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Age-correlated tissue preferences of *Heliothis armigera* (Hubner) and *Spodoptera litura* (F) with special reference to phenolic substances

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Abstract. Age correlated tissue preferences of *Heliothis armigera* (Hubner) and *Spodoptera litura* (F) were investigated on *Gossypium hirsutum*, *Abelmoschus esculentus*, *Ricinus communis* and *Cajanus cajan*. The food utilization and reproductive potential of these two noctuids are correlated with the host plant nutritional and non-nutritional phenolic substances, separated and characterised through thin-layer chromatography and UV spectra. The non-nutritional phenolic substances such as phloroglucinol, resorcinol, protocatechuic acid, gallic acid, pyrogallol and vanillic acid tend to vary within the host plant parts as well as in different host plant species.

Keywords. *Spodoptera litura*; *Heliothis armigera*; age-correlated tissue preferences; non-nutritional phenolic substances.

1. Introduction

Plants vary greatly in their food value for different insects and the host plant specificity is based on the insect's nutritional requirements. The nutritional quality of plant tissues does not differ considerably qualitatively, but varies quantitatively. Fraenkel (1959) suggested that all primary nutrients for insects are found in all higher plants, but the attractancy and repellency are governed by the elaborated secondary plant products. The distribution of the secondary plant products particularly phenolic substances tend to vary in different host plant tissues. Reese and Beck (1976), Carter and Lyman (1969) and Van Sumere (1975) have indicated the allelochemical effect of various phenolic substances. The influence of nutritional factors coupled with the non-nutritional phenolic substances on food preference, food utilization, growth and reproduction of two economically important noctuids viz. *Spodoptera litura* (F) and *Heliothis armigera* (Hubner) are presented in this paper.

2. Materials and methods

Larvae of *S. litura* and *H. armigera* were reared from eggs collected from the field. In all experiments newly ecdysed third instar larvae were chosen and subjected to feeding experiments. Freshly excised tissues of various host plants viz. *Gossypium hirsutum* L., *Ricinus communis* L., *Cajanus cajan* (L.) Millspaugh and *Abelmoschus esculentus* (L.) Moench. were utilised for estimation of food consumption as well as biochemical components. Semi-synthetic diet was also tested for nutritional indices and reproductive potential for both the species as indicated by Ananthakrishnan *et al* (1990).

2.1 *Quantitative food utilization*

The gravimetric method was adopted for assessing the quantity of food utilised. Experimental larvae were separated from the stock culture and the initial weights recorded; then introduced to individual feeding chambers where they were allowed to feed on weighed quantity of the chosen plant tissues for 24 h. At the end of the experiment, the left over food, excreta and the instars were weighed. The difference in the weight of the instar gave the weight gained during the period of study. Comparison of food utilisation was carried out by studying the growth parameters of Waldbauer (1968).

2.2 *Biochemical estimations*

The various host tissues were subjected to biochemical estimation for their total proteins (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1956), nitrogen (Vogel 1963) and phenol (Hori 1974).

2.3 *Extraction and separation of phenolic substances*

About 15 g of each selected plant tissue was subjected to acid hydrolysis with 2 M HCl for 30 min. The solution was later cooled and filtered. The phenols were subsequently taken into ether and the ether extract was dried. The residue was then dissolved in ether and chromatographed on silica jel in acetic acid chloroform; ethyl acetate-benzene; and benzene-methanol acetic acid. The R_f values and spectral ranges of standard phenols and phenolic acids were compared with the eluted fractions (Harborne and Williams 1969).

2.4 *Fecundity studies*

In order to assess the impact of leaf quality on the fecundity of adult *S. litura* and *H. armigera*, larvae reared from the different feeding regimes were allowed to pupate separately till eclosion. Males and females emerging from similar feeding regimes were allowed to mate and the number of eggs laid till death was taken as fecundity with 10 replications.

3. Results

3.1 *Age-correlated tissue preference of H. armigera*

The differential quantitative food preferences of *H. armigera* are depicted in figure 1. Of all the host plant tissues, the mature bolls of *G. hirsutum* were consumed more (2·092 g) by the 10-day old larva of *H. armigera*. The 10-day old larva when reared on the pods of *C. cajan* consumed 1·057 g but it egested only 0·018 g. The growth rate was very high (1·35 g/day) in the case of 7-day old larva when fed with pods of *C. cajan*. *G. hirsutum* squares supported growth faster than the bolls. A comparison of the total amount of food consumed during the larval stages among the different

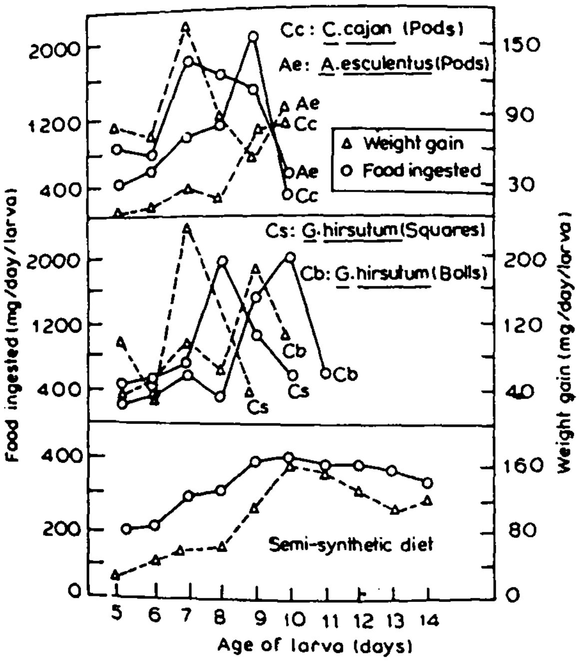


Figure 1. Host plant tissue preference of *H. armigera*.

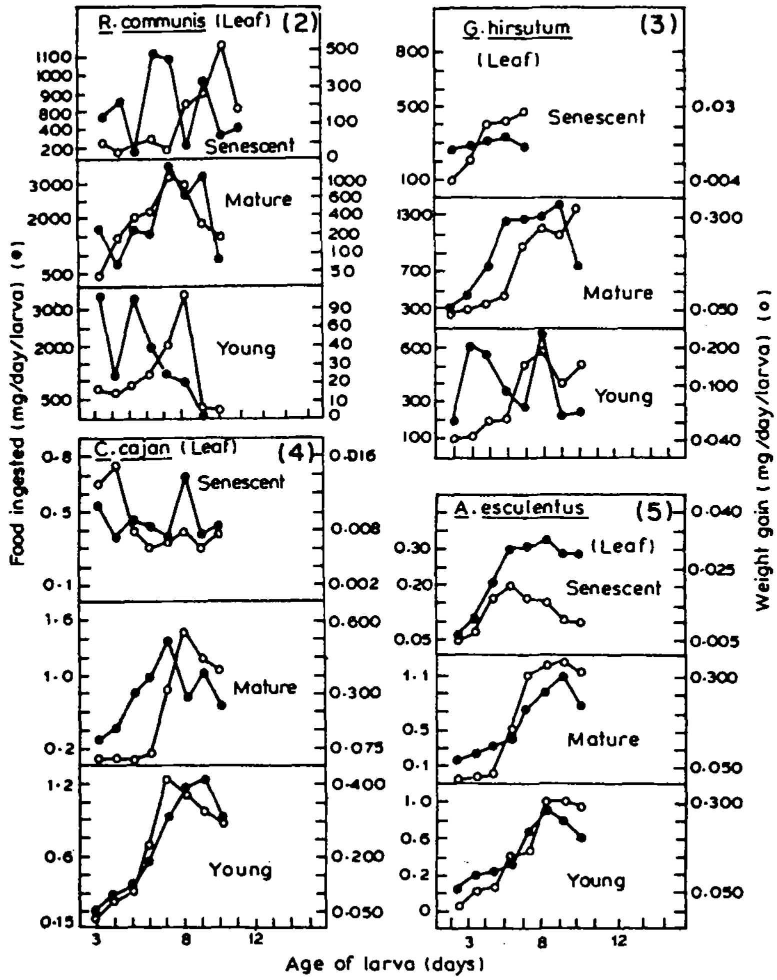
host tissues indicate some interesting results. Maximum amount of *A. esculentus* pods were utilised (7·505 g) followed by cotton bolls (5·779 g), squares (5·270 g) and *C. cajan* pods (4·345 g). However, the body weight gained was maximum only in the case of cotton bolls (0·552 g) followed by squares (0·524 g), *C. cajan* pods (0·388 g) and *A. esculentus* pods (0·365 g).

3.2 *Host tissue preference of S. litura*

Of the 4 host plants tested for *S. litura*, mature leaves of *R. communis* were preferred as compared to young and senescent leaves, the order of preference for other hosts being *G. hirsutum*, *C. cajan* and *A. esculentus*. The consumption efficiency in terms of body weight gain on all host tissues is illustrated in figures 2–5. Maximum food was consumed by 7-day old larva (3·957 g) on mature larvae of *R. communis* and the body weight gain was 1·220 g/day. By the 10th day the food consumption declined drastically (0·341 mg). In the case of mature leaves of *G. hirsutum*, the maximum quantity was utilised on the 10th day (1·146 g). Six-day old larvae consumed 1·4 g of mature leaves when compared to young and senescent leaves.

3.3 *Host tissue influenced fecundity*

The food plant tissue quality was clearly reflected in the reproductive capacity of the moths. Individuals maintained on *G. hirsutum* bolls laid a maximum of 1865 ± 155 (mean ± SD) eggs with an oviposition period of 7 days. Individuals maintained on *C. cajan* pods laid a maximum of 1335 ± 128 eggs within 5 days



Figures 2–5. Host plant tissue preference of *S. litura* on (2) *R. communis*, (3) *G. hirsutum*, (4) *C. cajan* and (5) *A. esculentus*.

while 1255 ± 125 eggs were laid by individuals maintained on *G. hirsutum* squares. *A. esculentus* pods seem to be a poor quality diet since only 1210 ± 108 eggs were laid.

Figure 6 indicates the total egg output of *S. litura* when reared on the leaves of different ages of *R. communis*, *A. esculentus*, *G. hirsutum* and *C. cajan* as well as on the semi-synthetic diet. A maximum of 1966 ± 103 eggs were laid when reared on mature leaves of *R. communis*. When reared on young leaves they laid only 1744 ± 177 eggs and individuals reared on senescent leaves laid only 833 ± 112 eggs. Individuals reared on semisynthetic diet were able to lay as much as 1605 ± 127 eggs. Mature leaves of cotton, seemed to support maximum fecundity (1802 ± 130 eggs) next to *R. communis* and in this case also the young leaves were next to mature leaves (1616 ± 115 eggs). Senescent leaves yielded only 773 ± 87 eggs and the reduction is less than 50%. *C. cajan* leaves appeared almost similar to *R. communis*

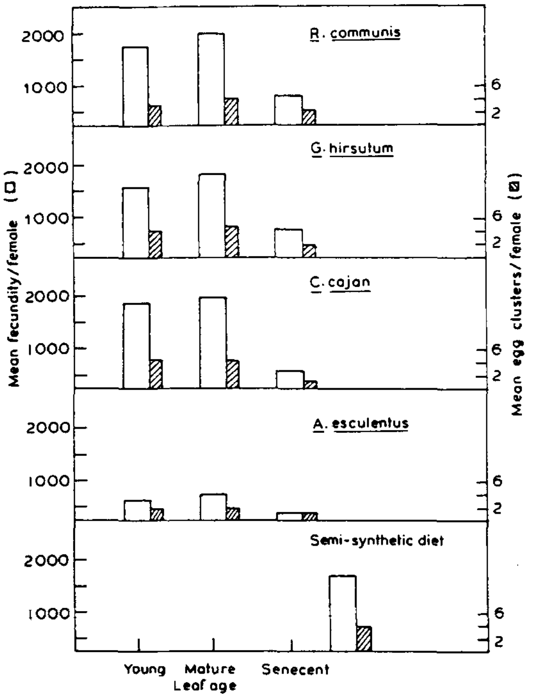


Figure 6. Reproductive potential of *S. litura* on the various host plant tissues.

deciding the egg output. However, in the case of *A. esculentus*, the egg output was reduced considerably and the maximum was attained on mature leaves (740 ± 83).

3.4 *Age-correlated host tissue chemical profile*

The various biochemical nutritional components such as total proteins, total carbohydrates, total nitrogen and water were analysed for the host tissues of different ages in addition to the non-nutritional plant substances such as phenolics. Table 1 provides the data relating to the concentration of various biochemical components of the host plant tissues tested. Mature leaves of *R. communis* had the highest concentration of protein (73 mg/g) and amino acids 0·46 mg/g when compared to all other host plants. The carbohydrate concentration was very high in the case of mature leaves 275 mg/g of *G. hirsutum*. Mature leaves of *A. esculentus* had a higher concentration of phenols (53 mg/g). The water/nitrogen (W/N) ratio was also assessed for determining the food plant preferences. Among the host leaves, W/N ratio was less in *R. communis*; while the ratio was the lowest in senescent leaves where the preference was also the least. Of all the hosts, *G. hirsutum* leaf tissues showed the highest W/N ratio. In order to identify the role of non-nutritional phenolic substances, extraction, separation and characterization were made of the different host plant tissues. Young *G. hirsutum* squares showed the presence of vanillic and salicylic acid while the mature bolls showed the presence of vanillic acid, phloroglucinol and protocatechuic acid. Protocatechuic acid was also identified in the young leaves of *G. hirsutum* in addition to pyrogallol. Mature

Table 1. Biochemical composition of host leaves/pods.

Host leaves/pods	Protein (mg/g)	Carbohydrates (mg/g)	Phenols (mg/g)	Amino acids (mg/g)	N2 (%)	Water (%)	W/N (%)
Ricinus communis							
Young	61	241	31·6	0·238	3·70	63·0	17·10
Mature	73	265	35·3	0·464	4·25	78·0	18·40
Senescent	27	170	28·8	0·132	2·63	35·0	13·30
Gossypium hirsutum							
Young	36	228	23·8	0·121	2·92	70·1	24·00
Mature	58	275	41·0	0·163	3·38	88·3	26·12
Senescent	19	153	28·6	0·081	2·23	45·2	20·27
Square	43	321	18·5	0·216	3·11	75·2	24·16
Bolls	63	435	19·8	0·259	3·52	91·5	25·99
Cajanus cajan							
Young	32	167	43·0	0·185	2·75	60·0	21·8
Mature	40	173	48·0	0·232	3·68	88·0	23·7
Senescent	18	081	31·3	0·106	2·25	41·0	18·2
Pods	51	285	21·6	0·213	3·81	93·0	24·27
Abelmoschus esculentus							
Young	29	152	46·2	0·286	3·02	65·0	21·52
Mature	35	168	53·0	0·358	3·63	85·0	23·41
Senescent	14	109	37·3	0·220	2·51	48·0	19·12
Pods	43	187	28·6	0·223	3·76	89·0	23·75

leaves contained gallic acid, hydroquinone and protocatechuic acid. Senescent leaves showed the presence of 2-methyl resorcinol, hydroquinone, phloroglucinol and resorcinol (table 2). The R_f values and spectral maxima of the substances identified are presented in table 3.

In the mature pods of *A. esculentus* the presence of vanillic and protocatechuic acids were noticed. The 3-leaf stages showed the presence of protocatechuic acid, while young and mature leaves possessed gentisic acid in addition to resorcinol. The mature pods, young leaves, mature leaves and senescent leaves of *C. cajan* had phloroglucinol, protocatechuic acid, 2-methyl resorcinol and resorcinol respectively (table 2).

4. Discussion

S. litura and *H. armigera* when tested for their age correlated tissue preference, fecundity and biochemical interaction indicated that the larvae of *S. litura* showed a higher food intake and better growth rate when fed on the mature leaves of *R. communis*. However larvae of *H. armigera* showed a higher food intake and growth when fed on the cotton bolls when compared with the different leaf stages. Pretorius (1976), based on the net reproductive rates of *H. armigera*, reported that the best food was cotton buds and leaves, followed by groundnut leaves, sunflower heads, green bean, rose petals, potato leaves, lucerne leaves, grains, sorghum

Table 2. Phenolic substances identified in the host tissues of *H. armigera*.

Host plants	Tissue	Phenolic substance
Gossypium hirsutum	Young squares	Vanillic acid, salicylic acid
	Mature bolls	Vanillic acid, phloroglucinol, protocatechuic acid
	Young leaves	Pyrogallol, protocatechuic acid
	Mature leaves	Gallic acid, hydroquinone, protocatechuic acid
	Senescent leaves	2-Methyl resorcinol, hydroquinone, phloroglucinol, resorcinol
Abelmoschus esculentus	Mature pods	Vanillic acid, protocatechuic acid
	Young leaves	Protocatechuic acid, gentisic acid
	Mature leaves	Protocatechuic acid, resorcinol, gentisic acid
	Senescent leaves	Protocatechuiç acid
Cajanus cajan	Mature pods	Phloroglucinol
	Young leaves	Protocatechuic acid
	Mature leaves	2-Methyl resorcinol
	Senescent leaves	Resorcinol

Benzene derivatives	Benzoic acid derivatives
Resorcinol—1,3-dihydroxy benzene	Salicylic acid—2-hydroxy benzoic acid
Hydroquinone—1,4-dihydroxy benzene	Gentisic acid—2,6-dihydroxy benzoic acid
Phloroglucinol—1,3,5-trihydroxy benzene	Gallic acid—3,4,5-trihydroxy benzoic acid
Pyrogallol—1,2,3-trihydroxy benzene	Vanillic acid—4 hydroxy-3-methoxy benzoic acid
	Protocatechuic acid—3,4-dihydroxy benzoic acid

Table 3. Characterization of phenolic substances.

Phenol	Solvent system (mobile phase)	R_f value (×100)	Spectral maxima (nm)
Benzene derivatives			
Resorcinol—1,3-dihydroxy benzene*	HOAc-$CHCl_3$	19	291·2
Hydroquinone—1,4-dihydroxy benzene	Benzene-MeOH-HOAc	32	297·6
Phloroglucinol—1,3,5-trihydroxy benzene*	EtOAc-$CHCl_3$	47	247·0
Pyrogallol—1,2,3-trihydroxy benzene	Benzene-MeOH-HOAc	19	260·8
Benzoic acid derivatives			
Salicylic acid—2-hydroxy benzoic acid*	EtOAc-benzene	80	235·0
Gentisic acid—2,6-hydroxy benzoic acid	Benzene-MeOH-HOAc	42	332·0
Gallic acid—3,4,5-trihydroxy benzoic acid	EtOAc-benzene	42	272·0
Vanillic acid—4-hydroxy-3-methoxy benzoic acid	Benzene-MeOH-HOAc	71	256·0
Protocatechuic acid—3,4-dihydroxy benzoic acid	HOAc-$CHCl_3$	19	248·4 254·5

*Compounds characterized based on the best separation achieved among 3 different solvent systems.

panicles, young maize cobs and tomato leaves. Adult production on *G. hirsutum* was about 4 times more compared to maize. However Sparks *et al* (1971) reported that corn particularly a multieared sweet corn, produced 4 times as many corn earworm on *G. hirsutum*. Scriber and Feeny (1979) and Scriber (1978) have concluded that chemical factors correlated with plant growth account for the major portion of the variation in larval efficiency. Scriber (1978) reported no difference in consumption rate, assimilation. efficiency of utilization of plant biomass of *S. eridania* when fed with acynogenic and cynogenic leaves of *Lotus corniculatus*. Larvae of *S. litura* and *H. armigera* when fed with the different host plant tissues of different ages show clear variation with respect to growth, food consumption and fecundity. These differences may be attributed to the differential biochemical composition of the host tissues particularly the phenolic substances. In the present study, it was possible to characterise the various phenolic substances in the different host tissues. Reese and Beck (1976) have indicated that resorcinol inhibited ingestion and growth but did not inhibit pupation or pupal weight of the black cutworm, *Agrotis ipsilon.* Phloroglucinol inhibited survival, growth and pupation. It inhibited growth by reducing the efficiency of conversion of assimilated and ingested food. The presence of phloroglucinol in the mature bolls of *G. hirsutum* and mature pods of *C. cajan* may account for the higher preference of *H. armigera* to these tissues since phloroglucinol stimulates growth, survival and pupation at low concentrations even though it is a harmful stress agent apparently due to hormoligosis (the phenomenon in which harmful quantities of many stress agents may be stimulating) (Luckey 1968). The presence of resorcinol in the leaves of *C. cajan* and *G. hirsutum* may be the reason for the reduced rate of ingestion of the senescent leaves in both the species. It is possible that the presence of any one of these substances singly or in combination with other substances may play an attractant or repellent role and it is crucial to indicate their role before ascertaining their absolute quantities in plant tissues.

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References

Ananthakrishnan T N, Senrayan R, Annadurai R S and Murugesan S 1990 Antibiotic effects of resorcinol, gallic acid and phloroglucinol on *Heliothis armigera* Hubner (Insecta: Noctuidae); *Proc. Indian Acad. Sci.* (*Anim. Sci.*) **99** 39–52

Carter C M and Lyman C M 1969 Reactions of gossypol with amino acids and amino compounds; *J. Am. Oil Chem. Soc.* **46** 649–653

Dubois M, Giller K Å, Hamilton J K, Rebers P A and Smith F 1956 Calorimetric determination of sugars and related substances; *Annal. Chem.* **28** 351–356

Fraenkel G 1959 The Raisen d' etre of secondary plant substances; *Science* **12** 1466–1470

Harborne J B and Williams C A 1969 The identification of Orcinol in higher plants in the family Ericaceae; *Phytochemistry* **8** 2223

Hori K 1974 Studies on the feeding habits of *Lygus disponsi* L. (Hemiptera: Miridae) and the injury to its host plants. V Phenolic compounds, acid phosphatase and oxidative enzymes in artificially infested tissue of the sugar beet leaf; *Annal. Appl. Entomol. Zool.* **9** 225–230

Luckey T D 1968 Insecticide hormoligosis; *J. Econ. Entomol.* **61** 7–12

Lowry O H, Resegrough N J, Farr A L and Randol R J 1951 Protein measurements with folin phenol reagents; *J. Biol. Chem.* **193** 265–275

Pretorius L M 1976 Laboratory studies on the developmental and reproductive performance of *Heliothis armigera* on various food plants; *J. Entomol. Soc. Africa* **39** 337–343

Reese J C and Beck S D 1976 Effect of allelochemicals on the black cutworm, *Agrotis ipsilon*: Effect of *p*-benzoquinone, hydroquinone and duroquinone on larval growth, development and utilization of food; *Ann. Entomol. Soc. Am.* **69** 50–67

Scriber J M 1978 Cynogenic glycosides in *Lotus corniculatis*: Their effect upon growth, energy budget and nitrogen utilization of the southern army worm, *Spodoptera eridania*; *Oecologia* **34** 143–155

Scriber J M and Feeny P P 1979 Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants; *Ecology* **60** 829–850

Sparks A N, Wiseman B R and Mac millian W W 1971 Production of corn earworms on several hosts in field cages; *J. Econ. Entomol.* **64** 540–541

Van Sumere C F 1975 Plant proteins and phenolics: in *The chemistry and biochemistry of plant proteins* (eds) J B Harborne and C F VanSumere, (New York: Academic Press) pp 211–256

Vogel I A 1963 Determination of nitrogen by Kjeldahl's method; in *A text book of quantitative elementary instrumental analysis* (ed.) Osner (Hawk's physiological chemistry) pp 256–257

Waldbauer G P 1968 The consumption and utilization of food by insects; *Adv. Insect Physiol.* **5** 229–288