The impact of spray application methodology on the development of resistance to cypermethrin and spinosad by fall armyworm *Spodoptera frugiperda* (J. E. Smith).

Ali Al-Sarar[1] and Franklin R. Hall[2]*

[1]/ King Saud University, Faculty of Food & Agricultural Sciences College,Department of Plant Protection, P.O. Box 2460, Riyadh 11451. Saudi Arabia, E-mail: Asarar@ksu.edu.sa

[2]/ The Ohio State University, Laboratory for Pest Control Application Technology, OARDC, Department of Entomology, Wooster, OH 44691, USA, E-mail: Hall.1@osu.edu

ABSTRACT:

The development of resistance to an insecticide under various type of application methods has yet to be reported in the literature. Colonies Five fall armyworm reared in the chamber for 10 generations before starting topical application bioassay. From each FAW colony, 200-500 3rd –4th –instar larvae were fed for 72 hours on corn plant sprayed with cypermethrin or spinosad at minimum application rate (20 g ha^{-1}) using the small droplet size nozzle XR8001vs (VMD 163µm) the large droplet nozzle XR8008vs (MD = 519.1µm). surviving larvae were transferred to untreated corn leaves to complete their life cycle. Next generation 3rd instar larvae of each colony were topically dosed with technical cypermethrin or spinosad at 1µl/larvae and mortality recorded 24 hours post treatment. The results indicated that cypermethrin demonstrated an insecticidal activity greater than spinosad and the cypermethrin LD_{50} lines moved to the right faster than spinosad indicating an increased tolerance of cypermethrin. Generally, larvae from all generations (F1-F7) under the XR8008vs treatments were less susceptible to cypermethrin and developed resistance faster and to higher levels than larvae from the XR8001vs treatments. The confidence limits (95%) of LD50 for all spinosad treatments indicated there was no significant difference from the LD_{50} value of the susceptible reference strain. The results are a first indicating that application technology/ insecticide reaction may affect the rapidity of resistance development in certain pest/ plant scenarios but field studies are needed to confirm this conclusion

1 / INTRODUCTION:

Many insecticides have been used to control fall armyworm (FAW) including pyrethroid insecticides. Resistance of fall armyworm was first reported in a strain of FAW collected from corn in Georgia was resistant to carbaryl[1]. Later field strains of FAW collected from corn in central and south Florida showed resistance to commonly used insecticides as compared with a laboratory susceptible strain[2].

It is well known that the impact of spray application methods in the pesticide dose-transfer processes is dynamic and multifaceted with numerous opportunities for loss of active ingredient. There are major gaps in our knowledge of the interaction between pesticides and their targets at the level of the canopy and foliage surface. Different application equipment can provide a wide variation in spray distribution and deposition over the plant canopy. Consequently, the impact of various application techniques on insect populations will be quite variable between various deposition patterns (i.e. small droplet deposits vs. large droplet deposits). Furthermore, when infested plants are sprayed with an insecticide, only a very small proportion of the spray solution is deposited where the insect will encounter it. In reality, the efficiency with which pesticides are utilized in crop protection is extremely poor[3]. The impact of different spray application methods and resulting deposits contributing to selection for insect resistance has not been investigated

Pesticide deposit variability could be important in pesticide resistance development[4]. However, it remains unclear whether such deposit variability can impose a significant pressure on the resistance selection process. In conclusion, failure to achieve appropriate pesticide dosage results in poor pest and disease control, and may contribute to the development of pesticide resistance. On the other hand, improvement in the accuracy and efficiency of spray applications, which result in more uniform and/or better targeting spray deposits, should help to ensure effective control while reducing selection for resistance. In contrast, Ebert[5] observed that (1) uniform deposit structures provide greater opportunity for insects to acquire a sub-lethal dose; (2) encounter probabilities and mortality are a function of insect behavior and deposit structure; and (3) uniform deposit structures could give individual insects a chance to develop physiological resistance. In other words, a variable deposit structure is likely to reduce resistance development[5]. We therefore hypothesize that selection pressure for resistance will vary with different insecticides, insect behavior, as well as the insecticide deposition structure. In this study, we will focus on development of resistance to cypermethrin and spinosad on fall armyworm (FAW) *S. frugiperda* larvae using contrasting droplet size deposit patterns produced by different nozzles.

OBJECTIVE:

Explore the impact of using various deposition patterns (small droplet deposits vs. large droplet deposits) on the development of resistance to cypermethrin and spinosad on fall armyworm (FAW) *S. frugiperda* larva

Table 1. Five fall armyworm colonies fed on corn plant leaves, which were treated with cypermethrin and Spinosad using XR8001vs and XR8008vs, under different application conditions

Colony	Insecticide	Nozzle	Pressure (kPa)	Volume (L/ha)	Speed (Kph)
1	Cypermethrin	XR 8001vs	276	187	2.38
2	Spinosad	XR 8001vs	276	187	2.38
3	Cypermethrin	XR 8008vs	138	187	13.62
4	Spinosad	XR 8008vs	138	187	13.62
5	Control (untreated)				

Table 2. Toxicity of topically applied cypermethrin on seven generation of Fall armyworm populations fed on corn plant sprayed with cypermethrin using two different flat nozzle XR8008 vs and XR8001vs.

Nozzle	Generation	LD_{50} µg/Larva	95% F.L. a	Slope ± S.E	X^2	n	d.f	RR b
Control	F0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1
XR8001vs	F1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001vs	F2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001vs	F3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001vs	F4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001vs	F5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001vs	F6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001vs	F7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

a F.L. Fiducial limits. b RR: resistance ratios based upon comparisons to susceptible reference strain

Table 3. Toxicity of topically applied spinosad on seven generation of Fall armyworm populations fed on corn plant sprayed with spinosad using two different flat nozzle XR8008 vs and XR8001vs

Nozzle	Generation	LD_{50} µg/Larva	95% F.L. a	Slope ± S.E	X^2	n	d.f	RR b
Control	F0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1
XR8001	F1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001	F2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001	F3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001	F4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001	F5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8001	F6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
XR8008vs	F6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

a F.L. Fiducial limits. b RR: resistance ratios based upon comparisons to susceptible reference strain

2 MATERIALS AND METHODS

2.1 / Insects

Fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) larvae and an artificial fall armyworm diet were shipped by the Agripest Company. The larvae were reared on artificial diet in 30 ml plastic cups in a growth room held at 27 °C and 70% RH under 14/10 hour light: dark photoperiod for 10 generations without exposure to insecticides, before starting the experiment. Resulting pupae were placed into aluminum cages, and resulting eggs were used to establish the parent generation for five different colonies placed on five different insecticide application scenarios (Table 1).

2.2 / Plants

Corn plants were placed in a greenhouse in 10 cm pots under ambient lighting condition, and temperatures, which ranged from 25-35 °C (day) and 18-20 °C (night). Plants were sprayed approximately 30-40 days after planting when they became 130-150 cm in height.

2.3 / Chemicals

cypermethrin 2.5 EC (Ammo) [30.6 % cypermethrin] provided by FMC (Corporation, Chemical Research and Development center, Princeton, NJ) was sprayed at the minimum recommended application rate (20 gm ha^{-1}), and spinosad 22.8% SC provided by Dow AgroSciences™ (DowAgroSciences LLC, Indianapolis, In) was sprayed at 187 l ha^{-1}. Both pesticides were delivered via two nozzles at 187 l ha^{-1}. Technical grade (95%) cypermethrin was provided by Chem. Service (660 Tower Lane, P.O.Box, 599, West Chester, Pa) and technical grade (88%) spinosad (76.1 spinosyn A and 11.5 spinosyn D), was kindly provided by Dr. Gary Thompson, Dow AgroSciences. The compounds were dissolved in analytical grade acetone and used for the topical bioassays.

2.4 / Insecticide application

Two flat fan nozzles (XR8001vs and XR8008vs) were used to produce a small droplet size pattern (VMD 163µm) and a large droplet size pattern (VMD 519.1µm). The plants (three plants) were sprayed at the Laboratory of Pest Control Application Technology (LPCAT) track room at Ohio Agricultural Development Center (OARDC) at Ohio State University. The sprayed corn plants were placed on the bench for two hours until they dried before placing feeding larvae on them at approximately 100 3rd instar larvae / plant.

2.5 / Bioassays

Five fall armyworm colonies (Table 1) were reared in the chamber room for 10 generations before starting topical application bioassays. In each FAW colony, 200-500 3rd-4th-instar larvae were fed for 72 hours on corn plants sprayed with cypermethrin 2.5 EC or spinosad 22.8% SC at the minimum dosage rate (20 g ha^{-1}) using the small (XR8001vs) or large (XR8008vs) droplet nozzle. Surviving larvae were transferred to untreated corn leaves to complete their life cycle.

Following generation of each colony was divided into two groups and the first group fed on treated leaf corn for 72 hours and the surviving larvae were transferred to untreated leaf corn. The second group of 3 rd instar larvae was randomly selected for topical application on the dorsal thorax with 1.0 µl using a micro-applicator (ISCO, Inc., Lincoln, NE). Nine serial concentrations of each insecticide Technical grade were used with concentration increased for every generation. Three replicates per concentration (three per petri dish) and 10 larvae per replicate were treated and mortality recorded 24 hours post treatment. Seven successive generations were treated (F1-F7) for cypermethrin and up to six generations (F6) for spinosad. Initially, tests were performed with a wide range of concentrations in order to determine the range needed to obtain 0 to100% mortality of FAW larvae. After the proper range was obtained, 9 serial concentrations of technical cypermethrin and spinosad were prepared within this range plus a control (treated with acetone), using three replicates of 10 larvae per replicate. After treatment, the larvae were held individually in 30 ml plastic cups, containing artificial diet in the growth chamber room for 24 hours after which mortality was recorded.

The LD50 was computed and plotted using the computer program Lpd line (http://www.ehabsoft.com) probit analysis according to[5], and expressed as micrograms a.i. per larva. Two populations were considered significantly different in their susceptibility to insecticide if fiducial limits (95%) of the LD_{50} of an insecticide did not overlap.

3 / RESULTS

The results of the standard topical application assays for cypermethrin and spinosad are summarized in Table 2, and Figure 2-3 and Table 3, Figure 4-5 respectively. The results on the susceptible reference strain indicated that spinosad has an insecticidal activity that is less than cypermethrin. The LD_{50} estimates of susceptibility to cypermethrin and spinosad of parental generation (F0) as the susceptible reference strain was 0.0055 µg/larva and 0.053 µg/larva, respectively. In addition, the slopes with cypermethrin populations were steeper than in spinosad populations.

As shown in table 2 treated colonies were less susceptible to cypermethrin than was the reference strain (F0). Moreover, resistance ratios (RR) increased as the generations increased. The LD_{50} values for XR8001vs treatment were not significantly different for the F1 and F2 generations in comparison to the 0.0055 µg/larva by the susceptible reference strain (F0). However, it was significantly different for all generations (F1-F7) in XR8008 treatments than the reference strain (F0) (Table 2). In comparison with the susceptible strain, the LD50 values for the XR8001vs treatment and the XR8008vs treatment with cypermethrin were 6.18-fold and 10.18-fold higher than the reference strain respectively (Table 2). Generally, larvae from all generations (F1-F7) under the XR8008vs treatments were less susceptible to cypermethrin and developed resistance faster and higher levels than larvae in XR8001vs treatments (see Figure 2 and 3).

The toxicity of spinosad was less variable for all treatments, and fell within a range of an LD50 of 0.048 to 0.077µg/larva (Table 3). Confidence limits (95%) of LD50 for all treatments indicated there was no significant difference from the value of 0.053 µg/larva of the susceptible reference strain (F0). The relatively shallow slope of 1.47 to 2.03 in the spinosad treatments and less variability in slope among treatments and susceptible strain suggest that the tested population was relatively homogenous population11. Moreover, as shown in the spinosad dosage-mortality curves (Figure 4and 5), there was slight difference between XR8001vs treatments and the XR8008vs treatments. However, under the XR8008vs treatments, the lines started to move faster to the right at F4, but kept the same slope with only slight changes.