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PHYSICAL AND CHEMICAL BASIS OF RESISTANCE TO INSECTS IN WILD PIGEONPEA

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Introduction

Pigeonpea, *Cajanus cajan* (L.) Millspaugh, an important source of high-quality dietary protein in the semiarid tropics (Reed et al., 1981) is attacked by more than 200 species of insect pests (Lateef and Reed, 1990) which cause yield losses up to US$ 420 million (ICRISAT, 1992). *Helicoverpa armigera* is one of the major primary yield constraints (Lateef and Pimbert, 1991) besides *Maruca testulalis, Melanagromyza obtusa,* and *Clavigralla* spp. So far, no cultivated pigeonpea accessions show resistance to either *Helicoverpa* or other vital insect pests. A few wild pigeonpea species (*Cajanus*

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(*Atylosia*) *scarabaeoides*, *C. sericeus*, *C. albicans*, and *C. platycarpus*) are reported to be the potential pod borer resistant genotypes (ICRISAT, 1980). In addition, wild pigeonpea species possess desirable features such as disease resistance, high protein content, and drought resistance (Remanandan, 1981). The antibiotic chemicals and pod physical factors could be the reason for *Helicoverpa* avoidance to *C. scarabaeoides* (Lateef et al., 1981). Conventional breeding techniques to hybridize *C. cajan* with various wild species have failed due to difficulties in separating desirable from undesirable traits (Reed and Lateed, 1990).

The mechanisms of resistance need to be understood for any genetic enhancement program. Plant physical characters (Southwood, 1986) as well as variations in concentration and proportion of plant nutrients (Waldbauer and Friedman, 1991) and allelochemicals (Ishaaya, 1986) are prime factors to be considered for host plant resistance. Since knowledge about pod infesting insects other than pod borer and pod wasp in wild pigeonpea is scanty, in the present study, the following objectives were taken : (i) to study the incidence of pod infesting insects on two wild *C. platycarpus* (ICPW 68) and *C. scarabaeoides* (ICPW 82) and one cultivated *C. cajan* (ICPL 87), (ii) to find out the most preferred part of *Cajanus* species for feeding taking *H. armigera* as a test insect, and (iii) physical nature of the pod wall and pod surface chemistry to find out the mechanism of resistance to insects in wild *Cajanus* species.

Materials and Methods

Host Plants and Insect Pest Abundance

Seeds of *C. cajan*, *C. platycarpus*, and *C. scarabaeoides* obtained from the Genetic Resource Division, ICRISAT Asia

Center (IAC) were sown at IAC, Hyderabad, India on the first week of July 1995 with a plot size of 45 x 0.6m. The plot was irrigated at regular intervals and free from insecticides. The data on pod infesting insects were recorded on weekly basis till October and gradations were made based on their abundance.

Larval performance of *Helicoverpa armigera*

One day old first instar larvae used to study the larval growth and survival were obtained from the laboratory culture maintained on a semi-synthetic diet containing chickpea flour (Armes et al., 1992). The leaves, pods, and flowers were fed to the larvae to find the best suitable parts for larval growth and survival. To avoid tissue water loss and insect escape, the petri dishes were covered with parafilm. Petri dish (12 cm dia) containing one day larva and the respective plant tissues was kept in an incubator at 25 ± 2°C with a 12-h photoperiod. Fifteen replicates were maintained for *C. cajan* and *C. platycarpus* and thirty for *C. scarabaeoides* tissues (due to poor larval success). The weight and percentage larval survival were computed on day 4.

Insect growth rate

The larval growth rate on *Cajanus* pods was determined using seven day old *H. armigera* larvae since their survival was better at this age. To identify the mechanism of resistance, larvae were fed with pods and seeds separately. Before initiating, the larvae were starved for four hours to clear the gut content and weighed. Relatively uniform sized mature pods were fed to the larvae for seven days. Ten to thirteen replicates were maintained for each species. In addition, three replicates per species were kept as control (without larvae) to measure the water loss. To find out the larval fitness on seeds, pods were split longitudinally along the sutures and the alternate seeds were used as treatment and control (without larvae) to minimize the error in water loss. The setup was kept in an incubator at 27 ± 2°C and 20 ± 2°C with 12-h photoperiod by giving the preweighed pods (with seeds) and seeds for feeding. The insect and unconsumed food were weighed in 24 - 36 hours and the consumption rate (CR), growth rate (GR), and the insect mean weight (IWG) were calculated according to Waldbauer (1968).

Physical and Chemical Characteristics of Pod wall

The pods were washed with distilled water, filtered through Whatman # 1 filter paper in a preweighed 50 ml conical flask and freeze dried. Difference in beaker weight was depicted as quantity of water soluble chemicals. To calculate the pod surface wax, the pods were washed with distilled water, air-dried at room temperature, and then washed with chloroform for 1– 1.5 min. The chloroform washings were filtered in 50 ml preweighed beaker, oven dried at 50°C and weighed again. Difference in beaker weight was expressed as the quantity pod surface wax/g pod dry weight by weighing the pod walls after drying them in an oven for three days.

Fine hand sections of pod walls were taken using a razor blade and the thickness was measured using a micrometer. The seed hardness tester (1-20k.g., Kiya seisakusho, Japan) was used with minor modifications to evaluate the pod toughness. A blunt end needle (11 mm long x 1mm dia.) was fixed at the top. To help the pod penetration by the needle without touching the platform, a cork (3mm thick) with 2mm hole at the center was fixed on the platform using scotch tape. Four pod walls of *C. platycarpus* and two pod walls of *C. cajan* and *C. scarabaeoides* were taken since the pod wall of the former is relatively softer. Toughness was recorded at three different pod sites and the mean was taken as replicate one. Thirty replicates were maintained per species.

Statistical analysis

The data on larval growth were analyzed using two way Anova (Mstat Package). Simple two tailed Student's 't' test (Quatropro Package) was used to analyze the larval growth and pod wall parameters.

Results

Pod infesting insect pests

The pod infesting pest complex was almost similar in *C. cajan* and *C. platycarpus* with considerable variations in percentage abundance (Table 1). In some cases, the percentage pest availability was higher on *C. platycarpus* than on *C. cajan* (eg : *Clavigralla obtusa, Exelastis atomosa* and *Melanagromyza obtusa*). *H. armigera,* a major pest of cultivated pigeonpea, was less on *C. platycarpus* followed by *C. scarabaeoides.* However, the pod wasp, *Tanaostigmodes cajaninae* was omnipresent on the pods of *C. scarabaeoides* than on *C. cajan.* It should also be mentioned that *T. cajaninae* was the only major insect pest recorded infesting *C. scarabaeoides* pods.

Table 1 : Some major pod infesting insect pests in relation to population abundance during 1995 kharif season

Pests	*C. cajan*	*C. scarabaeoides*	*C. platycarpus*
Helicoverpa armigera	++++	+	++
Adisura atkinsoni	– –	– –	+++++
Lampides boeticus	++++	– –	+ + +
Tanaostigmodes cajaninae	+	+ + + + +	+
Melanagromyza obtusa	+ +	– –	+ + + +
Exelastis atomosa	+ +	– –	+ + + +
Clavigralla spp.	+ + +	– –	+ + + +

Percentage population: + = 1-19%; + + = 20-39%; + + + = 40-59%; + + + = 60-79%; + + + + + = 80 - 100%

Larval growth and survival :

The neonate larvae of *H. armigera* when fed on different tissues of *Cajanus* species responded differently (Table 2 and Fig. 1). Larval survival was 100% when fed on the leaves of *C. platycarpus* and the flowers of *C. cajan*. However, the larval weight gain was significantly higher on the pods than on the flowers and leaves of *C. cajan* and *C. platycarpus*. The flowers of two wild pigeonpea species supported fewer larvae with significantly lower on the leaves and pods of *C. scarabaeoides* than on the other two *Cajanus* species (Fig. 1).

Table 2 : Larval weight of *H. armigera* on day 4 on different plant parts of three *Cajanus* species

Plant parts	wet weight(mg)			
	C. cajan	*C. platycarpus*	*C. scarabaeoides*	Mean
Leaves	0.264	0.413	0.220	0.310
Flowers	0.553	0.372	0.317	0.446
Pods	0.624	0.493	0.345	0.507
mean	0.487	0.455	0.294	
MSE for plant tissues		0.0385		
MSE for species		0.0418		

Larval weight is significantly different among the different plant tissues and species but not between the species and plant tissues (2 - way Analysis of Variance).

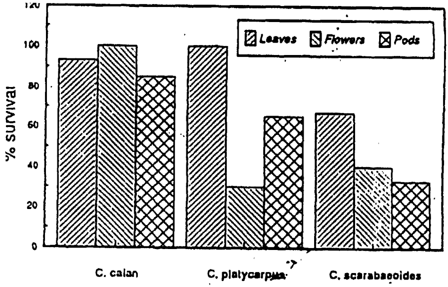
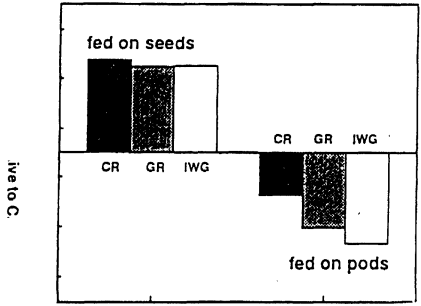


Fig. 1 : Larval survival of *H. armigera* on different parts of *Cajanus* species.

Larval food utilization

The feeding efficiency of 7 day old *H. armigera* larvae differed when they were fed with pods and seeds (Fig. 2). Larval consumption rate was significantly lower on *C. scarabaeoides* pods (43%) than on *C. cajan* pods. Larval weight gain and growth rate were lower on *C. scarabaeoides* than on *C. cajan* pods. However, the scenario was different entirely when the larvae were fed on the seeds of *Cajanus* species.

Food consumption was 94% higher when the larvae fed on the seeds of *C. scarabaeoides* than on *C. cajan* seeds (Fig. 2). The difference in consumption rate attributed the change in insect weight gain and growth rate that are relatively higher (87% and 88%) than the larvae fed on pods of *C. scarabaeoides*. From this it is evident that, the larval food consumption, weight gain, and growth rate were significantly lower when the larvae were allowed to feed on *C. scarabaeoides* pods than on the seeds, suggesting that the physical characteristics of the pod or chemical constituents act as barriers.



Rate of consumption (CR), growth (GR), and weight gained (IWG)

are significantly different (Students' 't' test)

Fig.2 : Food consumption and growth efficiency of *H. armigera* on pods and seeds of *C. scarabaeoides* relative to *C. cajan*

Pod wall thickness and toughness

The pod wall of *C. scarabaeoides* was thicker followed by the pod wall of *C. cajan* and *C. platycarpus*. The pod wall toughness was 0.20 times lower in *C. scarabaeoides* and 0.72 times higher in *C. platycarpus* than in *C. cajan*. A positive relationship was observed between the pod wall thickness and toughness within the three pigeonpea species (Table 3).

Table 3 : Physical and Chemical characeristics of pod walls with base value of 1.00 assigned to the check *C. cajan*

Characteristics	*C. cajan*	*C. platycarpus*	*C. scarabaeoides*
Soluble chemicals (mg/g)**	1.00a	1.60b	2.02c
Soluble chemicals/pod**	1.00a,b	0.94a	1.21b
Pod surface wax	1.00a	1.06a	0.47b
Pod wall thickness	1.00a	0.80b	1.72c

** Quantity of water soluble chemicals obtained from the respective pods of C=*Cajanus* species; Rows with different letters are significant at 51% level (Students' 't' test)

Pod surface chemistry

The total water soluble chemicals/pod were significantly higher (0.21 times) in *C. scarabaeoides* and 0.6 times lower (insignificant) in *C. platycarpus* than in *C. cajan* pods (Table 3). Since the pod-area varied between *Cajanus* species, the quantity was estimated on the basis of per miligram pod wall dry weight. The total chemical content per gram pod wall dry weight was significantly higher on *C. scarabaeoides* than *C. cajan* and *C. platycarpus* pods. The total pod surface wax did not differ significantly between the pods of *C. platycarpus* and *C. cajan*. However, pods of *C. scarabaeoides* contain significantly lower wax (0.47 times) than the pods of *C. cajan*.

Discussion

The pod infesting insect pest scenario obtained during 1995 kharif season show that the pods of *C. cajan* and *C. platycarpus* were attacked by similar insect pests. However, these pests were unable to attack the pods of *C. scarabaeoides*. The abundance of *H. armigera* was low on *C. scarabaeoides* (Table 1) which could be due to the presence of allomone or absence of kairomone and/or poor nutritional qualities coupled with allelochemical factors.

Larval survival and weight gain on the leaves of *C. cajan* were significantly lower than on the pods and flowers. Sison and Shanower (1994) reported four to eight fold lower larval weight on leaves than on pods and flowers. However, in this study, the superior larval performance on the leaves of *C. platycarpus* could partly be due to lesser trichome density on the abaxial and adaxial sides than the other two species (Peter and Shanower, unpublished data). Higher trichome density on the pods of *C. scarabaeoides* than on the *C. cajan* pods might have supported fewer larvae with small larval weight gain. Pod trichome density is lower on the pods of *C. platycarpus* than on *C. cajan* (Shanower *et al.*, 1995) and the present study suggests no relationship between larval performance and trichome density in *C. platycarpus*.

Larvae allowed to feed on the pods gained significantly higher weight with faster growth rate on *C. cajan* than on *C. scarabaeoides*. This was mainly because, the consumption rate of larvae fed on *C. scarabaeoides* pods decreased by 47% over *C. cajan*. Similarly, larvae were compelled to bore each locule of *C. scarabaeoides* separately which deplete considerable energy due to higher pod wall toughness and thickness (Table 3). Surprisingly, larvae fed on *C. scarabaeoides* seeds gained more weight with higher larval growth than on the seeds of *C. cajan*. Approximately 100% increase in consumption rate was recorded in *C. scarabaeoides* seed feeding larvae over the check. This can be further highlighted by the fact that, increased food consumption in response to reduced nutrient levels is a common occurrence in several phytophagous insects (Slansky and Wheeler, 1989). In the present study, the larval growth rate and weight gain were higher on *C. scarabaeoides* on *C. cajan* seeds indicating the superior nutritional quality of the seeds. From this, it is apparent that the pod wall of wild pigeonpea may have allomones or act as physical barrier that reduce the host acceptability and suitability.

The present results revealed higher total soluble chemicals and lower total wax on the pod surface of *C. scarabaeoides* than the other two species. Yoshida and Shanower (in prep.) reported that the filter paper dipped in *C. scarabaeoides* pod water washings induced the larval feeding in *H. armigera*. But, no substaintial evidence is available to justify pod wax with insect resistance. The pod wall toughness and thickness seem to play a major role in determining the degree of resistance besides trichome density. For example, pod wall toughness was 1.18 times higher in *C. scarabaeoides* than *C. cajan*. The same holds good for pod wall thickness too. *H. zea* larvae preferred to fed on leaves than on tougher walled maize (Cohen et al., 1988) and tomato fruits (Burkett et al., 1983) indicating the larval preference for soft plant tissues. In the present study, pod wall thickness and toughness were significantly lower in *C. platycarpus* than in the other two species, but larval performance was relatively lower to *C. cajan*. *C. platycarpus* pods may contain antibiotic chemicals that may not be suitable for larval growth as reported in soybean leaves (Wheeler and Slansky, 1991) and in silks of maize (Straub and Fairchild, 1970). The higher incidence of *T. cajanenae* on *C. scarabaeoides* pods could partly be due to the strong ovipositor that can insert the eggs by penetrating the pod wall.

Summary :

The pest incidence was lower in *C. scarabaeoides* than in *C. cajan* and *C. platycarpus*. *T. cajaninae* was the only major insect pest recorded in *C. scarabaeoides*. As far as pod borer resistant genotypes are concerned, *C. scarabaeoides* was the most suitable species for genetic enhancement program than *C.platycarpus*.

Larval response of *H. armigera* was different in different parts of the *Cajanus* species. Larvae preferred to feed on the reproductive parts of *C. cajan* and the leaves *C. platycarpus*. Larval survival and growth were significantly lower on the leaves, flowers, and pods of *C. scarabaeoides* prevented the larvae from

boring because of higher pod wall toughness and thickness. Rate of larval consumption and growth was higher when they were fed on the seeds than on the pods of *C. scarabaeoides*. Pod wall, thus acts as a resistant source in *C. scarabaeoides* against various insect pests including *H. armigera*. Further study in needed to substantiate the quality and quantity of pod wall chemistry as a resistant factor location against pod infesting insects. Finally, from this study, it could be concluded that pod physical factors, especially pod trichome, pod wall toughness and thickness play a significant role in restricting the pod infesting pests of *Cajanus* species.

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