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ROLES OF OXALIC AND MALIC ACIDS IN CHICKPEA TRICHOME EXUDATE IN HOST-PLANT RESISTANCE TO *Helicoverpa armigera*

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Abstract—Effects of malic acid and oxalic acid on oviposition of *Helicoverpa armigera* were investigated in a laboratory cage choice experiment. Malic acid stimulated oviposition at a concentration of 0.6 μmol/cm^2 but inhibited it at 3.4 μmol/cm^2. Oxalic acid showed neither stimulation nor inhibition of oviposition at 0.25–1.7 μmol/cm^2. Correlations between the amount of these acids in trichome exudate on leaf and pod surface and *H. armigera* populations and pod damage were investigated in a field experiment using 14 chickpea genotypes. Malic acid on the leaves stimulated oviposition during the vegetative and flowering stages, when its concentration was 0.1–0.7 μmol/cm^2. Later, during the podding stage there was no significant correlation between either egg density or pod damage and malic acid levels. However, there was a significant negative correlation between pod damage and oxalic acid levels. Oxalic acid, which had been reported to have an antibiotic effect on *H. armigera* larvae, has an important role in resistance to this pest in chickpea. The length of the podding period was also a factor influencing the extent of pod damage; a longer podding period resulted in prolonged exposure to *H. armigera* attack and more pod damage.

Key Words—*Helicoverpa armigera*, *Cicer arietinum*, chickpea, host-plant resistance, trichome, exudate, oxalic acid, malic acid, oviposition, antibiosis.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a grain legume adapted to dry and cool environments in southern and western Asia, northern and eastern Africa, Central

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and South America, and southern Europe. India produces more than 65% of the world's chickpeas (FAO, 1994), and the crop is an important source of protein for the local population. *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), the gram pod borer, is the major pest of chickpea and causes a serious production loss in most places where this crop is grown (Reed et al., 1987). *H. armigera* lays eggs on chickpea leaves, and the larvae feed preferentially on flowers and pods. They also eat leaves during the vegetative stage and when the reproductive structures have been destroyed. The methods for the management of the pest include manipulation of sowing and harvesting time, intercropping, and application of pesticide (Lal et al., 1986; Reed et al., 1987). The development of insecticide resistance in *H. armigera* populations in the Indian subcontinent (Armes et al., 1992b, 1996), coupled with an increasing awareness of the possible detrimental effects of intensive insecticide use, has stimulated interest in the development of integrated methods of pest control that reduce pesticide inputs and produce a more sustainable farming system.

Exploitable levels of resistance to *H. armigera* exist within the chickpea germ plasm (Dias et al., 1983; Lateef, 1985; Lateef and Sachan, 1990). Resistant genotypes frequently suffer lower pod damage compared with susceptible checks. Acidic exudate from trichomes on the surface of chickpea plants, in which oxalic acid and malic acid are major acidic components (Rembold et al., 1990a; Rembold and Weigner, 1990; Yoshida et al., 1995), has been correlated with reduced pod damage (Rembold, 1981; Rembold and Winter, 1982; Srivastava and Srivastava, 1989; Rembold et al., 1990a,b). The concentration of oxalic acid is higher on the leaf surface of resistant genotypes than on susceptible ones, and this acid retards the growth of *H. armigera* larvae (Yoshida et al., 1995). Material from resistant genotypes has an antibiotic effect on *H. armigera* (Srivastava and Srivastava, 1990). The inhibition of larval growth by oxalic acid in the trichome exudate, therefore, appears to be a component of host-plant resistance in chickpea. Malic acid has not been shown to affect larval growth.

Ovipositional antixenosis has also been reported as a mechanism of resistance to *H. armigera* in chickpea (Lateef, 1985; Srivastava and Srivastava, 1989; Cowgill and Lateef, 1996). However, a chemical factor for the antixenosis has not been identified. The aim of this study was to evaluate the effects of malic acid and oxalic acid on oviposition preference of *H. armigera* adults in a laboratory experiment and to investigate in a field experiment the effects of these compounds on egg and larval density of *H. armigera* and the pod damage caused by the pest.

METHODS AND MATERIALS

Insect Culture. All *H. armigera* adults used in cage experiments were obtained from a laboratory culture maintained at ICRISAT Asia Center, Patan-

cheru, Andhra Pradesh, India. It was established from and occasionally supplemented with field-collected eggs. Larvae were reared on a chickpea-based diet (Armes et al., 1992a) at 27°C. Several pairs of adults were kept in a cage at 25°C with 10% honey solution on absorbent cotton for purpose of collecting eggs.

Oviposition Cage Experiment. A sheet of tissue paper (34.5 × 24.0 cm) (One-Way Nappy Liners, Boots Co., Nottingham, England) was dipped in oxalic acid or L-malic acid solution, squeezed, and dried in an oven at 60°C for 1 hr. The amount of the acid retained on the tissue paper (micromoles per square centimeter) was calculated from the increase in dry weight of the paper after dipping and drying. The tissue paper was cut into three strips and hung on the wall of a transparent cylindrical plastic cage (23 cm diameter × 30 cm high). Control papers were identical except that they were treated with distilled water. They were hung between the acid-treated strips. A gravid female adult and a male adult, which had been kept in a cage for two days for mating, were released in the cage. Absorbent cotton soaked in 10% honey solution was placed in the cage for feeding of the moths. Oviposition was allowed to continue for 40 hr at 25°C under a photocycle of 2L:12D:12L:12D:2L hr. Individual pairs of moths were replicates, and eight replicates were used for each concentration of the acid treatments.

Design of Field Experiment. The field experiment was carried out at ICRISAT Asia Center, Patancheru, Andhra Pradesh, India, after the 1994 rainy season. A randomized block design with four replicates of 14 treatments (chickpea genotypes) was used. Each replicate consisted of four rows 9 m long with 60 cm between adjacent rows and 30 cm between plants within rows. Of the 14 chickpea genotypes, eight were *H. armigera*-resistant genotypes, five were susceptible checks, and one was a resistant × susceptible cross. They were divided into two groups, short-duration and medium-duration, according to the number of days from sowing to maturity (Table 1). The genotypes were sown on October 12, 1994. No pesticide was applied to the crop during the growing season.

Sampling of H. armigera *in the Field.* Ten chickpea plants in the second row of each replicate were examined for *H. armigera* eggs and larvae at weekly intervals from 28 days after sowing (DAS) to 91 DAS (short-duration genotypes) or to 112 DAS (medium-duration genotypes). Larvae were recorded as small (first to second instars), medium (third to fourth instars), and large (fifth to sixth instars). Medium and large larvae cause most of the damage to chickpea, while small larvae mainly scrape the surface of pods and leaves. As a result, analyses of larval density data were restricted to those of medium and large larvae only.

Five plant samples were collected from the first or fourth row of each replicate on the same day as the pest count. The plants were cut off at the roots, dried in an oven at 60°C for five days, and weighed. The egg and larval densities

TABLE 1. GROWTH OF 14 CHICKPEA GENOTYPES[a]

Genotype	Flowering (WAS)	Pod setting (WAS)	Seed maturity (WAS)
Short-duration			
Annigeri (S)	6	8	13
ICCV 2 (S)	5	7	12
ICCV 37 (S)	6	8	13
ICCX 730266 (S)	6	9	13
ICCV 93122 (S × R)	7	8	14
ICCV 7 (R)	7	8	13
ICC 506 (R)	6	8	13
ICCL 86101 (R)	6	8	13
ICCL 86102 (R)	6	8	13
ICCL 86111 (R)	7	8	14
Medium-duration			
ICC 3137 (S)	8	8	16
ICC 4935 (R)	9	10	16
ICCL 86106 (R)	10	11	16
ICCX 730041 (R)	8	10	16

[a]WAS: week after sowing; S: susceptible check; R: genotype selected for *H. armigera* resistance; S × R: cross between Annigeri and ICC 506.

are expressed on a gram plant dry weight (DW) basis to give a more precise estimate of their distribution among the 14 genotypes, which varied in plant size.

Analysis of Oxalic Acid and Malic Acid in Leaf and Pod Exudate. A branch with green leaves was collected from each of five plants in the third row of each replicate every two weeks from 28 to 70 DAS on the same day as the pest count. Two extra samples were taken for medium-duration genotypes 84 and 105 DAS. At the first sampling, when the plants were seedlings, whole plants were collected instead of branches. All the leaves were detached and washed with distilled water to collect the exudate. The exudate samples were filtered through a Millipore Filter (HVLP, pore size 0.45 μm) and injected in a Shimadzu LC-6A liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a Supelcogel C610-H column (7.8 mm ID × 30 cm) (Supelco Inc., Bellefonte, Pennsylvania), a SCL-6A system controller, and a C-R7A data analyzer. The mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.4 ml/min. The elutes were monitored at 210 nm on a SPD-6AV UV-VIS spectrophotometric detector. Oxalic acid and malic acid were identified from their retention times of 12.0 and 17.0 min, respectively, and quantified from the area of the

peaks, calibrated with authentic samples injected separately. The area of the washed leaves was measured with an LI-3100 area meter (LI-COR, Inc., Lincoln, Nebraska). The leaves were then dried in an oven at 60°C for two days, and the dry weight was measured. Concentration of the acids in the leaf exudate was calculated on the basis of the leaf area (micromoles per square centimeter) and dry weight (micromoles per gram dry weight).

A green tender pod was collected from each of 10 plants in the third row of each replicate. The pods were collected twice for each genotype at 49 and 63 DAS for genotype ICCV 2, at 63 and 77 DAS for other short-duration genotypes, and at 91 and 111 DAS for medium-duration genotypes. Green tender pods with small seeds were collected in the first sampling, and those with big seeds in the second sampling. Pods were washed with distilled water, and the exudate samples were analyzed for oxalic acid and malic acid as described above. The washed pods were opened, and seeds were discarded. The pod samples were dried in an oven at 60°C for two days, and the dry weight was measured. Concentration of the acids in the pod exudate was calculated on the basis of the pod dry weight (micromoles per gram dry weight).

Pod Damage Assessment. On January 25, 1995 (105 DAS), 25 plants were collected from the second row of each short-duration replicate, except ICCV 93122 and ICCL 86111. The sampling for the other genotypes was on February 13, 1995 (124 DAS). All the pods were removed from each sampled plant and placed individually into labeled paper bags. The pods were subsequently examined, and the number of pods with and without *H. armigera* damage was recorded.

Statistical Analysis. Oviposition preference between acid treated and control papers in cage experiments was evaluated by *t* test (10% significance level) on the angular transformed percentage of the eggs laid on the acid-treated papers by analyzing whether the percentage was different from 50%. The mean number of eggs (per gram dry weight), the mean number of medium and large larvae (per gram dry weight), and mean acid concentration (micromoles per gram dry weight) on the leaves were analyzed by a factorial ANOVA (GENSTAT Version 4.03) using date and chickpea genotype as factors. The data of the short-duration genotypes were analyzed as one group, and those of the medium-duration genotypes were analyzed separately. The angular transformed pod damage, mean concentration of the acids on the pods (micromoles per gram dry weight), and cumulative medium and large larval days were analyzed by SYSTAT one-way ANOVA (Wilkinson, 1990). For the one-way ANOVA, data of the short- and medium-duration genotypes were pooled. Means were separated at the 5% level using Fisher's least significant difference (LSD) test (Wilkinson, 1990). Results of Bartlett's test showed that variances of the data sets to which ANOVA was applied were homogeneous. The significance in correlation of *H. armigera* pop-

ulations, acid concentrations, and angular transformed pod damage was evaluated by the F test of the regression coefficient at the 1% level.

RESULTS AND DISCUSSION

Oviposition Cage Experiment. The fecundity of *H. armigera* females was highly variable. The total number of eggs laid per cage ranged from 109 to 1272. As a result, the percentage of the total number of eggs laid on acid-treated papers was calculated and used in subsequent analyses instead of egg number. When the concentration of malic acid on the papers was 0.6 μmol/cm^2, significantly more than 50% of the total number of eggs were laid on the acid-treated papers (Figure 1). At 3.4 μmol/cm^2 significantly less than 50% of the total number of eggs were laid on the acid treated papers. These results indicate that malic acid stimulates oviposition at the lower concentration but inhibits it at the higher concentration.

When the concentration of oxalic acid on the papers was 0.25–1.7 μmol/cm^2, neither stimulation nor inhibition of oviposition was observed (Figure 1).

Growth of Chickpea Genotypes and H. armigera *Populations in the Field.* The growth stages of the 14 chickpea genotypes used in this study are listed in

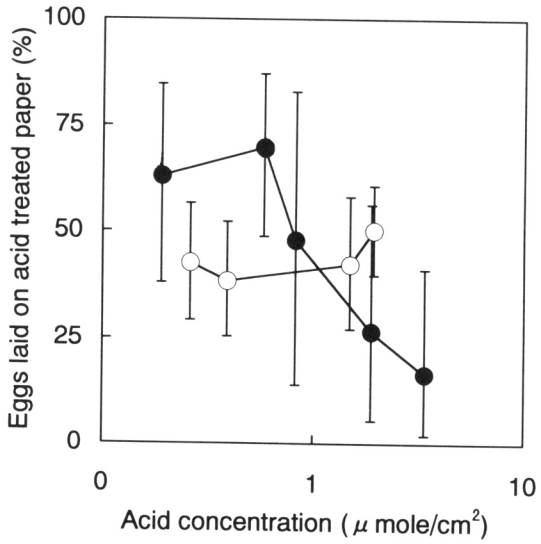

FIG. 1. Effect of malic acid and oxalic acid on oviposition of *H. armigera* in laboratory cage experiment. Bars show 95% confidence interval (t test); ●: malic acid; ○: oxalic acid.

Table 1. Short-duration chickpea genotypes started flowering 6–7 weeks after sowing (WAS), set pods 8–9 WAS, and matured 13–14 WAS, except for ICCV 2, which was one to two weeks more advanced (extra-short duration) than the other short-duration genotypes. Medium-duration genotypes, with the exception of ICC 3137, started flowering 8–10 WAS and set pods 10–11 WAS. ICC 3137 had tender pods 8 WAS. Seed maturity of the medium-duration genotypes was 16 WAS.

There was significant variation in egg density (per gram dry weight) among the genotypes in the two duration groups (Table 2: short-duration group: $F = 5.35$; $df = 9, 270$; $P < 0.001$; medium-duration group: $F = 2.98$; $df = 3, 117$; $P < 0.05$). There was also a significant date-genotype interaction in the short-duration group ($F = 1.67$; $df = 81, 270$; $P < 0.005$) because relative egg density was not constant during the sampling period. The egg density on ICCV 2, ICCV 37, ICCV 93122, and ICCV 7 peaked at 49 DAS, late flowering

TABLE 2. MEAN POPULATION OF *H. armigera* AND MEAN CONCENTRATION OF OXALIC ACID AND MALIC ACID ON LEAVES OF CHICKPEA GENOTYPES[a]

Genotype	Egg density (per g DW)	Larval density[b] (per g DW)	Oxalic acid (μmol/g DW)	Malic acid (μmol/g DW)
Short-duration				
Annigeri (S)	0.406 ab	0.212 bcd	44.6 c	67.3 e
ICCV 2 (S)	0.145 e	0.188 cd	33.8 d	80.0 cde
ICCV 37 (S)	0.455 a	0.226 bcd	28.9 d	122.3 b
ICCX 730266 (S)	0.368 abc	0.285 ab	54.6 b	86.9 c
ICCV 93122 (S × R)	0.429 a	0.275 abc	31.6 d	175.9 a
ICCV 7 (R)	0.288 bcd	0.226 bcd	63.6 a	69.9 de
ICC 506 (R)	0.156 de	0.153 d	64.1 a	76.3 cde
ICCL 86101 (R)	0.327 abc	0.256 abc	66.9 a	81.4 cde
ICCL 86102 (R)	0.260 cde	0.186 cd	63.7 a	71.3 de
ICCL 86111 (R)	0.401 ab	0.328 a	63.8 a	83.7 cd
SEM	0.047	0.033	2.7	5.1
Medium-duration				
ICC 3137 (S)	0.340 b	0.388 a	32.0 a	115.9 c
ICC 4935 (R)	0.399 b	0.276 b	34.4 a	134.4 b
ICCL 86106 (R)	0.569 a	0.210 b	25.0 b	182.1 a
ICCX 730041 (R)	0.445 ab	0.223 b	30.7 a	169.1 a
SEM	0.057	0.033	1.3	5.9

[a] Means within a duration group within a column followed by the same letter are not significantly different at the 5% level (LSD test). S, susceptible check; R, genotype selected for *H. armigera* resistance; S × R, cross between Annigeri and ICC 506; SEM, standard error of mean.
[b] Medium and large larvae.

stage, while on the other short-duration genotypes peak egg density occurred at 56 DAS, early podding stage. Peak egg density on the medium-duration genotypes occurred 49–56 DAS. The peak egg density was largest on the medium-duration genotypes and smallest on the *H. armigera*-resistant short-duration genotypes and ICCV 2. A second, smaller oviposition peak was observed approximately 70 DAS for both duration groups. At this time the medium-duration genotypes had tender pods.

There was significant variation in the density of medium and large larvae (per gram dry weight) among the genotypes in both duration groups (Table 2: short-duration group: $F = 2.65$; $df = 9, 270$; $P < 0.01$; medium duration group: $F = 6.06$, $df = 3, 117$; $P < 0.001$). There was a significant date–genotype interaction in the medium-duration group ($F = 2.03$; $df = 36, 117$; $P < 0.005$). Although the larval density generally declined along with the growth of the crop, a peak in the larval density occurred during the flowering–early podding stage of the short-duration genotypes. With the medium-duration genotypes a second peak was also observed during their pod-setting stage. With the exception of ICC 3137, larval density at the second peak was lower than that at the first peak. ICC 3137 had the highest second peak among the medium-duration genotypes, although the first peaks of ICC 3137, ICC 4935, and ICCX 730041 were similar. ICCL 86106 had the lowest larval density at these peaks.

Oxalic Acid and Malic Acid Concentration on Leaves. There was significant variation in oxalic acid concentration on a plant dry weight basis (micromoles per gram dry weight) among the short-duration genotypes from vegetative to podding stage (Table 2: $F = 32.73$; $df = 9, 108$; $P < 0.001$), and a significant date–genotype interaction ($F = 5.63$; $df = 27, 108$; $P < 0.001$). The level of oxalic acid was highest at 56 DAS, except for ICCV 93122. It decreased as the leaves senescenced during the podding stage. ICCV 93122 retained flowers and green leaves approximately one week longer than the other short-duration genotypes and had the maximum oxalic acid concentration at 70 DAS. The resistant genotypes retained significantly higher concentrations of oxalic acid on the leaf surface than the susceptible genotypes throughout the sampling period.

There was significant variation in the amount of oxalic acid (micromoles per gram dry weight) among the medium-duration genotypes (Table 2: $F = 8.85$; $df = 3, 54$; $P < 0.001$), and a significant data-genotype interaction ($F = 3.16$; $df = 15, 54$; $P < 0.001$). The pattern of change in the oxalic acid level was similar to that of the short-duration genotypes; the maximum level was observed at 70 DAS (flowering–early podding stage), and the concentration decreased towards senescence. The oxalic acid level on ICCL 86106 was significantly lower than those on the other medium-duration genotypes in the vegetative stage, but the levels were similar for all the genotypes in the podding stage. There was no significant difference in oxalic acid levels between the

susceptible check, ICC 3137, and the resistant genotypes, except ICCL 86106, which exhibited lower oxalic acid levels.

Significant variation was also observed in malic acid level (micromoles per gram dry weight) among the short-duration group (Table 2: $F = 43.80$, $df = 9, 108$; $P < 0.001$) and medium-duration group (Table 2: $F = 26.95$, $df = 3, 54$; $P < 0.001$). A significant date-genotype interaction was found in the former group ($F = 2.53$; $df = 27, 108$; $P < 0.001$). The malic acid level was highest at 70 DAS for all the genotypes, regardless of their duration. A decrease in the level of malic acid was observed in the podding stage of the medium-duration genotypes. The malic acid levels on ICCV 93122 and ICCV 37 were significantly higher than those on the other short-duration genotypes throughout the sampling period. There was no clear difference in the malic acid level between the resistant and susceptible short-duration genotypes. Within the medium-duration genotypes, significantly more malic acid was detected on ICCL 86106 and ICCX 730041 than on ICC 4935 and ICC 3137.

Relationship Between Leaf Malic Acid Level and H. armigera *Population Density.* Analysis of the relationship between mean malic acid level on a leaf area basis (micromoles per square centimeter) and mean egg density (per gram dry weight) during the vegetative and flowering stage, 28–49 DAS, showed a significant correlation ($r = 0.906$) between the two variables for all 14 chickpea genotypes (Figure 2). Hence malic acid on the chickpea leaves in the field acts as an oviposition stimulant for *H. armigera* at a concentration of 0.1–0.7 μmol/

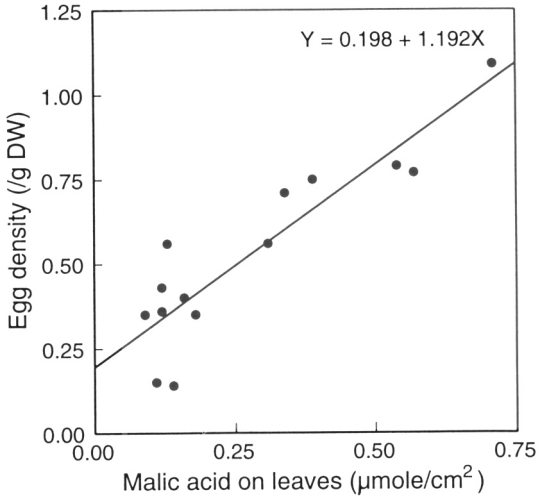

FIG. 2. Correlation between concentration of malic acid on leaves and *H. armigera* egg density during vegetative and flowering stage of chickpea genotypes.

cm^2. This observation is consistent with the result from the laboratory cage experiment (Figure 1). In the podding stage in the field, when malic acid concentration was 1–2 $\mu mol/cm^2$, there was no significant correlation between the malic acid level and the egg number, although malic acid tended to inhibit oviposition around this range of concentration in the cage experiment (Figure 1). This discrepancy may be due to other factors that may affect oviposition, e.g., the presence of leaf surface volatiles (Rembold et al., 1990a).

There was no significant correlation between the level of malic acid on the leaves (micromoles per gram dry weight) and the density of medium and large larvae (per gram dry weight).

Relationship Between Leaf Oxalic Acid Level and H. armigera *Population Density.* There was a significant negative correlation ($r = -0.840$) between mean leaf oxalic acid level (micromoles per square centimeter) and mean egg density (per gram dry weight) in the vegetative–flowering stage, when the oxalic acid concentration was 0.05–0.25 $\mu mol/cm^2$. This negative correlation is not considered to be due to oviposition antixenosis by oxalic acid because the correlation was not observed later in the podding stage, when the oxalic acid level was higher (0.25–0.75 $\mu mol/cm^2$). Even at the higher concentration of 1.5–1.7 $\mu mol/cm^2$, oxalic acid did not have a significant effect on oviposition in the laboratory cage experiment (Figure 1). It was not possible to test concentrations lower than 0.25 $\mu mol/cm^2$ in the laboratory study because the amount of acid on the tissue paper was too small to be detected. There was a significant negative correlation ($r = -0.877$) between the levels of leaf malic acid and leaf oxalic acid in the vegetative–flowering stage of chickpea; genotypes with higher malic acid levels had lower oxalic acid levels. The negative correlation between the leaf oxalic acid level and the egg number was, therefore, considered to be a result of the stimulation of oviposition by the leaf malic acid.

A negative correlation ($r = -0.614$, $P = 0.05$) between the leaf oxalic acid level (micromoles per gram dry weight) and the density of medium and large larvae (per gram dry weight) was observed 56 DAS, corresponding to pod setting and late vegetative–flowering stage in the short- and medium-duration genotypes, respectively. Before and after this date there was no correlation between the two variables. This is surprising as oxalic acid has been reported to have an antibiotic effect on *H. armigera* larvae (Yoshida et al., 1995). The frequency of sampling may have been insufficient to detect such a relationship. Alternatively, relative larval density may have been underestimated during the late podding stage as many larvae were concealed inside the pods.

Oxalic Acid and Malic Acid Concentration on Pods. There was significant variation in mean oxalic acid level (micromoles per gram dry weight) on the pods on the two sampling dates among the 14 genotypes (Table 3; $F = 23.46$; $df = 13, 55$; $P < 0.001$). The medium-duration genotypes had significantly lower oxalic acid levels on the pods than the short-duration genotypes, except

TABLE 3. MEAN CONCENTRATION OF OXALIC ACID AND MALIC ACID ON PODS, CUMULATIVE LARVAL DAYS OF PODDING STAGE, AND POD DAMAGE OF CHICKPEA GENOTYPES[a]

Genotype	Oxalic acid (μmol/g DW)	Malic acid (μmol/g DW)	Cumulative larval days[b]	Pod damage (%)
Short-duration				
Annigeri (S)	59.2 de	249.1 cd	2.75 cde	31.2 b
ICCV 2 (S)	77.8 b	278.5 abc	3.92 bcd	25.9 bcd
ICCV 37 (S)	55.2 e	300.2 a	3.93 bcd	28.8 bc
ICCX 730266 (S)	66.3 cd	282.1 abc	2.78 cde	25.7 bcd
ICCV 93122 (S × R)	51.1 ef	283.7 ab	2.59 bc	22.2 cd
ICCV 7 (R)	75.1 bc	233.9 de	4.13 cde	13.7 e
ICC 506 (R)	77.9 b	291.4 a	1.98 de	12.7 ef
ICCL 86101 (R)	77.5 b	275.4 abc	1.37 e	10.9 ef
ICCL 86102 (R)	90.3 a	289.3 a	1.18 e	6.9 f
ICCL 86111 (R)	66.7 cd	255.7 bcd	2.97 bcde	19.7 de
Medium-duration				
ICC 3137 (S)	43.6 f	208.3 e	10.20 a	64.4 a
ICC 4935 (R)	44.1 f	252.0 bcd	4.87 b	32.8 b
ICCL 86106 (R)	44.5 f	268.4 abc	2.34 cde	28.3 bc
ICCX 730041 (R)	45.0 f	253.8 bcd	3.13 cde	34.2 b
SEM	3.2	11.6	0.71	2.8

[a]Means within a column followed by the same letter are not significantly different at the 5% level (LSD test). S, susceptible check; R, genotype selected for *H. armigera* resistance; S × R, cross between Annigeri and ICC 506; SEM, standard error of mean.
[b]Medium and large larvae.

ICCV 93122. Among the short-duration genotypes, the three *H. armigera*-susceptible checks, Annigeri, ICCV 37, and ICCX 730266, had significantly lower levels of oxalic acid than the resistant genotypes, except ICCL 86111 and ICCV 7. ICCV 2 had an oxalic acid concentration comparable to that of the short-duration resistant genotypes.

Mean malic acid levels (micromoles per gram dry weight) on the pods were much higher than the mean pod oxalic acid levels (Table 3). There was significant variation in the malic acid level among the genotypes ($F = 4.73$; $df = 13, 55$; $P < 0.001$), but the genotypic variation was smaller than that of oxalic acid. There was no significant difference in the malic acid level between the *H. armigera* resistance and susceptible genotypes.

Pod Damage. There was significant variation in the angular transformed percentage pod damage among the 14 genotypes (Table 3; $F = 22.83$; $df = 13, 55$; $P < 0.001$). Highest pod damage was recorded on ICC 3137, a medium-

duration *H. armigera*-susceptible check. Other medium-duration genotypes exhibited significantly lower pod damage than ICC 3137, but the levels of the damage were similar to those in the *H. armigera*-susceptible short-duration genotypes. The *H. armigera*-resistant short-duration genotypes, except ICCL 86111, had significantly lower pod damage than the corresponding susceptible checks. These pod damage data confirmed the reduced damage in the genotypes that had previously been selected for *H. armigera* resistance. ICCV 93122, which is a new variety obtained from a cross between Annigeri, a *H. armigera*-susceptible genotype, and ICC 506, a *H. armigera*-resistant genotype, showed pod damage intermediate between the two parents.

The density of medium and large larvae (per gram dry weight) was plotted against time (DAS), and the area under the curve was calculated to provide an index of the intensity of larval attack, i.e., cumulative larval days, for each genotype from pod setting to maturity. There was significant variation in the cumulative larval days among the genotypes (Table 3; $F = 9.55$, $df = 13, 55$; $P < 0.001$). There was also a significant correlation between the cumulative larval days and the angular transformed percentage pod damage ($r = 0.879$) (Figure 3). Wightman et al. (1995) showed a negative correlation between cumulative larval days and grain yield and a strong negative correlation between grain yield and pod damage of chickpea in southern India. Our results confirm that pod and seed damage are mainly caused by medium and large *H. armigera*

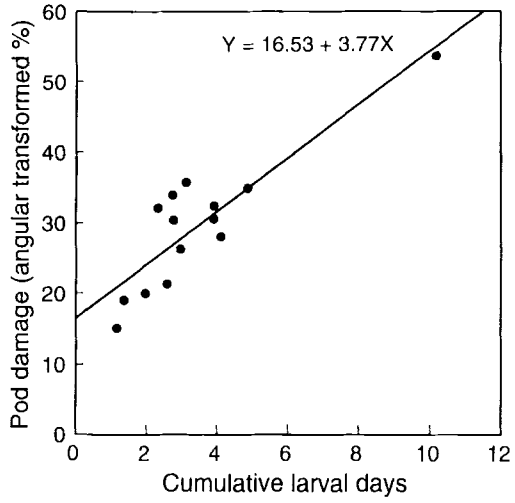

FIG. 3. Correlation between cumulative larval days for medium and large larvae of *H. armigera* during podding stage and pod damage of chickpea genotypes.

larvae in the podding stage and that their control should result in lower pod damage and higher yield in chickpea (Fitt, 1989).

In the present study genotypes that had high numbers of eggs did not necessarily have high numbers of larvae. This suggests that the factor(s) that stimulate(s) or inhibit(s) oviposition is different from the factor(s) that affect(s) larval growth and survival. In this study more oxalic acid (micromoles per gram dry weight) was detected, in general, on the leaves and pods of the short-duration *H. armigera*-resistant genotypes than on the susceptible checks or on the medium-duration genotypes (Tables 2 and 3). Angular transformed percentage pod damage was negatively correlated with the mean oxalic acid level on the pods ($r = -0.841$), when the data of ICC 3137 were excluded (Figure 4). The mean leaf oxalic acid level, which was correlated with the pod oxalic acid level ($r = 0.800$), was also negatively correlated with the angular transformed percentage pod damage ($r = -0.815$), when ICC 3137 was excluded from analysis (Figure 5). Oxalic acid has been reported to have an antibiotic effect on larvae (Yoshida et al., 1995), and it is possible that the antibiotic properties of oxalic acid may negate differences due to ovipositional antixenosis and determine the size of the larval population, and therefore pod damage, on a particular genotype.

The pod damage of ICC 3137, a susceptible check for medium-duration genotypes, was extremely high despite the levels of oxalic acid on both the leaves and pods being similar to those of other medium-duration genotypes.

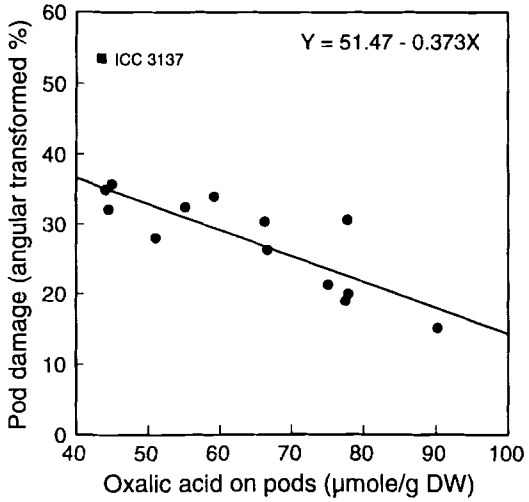

FIG. 4. Correlation between concentration of oxalic acid on pods and *H. armigera* pod damage of chickpea genotypes.

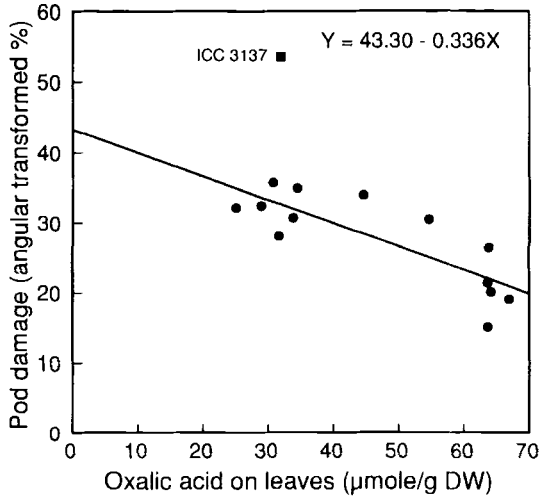

FIG. 5. Correlation between concentration of oxalic acid on leaves and *H. armigera* pod damage of chickpea genotypes.

This observation indicates that, in addition to oxalic acid, another factor also determines *H. armigera* susceptibility in chickpea. ICC 3137 began podding earlier than the other medium-duration genotypes but retained green leaves and tender pods for as long as the other genotypes (Table 1) and, as a result, had a more prolonged exposure to *H. armigera* attack than the other genotypes. The length of the podding period is, therefore, also an important factor in resistance to *H. armigera*.

There was no significant correlation between the pod damage and mean pod malic acid level (micromoles per gram dry weight). Although previous studies proposed malic acid in the chickpea leaf exudate to be a *H. armigera* resistance factor (Rembold, 1981; Rembold and Winter, 1982; Rembold et al., 1990a,b), we did not find any evidence that malic acid acts as a resistance factor in the field experiment.

The results of our study have practical implications for chickpea germ plasm enhancement programs. Oxalic acid levels could be used to select material for further screening. Leaves in the flowering–early podding or tender pod stage would be the most appropriate sample unit, as the differences in the oxalic acid level between resistant and susceptible genotypes are most marked at this time. In addition, the duration of the podding period could also be used as a selection criterion. This would be particularly useful for medium-duration genotypes where plants with shorter podding periods should be selected to minimize the period of exposure to the pest.

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