BIOLOGICAL CONTROL

Influence of Parasitism by *Chelonus insularis* Cresson (Hymenoptera: Braconidae) on the Susceptibility of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) to Insecticides

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Influencia del Parasitismo por *Chelonus insularis* Cresson (Hymenoptera: Braconidae) sobre la Susceptibilidad de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) a Insecticidas

RESUMEN - El parasitoide huevo-larva *Chelonus insularis* Cresson es un parasitoide clave del cogollero del maíz, *Spodoptera frugiperda* (J.E. Smith) durante la segunda generación de campo en el Valle Superior del Río Magdalena de Colombia. A pesar de la presión de selección se encuentra una susceptibilidad mayor a insecticidas en la segunda generación, comparado con la primera, siendo para esto la única diferencia aparente el parasitismo. Por esta razón, la susceptibilidad a clorpirifos, metomyl, cipermetrina y *Bacillus thuringiensis* de larvas parasitadas y no parasitadas del segundo instar del cogollero fueron evaluadas, utilizando el test de inmersión foliar. Las larvas parasitadas fueron 3,93 veces más suscptibles a clorpirifos, 3,71 veces más a metomyl y 14,11 veces más a cipermetrina que las no parasitadas. El menor efecto del parasitismo sobre la susceptibilidad se encontró con *B. thuringiensis*. Se discute la influencia negativa de las aplicaciones de insecticidas sintéticos sobre la dinámica poblacional del parasitoide, lo mismo que sus implicaciones en estudios de resistencia.

PALABRAS CLAVE: Cogollero del maíz, parasitoide huevo-larva, resistencia a insecticidas, manejo integrado de plagas

ABSTRACT - The egg-larval parasitoid *Chelonus insularis* Cresson is a key parasitoid of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) during the second field generation in the upper Magdalena River Basin, Colombia. In spite of selection pressure, the higher susceptibility of the second generation fall armyworm larvae to insecticides, compared with that of the first generation, suggests that the parasitism may be responsible for the apparent difference in susceptibility. Parasitized and non-parasitized 2nd-instar larvae of the fall armyworm were tested for susceptibility to chlorpyriphos, methomyl, cypermethrin, and *Bacillus thuringiensis* in the laboratory, using the leaf dip test. Parasitized larvae were up to 3.93 times more susceptible to chlorpyriphos, 3.71 times to methomyl, and 14.11 times to cypermethrin than non-parasitized larvae. The least effect of parasitism on susceptibility was found for *B. thuringiensis*. We discuss the negative influence of synthetic insecticide on the parasitoid population dynamics and its impact on insecticide resistance.

KEY WORDS: Fall armyworm, egg-larval parasitoid, insecticide resistance, integrated pest management

One of the most frequently observed parasitoids of the fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith), a pest of corn, sorghum, rice and cotton in the upper Magdalena Valley (Tolima, Colombia), is the egg-larval parasitoid *Chelonus insularis* Cresson. Females of *C. insularis* lay their eggs in host egg masses, emerge as adults usually during the 4th instar, and lead to host death. In the upper Magdalena Valley, higher parasitism is observed during the second FAW generation (Medina *et al.* 1988, Zenner & Borrero 1993).

The susceptibility of *S. frugiperda* larvae to synthetic insecticides, when using second generation egg masses collected in corn fields of El Espinal (Tolima), was different from the susceptibility found in first generation masses (Ingeborg Zenner, data not published). Based on this observation, we hypothesized that susceptibility could be caused by the parasitism of *C. insularis*, which was not detected during the fall armyworm egg stage nor during the first two instars. Fix & Plapp (1983) found that larvae of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) parasitized by *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) were 14.2 times more susceptible to methyl parathion and 2.5 times more susceptible to permethrin than non-parasitized larvae, and concluded that parasitism interferes with host ability to detoxify the insecticides. Subsequently, Fix & Plapp (1987) proved that the difference in enzyme activity was due to the lower aliesterase activity in parasitized than in non-parasitized larvae.

Wasps of the subfamily Cheloninae can induce a precocious metamorphosis in its natural host, that is, they are able to regulate host physiology, disturbing its endocrine system (Jones 1985). Furthermore, parasitized larvae decrease their feeding rhythm and exhibit smaller head capsules than nonparasitized larvae (Ashley 1983). Finally, the instar in which host death occurs is longer (Ashley 1983).

Our study was conducted in the laboratory, to verify the influence of parasitism on the insecticide susceptibility of *S. frugiperda* larvae parasitized by *C. insularis.* The insecticides were chlorpyrifos, methomyl and cypermethrin, which are frequently used to control the pest. Our trials included a commercial formulation of *Bacillus thuringiensis* subsp. *kurstaki* (Dipel 2X, 32.000 I.U./mg, Abbott Laboratories), to account for the future introduction of transgenic cotton and corn cultivars in the study area.

Material and Methods

Egg masses of *S. frugiperda* were obtained from commercial corn fields located in San Luis and El Espinal (Tolima) (4°03'N 75°04'W, 450 m.a.s.l., and 4°24'N 75°13', 300 m.a.s.l., respectively), during the second growing season of the year. The *C. insularis* population was brought from El Espinal. Second generation FAW egg masses were collected and the larvae were reared on corn leaves up to F_1 free of parasitism, in the laboratory (U.D.C.A. - Universidad de Ciencias Aplicadas y Ambientales), at 24 ± 2°C, RH between 65% and 75%, and 12h photoperiod to simulate local field conditions. Simultaneously, the parasitoid was mass reared in transparent plastic cages measuring 15 x 10 x 8 cm. Ten recently laid FAW egg masses were placed on humid absorbent paper at the bottom of the cage and exposed to five pairs of adult parasitoids. Newly hatched FAW larvae were reared on corn leaves until the parasitoids developed and emerged. The same methods were used to obtain parasitized and non-parasitized, 2nd-instar FAW larvae.

With the standard dip test (Gifap 1990) we determined FAW susceptibility to the selected insecticides: methomyl 90WP (0.5, 1.0, and 2.0 ml/l), chlorpyriphos 480EC (0.5, 1.0, 2.0, and 5.0 ml/l), cypermethrin 24.8EC (0.1, 1.0, and 10 ml/l), and *B. thuringiensis*. (250, 500, and 1000 ml/l). Next, the treated corn leaves were dried at room temperature. The tests consisted of feeding parasitized and non-parasitized, 2nd-instar larvae of both collection sites with pieces of corn leaves (25 ± 1 cm long and 5 ± 0.5 cm wide), which were previously dried at room temperature. The corn leaves were dipped into aqueous solutions of commercial formulations of the selected insecticides or into water (control trial).

The estimate percentage of parasitism was based on 50, theoretically parasitized FAW larvae of each collection site, reared until the emergence of adult *C. insularis.* A random block experimental design with five replications was used, considering potential changes in laboratory condition even within short distances. Each replication was conducted in a 15 cm^3 plastic vial with a perforated lid, where the treated piece of corn leaf was provided to the larvae. Each vial held five, 2nd-instar FAW larvae from egg masses, exposed or not to the parasitoid. Larval mortality was assessed after 48h for the synthetic insecticides, and after 96h for *B. thuringiensis*. The results were analyzed using the SAS Probit program. For each case, we estimated the lethal dose (LD_{50}), regression line, slope values, and response-dose with the transformed data (Borrero & Zenner 1998). The lethal doses of parasitized and non-parasitized FAW strains were compared by means of the 95% confidence interval of slopes obtained by the Probit analysis, the corresponding regression statistics, and the ANOVA. We estimated the difference in induced parasitism for both collection sites by analyzing the proportions and by calculating and comparing the Z values.

Results

The estimated FAW larvae parasitism by *C. insularis* differed slightly in the two *S. frugiperda* populations, even though both egg masses were submitted to the same procedure. Parasitism rate on FAW egg masses of the El Espinal population corresponded to 92.5% ($P_2 = 0.92$); for the San Luis population, 86.1% ($P_1 = 0.86$). Our H_0 was that $P_1 = P_2$, whereas H_1 meant that $P_1 \neq P_2$. The calculated Z = -0.9632, and at 95% confidence level, Z = -1.96. Therefore, H_0 was accepted: the proportions of parasitism obtained for both populations were statistically equal.

The effect of parasitism on FAW susceptibility to the insecticides and *B. thuringiensis* are represented in Table 1. For each product, except for *B. thuringiensis*, LD_{50} was statistically different ($P < 0.05$, indicated by the confidence interval) and lower for parasitized larvae, with the same trend in both populations. For the El Espinal FAW population, we found a 2.76-fold increase in susceptibility of parasitized larvae compared to the LD_{50} for chlorpyriphos. The increase for methomyl, cypermethrin, and *B. thuringiensis* was 3.26, 9.75, and 1.18 times, respectively. Similar data were obtained for the San Luis FAW population: a 3.93-fold increase for chlorpyriphos, whereas for methomyl, cypermethrin, and *B. thuringiensis,* the increases were 3.71, 14.11, and 1.21 times greater, respectively. The highest LD_{50} increase in both populations was for cypermethrin, and the lowest for *B. thuringiensis*.

Based on the LD_{50} data and the calculated confidence intervals (Table 1), there is an apparent difference in tolerance to at least chlorpyriphos, in both FAW populations. The El Espinal strain was less susceptible to chlorpyriphos than the strain collected in San Luis. The LD_{50} also suggests differences in susceptibility to methomyl and cypermethrin between the two populations, the El Espinal strain always being more tolerant. The methomyl and cypermethrin confidence intervals overlap and therefore, the differences were not statistically confirmed. No difference in susceptibility to *B. thuringiensis* was found for LD_{50} or CI.

Toxicity of chlorpyriphos, methomyl, cypermethrin, and *B. thuringiensis*, expressed as LD_{50} to parasitized and non-parasitized FAW larvae are also presented in Table 1.

Table 1. Effect of parasitism by *C. insularis* on FAW susceptibility to three synthetic insecticides and *B. thuringiensis* (24 ± 2°C; RH 65-75%).

Insecticide	Parasitism %	Origin of population	Difference	LD_{50}	95% C I		Slope	F	Critic F value	R^2
Chlorpyriphos	92.5	El Espinal		1.31	1.11	1.54	1.85	24.6	0.04	0.91
			2.31							
Chlorpyriphos	0.0	El Espinal		3.62	2.60	5.04	2.78	21.2	0.04	0.92
Chlorpyriphos	86.1	San Luis		0.29	0.21	0.40	2.82	11.8	0.07	0.85
			0.85							
Chlorpyriphos	0.0	San Luis		1.14	1.02	1.28	3.85	10.6	0.08	0.84
Methomyl	92.5	El Espinal		5.06	2.00	12.81	0.93	912	0.02	0.99
			11.46							
Methomyl	0.0	El Espinal		16.52	2.58	105.79	1.17	89.2	0.07	0.99
Methomyl	86.1	San Luis		0.99	0.72	1.38	1.23	1.4	0.44	0.89
			2.69							
Methomyl	0.0	San Luis		3.68	1.73	7.82	1.59	8.7	0.20	0.59
Cypermethrin	92.5	El Espinal		1.65	0.74	3.69	0.80	16.3	0.15	0.99
			14.44							
Cypermethrin	0.0	El Espinal		16.09	2.15	120.09	0.73	1015	0.02	0.94
Cypermethrin	86.1	San Luis		0.18	0.06	0.49	0.58	37.6	0.10	0.67
			2.36							
Cypermethrin	0.0	San Luis		2.54	0.73	8.86	1.05	2.0	0.40	0.97
B. thuringiensis	92.5	El Espinal		1272.0	994.5	1626.9	2.13	11.9	0.17	0.94
B. thuringiensis	0.0	El Espinal		1504.2	1142.1	1981.1	2.31	17.2	0.15	0.92
B. thuringiensis	86.1	San Luis		1227.9	913.62	1650.4	1.74	16.4	0.15	0.96
B. thuringiensis	0.0	San Luis		1486.4	1061.3	2081.7	1.91	26.6	0.12	0.94

LD = Lethal dose (ml/l)
C I = Confidence interval
R^2 = Determination coefficient

Results for all insecticides tested show similar trends for the slope and R^2 values obtained with the regression analysis by Probit transformation. The regression lines for all synthetic insecticides were significantly different ($P < 0.05$), representing the clear influence of parasitism by *C. insularis* on the susceptibility to insecticides. The regression lines obtained for *B. thuringiensis* were not significantly different ($P > 0.05$), meaning that the bacterium efficacy in causing death among *S. frugiperda* larvae was not influenced by the parasitism up to the 96th hour of trial.

When comparing the slopes (Table 1) of regression lines for non-parasitized larvae and all synthetic insecticides, the San Luis FAW population was more susceptible than the El Espinal (Tolima) population. Tolerance to Bt was considered equal for both strains.

The determination coefficients R^2 and the F-test for the hypothesis (H_0) indicate a regression coefficient equal to 0, and H_1 means ≠ (Table 1). This suggests that the data are satisfactorily explained by the calculated regression lines. However, the San Luis non-parasitized FAW population submitted to methomyl (59%) and the same strain, parasitized and submitted to cypermethrin (67%) have determination coefficients equivalent to or higher than 84%. Therefore, at least 84% of the transformed data [Probit and log (100 d)] adjust significantly to the regression line.

Discussion

The slight variation in parasitism by *C. insularis* between the two populations, during the process of egg mass parasitization, did not affect the results. The percentage of parasitism in this study coincides with that achieved by Cruz *et al*. (unpublished) and cited by Silva Jr. *et al.* (2000), under similar laboratory conditions (ca., 86%), showing that the method employed was reliable. The significant difference in susceptibility to chlorpyriphos, methomyl, and cypermethrin between parasitized and control FAW larvae might therefore be attributed not to the difference of parasitism yielded in the laboratory, but to the direct influence of the parasitoid effect on the larvae. Consequently, parasitism can diminish or even inhibit FAW detoxification mechanisms for the three insecticides tested. This, however, still has to be proven after an evaluation of enzyme activities in parasitized and non-parasitized FAW larvae, for each insecticide.

The LD_{50} estimated for the El Espinal FAW population agrees with that registered by Zenner & Borrero (1993): 16.5ml/l for methomyl. The LD_{50} calculated by these authors for chlorpyriphos and for cypermethrin, however, were much lower (2.35 ml/l and higher than 12.0 ml/l, respectively), which means that FAW tolerance increased after eight years of constant selection pressure by the insecticides in the area. In San Luis, a relatively isolated valley area, data on the monitoring of FAW resistance are not available. Corn is normally planted in small plots and almost no pesticides are used to control FAW. Therefore, at first glimpse we would not expect great pressure on resistance selection, as suggested by the results expressed in LD_{50} and compared with the increase observed in the El Espinal population.

The only local data available regarding concentration-mortality (LD_{50}) of FAW larvae was 925.93 ml/l (Borrero & Zenner 1998), obtained from a population collected in a different site in Colombia. The larvae were treated with *B. thuringiesis* subsp. *kurstaki* and recorded under laboratory conditions, after four generations of selection pressure. The greater LD_{50} estimated for the San Luis and the El Espinal populations can be due to the applications of commercial formulations of *B. thuringiensis* to control this insect in corn and other crops, such as cotton, rice and sorghum, and alternate FAW hosts.

The increased mortality rate of parasitized larvae in this study, when compared with the control trials, can only be attributed to the deleterious effect of parasitism. Fix & Plapp (1983) obtained similar results with the tobacco budworm (TBW) *H. virescens* parasitized by *Ca. nigriceps* Viereck. In a subsequent work, Fix & Plapp (1987) found that the difference in TBW susceptibility to the tested insecticides was a result of enzymatic activity of microsomal monooxygenases and glutathione-*S* transferases to detoxify the chemicals in the parasitized larvae.

An increase in host-larva susceptibility can also be partially due to the debilitating effect of parasitism stages on the host larva. Ashley (1983) found that parasitism of *S. frugiperda* by *C. insularis* reduced larval weight gain by 62%, affected frass production, and developed a smaller head capsule width compared to non-parasitized larvae. Similar results were obtained by Powers & Oatman (1994), when studying *Chelonus kellieae* Marsh and *Chelonus phthorimaeae* Gahan, parasites of *Phthorimaea oprculella* (Zeller). It is clear that parasitism debilitates the host larvae and that the weaker, parasitized larvae become more susceptible to synthetic insecticides than non-parasitized larvae. However, the apparent tolerance of parasitized larvae to *Bacillus thuringiensis* does not increase significantly, due to the slow bacterial action.

FAW susceptibility to the tested synthetic insecticides increased when they were parasitized by *C. insularis.* Host larvae mortality automatically causes parasitoid death, thus diminishing its natural population and causing an even higher lack of equilibrium in the local agroecosystem. Insecticide applications should therefore be avoided during the second FAW generation, when parasitism by *C. insularis* can reach 82% during the second planting season of the year (Medina *et al.* 1988), as opposed to the 28% to 73% variation during the first planting season (our field observations). In the upper Magdalena Valley, seasonal differences in parasitism are due to the planting of cotton and consequent insecticide applications during the first growing season. Likewise, monitoring resistance with field-collected populations during the second FAW generation, in areas where the parasitoid was registered, requires that parasitism is simultaneously evaluated to avoid misinterpretation of results.

During this study, no significant effect of parasitism was observed when larvae were treated with *B. thuringiensis* subsp. *kurstaki*. Feeding cessation of the larvae due to *B. thuringiensis* toxin action apparently did not interfere with the normal development of the parasitoid 1st-instar larva. According to Broodryk (1969), larvae of *Chelonus* spp. develop in the host larvae hemocel and feed on the hemolymph. Considering that the trial lasted 96h, the host larva intestine might not have been perforated to permit toxins to enter the hemocel, or the parasitoid 1st-instar larva did not ingest enough bacteria to be affected. Furthermore, the apparent absence of parasitism influence on FAW mortality, when FAW was treated with *B. thuringiensis* could be due to a concentration of the Bt-toxin in the hemolymph, which was not high enough to affect the parasitoid larva.

The negligible variation of FAW susceptibility when treated with *B. thuringiensis* corroborates with field observations made by Fernández & Clavijo (1984). Their results showed no difference in the parasitism rate of FAW by *C. insularis* in *B. thuringiensis*-treated or untreated plots. However, further interpretation of parasitoid survival cannot be done because the final, host-mortality counting was conducted only up to 96h. This period was not enough for FAW larvae to molt to the 4th-instar, which is when death occurs due to parasitism. Therefore, other studies are needed to determine the emergence, survival, and positive parasitism capacity of *C. insularis* adults when FAW larvae are treated with *B. thuringiensis* and when these larvae feed on transgenic cultivars.

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