-	-	-	30.0±2.4	40	1.0±0.01
<i>Adansonia digitata</i>	6.50 ±0.7	8.8 ±0.7	9.3 ±0.8	11.8 ±0.9	-	-	-	-	-	-	12.0±1.2	10	1.0±0.01
Termites	5.1 ±0.5	4.6 ±0.3	8.2 ±0.5	7.2 ±0.44	9.9 ±0.8	79	1:1.3	0.87	11.6±0.8	55.0±4.2	115.0±7.3	82	3.2±0.04
Snails	6.0 ±0.5	5.2 ±0.4	12.1 ±0.9	13.4 ±0.8	11.5 ±0.8	52	1:1.6	0.77	16.2±1.2	19.0±1.2	32.0±2.2	25	1.8±0.01
Ants	5.4 ±0.2	4.8 ±0.3	10.3 ±0.9	9.4 ±0.6	10.2 ±0.7	65	1:1.5	0.73	14.2±1.1	48.4±3.1	96.4±5.6	73	2.4±0.03
Beetles	5.0 ±0.2	4.5 ±0.3	9.0 ±0.8	7.8 ±0.5	9.2 ±0.7	71	1:1.4	0.81	12.4±0.8	52.0±4.5	102.0±8.4	79	2.9±0.05
Mixed*	2.0 ±0.08	2.9 ±0.1	4.2 ±0.3	5.0 ±0.3	6.0 ±0.4	98	1:1	1.08	7.5±0.5	70.0±5.2	162.0±6.8	98	7.1±0.09

LSD (1%), \bar{X} ± SD of 5 replicates.

8.65 13.7

Among the animal hosts, freshly killed termites supported *P. sanguinolens* with maximum adult longevity (55 days), fecundity (115 per female), egg hatchability (82%) and number of oviposition (3.2 per female). Besides, the nymphal development (35.0 days) was also faster in the individuals reared on termites. Interestingly, in contrast to the results obtained from the plant hosts, all animal hosts supported the nymphs to complete their developmental stages. In addition when compared to the seeds of *Azadirachta indica* and *Adansonia digitata* all animal hosts supported the nymphs of *P. sanguinolens* with faster development rate in their early life stages.

In the case of a mixed diet containing both plant as well as animal hosts in equal proportions revealed a significant decline in the nymphal period (20.1 days) and a significant increase in adult longevity (70.0 days), fecundity (162.0 per female), percentage hatchability (98.0%) and number of ovipositions (7.1 per female) when compared to other two diets. The sex ratio was also found to be favourable (1:1) in the mixed diet fed individuals.

Biochemical profiles of the fat body, ovary and haemolymph showed a significant increase of primary metabolites in the mixed diet fed individuals irrespective of the tissues tested (figure 1). Interestingly enough,

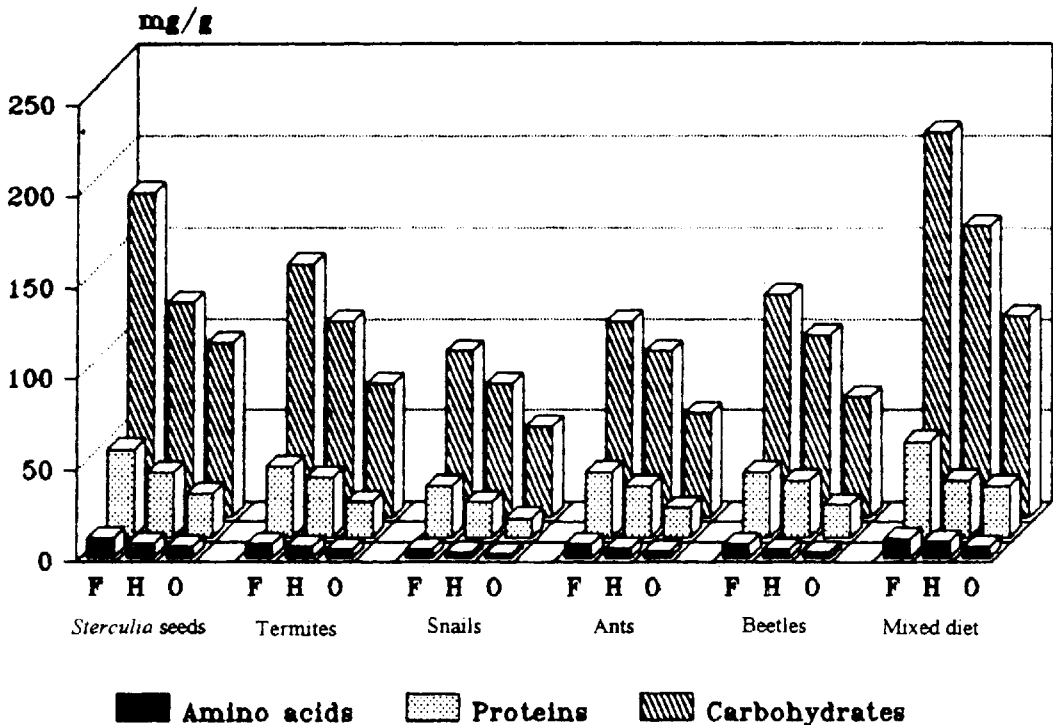


Figure 1 Levels of primary and secondary metabolites in the fat body (F), haemolymph (H), and ovary (O) of *Probergrothius sanguinolens*, fed on *Sterculia* seeds, termites, snails, ants, beetles and mixed diet (seed and animal tissue).

Table 2 Levels of primary and secondary metabolites in different host seeds of *Probergrothius sanguinolens*

Host seeds	Carbohy- drates (mg/g)	Pro- teins (mg/g)	Lipids (mg/g)	Amino acids (mg/g)	Phenols (mg/g)	OD Phenols (mg/g)	Mois- ture (%)	Nitro- gen (%)	W/N ratio	C/N ratio
<i>Sterculia foetida</i>	208.3	36.3	7.0	0.51	4.0	0.26	41	5.8	7.1	35.91
<i>Azadirachta indica</i>	187.2	27.5	6.4	0.43	4.4	0.32	38	5.1	7.4	36.71
<i>Adansonia digitata</i>	154.2	15.2	3.7	0.31	5.7	0.46	25	4.3	5.8	35.86

W/N-Water/Nitrogen; C/N-Carbon/Nitrogen; OD Phenols-Ortho Dihydroxy phenols

postembryonic development was incomplete when fed on seeds of *Azadirachta indica* and *Adansonia digitata*.

Primary metabolite levels were significantly higher in *S. foetida* seeds. Carbohydrates content in the 3 host seeds ranged from 208.3 to 154.2 mg/gm with the maximum in *S. foetida* and the lowest in *Adansonia digitata*. *Azadirachta indica* and *Adansonia digitata* showed 11.3% and 35.1% lesser carbohydrates than *S. foetida*. Levels of total proteins, lipids, amino acids, nitrogen and moisture content in *S. foetida* were markedly higher than in other hosts. Total phenolics and OD phenols varied inversely with the proportion of major nutrients. Total phenolic levels showed 42.5% increase in *Adansonia digitata*, 10% increase in *Azadirachta indica* as compared to *S. foetida*, water/nitrogen and carbon/nitrogen ratios varied in the 3 seed hosts and was highest in *Azadirachta indica* followed by *S. foetida* and *Adansonia digitata* (table 2).

p-Hydroxy benzoic acid, protocatechuic acid and salicylic acids were the phenolic fractions recorded from *S. foetida* while

Table 3 Phenolics and flavonoids profiles of different host seeds of *probergrothius sanguinolens*

Host seeds	Phenols	Flavonoids
<i>Sterculia foetida</i>	<i>p</i> -hydroxy benzoic acid, Protocatechuic acid, Salicylic acid	Apigenin Hesperidin
<i>Azadirachta indica</i>	Pyrogallol, Catechol, Gallic acid, Salicylic acid	Hesperidin Chrysoeriol
<i>Adansonia digitata</i>	Resorcinol, Gallic acid, Protocatechuic acid	Naringenin Apigenin

pyrogallol, catechol, gallic acid and salicylic acid were evident in *Azadirachta indica*, resorcinol, gallic acid and protocatechuic acid were the phenolic fractions in *Adansonia digitata*. Major flavonoids were apigenin and hesperidin in *S. foetida*, hesperidin and chrysoeriol in *Azadirachta indica* and naringin and apigenin in *Adansonia digitata* (table 3).

Volatile profiles of the host plants revealed the presence of a wide array of

volatiles in different concentrations. 2-Octylcyclopropene-1-heptanona methyl, pentacosane and pentadecanoic acid in *S. foetida*, hexacosane, 1,2-benzene dicarboxylic acid in *Azadirachta indica* and 2,6-Octadiene in *Adansonia digitata* were major volatile fractions detected (table 4 and figure 2).

Discussion

Variation in the quantity and quality of host plant nutrients alter the life cycle performance and fitness of the insects (Slansky & Rodriquez 1987). Nutritional deficiencies in some plant foods place the insects at risk and their inability to obtain adequate nutrients results in low growth rate, body size, nutrient storage, reproductive output and finally increasing the mortality rate (Price 1984, Slansky & Scriber 1985).

However, the quantity of primary nutrients such as carbohydrates, proteins, lipids and amino acids were reported to be responsible for better life cycle performance and reproduction (Singh & Agarwal 1988, Saxena 1965, Hedin & Mc Carthy 1990). Similarly the seeds of *S. foetida* also showed higher levels of primary metabolites and low levels of secondary metabolites, which may be the reason for *P. sanguinolens* to select *S. foetida* as the main host among the three plant hosts. Chemicals of secondary metabolism and their oxidation products such as phenols, flavonoids and OD phenols are very important because of their manifold effect on the insects including growth rate, biomass accumulation, survival rate and fecundity (Klocke & Chan 1982). A similar trend in the life cycle performance especially low growth indices, high mortality rate and low reproductive potential were observed in *P. sanguinolens* when fed on the secondary

Table 4 GC-MS analyses of volatile compounds in different host seeds of *Probergrothius sanguinolens*

Host seeds	Volatile compounds	Quality (%)	Area (%)	Retention Time (min)
<i>Sterculia foetida</i>	Butyl 2-1, 2-Benzene dicarboxylic acid	56	13.81	8.41
	Pentadecanoic acid	74	14.23	6.02
	2-Octylcyclopropene-1-heptanona methyl	83	17.29	3.52
	Pentacosane	78	19.93	4.99
	Hexatriacontane	62	22.40	7.92
<i>Azadirachta indica</i>	9-methyl nonadecane	54	24.37	5.08
	1,2-Benzene dicarboxylic acid	72	24	13.79
	Tridecanoic acid	50	47	14.23
	Cyclopentane	53	-	17.29
<i>Adansonia digitata</i>	Hexacosane	83	-	19.91
	Benzoyl chloride	50	-	9.60
	1,2-Benzene dicarboxylic acid	53	-	13.81
	2,6-Octadiene	58	-	22.49

metabolite rich seeds of *Azadirachta indica* and *Adansonia digitata*.

High moisture content in the seeds of *S. foetida* was also one of the reason for

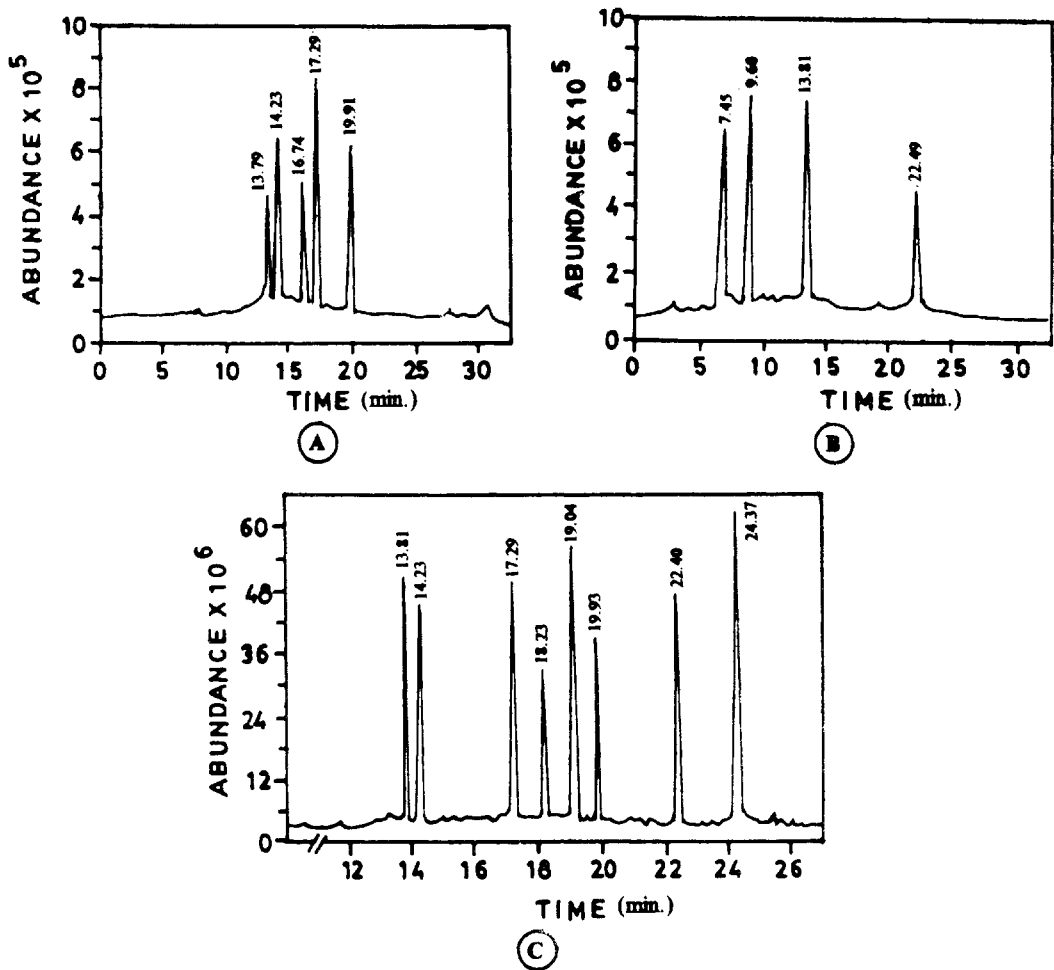


Figure 2 Volatile profiles in the seeds of *Azadirachta indica*(A), *Adansonia digitata* (B) and *Sterculia foetida* (C).

its susceptibility towards *P. sanguinolens*, because preference of wet food is probably for compensating their water deficit (Roesingh et al. 1985). Higher percentage of nitrogen in the preferred host of *P. sanguinolens* can also attributed to its higher growth rate and reproduction (Mc Neil & Southwood 1978). Chemical composition of haemolymph depends on the type of food that particular insects feed upon and haemolymph serve as the via media for the

transport of synthesized materials from fat body to ovary, besides playing a key role in the storage of nutrients continuously derived from digested food in the gut (Wyatt 1980). The present investigation also revealed high amounts of carbohydrates, proteins and amino acids in the haemolymph, fat body and ovary of the insects fed on *S. foetida* when compared to the seeds of other plant hosts whereas, it was significantly high in the mixed diet among the three diets tested.

Studies on life cycle parameters also revealed better performance of *P. sanguinolens* on mixed diet. Thus occasional carnivory might allow *P. sanguinolens* to obtain essential nutrients that are deficient in plant diets as reported by Whitman (1994). In addition, Adis and Froeschner (1982) suggested that *Dysdercus urbhani* turned to animal matter when seeds are unavailable because of dry condition. This supports the facultative carnivory of *P. sanguinolens* towards the animal matter. Early instars of *P. sanguinolens* highly preferred the dead animals which may be due to the reason that animal tissue serve as the last water resource for *P. sanguinolens* when moisture content in their normal plant foods are in short supply as observed by Joyner and Gould (1985). In addition facultative carnivory in the early stages might give a nutritional 'jump start' providing extra energy, endurance and power to disperse and

search for suitable feeding sites, to cut through tough seed coat, to withstand climatic effects, to deter predators and to compete against both con- and allelo specific (Whitman 1994).

The presence of gallic acid in the food was reported to reduce survival percentage of *Heliothis armigera* (Ananthakrishnan et al. 1990). The present investigation also showed the presence of gallic acid in *Azadirachta indica* and *Adansonia digitata* which may be the reason for the mortality of *P. sanguinolens* when fed on these host seeds.

Acknowledgements

This work was carried out during the tenure of a DST Project (SP/SO/C/56/90/1993) for which thanks are due. Our thanks are due to Professor T N Ananthakrishnan, Principal Investigator and Director, Entomology Research Institute, for necessary guidance and interest.

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