

Host selection and food utilization of the red pumpkin beetle, *Raphidopalpa foveicollis* (Lucas) (Chrysomelidae: Coleoptera)

K RAMAN and R S ANNADURAI

Entomology Research Institute, Loyola College, Madras 600 034, India

MS received 2 January 1985; revised 22 February 1985

Abstract. An analysis of the host plant relationships with respect to the red pumpkin beetle, *Raphidopalpa foveicollis* Lucas is presented based on the role of receptors involved in host selection, the quantitative food utilization on different cucurbitaceous host plants and the biochemical parameters involved in food plant selection. Orientation of the beetles towards the host plants appeared to be profoundly affected when the receptors present on the antennae and mouthparts were ablated or coated. Though significant differences were observed with regard to the quantity of food ingested among different host plants, ingestion of food was higher for mature leaves and flowers compared to young and senescent leaves. Accordingly, mature leaves and flowers showed high nitrogen and proteins, low sugars, moderately high phenols and narrow C/N ratio compared to other plant parts. The chemosensory receptors present on the antennae and mouthparts were also studied using scanning electron microscope.

Keywords. Host selection; food utilization; *Raphidopalpa foveicollis*.

1. Introduction

An individual plant is heterogenous to any phytophagous insect and since the plant parts vary in their nutritional value, the food utilization on different parts of the same plant appears to be important in order to determine the pest status of the concerned species. The red pumpkin beetle, *Raphidopalpa foveicollis* Lucas feeds on a variety of cucurbitaceous crops causing economic losses. Observations by Sihna and Krishna (1971) and Grewal and Sandhu (1982) have shown the relative preference of the beetle towards different parts of the cucurbits, *Lagenaria vulgaris* Ser and *Cucumis melo* L. In view of the fact that the information on the comparative utilization of the different plant parts viz flowers, young, mature and senescent leaves is meagre, an attempt has been made to study the relative preference and quantitative food utilization of some economically important cucurbits and a weed host by *R. foveicollis* in terms of some important chemical parameters. In addition, the role of sensory structures in host selection by *R. foveicollis* was also investigated using scanning electron microscope (SEM).

2. Material and methods

Adult individuals of mixed age and sex groups, collected from the college campus were used in this investigation. The different cucurbitaceous plants viz *Luffa cylindrica*

Roem, *Luffa acutangula* Roxb, *Trichosanthes anguina* L, *Cucurbita maxima* Duch, *Benincasa cerifera* Savi, *Momordica charantia* L and *Mukia scabrella* Arn were collected from the college farm.

2.1 *Role of receptors in host selection*

The role of receptors in host selection was studied by ablation of antennae, painting the eyes with nail polish, and coating the mouthparts with nail polish. In each of these tests ten adults of *R. foveicollis* were subjected to various treatments and another ten adults were used as control. The beetles were released into a glass trough containing the preferred host plant at the centre and covered by a glass plate. The number of beetles (control and experimental) orienting towards the host plant was monitored for 15 min. Each set of experiment was repeated three times.

2.2 *SEM studies*

To get a detailed picture of the sensilla on the antennae and mouthparts SEM studies were made. The mouthparts and antennae were detached from etherized specimens, dried in a dust-free desiccator for 1–2 days, fixed to aluminium stubs using double adhesive tapes and then coated with gold for 2–3 min in a standard ion-coater. Micrographs were made using a Hitachi scanning electron microscope, table top model S 415 A under 15 kV emission current.

2.3 *Quantitative food utilization*

To study the quantitative food utilization by *R. foveicollis*, leaf discs (7.5 cm dia) or an entire leaf were placed inside a petri dish over a moist filter paper of the same dimension. The initial weight of the leaf disc and the filter paper was determined. The test insects were fed with water for 2–3 hr by providing a moist cotton to clear their guts before the start of the experiment. A pair of beetles (male and female) were released into the dish and covered by another petri plate. A control was also maintained without the test insects. Twentyfour hours after the release of the insect, the leaf discs and filter paper were removed and reweighed. Before reweighing the filter paper, the sides of the petri dishes were carefully wiped with it to collect any excrement left on the dishes. After correcting for the weight loss due to transpiration by the leaf, the comparative food utilization on different host plants was assessed by determining the quantity of food ingested/24 hr and the coefficient of digestibility (Waldbauer 1968). Each set of experiment was repeated three times.

2.4 *Biochemical analysis*

The different cucurbitaceous host plant parts *viz* flowers (1-day old), young (3–5 days old), mature (5–10 days old), and senescent leaves (more than 10 days old) were analysed for various biochemical components. The total nitrogen was estimated (Humphries

1956) and multiplied by 6.25 to estimate the total protein content. In addition, the total carbohydrates (Dubois *et al* 1956) and phenols (Bray and Thorpe 1954) were also estimated. The C/N ratio was computed for the different host plants.

3. Results

3.1 Role of receptors in host selection

It was observed that orientation towards the host plant by *R. foveicollis* was profoundly affected when subjected to various tests such as antennal ablation, blinding of eyes and coating of mouthparts. Antennectomized and blinded beetles took a longer time in moving near the host plant. The mean numbers of antennectomized beetles that located the host plant and involved in active feeding were 2.0 and 0.67 respectively. When eyes were blinded, the mean number of beetles which located the host plant was only 0.33 and they did not involve in active feeding. However, in control, the number of beetles involved in host location and active feeding was 7.00 and 5.00 respectively. The beetles, whose mouthparts have been suitably coated, did not show any positive response to initiate feeding even after locating the host plant (table 1).

3.2 SEM studies

Figure 1 depicts the sensory structures involved in perceiving the odours/stimulus emanating from the host plants and are located on the antennae and mouthparts of *R. foveicollis*, involving (1) sensilla trichoidea: four long sensory hairs at the apex of each antennae, functioning as olfactory chemoreceptors, (2) sensilla basiconica: short sensillae distributed all along the antenna and designated as trichoid sensillae involved in olfaction, and (3) sensilla chaetica: bristles with blunt tips or sometimes with sharp pointed ends serve as mechanoreceptors. Ablation of antennae in *R. foveicollis* deprived the insect of the ability to perceive stimuli and interfered with the normal orientation towards the host plant. The outer and inner face of the labrum bears sparsely distributed trichoid sensillae. While the mandibles did not bear any sensory hairs, on

Table 1. Effect of antennectomy, blinding of eyes and coating of maxillae on the host plant location and feeding by *R. foveicollis*.

Treatments	Mean number of beetles orienting towards the host plant			
	Host location	<i>t</i> value	Active feeding	<i>t</i> value
Antennectomized	2.00	6.10*	0.67	6.69*
Eyes blinded	0.33	10.30**	0.00	12.20**
Maxilla coated	0.33	10.30**	0.00	12.20**
Control	7.00		5.00	

Data represent mean of three replicates. *Significant at 5% level, **significant at 1% level.

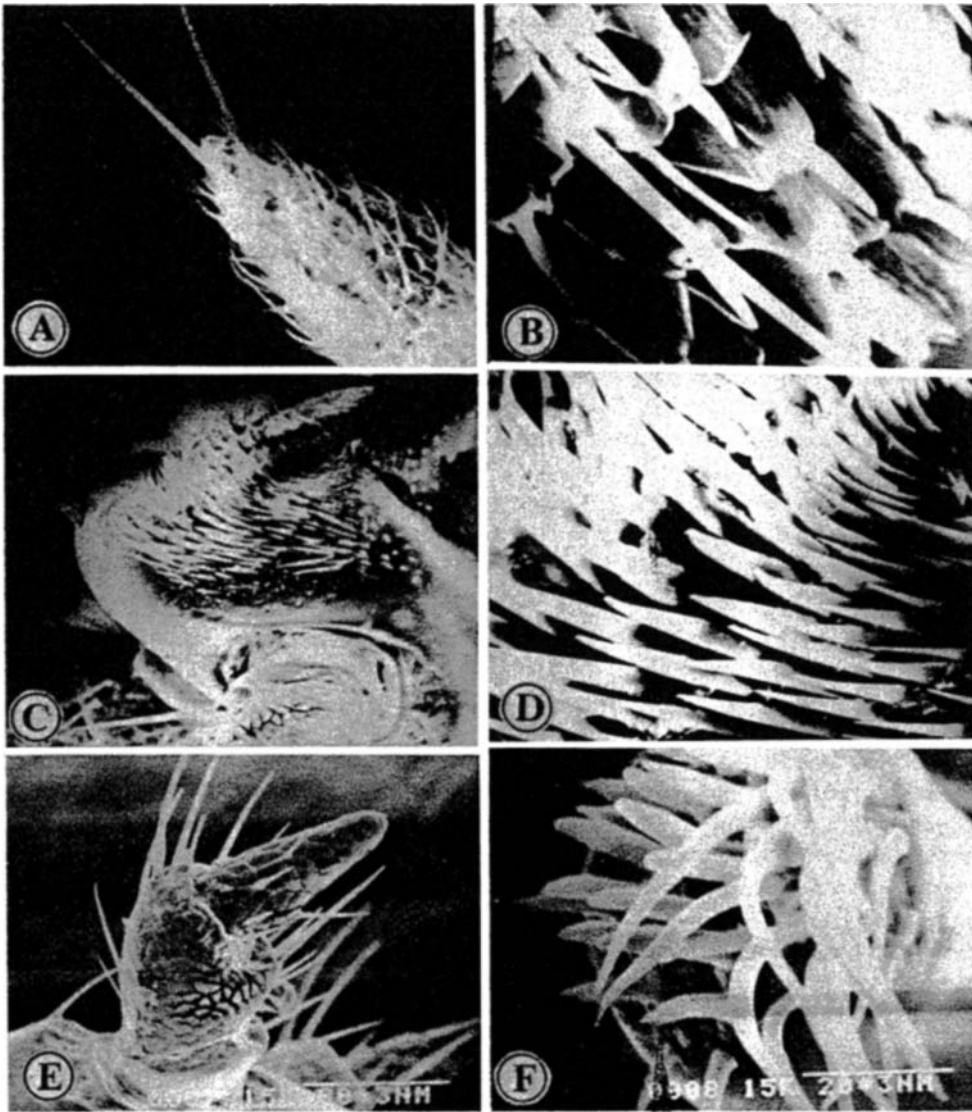


Figure 1. A. Antenna showing four long *Sensilla trichoidea* at the tip. B. Distribution of *sensilla basiconica* (short sensillae) and *Sensilla chaetica* (bristles with blunt tips) on the antenna. C. Stipes of maxilla showing the campaniform sensilla. D. Enlarged view of the campaniform sensilla on the stipes. E. Labial palp. F. Labial palp showing the presence of two types of sensillae at the tip.

the maxilla, the stipes carried large number of sensory hairs (figure 1). The maxillary palp did not carry any sensilla but the labial tips showed the presence of two types of sensillae, one group with sharp and pointed ends and the other with blunt ends. These sensillae appear to be the campaniform sensillum and act as gustatory chemoreceptors.

3.3 Quantitative food utilization

The quantity of food ingested by *R. foveicollis* varied considerably among different cucurbitaceous host plant parts (table 2). On young leaves, maximum consumption was observed on *B. cerifera* (0.367 mg) followed by *T. anguina* (0.362 mg), *L. cylindrica* (0.342 mg), *L. acutangula* (0.259 mg) and *C. maxima* (0.256 mg). The food ingested was the least on the weed host *M. scabrella* (0.199 mg). However, the quantity of food ingested on mature and senescent leaves was the highest for *T. anguina* (0.510 and 0.427 mg) as against 0.469, 0.335 mg; 0.385, 0.339 mg; 0.350, 0.307 mg; 0.295, 0.272 mg and 0.188, 0.178 mg on *L. cylindrica*, *B. cerifera*, *L. acutangula*, *C. maxima* and *M. scabrella* respectively. With regard to the flowers, the highest consumption was observed for *C. maxima* (0.395 mg) compared to other host plants. Thus an overall assessment of the pattern of food ingestion by *R. foveicollis* showed that apart from the

Table 2. Quantitative food utilization by *R. foveicollis* on certain cucurbitaceous hosts

Plant parts	Host plants	Quantity of food ingested (mg)	Coefficient of digestability (%)
Young leaves	<i>L. cylindrica</i>	0.342 ± 0.000*	92.950 ± 0.562
	<i>T. anguina</i>	0.362 ± 0.038	93.770 ± 1.490
	<i>C. maxima</i>	0.256 ± 0.033	89.456 ± 2.919
	<i>L. acutangula</i>	0.259 ± 0.008	89.575 ± 1.219
	<i>B. cerifera</i>	0.367 ± 0.027	94.005 ± 1.041
	<i>M. charantia</i>	— — —	— — —
	<i>M. scabrella</i>	0.199 ± 0.001	85.929 ± 1.489
Mature leaves	<i>L. cylindrica</i>	0.469 ± 0.005	94.570 ± 0.271
	<i>T. anguina</i>	0.510 ± 0.001	96.275 ± 0.873
	<i>C. maxima</i>	0.295 ± 0.027	91.985 ± 1.983
	<i>L. acutangula</i>	0.350 ± 0.039	94.000 ± 0.481
	<i>B. cerifera</i>	0.385 ± 0.007	94.805 ± 1.292
	<i>M. charantia</i>	— — —	— — —
	<i>M. scabrella</i>	0.188 ± 0.036	86.705 ± 0.708
Senescent leaves	<i>L. cylindrica</i>	0.335 ± 0.004	90.290 ± 0.572
	<i>T. anguina</i>	0.427 ± 0.065	95.316 ± 0.053
	<i>C. maxima</i>	0.272 ± 0.065	88.971 ± 1.021
	<i>L. acutangula</i>	0.307 ± 0.053	90.228 ± 0.553
	<i>B. cerifera</i>	0.339 ± 0.022	93.805 ± 0.512
	<i>M. charantia</i>	— — —	— — —
	<i>M. scabrella</i>	0.178 ± 0.016	86.516 ± 0.038
Flowers	<i>L. cylindrica</i>	0.347 ± 0.049	84.725 ± 0.802
	<i>T. anguina</i>	0.355 ± 0.010	85.070 ± 0.140
	<i>C. maxima</i>	0.395 ± 0.066	87.848 ± 0.539
	<i>L. acutangula</i>	0.349 ± 0.022	85.673 ± 0.138
	<i>B. cerifera</i>	0.372 ± 0.071	85.022 ± 0.412
	<i>M. charantia</i>	— — —	— — —
	<i>M. scabrella</i>	0.165 ± 0.040	65.455 ± 0.412

*Indicate mean ± S.E.

preference among different cucurbitaceous host plants, the preference was more for mature leaves and flowers compared to young and senescent leaves. Observations on the feeding behaviour of the beetle under field conditions also revealed a similar picture. The non-utilization of the cucurbitaceous host, *M. charantia* is of interest since the beetles were not found feeding on this plant in the field as well as under laboratory conditions. The computation of the coefficient of digestibility of various plant parts also showed a direct proportionality with the quantity of food ingested. Moreover, the digestibility was comparatively higher when *R. foveicollis* was fed on mature leaves as against other host plant parts.

3.4 Biochemical analysis

Data on the biochemical analysis of different cucurbitaceous host plants are furnished in table 3. With regard to water content, it was found to be higher in young leaves and flowers compared to mature and senescent leaves, but significant differences were not observed among different host plants, excepting in the case of senescent leaves. The estimation of nitrogen and protein content showed significant differences between different parts of the plant as well as among different host plants. While the nitrogen and protein content was appreciably high for *B. cerifera* and *L. cylindrica*, on other host plants they were comparatively lower. It was maximum in mature leaves compared to young and senescent leaves and moderately high in flowers.

Estimation of carbohydrates showed that it was lower in *L. acutangula*, *T. anguina* and *M. scabrella* when compared to *C. maxima*. Variation was evident between the different parts of the plant such as mature and senescent leaves exhibiting lower carbohydrates as compared to young leaves and flowers. Similarly, the phenol content in *M. scabrella* and *T. anguina* was less when compared to other host plants and young leaves showed higher phenolic contents compared to other parts. Computation of C/N ratio also indicated considerable differences among different cucurbitaceous host plants as well as between plant parts. The ratio was narrow for mature leaves followed by senescent leaves, flowers and young leaves. However, it was high in *M. charantia* compared to other host plants.

3.5 Host plant preference in terms of food utilization and biochemical parameters

An assessment of the host plant preference of *R. foveicollis* in terms of the quantity of food ingested and various biochemical components also revealed some interesting results. With regard to the utilization of flowers, the preference among different plants is as follows: *C. maxima* > *B. cerifera* > *T. anguina* > *L. acutangula* > *L. cylindrica* > *M. scabrella*. Although the percentage of nitrogen and protein was maximum in *L. cylindrica*, the quantity of food ingested was comparatively lower when compared to *C. maxima*, probably due to the increased carbohydrates and phenols. A similar trend was also evident for *L. acutangula*. Moreover, the low nitrogen and protein in *C. maxima* have been compensated by high food intake by the beetles.

The presence of increased amounts of nitrogen and protein, low phenols and moderate carbohydrate in the young leaves of *B. cerifera* resulted in an increased quantity of food intake by *R. foveicollis*. On the contrary, the high concentration of

Table 3. Chemical composition of certain cucurbitaceous host plants

Host plants	Plant parts	Moisture (%)	Nitrogen (%)	Proteins (%)	Carbohydrates (mg/g fresh wt)	Phenols (mg/g fresh wt)	C/N ratio
<i>L. cylindrica</i>	Y	83.28	5.32	33.25	2.38	2.60	0.45
	M	77.76	6.44	40.25	1.25	2.53	0.19
	S	62.73	4.79	29.94	1.08	2.19	0.23
	F	80.43	5.95	37.19	2.13	2.60	0.36
<i>T. anguina</i>	Y	84.39	3.64	22.75	1.13	1.85	0.31
	M	84.00	5.18	32.38	1.00	1.63	0.19
	S	76.84	3.15	19.69	0.93	1.80	0.29
	F	83.10	3.41	21.81	2.00	2.10	0.59
<i>C. maxima</i>	Y	82.24	1.61	10.06	2.60	2.00	1.61
	M	72.94	3.15	19.69	1.48	1.83	0.47
	S	68.32	2.10	16.63	1.13	1.35	0.54
	F	80.10	2.66	16.63	1.18	1.35	0.44
<i>L. acutangula</i>	Y	78.14	2.17	13.56	1.63	2.60	0.75
	M	73.75	2.94	18.38	1.13	2.53	0.38
	S	66.00	2.01	18.81	0.85	2.30	0.28
	F	79.75	4.69	29.31	1.73	1.56	0.37
<i>B. cerifera</i>	Y	84.00	6.09	38.06	2.23	3.03	0.37
	M	79.26	6.58	41.13	2.13	2.60	0.32
	S	77.62	4.34	27.13	2.23	2.18	0.51
	F	80.00	2.38	14.88	1.75	2.18	0.74
<i>M. charantia</i>	Y	82.34	1.96	12.25	2.39	2.61	1.22
	M	78.84	2.17	13.56	1.64	2.00	0.75
	S	73.92	1.26	7.88	1.08	1.08	0.85
	F	81.42	1.61	10.06	2.28	1.75	1.42
<i>M. scabrella</i> *	Y	84.00	1.68	10.50	1.25	1.50	0.74
	M	78.77	1.75	10.94	0.95	1.55	0.54
	S	76.63	1.96	12.25	0.93	1.55	0.47
	F	82.50	1.12	7.00	1.10	2.15	0.98

*Indicates cucurbitaceous weed host. Y: Young leaves; M: Mature leaves; S: Senescent leaves; F: Flowers

phenols and carbohydrates in *L. cylindrica* reduced the food consumption. The food intake in *L. acutangula*, *C. maxima* and *M. scabrella* was sufficiently low presumably due to low nitrogen and protein. Thus the preference for young leaves indicate the following sequence: *B. cerifera* > *T. anguina* > *L. cylindrica* > *L. acutangula* > *C. maxima* > *M. scabrella*.

The pattern of food consumption on mature and senescent leaves also indicate a striking relationship between leaf nitrogen, protein, carbohydrate and total phenolic contents. High food intake was evident in the presence of increased nitrogen and protein, low carbohydrate and phenols, as in the case of mature leaves of *T. anguina* followed by *L. cylindrica* and *B. cerifera* respectively. In the case of low nitrogen and low phenols, compensation was achieved by a relatively high food intake as seen in the senescent leaves of *T. anguina*.

4. Discussion

Chemoreceptors of insects form a complicated and subtle sensory system that enables them to differentiate between many natural stimuli of fairly great diversity (Schoonhoven 1977). In the present study three types of sensory hairs were observed on the antennae of *R. foveicollis* viz sensilla trichoidea, sensilla basiconica and sensilla chaetica. In addition, the sensillum present on the stipes of the maxillae and tip of the labial palps appears to be gustatory receptors. Schneider (1964) opined that the sensilla present on the antennae acts as olfactory and mechanoreceptory chemoreceptors. In acridids, the campaniform and trichoid sensilla located on the maxilla and labium were found to act as gustatory chemoreceptors (Chapman and Thomas 1978). Thus the differences in sensitivity of chemoreceptors to different host plant odours enable the insect to orient towards its food plant and the delay in location of host plants and the failure to initiate feeding by *R. foveicollis* could be attributed to the inability to perceive the plant odours as a result of antennal ablation and coating of the sensory areas located on the mouthparts.

It is well known that both primary and secondary plant chemicals are indispensable in the regulation of feeding behaviour of phytophagous insects. While primary plant chemicals serve as essential phagostimulants to elicit initial feeding response and to maintain continuous feeding on the host plant, many secondary plant chemicals exhibit repellent, deterrent and toxic effects, and their presence provides the basis of insect resistance in the majority of plants (Hsiao 1974). The greater preference of *R. foveicollis* towards leaves and flowers was attributed to some chemical stimuli present in these regions (Sinha and Krishna 1971). In the present investigation, the differences in the relative preference and quantitative food utilization of *R. foveicollis* among different cucurbitaceous host plants as well as between different plant parts appear to be due to variations in their nutritive qualities, *M. charantia* being an exception. Mature leaves and flowers showed high nitrogen and proteins, low carbohydrates, moderately high phenols and narrow C/N ratio compared to other plant parts. The importance of organic nitrogen (McNeill and Southwood 1978), sugars and proteins (Beck 1956), and phenols (Hartfield *et al* 1982), in the host plant selection by phytophagous insects is very well established. Moreover, a lower C/N ratio is also associated with greater susceptibility of the host plant to insect attack (Jayaraj 1967). With increasing plant age, the total soluble nitrogen decreased appreciably thereby interfering with the development of the aphid, *Myzus persicae* Sulz on Brussels sprout plants (van Emden and Bashford 1971). Marian and Pandian (1980) showed that the consumption, assimilation and net-conversion of leaf proteins by the *Danaus chrysippus* L larvae fed on senescent leaf of *Calotropis gigantea* R. Br. were significantly less than those fed on normal leaf. However, in the present study it was observed that a definite relationship existed between the quantity of food ingested and the different biochemical parameters such as the nitrogen, protein, carbohydrate and phenolic contents of different plant parts. The presence of increased nitrogen and protein, low carbohydrate and phenol in the host plant resulted in increased food consumption by *R. foveicollis*. Similarly, the food intake was also relatively high in the case of host plants with low nitrogen and low phenols. This emphasises the fact that *R. foveicollis* exhibited a compensatory behavioural and physiological response i.e. altering the food consumption and utilization in response to variation in the nutritional suitability of their food (Slansky 1982).

Chandaravadana and Pal (1983) reported the presence of a triterpenoid feeding deterrent from *M. charantia* which could also be the reason for the non-utilization of this plant by *R. foveicollis*. In addition, Sinha and Krishna (1970) showed that for an optimum level of the alkaloid, cucurbitacin E (a feeding incitant widely distributed in cucurbitaceous plants) was essential to stimulate feeding in *R. foveicollis*. While a lower concentration of this compound will not initiate the beetle feeding, higher concentrations can also act as a feeding deterrent. Thus the differential feeding behaviour of *R. foveicollis* on different parts of the cucurbitaceous plants could also be due to the distribution and varied concentrations of such secondary substances. It could therefore be concluded that the qualitative and quantitative differences in the primary and secondary chemicals coupled with the discriminating powers of the various chemosensory receptors (Visser 1983) located in the antennae and mouthparts determine the selection of food plants/plant parts by *R. foveicollis*.

Acknowledgement

The authors are grateful to Prof. T N Ananthkrishnan, for valuable suggestions and critical perusal of the manuscript.

References

- Beck S D 1956 A bimodal response to dietary sugars by an insect; *Biol. Bull.* **10** 219–228
- Bray H C and Thorpe W V 1954 Analysis of phenolic compounds of interest in metabolism; *Methods Biochem. Anal.* **1** 27–52
- Chandaravadana M V and Pal A B 1983 Triterpenoid feeding deterrent of *Raphidopalpa foveicollis* L from *Momordica charantia*; *Curr. Sci.* **52** 87–88
- Chapman R F and Thomas G 1978 The number and distribution of sensilla of the mouthparts of Acrididoidea; *Extrait. d'Acrida* **7** 115–148
- Dubois M, Gilles K A, Hamilton J K, Robers P A and Smith F 1956 Colorimetric determination of sugars and related substances; *Anal. Chem.* **28** 351–356
- Grewal S S and Sandhu G S 1982 The feeding preference of red pumpkin beetle for different leaves of cucurbits; *Sci. Cul.* **48** 389–390
- Hartfield L D, Frazier J L and Ferreira J 1982 Gustatory discrimination of sugars, amino acids and selected allelochemicals by the tarnished plant bug, *Lygus lineolaris*; *Physiol. Entomol.* **7** 15–23
- Hsiao T H 1974 Chemical influence on feeding behaviour of *Leptinotarsa* beetles; in *Experimental analysis of insect behaviour* (ed.) L B Browne (Berlin: Springer-Verlag) pp. 237–248
- Humphries E C 1956 Mineral compounds and ash analysis; in *Modern methods of plant analysis* (eds) K Peach and M V Tracey (Berlin: Springer-Verlag) Vol. 1 pp. 542
- Jayaraj S 1967 Effect of leafhopper infestation on the metabolism of carbohydrates and nitrogen in castor varieties in relation to their resistance to *Empoasca flavescens* F. (Homoptera: Jassidae); *Indian J. Exp. Biol.* **5** 156–162
- Marian M P and Pandian T J 1980 Effects of feeding senescent leaf of *Calotropis gigantea* on food utilization in the Monarch butterfly, *Danaus chrysippus*; *Entomon* **5** 257–264
- McNeill A and Southwood T R E 1978 The influence of nitrogen in the development of insect host plant relationship; *Symp. Phyto-Chem. Soc.*, Reading 1977
- Schneider D 1964 Insect antennae; *Annu. Rev. Entomol.* **9** 103–123
- Schoonhoven L M 1977 Insect chemosensory responses to plant and animal hosts; in *Chemical control of insect behaviour—Theory and application* (eds) H H Shorey and J R McKelvey Jr. (New York: John Wiley) pp. 1–5

- Sinha A K and Krishna S S 1970 Further studies on the feeding behaviour of *Aulacophora foveicollis* on Cucurbitacin; *J. Econ. Entomol.* **30** 333-334
- Sinha A K and Krishna S S 1971 Feeding behaviour of *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) on *Lagenaria vulgaris* Ser. (Cucurbitaceae); *Bull. Entomol. Soc. Nigeria* **3** 60-63
- Slansky Jr F 1982 Toward a nutritional ecology of insects, in *Proc. 5th Int. Symp. Insect-Plant Relationships*, Wageningen, 1982 pp. 253-259
- van Emden H F and Bashford M A 1971 The preference of *Brevicoryne brassicae* and *Myzus persicae* in relation to plant age and leaf amino acids; *Entomol. Exp. Appl.* **14** 349-360
- Visser J H 1983 Differential sensory perception of plant compounds by insects; in 'Plant resistance to insects, *Proc. Symp. Am. Chem. Soc.* pp. 215-230
- Waldbauer G P 1968 The consumption and utilization of food by insects, in *Advances in insect physiology* (eds) J W L Beament, V B Treherne and V B Wigglesworth, (New York: Academic Press) **5** 229-288