Title:

Monitoring resistance of *Helicoverpa armigera* to different insecticides used in cotton in Spain.

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Abstract

Helicoverpa armigera is the key pest of cotton in Spain, resulting in many insecticide treatments against it. The resistance status of *Helicoverpa armigera* to different insecticides currently used in cotton was evaluated in Spain in two different seasons, 1999 and 2004. Four populations were tested in total, two in each season. Toxicological bioassays were conducted in the laboratory, and performed on third instar larvae by topical application of the insecticides. LD_{50}'s were estimated by probit analysis and resistance factors (RF) were calculated at the LD_{50} level. Four insecticides were evaluated, but only endosulfan reached a moderate resistance level (RF=11.4), and the others (methomyl, chlorpyrifos and lambda-cyhalothrin) showed low resistance (RF between 1.9 and 6.0). Such results indicate the generally low resistance of *H. armigera* to most of the insecticides used against this pest in cotton in Spain. Possible explanations for this situation are discussed.

Key words: insecticide resistance, cotton, *Helicoverpa armigera*, endosulfan, methomyl, chlorpyrifos, lambda-cyhalothrin.

1. Introduction

Cotton is an important crop in the Guadalquivir river valley (Andalucía, southern Spain), especially in the provinces of Seville, Córdoba and Cádiz. One of its main economic costs comes from insecticide treatments against pests like *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), the key pest of cotton in southern Spain (Durán, 1999). *H. armigera* can severely reduce cotton yields, mainly because it feeds on flower buds, flowers and on the boll. Its control is based mainly on an IPM schedule, which involves estimating populations of the pest and its natural enemies: predadory bugs (Heteroptera) and lacewings (Neuroptera), and recommending insecticides only when *H. armigera* populations surpass a threshold and natural enemies are scarce (Durán, 1999; 2003).

The control of this pest in Spanish cotton may require several applications through the season (the IPM cotton is sprayed with insecticides between 2 and 9 times, range obtained from 23 seasons, Durán (1999; 2003)), mainly of endosulfan and methomyl (2-6 treatments), complemented with other products including pyrethroids (e.g. lambda-cyhalothrin), organophosphates (e.g. chlorpyrifos) or other carbamates (e.g. thiodicarb) (Durán, 2003). The continuous use of these products can lead to the development of resistant populations of *H. armigera*, and in the 2003 season important control failures of *H. armigera* were observed in cotton fields. The failure reports involved mainly the Seville province and the two products that were most used against this caterpillar, endosulfan and methomyl. There are many reports of the resistance of *H. armigera* (and other Heliothinae) to insecticides around the world, especially to pyrethroids, but also to other groups of insecticides, like cyclodienes (such as endosulfan), carbamates (such as methomyl and thiodicarb) and organophosphates (such as clorpyriphos) (McCaffery, 1999; Bues et al., 2005; Ahmad et al., 2007; Pietrantonio et al., 2007; Ugurlu and Gurkan, 2007; Saleem et al., 2008).

Cotton is a good crop in which to study the presence of resistance to insecticides due to the high number of treatments that may be applied (Deguine et al., 2008), but until now no specific study has been done in this crop in Spain, even though, with Greece and Bulgaria, it is one of the few countries in the European Union where cotton is cultivated. On the other hand, our study complements others carried out in Spain to analyze the status of resistance in *H. armigera* in different crops (Torres-Vila et al., 2002a,b). This study covered two seasons: the 1999 season, in which the baseline resistance to the insecticides that are most frequently used against *H. armigera* in cotton was established with a laboratory strain, and compared with a field population, and the 2004 season, in which the evolution of susceptibility to the same insecticides over the period of 5 years, and control failures of *H. armigera* in cotton reported in the previous 2003 season, were analysed.

2. Material and methods

The *H. armigera* populations used in this study were obtained from cotton fields in Seville and Córdoba (Andalucía, southern Spain) from larvae collected in different plots. In 1999, the laboratory strain was created from a number of pupae from a colony that had been maintained in the University of Córdoba by Professor E. Vargas-Osuna since 1996, with periodical reintroductions of individuals from cotton fields, and with no contact with any kind of insecticide. The 1999 field strain was obtained in June by collecting larvae from different cotton fields from the province of Seville. In the 2004 season, two populations were collected, the first in June (prior to application of insecticides against *H. armigera* in that season) from larvae from different cotton fields in the province of Seville in which control failures of *H. armigera* had been observed in the previous 2003 season, and the other in September, from the same or nearby cotton plots.

Larvae were brought to the laboratory and reared following the methodology of Poitout and Bues (1974), at 26±1 ºC and 55±10 %RH with a 16:8(L:D) photoperiod. Adults were reared in vertical cylindrical tubes of 11 cm in diameter and 26 cm in height, constructed from laboratory drying paper and covered with paper. Three to five pairs of adults were placed in each tube for mating and oviposition in the conditions described above, and were fed with a 10% honey solution. Eggs were collected in cotton bundles and placed in containers. After hatching, larvae were fed with a semi-synthetic diet (Poitout and Bues, 1974) and reared in the conditions described above. Third instar larvae were transferred to individual cages of 4 cm in diameter and 2 cm in height, with a portion of diet, for insecticide bioassays.

Laboratory testing of the insecticides was carried out with the first or second generation of larvae reared in the laboratory. Toxicological bioassays were performed on third instar larvae using the standard topical application procedure as recommended by the Entomological Society of America (Anonymous, 1970) and following the guidelines of Robertson and Preisler (1992). Four products were used:

endosulfan (Thiodan-35®, 350 g l^{-1} a.i. from Bayer CropScience), methomyl (Bonsul®, 200 g l^{-1} a.i. from Bayer CropScience), chlorpyrifos (Cuspide-48®, 480 g l^{-1} a.i. from Comercial Química Massó), and lambda-cyhalothrin (Karate King®, 25 g l^{-1} a.i. from Syngenta). For each insecticide, serial dilutions in acetone [or 0.075% humectant (Agridexa, from Bayer CropScience) in water for lambda-cyhalothrin] were prepared so that each was one-half of the previous dose. A drop of solution (2 µl) was applied with a 0.5–10 µl micropipette to the thoracic dorsum of each third instar larvae. Controls for each replicate were treated with acetone (or water with Agridexa) alone. The cages were closed with a lid ventilated by a hole covered with mesh, and left in the same rearing conditions described above. For each strain, at least 240 larvae were treated per insecticide, usually with 4 replicates of 10 larvae at each of 5 or more insecticide concentrations. After 48 hours mortality was assessed: larvae were considered dead if they were desiccated, or unable to move when disturbed with a brush.

Dose-mortality regressions, LD_{50}'s and their fiducial limits were estimated by probit analysis (Finney, 1971) using the POLO Plus 1.0 software (LeOra Software, Berkeley, CA, USA). Resistance factors (RF) were estimated at the LD_{50} level as RF= LD_{50} collected strain/ LD_{50} reference strain (either the 1999 laboratory strain or the strains collected in the field in 1999 or June 2004). The insecticide resistance level was classified according to the criteria reported in Torres-Vila et al. (2002a,b): susceptibility (RF=1), low resistance (RF=2–10), moderate resistance (RF=11–30), high resistance (RF=31–100), and very high resistance (RF=>100).

3. Results and Discussion

The resistance in *Helicoverpa armigera* to different insecticides that are usually applied in Spain on cotton was tested in two seasons (Table 1). In all cases, the *t*-ratio of the slope was significant ($P<0.05$), so that a significant dose-response line was obtained.

Both inter-season and intra-season comparisons can be made with the data. In the inter-season comparison, the results showed that the LD_{50} of all products tested had increased at the end of the 2004 season (in September), when compared against the LD_{50} of the 1999 laboratory strain, with the fiducial limits not overlapping, except in chlorpyrifos. This increment was most obvious with endosulfan, which reached the category of moderate resistance (RF=11.4), whereas the other products were placed in the category of low resistance (RF range between 1.9 and 6.0). Comparison of the data from September 2004 with the field population of 1999 produced RFs that ranged from 1.1 to 3.1, i.e., from susceptibility to low resistance, but only endosulfan had fiducial limits that did not overlap, indicating an increment in its LD_{50} in this period.

The second kind of comparison is intra-season. The only comparison that is meaningful is for the year 2004, when field populations were collected at the beginning and at the end of the season. The September population showed susceptibility/low resistance (RF between 0.6 and 1.7) when compared with the June population, but no increment of the LD_{50} was detected, with the fiducial limits overlapping for all the insecticides.

The results obtained in this work are similar to those obtained in a study by Torres-Vila et al. (2002a,b) during 1995 to 1999, which included the same insecticides tested here, but in different crops (excluding cotton). They found the LD_{50} of endosulfan to be between 0.93 and 6.70 µg/larva, which includes our result. In terms of the RF, the value obtained in this study was higher (RF=11.4, included in the moderate resistance category), but this is probably because it was compared with a more susceptible laboratory strain. If the less susceptible strain of Torres-Vila et al. (2002a) had been used instead, then the RF would be 3 (included in the low resistance category).

The other insecticides tested in this study (i.e., methomyl, chlorpyrifos and lambda-cyhalothrin) followed the same pattern as endosulfan: their LD_{50} is included in the range of values reported in Torres-Vila et al. (2002a,b), and with RF values that are always within the susceptibility to low resistance category.

A clear increment of the LD_{50} has been detected in most of the insecticides tested between 1999 and 2004, compared with the laboratory strain. Only a small change was observed in the LD_{50} in the period from 1999 to 2004 in the field strains (only significative in the case of endosulfan), and no significant increment was detected in the 2004 season. Overall, our results showed little or no difference from the results obtained in the general study carried out from 1995 to 1999 by Torres-Vila et al. (2002a,b) in Spain, even though the study presented here included two seasons, 1999 and 2004, that were very separate in time. Such results indicate a general scenario of low resistance of *H. armigera* to almost all the products tested in Spain, except for some pyrethroids (cypermethrin, lambda-cyhalothrin and deltamethrin), for which Torres-Vila et al. (2002b) found high or very high levels of resistance, although only in 4 out of 111 insecticide–strain combinations tested, involving 2 out of 35 *H. armigera* strains.

The level of resistance in *H. armigera* to endosulfan in our study can be considered as low to moderate. This is similar to the situation in other cotton-growing regions such as those in Turkey (Ugurlu and Gurkan, 2007) and India (Kranthi et al., 2002; Suryawanshi et al., 2008). In terms of other insecticides, like methomyl, several organophosphates, and especially pyrethroids (such as cypermethrin, deltamethrin, lambda-cyhalothrin, and others), there are many studies from different countries reporting high levels of resistance in this species (Kranthi et al., 2002; Pietrantonio et al., 2007; Ugurlu and Gurkan, 2007; Suryawanshi et al., 2008). Studies of insecticide resistance in *Helicoverpa/Heliothis* in Europe have not been limited to Spain, and in France there is also evidence of significant levels of resistance to pyrethroids (deltamethrin), and moderate resistance to methomyl in *H. armigera*, but no resistance has been detected against endosulfan (Bues et al., 2005).

The development of insecticide resistance is primarily the result of the selection pressure exerted on sprayed populations, which increases the frequency of resistant individuals. However, the cotton crop in Spain is sprayed with insecticides against *H. armigera* from 2 to 6 times per season (Durán, 2003), which does not help to explain the low level of resistance observed in this study. Other mechanisms (such as the behaviour and physiology of target individuals) can modulate insecticide resistance, and agroecological factors, like the presence of refugia that harbour susceptible or at least less resistant individuals, may dilute resistant gene frequencies, allowing them to remain at an acceptable level for successful control. Furthermore, there is a fitness cost associated with resistance genes (Sayyed et al., 2008). All these factors (and others) have been considered in an attempt to understand the low level of resistance to insecticides in Spain (Torres-Vila et al., 2002a,b), but the same authors considered the most important factor in the low level of resistance to be the gene flow that happens during the seasonal migrations of *H. armigera*. The capacity for migration of *H. armigera* is well known (Feng et al., 2005; Feng et al., 2009), and it may be of great importance for IPM programmes, given its potential effects on insecticide resistance dynamics. In Australia it is considered a key factor in the variation in resistance frequencies from year to year (Forrester et al., 1993; Scott et al., 2005), and in India, the migratory movements of resistant individuals is considered to be the origin of the high pyrethroid resistance that is prevalent in the subcontinent (Armes et al., 1996), as well as in Central Africa (Brevault et al., 2008). In the case of Spain, Northern Africa is the origin of *H. armigera* immigrants, and these populations may act as refugia that dilute insecticide resistance in northern countries and help to explain the general low level of resistance found (Torres-Vila et al., 2002a,b). Equally, the recently developed resistance in *H. armigera* to some pyrethroids in southern France could be explained by the migration of resistant populations from Spain (Bues et al., 2005).

The study presented here focused on the insecticides used in cotton. The general conclusion is that there was no substantial increase in resistance to the insecticides tested in the two years of the study (1999 and 2004), and only when both seasons were compared was there an indication of an increase in resistance to endosulfan, and a slight increase in resistance to methomyl and lambda-cyhalothrin. For that reason, it is unlikely that the failure to control *H. armigera* that was observed in cotton fields was due to resistance. Regarding endosulfan and methomyl, there is evidence of a significant positive correlation between the LD_{50} values of both insecticides, which could indicate cross-resistance ($r_s=0.383$, $P<0.05$, Torres-Vila et al., 2002a; $r=0.516$; $P\leq0.1$, Ahmad et al., 2007). As methomyl and endosulfan are the most frequently used insecticides against *H. armigera* in Spanish cotton, more attention should be given to evaluating possible control failures in field conditions, complemented by periodical evaluations of the susceptibility of *H. armigera* to both (and other) insecticides.

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References

Ahmad, M., Arif, M.I., Ahmad, M., 2007. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot. 26, 809-817.

Anonymous, 1970. Standard method for the detection of insecticide resistance in *Heliothis zea* (Boddie) and *H. virescens* (F.). Bull. Entomol. Soc. Am. 16, 147-153.

Armes, N.J., Jadhav, D.R., DeSouza, K.R., 1996. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. Bull. Entomol. Res. 86, 499-514.

Brevault, T., Achaleke, J., Sougnabe, S.P., Vaissayre, M., 2008. Tracking pyrethroid resistance in the polyphagous bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), in the shifting landscape of a cotton-growing area. Bull. Entomol. Res. 98, 565-573.

Bues, R., Bouvier, J.C., Boudinhon, L., 2005. Insecticide resistance and mechanisms of resistance to selected strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in the south of France. Crop Prot. 24, 814-820.

Deguine, J., Ferron, P., Russell, D., 2008. Sustainable pest management for cotton production. A review. Agron. Sustain. Dev. 28, 113-137.

Durán, J.M., 1999. La producción integrada y el algodón en Andalucía. *In* La producción integrada y el algodón en Andalucía. 6º Symposium Nacional De Sanidad Vegetal. Junta de Andalucía. Consejería de Agricultura y Pesca, Sevilla (España). pp. 189-206.

Durán, J.M., 2003. El algodón: Nuevas perspectivas en su defensa fitosanitaria. *In* El algodón: Nuevas perspectivas en su defensa fitosanitaria. 8º Symposium

Nacional De Sanidad Vegetal. Junta de Andalucía. Consejería de Agricultura y Pesca, Sevilla (España). pp. 181-197.

Feng, H.Q., Wu, K.M., Ni, Y.X., Cheng, D.F., Guo, Y.Y., 2005. High-altitude windborne transport of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in mid-summer in northern China. J. Insect Behav. 18, 335-349.

Feng, H., Wu, X., Wu, B., Wu, K., 2009. Seasonal migration of *Helicoverpa armigera* (Lepidoptera: Noctuidae) over the Bohai Sea. J. Econ. Entomol. 102, 95-104.

Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London.

Forrester, N.W., Cahill, M., Bird, L.J., Layland, J.K., 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera, Noctuidae) in Australia. Bull. Entomol. Res. (Supp) 1, 1-132.

Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S., Russell, D.A., 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot. 21, 449-460.

McCaffery, A.R., 1999. Resistance to insecticides in heliothine lepidoptera: A global view. *In* I. Denholm, J.A. Pickett, A.L. Devonshire (eds.) Insecticide Resistance: From Mechanisms to Management. CAB International and The Royal Society, Wallingford (United Kingdom). pp. 59-74.

Pietrantonio, P.V., Junek, T.A., Parker, R., Mott, D., Siders, K., Troxclair, N., Vargas-Camplis, J., Westbrook, J.K., Vassiliou, V.A., 2007. Detection and evolution of resistance to the pyrethroid cypermethrin in *Helicoverpa zea* (Lepidoptera: Noctuidae) populations in Texas. Environ. Entomol. 36, 1174-1188.

Poitout, S., Bues, R., 1974. Élevage des chenilles de vingthuit especes de lépidoptères Noctuidae et de deux especes d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les especes. Ann. Zool. Écol. Anim. 6, 431-441.

Robertson, J.L., Preisler, H.K., 1992. Pesticide Bioassays with Arthropods. CRC Press, Boca Raton, Florida.

Saleem, M.A., Ahmad, M., Ahmad, M., Aslam, M., Sayyed, A.H., 2008. Resistance to selected organochlorine, organophosphate, carbamate and pyrethroid insecticides, in *Spodoptera litura* (Lepidoptera : Noctuidae) from Pakistan. J. Econ. Entomol. 101, 1667-1675.

Sayyed, A.H., Ahmad, M., Crickmore, N., 2008. Fitness costs limit the development of resistance to indoxacarb and deltamethrin in *Heliothis virescens* (Lepidoptera: Noctuidae). J. Econ. Entomol. 101, 1927-1933.

Scott, K.D., Lawrence, N., Lange, C.L., Scott, L.J., Wilkinson, K.S., Merritt, M.A., Miles, M., Murray, D., Graham, G.C., 2005. Assessing moth migration and population structuring in *Helicoverpa armigera* (Lepidoptera: Noctuidae) at the regional scale: example from the Darling Downs, Australia. J. Econ. Entomol. 98, 2210-2219.

Suryawanshi, D.S., Bhede, B.V., Bhosale, S.V., More, D.G., 2008. Insecticide resistance in field population of American bollworm, *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae). Indian J. Entomol. 70, 44-46.

Torres-Vila, L.M., Rodriguez-Molina, M.C., Lacasa-Plasencia, A., Bielza-Lino, P., 2002a. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. Crop Prot. 21, 1003-1013.

Torres-Vila, L.M., Rodriguez-Molina, M.C., Lacasa-Plasencia, A., Bielza-Lino, P., Rodriguez-del-Rincon, A., 2002b. Pyrethroid resistance of *Helicoverpa armigera* in Spain: current status and agroecological perspective. Agric. Ecosyst. Environ. 93, 55-66.

Ugurlu, S., Gurkan, M.O., 2007. Insecticide resistance in *Helicoverpa armigera* from cotton-growing areas in Turkey. Phytoparasitica 35, 376-379.

Table 1. Toxicity of topically applied insecticides, currently used in cotton in Spain, to different strains of *Helicoverpa armigera* larvae (F1 and F2, third instar), in two years.

Insecticide	Year–Strain	Intercept	Slope±S.E.	LD_{50} (µg/larva)	95% fiducial limits	RF[a]
Endosulfan						
	1999–Laboratory	6.31	2.19±0.34	0.25	0.18–0.32	--
	1999–Field	5.05	1.81±0.26	0.93	0.72–1.28	3.7
	2004–June	4.54	2.11±0.27	1.64	1.30–2.05	6.6
	2004–September	4.10	1.97±0.33	2.86	1.44–5.03	11.4
Methomyl						
	1999–Laboratory	6.57	1.43±0.28	0.08	0.05–0.12	--
	1999–Field	6.14	1.92±0.27	0.26	0.19–0.33	3.3
	2004–June	5.46	1.01±0.16	0.35	0.22–0.50	4.4
	2004–September	5.47	1.48±0.22	0.48	0.19–0.90	6.0
Chlorpyrifos						
	1999–Laboratory	7.83	3.06±0.40	0.12	0.09–0.14	--
	1999–Field	7.16	3.21±0.46	0.21	0.17–0.25	1.8
	2004–June	5.88	2.21±0.28	0.40	0.32–0.50	3.3
	2004–September	6.41	2.22±0.20	0.23	0.11–0.41	1.9
Lambda-Cyhalothrin						
	1999–Laboratory	7.88	1.63±0.28	0.02	0.00–0.03	--
	1999–Field	7.12	1.40±0.25	0.03	0.02–0.04	1.5
	2004–June	6.87	1.64±0.19	0.07	0.06–0.09	3.5
	2004–September	7.08	1.85±0.27	0.08	0.05–0.12	4.0

In all strain–insecticide combinations the probit dose-response lines were significant (*t*-ratio test, $P<0.05$).

[a] Resistance factor (RF) estimated as RF= LD_{50} specific strain/LD_{50} 1999 laboratory strain.