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Comparison of the efficacy of chemical control and *Helicoverpa* NPV for the management of *Helicoverpa armigera* (Hübner) on resistant and susceptible chickpea

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The influence of host-plant resistance on the efficacy of NPV and quinalphos as mortality factors in *Helicoverpa armigera* Hübner populations on chickpea was examined in the field in 1993 and 1994. The effects of chickpea genotype and NPV or quinalphos were not independent. In 1994 quinalphos had a greater effect on the density of large *H. armigera* larvae on susceptible genotypes than on the resistant genotype (ICC 506). In 1993, NPV had greater effect on the density of large larvae on susceptible genotypes than on ICC 506. In 1993, the yields of NPV-treated susceptible genotypes were significantly greater than those in the quinalphos treatment or control. In 1994, the yields of susceptible genotypes treated with NPV or quinalphos were similar and significantly greater than those in the control. Yields of ICC 506 were similar in the treatments and control. Further studies are required to determine the factors influencing the compatibility of host-plant resistance with quinalphos or NPV; and to examine the potential for increasing the efficacy of these mortality factors when they are used in conjunction with *Helicoverpa* resistant chickpea. Copyright © 1996 Elsevier Science Ltd.

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Helicoverpa armigera Hübner is a major crop pest in Asia. In India it is the dominant pest of several legume crops including chickpea (*Cicer arietinum* L.) (Reed *et al.*, 1987) and pigeonpea (*Cajan cajanus* [L.] Millspaugh) (Bhatnagar *et al.*, 1982) and can cause serious losses to sorghum (*Sorghum bicolor* L.) (Mote and Murthy, 1990) and cotton (Kishor, 1992). Its high pest status arises from the preference of foraging larvae for plant structures rich in nitrogen (Fitt, 1989) such as flowers, pods and panicles.

The principal means of controlling *H. armigera* on crops has been the use of conventional insecticides. However, in 1987 farmers in parts of southern India were unable to control populations of *H. armigera* on cotton, chickpea and pigeonpea crops with insecticides. High levels of resistance to synthetic pyrethroids were subsequently confirmed by Dhingra, Phokela and Mehrotra (1988) and McCaffery *et al.* (1989) as a major cause of the control failures.

The development of insecticide resistance 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, which reduce pesticide inputs and produce a more sustainable farming system (Carter, 1989). A central component of integrated approaches to *H. armigera* management on chickpea could be the adoption of *Helicoverpa* resistant genotypes.

Screening chickpea germplasm accessions has identified several lines with exploitable levels of resistance to *H. armigera* (Singh and Sharma, 1970; Dias, Lal and Yadava, 1983; Lateef and Sachan, 1990). Currently there is little information on the compatibility of host plant resistance with other *H. armigera* management options for chickpea. Results of studies of other systems indicate that, in general, host plant resistance is compatible with, and complementary to, the action of biological control organisms (Kogan, 1975; Adkisson and Dyck, 1980; Beach and Todd, 1988; Meade and Hare, 1994) and chemical control options (Robinson *et al.*, 1978; van den Berg, van Rensburg and van Westhuizen, 1994) although exceptions do exist (Campbell and Duffey, 1979; Felton *et al.*, 1987; Rabindra, Sathiah and Jayaraj, 1992.

The aim of the present study was to examine the effect of variation in host plant suitability on the efficacy of chemical control and *Helicoverpa* NPV for the management of *H. armigera* on chickpea.

Materials and methods

The experiment was carried out at ICRISAT Asia Centre, Andhra Pradesh, India, during the 1993 and 1994 post rainy seasons. The experiment was designed as a split-plot with three replicates. Spray treatment

(NPV application, quinalphos application or control) was the main plot and chickpea genotypes the subplots.

In the 1993 season the chickpea genotypes comprised Annigeri (a local, non-improved variety, susceptible to *Helicoverpa*), ICC 506 (a germplasm line with approximately the same yield potential as Annigeri, *Helicoverpa* resistance and *Fusarium* wilt susceptibility) and ICCC 37 (a variety with higher yield potential than Annigeri in Deccan India, some *Fusarium* wilt resistance but susceptibility to *Helicoverpa* attack). In the 1994 season the genotypes comprised Annigeri, ICC 506, ICC 4958 (a germplasm line with *Fusarium* wilt and *Helicoverpa* susceptibility which is high yielding in low productivity terminal drought environments) and ICCV 93122 (an advanced breeding line combining the characters of Annigeri and ICC 506 but with greater susceptibility to *Helicoverpa* attack than ICC 506).

In both years replicates were prepared as 1.5 m broad beds with 30 cm between rows and 30 cm between plants within a row. In the 1993 season the experiment was sown on 11 October. Each replicate consisted of four beds, each of 9 m length. In the 1994 season, the experiment was sown on 29 September. Each replicate consisted of seven beds, each of 18 m length.

Sampling *H. armigera* populations

In the 1993 season, the number of small (I–II instar), medium (III–IV instar) and large (V–VI instar) *H. armigera* larvae on 24 plants per replicate was recorded at weekly intervals from 27 October to 30 November. From 3 December until harvest the frequency of sampling was increased to twice a week. In the 1994 season, larval counts began on 24 October and were continued at weekly intervals until 9 January 1995.

Spray applications

Because of the different modes of action of *Helicoverpa* NPV and quinalphos the two treatments were not applied simultaneously. An economic threshold of two larvae (all sizes) per plant (Reed *et al.*, 1987) from 5% flowering onwards was used to determine the timing and frequency of quinalphos applications. NPV was applied on a calendar basis at approximately 10 day intervals from 5% flowering onwards.

In the 1993 season, quinalphos was applied at a rate of 0.5 kg a.i./ha (1501/ha) on 10 December, 17 December and 7 January. Applications were made using a lever operated knapsack sprayer with hollow cone jet operated at 3 bar pressure. It was not possible to respond to the individual variation in larval density among genotypes because of the close proximity of the subplots in the quinalphos treated main plots, therefore, on each date all three genotypes were treated.

In the 1994 season, the larger replicate size made it possible to respond to variation in larval density among the genotypes. Quinalphos was applied to all four genotypes on 17 November, 8 December and 15 December; plus an additional application to Annigeri, ICC 4958 and ICCV 93122 on 23 December. Application rate and spray equipment were the same as in the 1993 season.

In the 1993 season, NPV was applied on 9, 14 and 21 December and 12 January. A liquid formulation of NPV was applied at a rate of 250 larval equivalents (LE)/ha (1.5×10^{12} PIB/ha) using a battery operated spinning disc sprayer. NPV suspensions were applied in water with 20% jaggery and 5% 'Robin Blue' as an adjuvant. Applications took place between 17.30 and 18.00 h. In the 1994 season, NPV was applied on 17 and 27 November and 5, 15 and 24 December. The first two applications were of a liquid formulation, the remaining three were of a wettable powder. The NPV suspensions were applied with water and no adjuvant.

In the 1993 season, chickpea leaves were collected from the NPV treatment immediately following each application to test the biological activity of the virus. On each occasion 40 II instar *H. armigera* larvae, obtained from laboratory cultures maintained at ICRISAT centre, were transferred to individual plastic tubs (4.5 cm diameter × 3 cm height) using a sterilized paint brush. Chickpea leaves were collected from a total of 30 randomly selected plants from the NPV treatment and from 10 randomly selected plants in the control. The leaves were placed in individual plastic bags and brought to the laboratory where they were transferrd to the tubs using forceps. The larvae were allowed to feed on the leaves for 48 h before being transferred to individual, sterilized glass tubes containing a chickpea based diet (Armes, Bond and Cooter, 1992). The larvae were observed for five days and the number of diseased larvae recorded.

Pod damage and yield assessment

In the 1993 season, 25 plants were collected from each replicate on 1 February. All the pods were removed from each plant by hand and transferred to individual paper bags. The number of damaged and undamaged pods, seed number and seed weight were recorded for each plant. During the period 2–4 February 1994, plants were harvested from the central 7 m of each of 14 rows per replicate. The plants were threshed and the net plot yield recorded. Yield data for ICC 506 were not included in statistical analysis because the plant stand had been significantly reduced in several replicates by *Fusarium* wilt. In the 1994 post rainy season, 50 plants were collected from each replicate for pod damage assessment on 24 January. Plants were collected from the central 16 m of each of five beds per replicate during 28 January to 1 February and threshed for the calculation of net plot yield.

Statistical analysis

Larval counts for each year were analysed separately using GENSTAT (version 4.04), split-plot ANOVA with genotypes as the subplot and sampling date as the sub subplot. The angular transformed percentage pod damage and yield (kg/ha) were analysed using split-plot ANOVA with genotype as the subplot.

In the 1993 season, analyses were performed on the mean number of all larvae per eight plants and the $n + 1$ square root transformed number of large larvae per eight plants. In the 1994 season, analyses were performed on the $n + 1$ square root transformed mean

number of all larvae per plant and the $n + 1$ square root transformed number of large larvae per plant.

Results

In 1993, the mortality of larvae exposed to NPV treated leaves in the laboratory bioassay ranged from 11.5 to 30.8%. There was no corresponding control mortality.

In both years, chickpea genotype and treatment had a significant effect on the density of all *H. armigera* larvae (*Table 1*). In 1993, there was a significant treatment × genotype interaction; both NPV and quinalphos had greater effect on *H. armigera* populations on the two susceptible genotypes than on ICC 506. There were significantly more larvae on the susceptible genotypes in the control than the NPV or quinalphos treatments, whereas there was no significant variation in larval density on ICC 506 among the treatments and control.

In both years, chickpea genotype and treatment had a significant effect on the density of large *H. armigera* larvae and there was a significant treatment × genotype interaction (*Table 2*). In both years, there were significantly more larvae on the susceptible genotypes in the control than in the quinalphos treatment. In 1994 the density of large larvae on ICC 506 was similar in the treatments and control.

Chickpea genotype and treatment both had a significant effect on pod damage. In 1993, there was a significant treatment × genotype interaction (*Table 3*); the treatments were not equally effective in reducing pod damage on all genotypes. Pod damage on the two susceptible genotypes was significantly reduced in the NPV treatment compared to the quinalphos treatment

Table 1. Mean density of all larvae/eight plants and mean number of all larvae/plant (1994) in each genotype × treatment combination. Pooled data from 15 (1993) or 11 (1994) sampling dates

	1993			1994			
	Annigeri	ICC 506	ICCC 37	Annigeri	ICC 506	ICC 4958	ICCV 93122
Control	21.00	6.38	19.59	3.27	2.22	3.60	3.06
Quinalphos	13.12	5.48	13.78	2.31	1.49	2.34	1.87
NPV	13.20	5.96	12.10	2.86	2.01	3.66	2.72
Effective standard errors of means:		0.58*	0.554		0.204*	0.202*	

*Comparisons with the same spray treatment
†Comparison with different spray treatments

Table 2. Mean number of large larvae/eight plants (1993) and mean number of large larvae/plant (1994) in each genotype × treatment combination. Pooled data from 15 (1993) or 11 (1994) sampling dates

	1993			1994			
	Annigeri	ICC 506	ICCC 37	Annigeri	ICC 506	ICC 4958	ICCV 93122
Control	3.28	0.96	2.84	0.21	0.16	0.32	0.26
Quinalphos	1.62	0.57	1.58	0.07	0.09	0.11	0.09
NPV	0.69	0.40	0.71	0.13	0.09	0.35	0.18
Effective standard errors of means:		0.102*	0.190†		0.035*	0.037†	

*Comparisons with the same spray treatment
†Comparisons with different spray treatments

Table 3. Mean percentage pod damage

	1993			1994			
	Annigeri	ICC 506	ICCC 37	Annigeri	ICC 506	ICC 4958	ICCV 93122
Control	31.6	10.4	33.2	35.3	21.3	42.1	23.9
Quinalphos	29.9	3.1	28.24	12.0	9.6	16.5	7.6
NPV	8.1	14.5	13.37	14.7	6.83	17.0	11.2
Effective standard errors of means:		2.46*	2.24†		2.10*	1.88†	

*Comparisons with the same spray treatments
†Comparisons with different spray treatments

Table 4. Mean yield (kg/ha)

	1993		1994			
	Annigeri	ICCC 37	Annigeri	ICC 506	ICC 4958	ICCV 93122
Control	619	804	1440	1417	1296	1467
Quinalphos	781	558	2173	1448	1827	2044
NPV	1268	1358	1872	1747	1940	1870
Effective standard errors of means:	144.2*	106.7†		101.5*	91.9†	

*Comparison with the same spray treatments
†Comparison with different spray treatments

or control. Pod damage in ICC 506 was greatest in the NPV treatment and least damage was recorded in the quinalphos treatment.

In 1993, treatment had a significant effect on yield. There was no effect of genotype and no treatment × genotype interaction. In 1994, treatment and genotype both had a significant effect on yield and there was a significant treatment × genotype interaction (*Table 4*). The mean yield of ICC 506 was similar in the two treatments and the control. The mean yields of Annigeri and ICC 4958 were similar in the NPV and quinalphos treatments and were significantly greater than the control. The mean yield of ICCV 93122 in the NPV treatment was significantly less than that in the quinalphos treatment and was not significantly different from that in the control.

Discussion

The results of the present study have shown that NPV can provide control of *H. armigera* larval populations which is comparable with, or superior to, that provided by a synthetic insecticide. This observation confirms that previously reported by Rabindra and Jayaraj (1988). However, in the present study NPV was not consistently more effective than quinalphos. In 1993, the number of large larvae was observed to be lowest in the plots which had been sprayed with NPV. In 1994 the density of large larvae on NPV treated susceptible genotypes was similar to that in the controls and significantly greater than that in the quinalphos treatment.

With the exception of ICC 4958, the differences in the density of large larvae among the treatments and genotypes were reflected in the pod damage and yield data. In 1993, pod damage was lowest and yield highest on the susceptible genotypes which had been treated with NPV. In 1994, yields of ICC 506 were similar in all treatments while the yields of Annigeri, ICC 4958 and ICCV 93122 were highest in the quinalphos or NPV treatments. In the case of ICC 4958, high yields were obtained in the NPV treatment despite a relatively high density of large larvae recorded in this treatment.

The poor performance of NPV in 1994 compared to the previous season may have been the result of differences in NPV formulations between the seasons or the absence of the jaggery + 'Robin Blue' adjuvant in the second year. In 1993, liquid formulations were applied throughout the trial; in 1994 the initial spray was with a liquid formulation and the remainder with a wettable powder formulation.

Comparisons of the effectiveness of adjuvant treatments have shown that the incorporation of certain products, e.g., soybean flour (Smith and Hostetter, 1982) or selected optical brighteners (Shapiro, 1992) can increase mortality due to nuclear polyhedrosis viruses. However, many adjuvants, including jaggery, have been shown to have no effect on pest morality in field conditions (Rabindra and Jayaraj, 1988). Further studies are required to examine the potential for increasing the effectiveness of *Helicoverpa* NPV on chickpea via product formulation and the inclusion of adjuvants.

In both years of the present study there was significant variation in the effectiveness of both treatments among chickpea genotypes. In 1993, NPV and quinalphos both produced significant reductions in *H. armigera* density (all and large larvae) on susceptible genotypes. However, neither NPV or quinalphos provided significant additional reductions in pest density (all larvae) when used in conjunction with ICC 506. A similar trend was observed with large larvae in 1994: the density of large larvae on ICC 506 was similar in the treatments and control whereas on more susceptible genotypes there were significantly fewer larvae in the quinalphos treatment than the NPV treatment or control. These observations indicate that the efficacy of both NPV and quinalphos as mortality factors can be influenced by the use of *Helicoverpa* resistant genotypes.

Previous studies of the effect of diet on susceptibility to baculoviruses have shown significant variation in mortality among larvae reared on different host plants (Richter, Fuxa and Abdel-Fattah, 1987; Keating, Yendol and Schultz, 1988; Forschler, Young and Felton, 1992) or resistant and susceptible genotypes of the same species (Beach and Todd, 1988). The differential mortality has been attributed to variation in leaf acidity and tannin content (Keating *et al.*, 1988) and rutin or chlorogenic acid content (Felton *et al.*, 1987; Felton and Duffey, 1990). In chickpea there is a negative correlation between the malic acid content of the plant surface exudate and *H. armigera* susceptibility (Rembold, 1981). The incompatibility of host plant resistance and NPV may be the result of a negative effect of tissue pH on the process of NPV infection. Both PIB dissolution and virion survival are strongly

influenced by larval midgut pH (Ignoffo and Garcia, 1966; Gudauskas and Canerday, 1968), which in turn is affected by the foliar constituents and the pH of tissues entering the gut. In addition, the retention time of tissue in the gut, and, therefore, infection time, is also influenced by tissue pH. *Lymantria dispar* L. larvae passed high tannin, low pH tissue faster than low tannin, high pH tissue (Keating *et al.*, 1988). Either, or both of these factors could have contributed to the genotypic differences in larval mortality observed in the present study. The mechanism of resistance to *H. armigera* in chickpea does not appear to involve an antifeedant effect (Yoshida, pers. commun.), therefore, it seems unlikely that differential rates of ingestion of NPV among the genotypes was responsible for the differences in susceptibility to the virus.

Previous studies of the interaction of host plant resistance and synthetic insecticides have generally shown that insecticide efficacy is increased against insects feeding on resistant genotypes (Kea, Turnispeed and Carner, 1978; van den Berg *et al.*, 1994). The increased susceptibility is attributed to stress caused by antibiosis or antixenosis. In the present study, application of quinalphos to ICC 506 produced little benefit in terms of reducing the density of all larvae (1993) or large larvae (1994). This may have been the result of differential exposure to the chemical among larvae on the different genotypes. Quinalphos is a contact and ingested insecticide (Worthing and Hance, 1991). It seems unlikely that there were differences in tissue consumption, and, therefore, rates of ingestion of quinalphos among the genotypes. However, it is possible that there were physical differences in the location of larvae on resistant and susceptible plants which resulted in differences in exposure to the chemical among the genotypes.

The results of the present study have important implications for the development of IPM strategies for *H. armigera* on chickpea and have highlighted the need to examine the compatibility of single component pest management options before they are recommended for inclusion in such strategies. Further studies are now required to determine the factors which influence the compatibility of host plant resistance with NPV or quinalphos and to examine the potential for increasing the efficacy of these mortality factors when they are used in conjunction with *Helicoverpa* resistant chickpea.

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