MORTALITY AND GUSTATORY RESPONSE OF BOLL WEEVIL FED SPINOSAD AND ABAMECTIN

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Abstract

Commercial formulations of spinosad (Tracer® 4 SC) and abamectin (Agri-Mek® 0.15 EC) mixed with 10% sucrose on a ppm ai weight:volume basis were evaluated as toxicants when fed to boll weevils (BW), *Anthonomus grandis grandis* Boheman, captured in pheromone traps during spring and fall, 2000. For both spinosad and abamectin, there was no significant difference in lethal concentration (LC) values between spring- and fall-captured BW of mixed sexes. The pooled 24 h LC_{90}s for spinosad and abamectin were 28.0 ppm (95% CLs [confidence limits] of 23.31 to 35.62) and 6.5 ppm (95% CLs of 5.37 to 8.44), respectively. When compared with 10% sucrose alone as control and relative to LC_{90} insecticide concentrations, abamectin significantly inhibited gustatory response of male and female BWs more than spinosad, but there were some differences in response to the same insecticide concentration due to sex. Females ingested significantly more spinosad than males at 28 and 140 ppm. Spinosad significantly reduced gustatory response for both males and females at concentrations higher than 10X $LC_{90.}$ Males ingested significantly more abamectin than females at 65 ppm (10X LC_{90}), while females ingested significantly more abamectin than males at 650 ppm (100X LC_{90}). Abamectin has limited potential for use as a toxicant because although it is more toxic than spinosad, it inhibits gustatory reponse at the LC_{90} concentration and higher. These data indicate that spinosad does have potential for use as a toxicant mixed with a feeding stimulant and with pheromone as an attractant in an adult control system because it is toxic at relatively low concentrations and does not inhibit gustatory response even at high concentrations.

Introduction

Successful long-term maintenance of eradication of boll weevil (BW), *Anthonomus grandis grandis* Boheman, in the Cotton Belt of the United States largely depends upon prevention and suppression of resurgent weevil populations in post-eradication zones. The development of control technologies designed to prevent re-entry and subsequent reproduction is essential for sustaining eradication. Adult control technology through the use of pheromone as an attractant and a feeding stimulant mixed with toxicants may be a viable suppression technique in post-eradication zones. Already success in adult control has been achieved for corn rootworm, *Diabrotica* spp., through use of a bait formulation (SLAM, Microflow, Lakeland, FL) which consists of a semiochemical, cucurbitacin contained in buffalo gourd root powder, mixed with carbaryl as a toxicant (Comis 1997). Furthermore, feeding-based adult control technology for corn earworm, *Helicoverpa zea* (Boddie) has been developed, but additional research will be needed for field implementation (Joyce and Lingren 1998; Lopez et al. 1999; Younger 2000). There is a need to identify selective chemicals that are effective toxicants in the development of feeding-based adult control technology for BW. Spinosad and abamectin are commonly used insecticides in the cotton ecosystem and have the potential to serve as toxicants in a feeding stimulant formula. Spinosad is derived from a naturally-occurring soil actinomycetes bacterium, *Saccharopolyspora spinosa* (Thompson et al. 1997). Similarly, abamectin is produced from disaccharide derivatives obtained from the naturally-occurring soil microorganism, *Streptomyces avermitilis* (Strong and Brown 1987; Lasota and Dybas 1991). These compounds are selective and are considered to be safe for the environment.

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We report here the results of a laboratory study to determine the lethal concentration (LC) of spinosad and abamectin mixed with a feeding stimulant when ingested by pheromone trap-captured BWs. Subsequent to LC determination, females and males were evaluated for gustatory response to toxic concentrations. Our objective was to ascertain whether or not spinosad or abamectin can be used in formulations for adult control technology using pheromone and a feeding stimulant for suppression of the BW in eradication zones or as a barrier between eradicated and non-eradicated zones.

Materials and Methods

Insecticides

Tracer® 4 SC, the commercial formulation of spinosad was supplied by Dow AgroSciences, Indianapolis, IN, USA. Agri-Mek®, 0.15 EC, the commercial formulation of abamectin, was supplied by Novartis Crop Protection, Inc., Greensboro, NC. We prepared test solutions of spinosad at 4, 8, 10, 12, 14, 16, 20, 25, 30 and 35 ppm (ai wt:vol) in 10% sucrose (Sigma, St. Louis, MO). Similarly, we also prepared test solutions of abamectin at 0.5, 1.5, 3, 4, 6 and 8 ppm. These concentrations were determined based on preliminary evaluations of a wide range of concentrations. The 10% sucrose solution alone served as the control. Test solutions were stored in a refrigerator and warmed to room temperature before each use.

Test Insects

Boll weevil pheromone traps baited with 10 mg lure (Hercon®, ai: grandlure 1.2%, Hercon Environmental Corp., Emigsville, PA) were placed adjacent to cotton fields in the Brazos Valley near College Station, TX during the spring and fall, 2000. Traps were emptied daily except during weekends and adult weevils were placed in Ziplock® plastic bags and held in an environmental chamber maintained at 55° F. Pheromone trap-captured BWs of both sexes were randomly removed from the bags for use in LC evaluations. Boll weevils were sexed for evaluating gustatory response to toxic concentrations of spinosad and abamectin. Boll weevils were within 7 and 3 days of being captured, respectively, when determining LC values and gustatory response to insecticides.

Determination of LC

A clear square plastic box (2½ X 2½ X 2¼ in.) with hinged lid was used as a feeding apparatus to determine LC values (Figure 1). Five notches were made on each of two opposite sides of the bottom portions of the box and the notches were reinforced with a thin strip of rubber padding with a small cut corresponding to each notch on the sides of the walls. Using the thumb and forefinger, an individual BW was picked up from a sample of pheromone trap-captured BWs and the snout was inserted into the tip of a 20 µl capillary tube broken in half and containing the test solution. Ten capillary tubes with BWs feeding at the orifice were placed in position through the notches and cuts in the rubber at 25 to 45° angles, and thereafter, the lid was closed and taped. Upon completion of feeding, BWs fell to the bottom of the box and all ten adults exposed to each treatment in a replicate were removed and placed inside a petri-dish containing four greenhouse-grown cotton squares. After 24 hours, BWs in each treatment in each petri-dish were examined for mortality by pinching the snout of each BW with a forcep. Weevils were scored dead if there was no movement of appendages after pinching the snout.

Determination of Gustatory Response

Solutions of spinosad at 1X, 2X, 5X, 10X, 100X, 1000X LC_{90} value with an upper limit of 10,000 ppm were prepared in 10% sucrose. Similarly, solutions of abamectin at 1X, 2X, 5X, 10X and 100X LC_{90} value were prepared. The upper limit for abamectin was set at 100X LC_{90} based on preliminary gustatory response studies.

The feeding apparatus used to determine gustatory response of the BW was similar to that described earlier (Lopez et al. 2000). Briefly, the feeding apparatus contained six 10 µl Hamilton® micro-liter syringes from which the needles had been cut and the tips shaped into a cone. Syringes were inserted into a Plexiglass® plate with holes of sufficient size to hold the syringes. The plate was mounted on a wooden frame to hold the syringes at 25 to 45º angles (Figure 2).

Female and male BWs were individually fed by inserting the tip of the snout into the orifice of the syringe. Before feeding was initiated the fluid level on each syringe was recorded. Boll weevils that stopped feeding and started wandering were considered to be satiated and were removed and killed. After feeding was completed, the fluid level on the syringe was again determined and the difference between the two readings was considered the amount ingested in µl.

Data Analysis

Lethal concentration data were analyzed by probit analysis (Finney 1971) as adapted for PC use (LeOra Software 1987). The goodness-of-fit of the curve was tested using the χ^2 statistic. Significant difference between any two LCs was determined by the criterion of whether or not the 95% CIs of the LCs overlapped. Slope values of probit mortality curves were tested for significant deviation from 0 using the *t ratio* statistic (Robertson and Preisler 1992). Analysis of variance of gustatory response data was conducted using SAS (1998). When F-values were significant at the 5% level, means were separated using the Least Significant Difference (LSD) test at $\alpha = 0.05$. Paired comparison of gustatory response to toxicants between male and female BW was conducted using the *t* test at $\alpha = 0.05$.

Results and Discussion

Lethal Concentration

Control mortalities in LC tests for spinosad and abamectin did not exceed 0.5%. The goodness-of-fit test for dosage response equations for both spinosad and abamectin indicated that the assumptions of the probit model were adequately described for both spring- and fall-captured BWs with χ^2 values significant at the 5% level (Tables 1 and 2). Furthermore, the regression coefficients for spinosad and abamectin were significant as the *t ratio* exceeded $t = 1.96$ ($\alpha = 0.05$; df = ∞). The LC values for the BWs captured in the spring and fall were not significantly different for either spinosad or abamectin; therefore, pooled seasonal LC values for the spring- and fall-captured BWs are presented in Tables 1 and 2. These data indicate that when ingested mixtures of both spinosad and abamectin in 10% sucrose are highly toxic to adult BWs. Wright (1984) reported that laboratory-reared BW adults exposed to abamectin treated- cotton squares had an LC_{50} of 0.0001% (1 ppm). Although we exposed BW to abamectin through direct ingestion, our seasonal LC_{50} of 2.33 ppm is comparable to the data obtained by Wright (1984).

Boll weevils ingesting spinosad lay on their sides without movement. However, BWs ingesting abamectin showed a variety of postures. Some BWs lay on their back with wings and elytra unfolded, making circular movements; some lay on their sides with wings protruding out and when helped with a forcep to roll over, they walked a few steps, stopped again and sat motionless. Wright (1984) reported that a swollen abdomen extending beyond the elytra was symptomatic of abamectin activity. He also reported that treatment of BWs with abamectin by topical application, immersion and ingestion through treated cotton squares decreased feeding and frass production. In this study, we found that BWs ceased feeding after ingesting abamectin at concentrations above 0.75 ppm and did not produce frass.

Gustatory Response

When compared with 10% sucrose solution alone as control, the amount of spinosad and abamectin ingested by male and female BWs was significantly different between concentrations (males [spinosad]: $F = 3.21$; $P < 0.01$; df = 6, 63; females [spinosad]: $F = 3.22$; $P < 0.05$; df = 6, 63; males [abamectin]: $F = 15.67$; $P < 0.001$; df = 5, 54; females [abamectin]: $F = 10.45$; $P < 0.001$; df = 5, 54).

There was no significant inhibition of gustatory response to spinosad by both male and female BWs up to 280 ppm (10X LC_{90}); however, spinosad inhibited feeding by males at 28 ppm (1X LC_{90}) (Table 3). Also, gustatory response for both male and female BWs was significantly reduced at 2800 and 10,000 ppm. Females ingested significantly more spinosad than males at 28 ppm and 140 ppm (5X LC_{90}), but the latter difference was significant only at the 10% level ($t = 1.78$; $P < 0.1$; df = 1, 18).

Abamectin significantly decreased male gustatory response at 6.5 ppm (1X LC_{90}) and higher, but reduced gustatory response significantly for female only at 32.5 (5X LC_{90}) and higher. Males ingested significantly more abamectin than females at 65 ppm (10X LC_{90}), while females ingested significantly more abamectin than males at 650 ppm (100X LC_{90}), but the latter difference was significant only at the 10% level ($t = 1.90$; $P < 0.1$; df = 1, 18).

Conclusion

Data presented here show that both spinosad and abamectin when mixed with 10% sucrose solution and ingested are highly toxic to BW adults. The inhibition of gustatory response of BWs to abamectin at low concentrations suggests that there is limited potential for its use as a toxicant mixed with a feeding stimulant and pheromone as an attractant in adult control technology. Gustatory response of BW to toxic concentrations of spinosad was less inhibitory and therefore, spinosad does have potential for use as a toxicant in adult control technology.

Disclaimer

Mention of a commercial or proprietary product does not constitute an endorsement for its use by the U. S. Department of Agriculture.

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References

Comis, D. 1997. Corn belt growers give areawide IPM a try. Agri. Res. 10: 4-7.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge.

Joyce, R. J. V. and P. D. Lingren. 1998. Potential for developing technology to control adult noctuids with chemical attractants: background and world perspective, pp. 9-24. *In* D. L. Bull [ed.], Potential for development of technology to control adult noctuid pests with plant attractants. Southwest Entomol. Suppl. 21. 65 pp.

Lasota, J. A. and R. A. Dybas. 1991. Avermectins, a novel class of compounds: Implications for use in arthropod pest control. Ann. Rev. Entomol. 36: 91-117.

LeOra Software. 1987. POLO-PC: A user's guide to probit or logit analysis. Berkeley, CA.

Lopez, J. D., Jr., M. A. Latheef and R. W. Meola. 1999. Effect of selected insect growth regulators on feeding response and reproduction of adult bollworm. Proc. Beltwide Cotton Prod. Res. Conf. 2: 1214-1221.

Lopez, J. D, Jr., M. A. Latheef and S. Li. 2000. Effect of hexaflumuron on feeding response and reproduction of adult boll weevil. Proc. Beltwide Cotton Conf. 2: 1321-1324.

SAS Inst. 1998. Statistical Analysis System for Windows, version 7, Cary, NC.

Strong, L. and T. A. Brown. 1987. Avermectins in insect control and biology: a review. Bull. Entomol. Res. 77: 357-389.

Robertson, J. L. and H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC Press. 127 pp.

Thompson, G. D., K. H. Michel, R. C. Yao., J. S. Mynderse, C. T. Mosburg, T. V. Worden, E. H. Chio, T. C. Sparks and S. H. Hutchins. 1997. The discovery of *Saccharopolyspora spinosa* and a new class of insect control products. Down To Earth 52: 1-5.

Wright, J. E. 1984. Biological activity of avermectin B_1 against the boll weevil (Coleoptera: Curculionidae). J. Econ. Entomol. 77: 1029-1032.

Younger, C. D. 2000. Mortality of adult *Helicoverpa zea* (Lepidoptera: Noctuidae) in corn and cotton treated with a feeding-based attracticide. M. S. Thesis, 74 p. Texas A&M University, College Station, TX.

Table 1. Lethal concentration (ppm ai wt:vol) data for 24-hour response for the toxicity of spinosad when mixed with 10% sucrose and ingested by mixed sexes of pheromone trap-captured boll weevils during spring and fall, 2000[1].

Regression Statistics	Spring[2]	Fall[3]	Seasonal[4]
Slope (±SE)	3.39±0.2985	2.88±0.2652	3.20±0.2132
t ratio	11.36	10.86	15.02
χ^2 (df)	7.69 (6)	6.24 (4)	11.00 (7)
LC_{10} (ppm)	4.72a	3.88a	4.40
(95% CIs)	(3.41-5.81)	(2.19-5.33)	(3.39-5.29)
LC_{50}	11.26a	10.82a	11.06
(95% CIs)	(10.0-12.59)	(8.79-13.00)	(9.93-12.29)
LC_{90}	26.90a	30.12a	27.79
(95% CIs)	(22.29-35.94)	(22.89-48.85)	(23.31-35.62)

[1]LC values were calculated using POLO-PC (LeOra Software 1987). LC values in the same row followed by same lower case letters are not significantly different based on the lack of overlap in 95% CI limits.
[2]Based on 693 weevils.
[3]Based on 548 weevils.
[4]Based on 1241 weevils.

Table 2. Lethal concentration (ppm ai wt:vol) data for 24-hour response for the toxicity of abamectin when mixed with 10% sucrose and ingested by mixed sexes of pheromone trap-captured boll weevils during spring and fall, 2000[1].

Regression Statistics	Spring[1]	Fall[2]	Seasonal[3]
Slope (±SE)	3.85±0.4860	3.56±0.4024	2.89±0.2772
t ratio	7.92	8.86	10.42
χ^2 (df)	2.29(3)	4.92(4)	1.55 (4)
LC_{10} (ppm)	0.63a	1.37a	0.84
(95% CIs)	(0.44-0.80)	(0.89-1.73)	(0.64-1.01)
LC_{50}	1.36a	3.13a	2.33
(95% CIs)	(1.14-1.56)	(2.63-3.84)	(2.10-2.60)
LC_{90}	5.47a	7.15a	6.48
(95% CIs)	(4.17-8.37)	(5.36-12.41)	(5.37-8.44)

[1]LC values were calculated using POLO-PC (LeOra Software 1987). LC values in the same row followed by same lower case letters are not significantly different based on the lack of overlap in 95% CI limits.
[1]Based on 227 weevils.
[2]Based on 381 weevils.
[3]Based on 608 weevils .

Table 3. Gustatotry response for spinosad when mixed with 10% sucrose and ingested by male and female boll weevils captured in pheromone traps during spring and fall, 2000[1].

	Mean[2] amount ingested (µl) ± SE	
Concentration (ppm)	Male	Female
0	4.05 ± 0.35aA	3.47 ± 0.45abA
28	2.28 ± 0.32cB	3.28 ± 0.20abcA
56	3.36 ± 0.21abA	3.85 ± 0.43abA
140	3.34 ± 0.23abB*	4.13 ± 0.38aA
280	3.29 ± 0.36abA	3.37 ± 0.29abcA
2800	2.57 ± 0.45bcA	2.38 ± 0.34cA
10,000	2.91 ± 0.30bcA	3.01 ± 0.41bcA

[1]Based on 10 weevils per sex per concentration.
[2]Means in the same column followed by the same lower case letter are not significantly different at 5% level (LSD test). Means in the same row followed by the same upper case letter are not significantly different at 5% level (t test). * Means in the same row followed by different upper case letter are significantly different at 10% level (t test).

Table 4. Gustatotry response for abamectin when mixed with 10% sucrose and ingested by male and female boll weevils captured in pheromone traps during spring and fall, 2000[1].

	Mean[2] amount ingested (µl) ± SE	
Concentration (ppm)	Male	Female
0	3.71 ± 0.42aA	3.50 ± 0.77aA
6.5	2.03 ± 0.18bA	2.48 ± 0.29abA
13	2.10 ± 0.48bA	2.73 ± 0.26abA
32.5	1.89 ± 0.24bcA	1.92 ± 0.23bA
65	1.18 ± 0.12cA	0.80 ±0.09cB
650	0.20 ± 0.03dB*	0.36 ± 0.08cA

[1]Based on 10 weevils per sex per concentration.
[2]Means in the same column followed by the same lower case letter are not significantly different at 5% level (LSD test). Means in the same row followed by the same upper case letter are not significantly different at 5% level (t test). * Means in the same row followed by different upper case letter are significantly different at 10% level (t test).

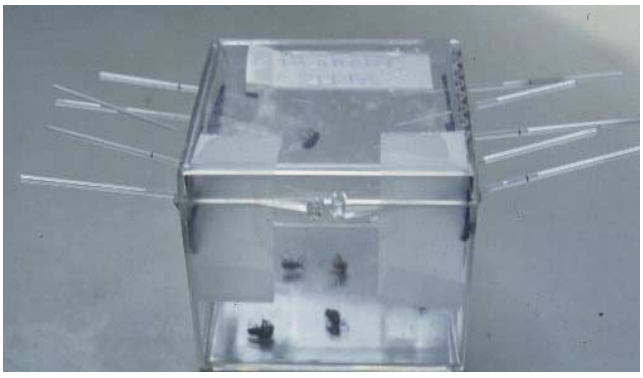

Figure 1. Square plastic box (2½ X 2½ X 2¼ in.) with hinged lid used in LC determination.

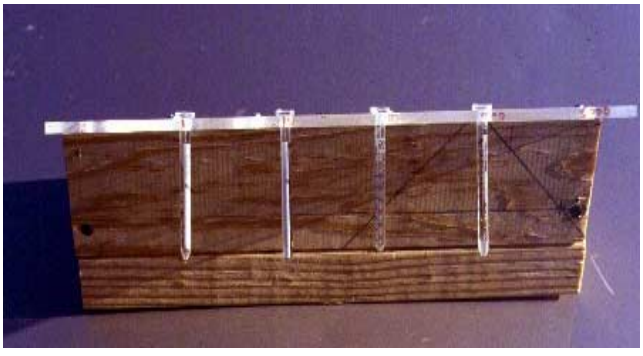

Figure 2. The feeding apparatus with 10 µl Hamilton ® syringes used in determining gustatory response.