EFFECT OF SELECTED INSECT GROWTH REGULATORS ON FEEDING RESPONSE AND REPRODUCTION OF ADULT BOLLWORM

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Abstract

Four insect growth regulators (IGRs), Confirm, Cyromazine, Fenoxycarb and Pyriproxyfen were evaluated as toxicants in mixtures with 1 M sucrose on a ppm AI weight:volume basis as a feeding stimulant for bollworm (BW), *Helicoverpa zea* (Boddie), adult control. Sex pheromone trap-captured males were evaluated for effects on proboscis extension response only and laboratory-reared females were evaluated for effects on gustatory response, mating frequency, fecundity, larval hatch and development. Confirm at 1, 10, 100, 1000, and 10,000 ppm concentrations did not significantly reduce proboscis extension and gustatory responses, mating frequency, larval hatch and development compared to control (sugar only). Cyromazine and Fenoxycarb significantly reduced gustatory responses at 10,000 and 1000 ppm, respectively. Cyromazine significantly depressed mating frequency at 10,000 ppm compared to 1 and 10 ppm, but there was no difference in mating frequency between 10,000 ppm and control. Fenoxycarb significantly reduced mating frequency at 1 ppm compared to 100 ppm, but mating frequency either at 1 or 100 ppm was not significantly different from control. Pyriproxyfen significantly reduced only proboscis extension response at 100 and 1000 ppm and mating frequency at 1000 ppm compared to control. Because none of the IGRs significantly reduced larval hatch or development, these results indicate that the potential for use of these IGRs as toxicants for adult control of BW is limited.

Introduction

Hazards associated with widespread pesticide applications have led many workers to develop alternate pest control methods that are more ecologically compatible. Lance and Sutter (1992) reported that semiochemical-based bait formulations containing the feeding stimulant (cucurbitacin) and the toxicant, carbaryl (0.3 %) effectively reduced *Diabrotica* spp. adult numbers in maize production systems. Lingren et al. (1990, 1998) and Lopez and Lingren (1994) reported on the use of feeding attractants and stimulants to develop techniques for areawide suppression of adult bollworm (BW), *Helicoverpa zea* (Boddie). Clemens (1996) studied the effect of feeding stimulant and insecticide mixtures on feeding response and mortality of adult BW and reported that a number of insecticides were suitable as toxicants for adult control. There is a need to broaden the scope of evaluation of toxicants to include insect growth regulators (IGRs) which interfere with insect metamorphosis and reproduction. IGRs are selective for pests, conserve beneficial arthropods (Elzen 1998) and have the potential to reduce use of conventional insecticides in integrated pest management programs (Graf 1993). On this basis, EPA approved use of the IGR, Confirm for control of beet armyworm on cotton in Mississippi and Alabama in 1994 (Duttle et al. 1997), and the IGRs, Buprofezin and Pyriproxyfen for whitefly control on cotton in Arizona in 1996 (Ellsworth et al. 1997) under Section 18 emergency exemptions.

Chandler et al. (1992) suggested that Fenoxycarb and Dimilin could be used to contain early-season populations of BW in their overwintering areas in corn before their dispersal to other crops. Carpenter and Chandler (1994) reported that sublethal doses of Dimilin and Confirm caused reduction in sperm transfer in male BW reared from larvae fed with pinto bean diet containing the IGRs. Kotze (1992) found that Cyromazine fed to adult Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) in water in concentrations up to 100 ppm did not interfere with oviposition or larval hatch, but larval development was inhibited in a dosage dependent manner. Ishaaya and Horowitz (1992) reported that Pyriproxyfen reduced whitefly larval hatch and produced LC_{50} and LC_{90} values of 0.026 and 0.049 ppm, respectively.

Our study objectives were to evaluate the effects of four IGRs, Confirm, Cyromazine, Fenoxycarb, and Pyriproxyfen on proboscis extension response of sex pheromone trap-captured males and gustatory response of laboratory-reared females when provided in a feeding stimulant solution. Laboratory-reared females were also evaluated for subsequent effects on mating frequency, fecundity, fertility, and survival of progeny to the pupal stage. Our intent was to determine if these IGRs could be used as toxicants in the development of adult control technology using feeding attractants/stimulants for areawide suppression of BW.

Materials and Methods

IGR Test Solutions

We formulated 1% stock solutions (10,000 ppm AI wt:vol) of Confirm 2F (RH-5992, 23.0%, Rohm & Haas, Philadelphia, PA), Cyromazine (Larvadex 2% SL, Ciba, Greensboro, NC), Fenoxycarb (Torus 2E, 23.9%, Ciba), Pyriproxyfen (Knack 0.84E, 11.23%, Valent USA, Walnut Creek, CA) in 1 M sucrose (grade II, Sigma Chemical Co., St. Louis, MO). Five serial dilutions ranging from 1 to 1000 ppm were prepared for each IGR. One M sucrose was prepared with deionized water. Test solutions were stored in a refrigerator and warmed to room temperature before each use.

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Test Insects

Sex pheromone trap-captured BW males were obtained using 75-50 Texas wire cone sex pheromone traps (Hartstack et al. 1979, Hartstack and Witz 1981) baited with laminated plastic Zealure (Hercon Environmental, Emigsville, PA). The traps were operated in farm areas in the Brazos River Valley near College Station, TX. Only males captured the previous night that had been provided deionized water *ad libitum* were used.

Bollworm females were reared in the laboratory from eggs obtained from the Southern Insect Management Laboratory, USDA-ARS; Stoneville, MS using similar techniques described previously (Lopez and Lingren 1994). Larvae were reared on soybean-wheat germ diet (ARTHRO FEEDS "*Manduca* Premix-*Heliothis* Premix", Stonefly Industries, Bryan, TX). All rearing and testing with laboratory-reared females were done in a laboratory maintained at 23.9°C ± 0.38 SE, RH 64.5 % ± 4.6 SE and a photoperiod of 14:10 h (L:D).

Proboscis Extension Response

Sex pheromone trap-captured males were evaluated after sunset in an insectary under red light. Males were pretested for response to deionized water alone and responders were excluded from further evaluation. Test solutions were placed in the wells of porcelain spot plates and males were allowed to rake test solutions with the front tarsi. A positive response was recorded when the proboscis was extended fully and touched the test solution. A different male was evaluated for response to each test concentration and all concentrations were evaluated consecutively. Means for each treatment in each replicate were based on response of groups of 10 males and a total of 10 replications for each IGR concentration were conducted.

Gustatory Respons

The conduct of gustatory response evaluations and the feeding apparatus used were similar to those described by Lopez and Latheef (1998). Only laboratory-reared females that had emerged during the previous night or the same day were used. To determine gustatory response, females were mounted individually on the feeding apparatus and were offered the test solutions contained in a disposable polystyrene microcentrifuge tube (0.5 ml). Gustation was determined based on weight of test solution ingested by the moth. Weights were taken before and after gustation and a correction was made for evaporation loss from an unfed sample for each test solution in each replicate. A total of at least ten moths were tested with each concentration that included a check of 1 M sucrose alone.

Reproduction Effects

Experimental procedures followed are similar to those described by Lopez and Latheef (1998). Each female was fed for one-half h, placed inside a 1-qt glass jar and paired with a male of the same age. The mouth of each glass jar was closed with a paper towel and a strip of paper towel was suspended from the mouth of each jar to provide a substrate for moths to rest and oviposit. Moths were provided with 10% sucrose solution in a 25 ml plastic souffle cup with a lid through which a cotton wick was inserted. Dead males were replaced during the test.

Mating Frequency

At the end of each test, females were dissected under a 30X stereozoom microscope to determine mating frequency by counting the number of spermatophores in the bursa copulatrix. Males transfer a spermatophore to the females during each mating (Callahan 1958).

Fecundity and Fertility

To determine fecundity, the number of eggs was counted from each jar beginning on the second day and for three consecutive days. The moths were placed in clean jars each day. In our test condition, bollworm females laid eggs indiscriminately on glass jar, cotton wick, plastic container and paper toweling. Accurate enumeration of eggs deposited was difficult and therefore, fecundity was determined only for Fenoxycarb and Pyriproxyfen. Ten and three replications were counted for Fenoxycarb and Pyriproxyfen, respectively. For Pyriproxyfen, eggs were counted for control, 1 and 100 ppm, because moths in other treatments were often found in *copula* during enumeration of eggs and were not able to separate from each other. A sample of about 30 eggs was collected from each jar for three consecutive days and was placed in a plastic cup to determine larval hatch. Each cup was checked for larval hatch starting on the second day after collection and for 3 consecutive days. Larval hatch data from unmated females were excluded from calculations of egg fertility.

Larval Development

During each larval hatch check, as many as ten larvae from each egg sample were collected and placed individually in cups with rearing medium until pupation. This provided a total of up to 30 larvae for each treatment in each replicate. Data were based on percentage survival of larvae to the pupal stage.

Data Analyses

Analyses of variance were conducted using SAS (1988). When *F*-values for treatment were significant at the 5% level, means were separated using Tukey's studentized range honestly significant (HSD) test at the 5% level of probability.

Results

Proboscis Extension Response

Confirm at 1, 10, 100, 1000, and 10,000 ppm concentrations did not significantly reduce the percentage of sex pheromone trap-captured males with a positive proboscis extension response compared to control (Fig. 1). Similarly, there was no significant difference in proboscis extension response to test concentrations of Cyromazine (Fig. 2) and

Fenoxycarb (Fig. 3) compared to control. Pyriproxyfen at 100 and 1000 ppm significantly reduced proboscis extension response when compared to control (Fig. 4) and there was an obvious dosage dependent effect.

Gustatory Response

The amount of each Confirm solution ingested by laboratory-reared females was not significantly different when compared to control (Fig. 5). Cyromazine significantly reduced gustatory response at 10,000 ppm when compared to control; however, there was no significant difference in amount of test solution ingested at 10, 000 ppm from that at 1, 10, 100 or 1000 ppm (Fig. 6). Fenoxycarb significantly depressed gustatory response of females at 1000 ppm; however, there was no significant difference in gustatory response between control and 1 ppm (Fig. 7). Also, the amount of Fenoxycarb ingested at 1000 ppm was not significantly different from that at 10 or 100 ppm. Pyriproxyfen significantly inhibited feeding at 1000 and 10, 000 ppm compared to that at 1 ppm and the effect was obviously concentration-related, but the amount ingested did not differ significantly from control (Fig. 8).

Mating Frequency

An analysis of mean number of spermatophores per female fed Confirm showed that there was no significant difference in mating frequency for any of the test concentrations compared to control (Fig. 9). Cyromazine at 10,000 ppm significantly depressed mating frequency compared to 1 and 10 ppm, but there was no significant difference in mating frequency between 10,000 ppm and control (Fig. 10). There was also no significant difference in mating frequency between 100 and 1000 ppm when compared to control. Fenoxycarb significantly reduced mating frequency at 1 ppm compared to 100 ppm, but mating frequency either at 1 or 100 ppm was not significantly different from control (Fig. 11) so the effect was not concentration-related. There was no significant difference in mean number of spermatophores per female between test concentrations up to 500 ppm of Pyriproxyfen and control (Fig. 12); however, mating frequency at 1000 ppm was significantly less than control. There was an obvious concentration- related effect.

Fecundity and Fertility

Fenoxycarb and Pyriproxyfen did not significantly affect the total number of eggs oviposited per female during the 3-day period compared to control (Figs. 13 and 14). The large number of total eggs oviposited per female during the 3-day test period indicates that the reproductive potential of each female was effectively evaluated. Confirm, Cyromazine, Fenoxycarb and Pyriproxyfen did not significantly affect percentage of larval hatch of eggs oviposited compared to control (Figs. 15 to 18).

Larval Development

Evaluation of survival to the pupal stage of larvae hatching from eggs oviposited by females fed Confirm indicated that there was no significant difference in larval survival between treatments when compared to control (Fig. 19). Similarly, ingestion of Cyromazine, Fenoxycarb and Pyriproxyfen by females did not significantly affect survival of larvae to the pupal stage compared to control (Figs. 20 to 22).

Discussion

An important initial concern in the evaluation of toxicants for use in a feeding-based attracticide is that they don't interfere with feeding. Proboscis extension is the first critical response in feeding because moths cannot feed if the proboscis is not extended. Our results show that only Pyriproxyfen inhibited proboscis extension response at concentrations as low as 100 ppm. Gustation is the next critical step in feeding, because the ultimate effect of a toxicant in this control approach is expected to be from ingestion. Both Cyromazine and Fenoxycarb inhibited gustation at 10,000 and 1000 ppm, respectively. Therefore, any consideration of these three IGRs for reproduction effects should be limited to concentrations below these critical concentrations.

Based on the feeding response concentration limitations, the only IGR with a significant inhibitory effect on mating frequency was Fenoxycarb at 1 ppm only and not at any of the higher concentrations. This effect may be important; however, because there was no evident concentration-related effect and the effect did not carryover to a reduction in larval hatch, the significance of this effect from a control viewpoint is reduced. None of the IGRs significantly reduced larval hatch or development, so the value of these IGRs for use in a feeding-based attracticide for adult control is limited. There were some obvious concentration-related effects of Cyromazine and Fenoxycarb on larval hatch which, although not statistically significant, may be noteworthy for further evaluation, but the concentrations needed for these effects may be too high to be practical.

Although several IGRs have produced toxic effects in *H. zea* treated as larvae, only Attia (1991) reported in a limited study that oral ingestion of the IGRs, PH-6040 and H-24108, decreased fecundity and fertility in the Old World BW, *Helicoverpa armigera* (Hübner) in a concentration dependent manner. Retnakaran and Wright (1987) reported that the ovicidal effect of IGRs via adults is probably caused by inhibition of chitin formation in the embryo, which usually dies inside the eggshell as a fully formed larva. In this study, the ingestion of IGRs did not significantly inhibit any of the reproduction parameters evaluated. However, percentage reductions in larval hatch caused by ingestion of Cyromazine and Fenoxycarb were numerically the highest among IGRs studied compared to control, although statistical significance at the 5% level could not be demonstrated. The reduction in larval hatch for Cyromazine was statistically significant at the 10 % level (P=0.0985). Inability to show a significant reduction in larval hatch may be due to age of the females tested.

Satyanarayana et al. (1992) reported that in BW, yolk deposition in oocytes begins 8 to 10 hs after adult emergence and is controlled by juvenile hormone. The bollworm females used may have been more than 12 h old and consisted of females which eclosed at different times of the night. We do not know if the time of eclosion is important for maximizing reduction in larval hatch by the IGRs evaluated and this may need to be researched.

Conclusion

The IGRs, Confirm, Cyromazine, Fenoxycarb and Pyriproxyfen did not significantly reduce fecundity, larval hatch, or survival of the progeny to the pupal stage. The potential for use of these IGRs as toxicants for adult control of BW in a feeding-based attracticide is, therefore, limited.

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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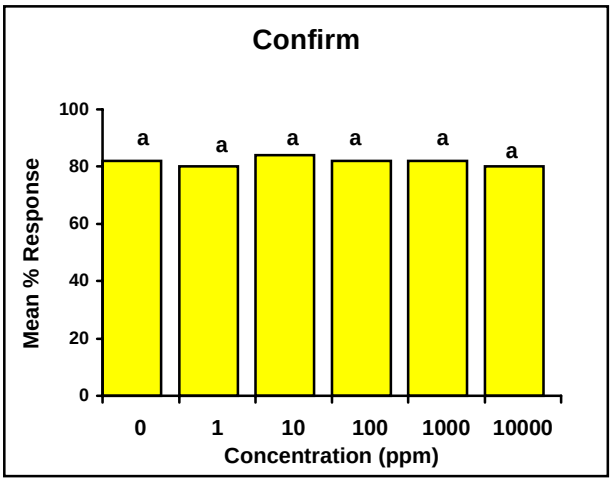

Figure 1. Percent of sex pheromone trap-captured bollworm males extending their proboscis to Confirm in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

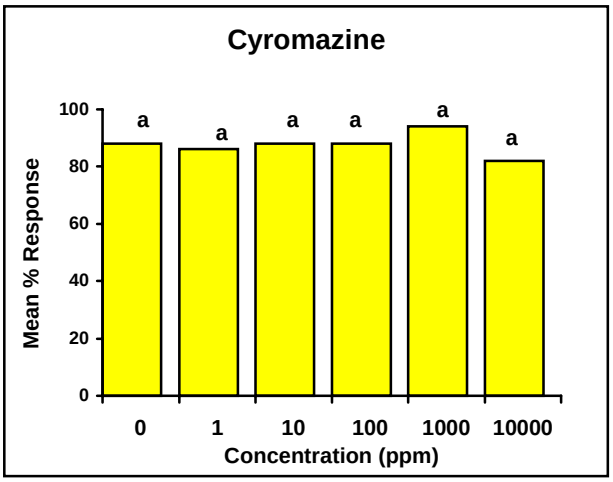

Figure 2. Percent of sex pheromone trap-captured bollworm males extending their proboscis to Cyromazine in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different(*P*=5%).

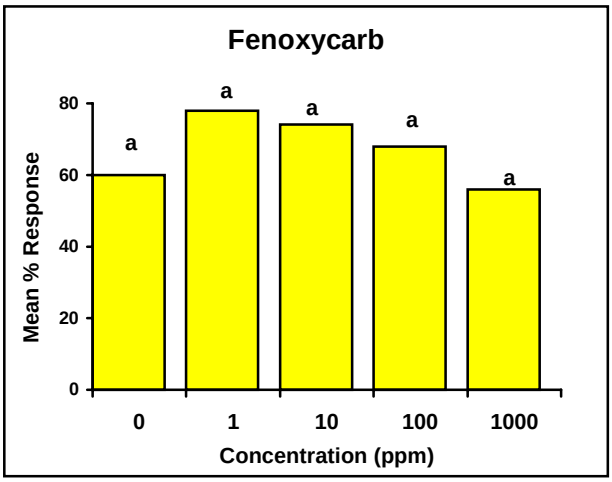

Figure 3. Percent of sex pheromone trap-captured bollworm males extending their proboscis to Fenoxycarb in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

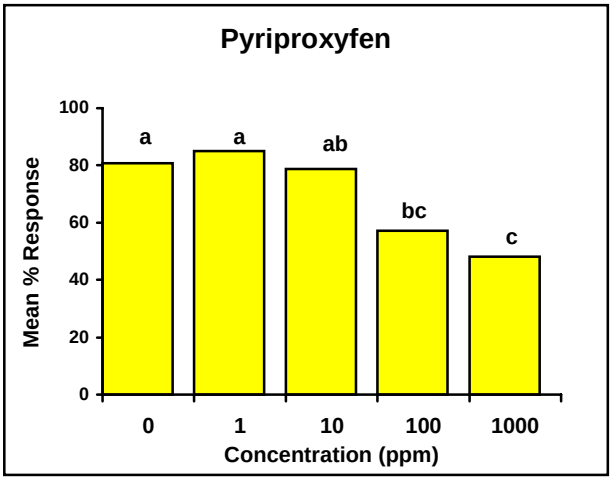

Figure 4. Percent of sex pheromone trap-captured bollworm males extending their proboscis to Pyriproxyfen in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

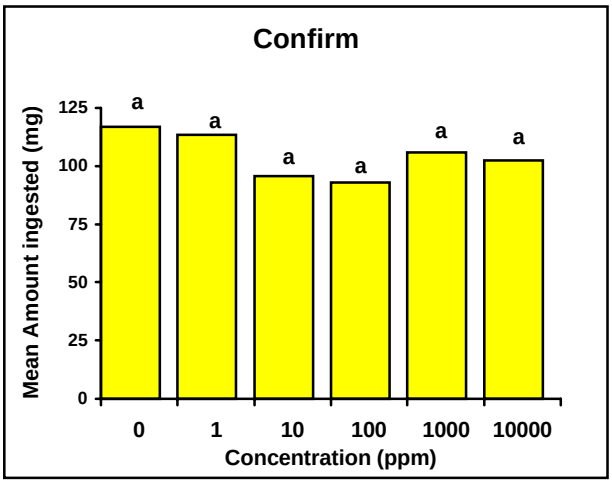

Figure 5. Gustatory response of laboratory-reared bollworm females to Confirm in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P=5%*).

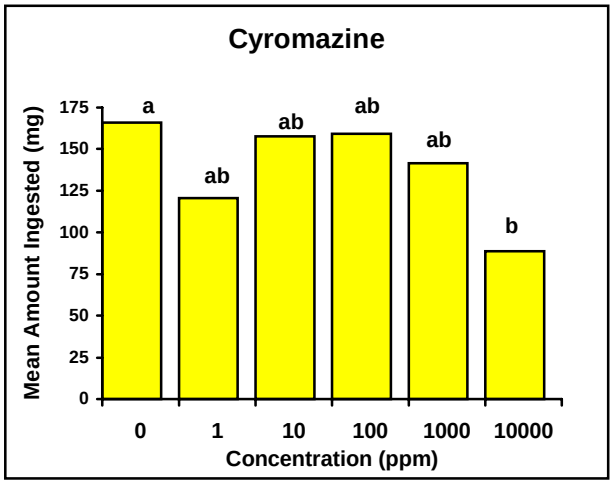

Figure 6. Gustatory response of laboratory-reared bollworm females to Cyromazine in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

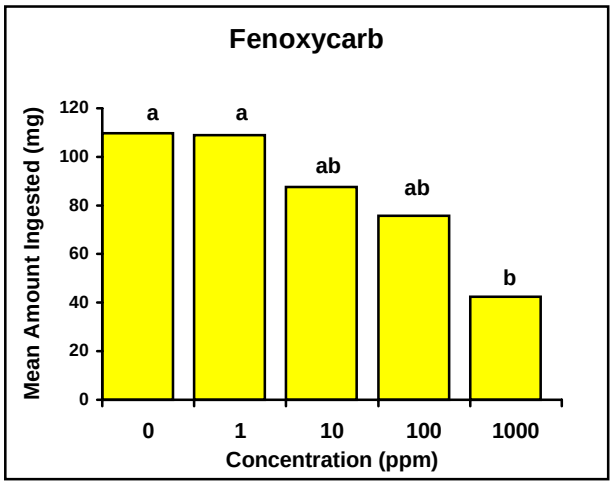

Figure 7. Gustatory response of laboratory-reared bollworm females to Fenoxycarb in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

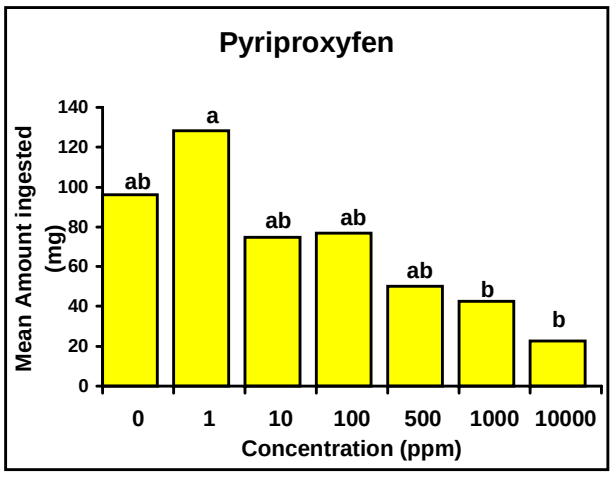

Figure 8. Gustatory response of laboratory-reared bollworm females to Pyriproxyfen in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

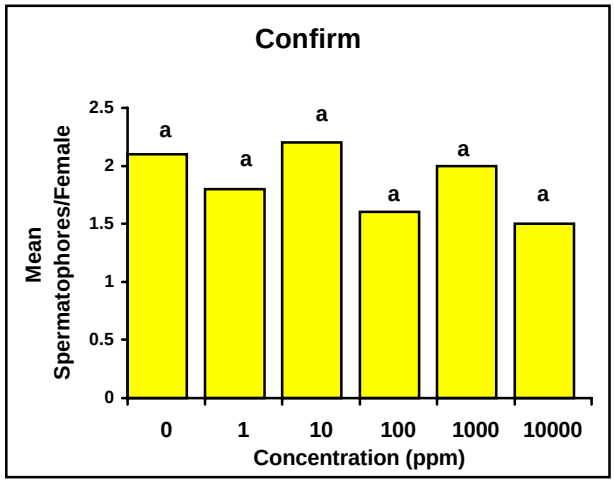

Figure 9. Mating frequency of laboratory-reared bollworm females fed Confirm 2F in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

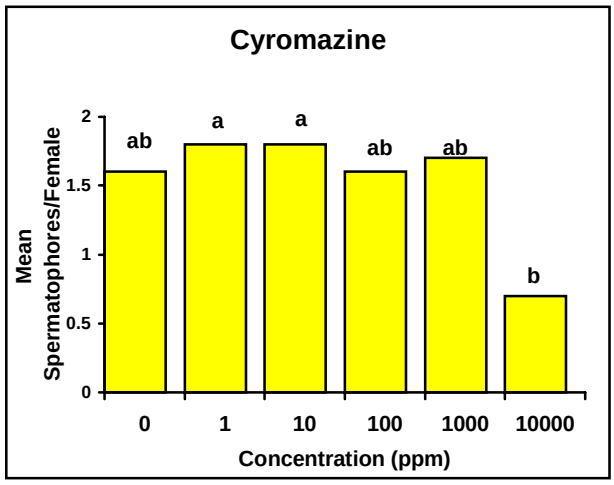

Figure 10. Mating frequency of laboratory-reared bollworm females fed Cyromazine in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

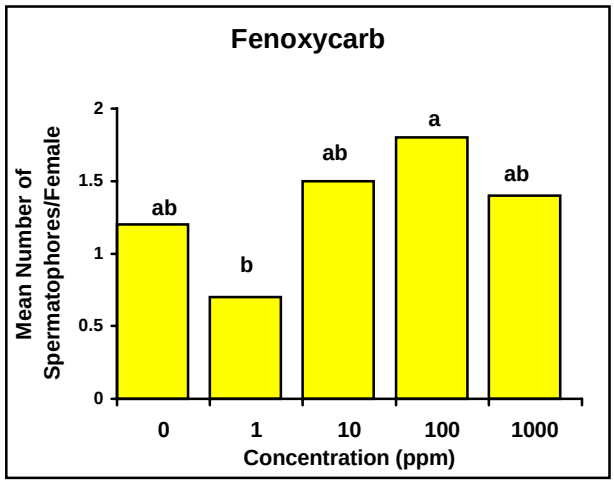

Figure 11. Mating frequency of laboratory-reared bollworm females fed Fenoxycarb in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

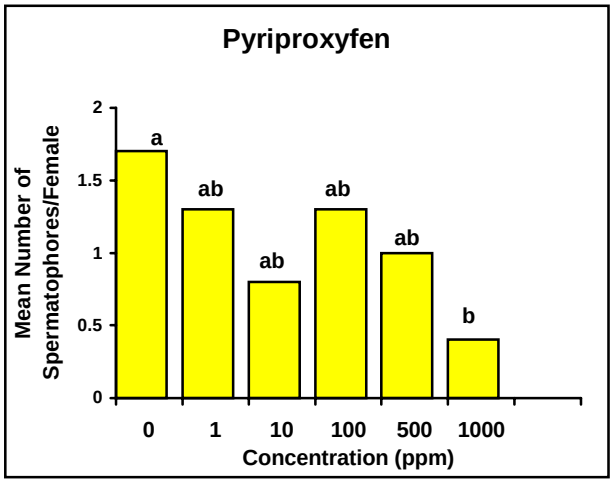

Figure 12. Mating frequency of laboratory-reared bollworm females fed with Pyriproxyfen in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

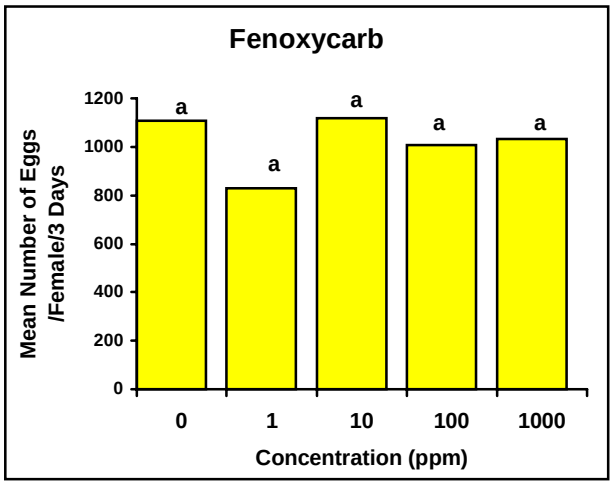

Figure 13. Mean number of eggs oviposited by laboratory-reared bollworm females fed Fenoxycarb in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

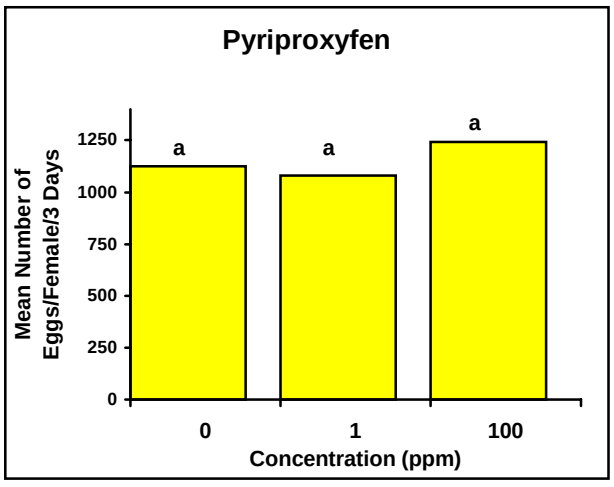

Figure 14. Mean number of eggs oviposited by laboratory-reared bollworm females fed with Pyriproxyfen in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

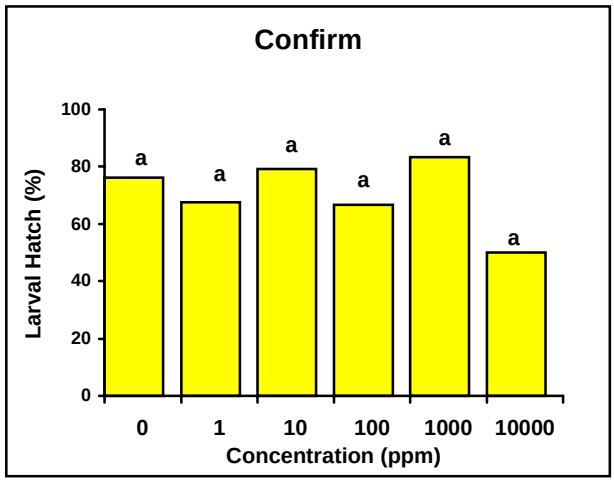

Figure 15. Percent of larval hatch from eggs oviposited by laboratory-reared bollworm females when fed Confirm 2F in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

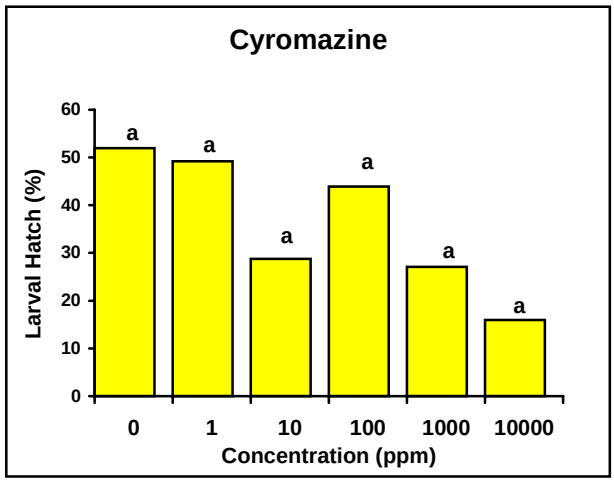

Figure 16. Percent of larval hatch from eggs oviposited by laboratory-reared bollworm females when fed Cyromazine in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

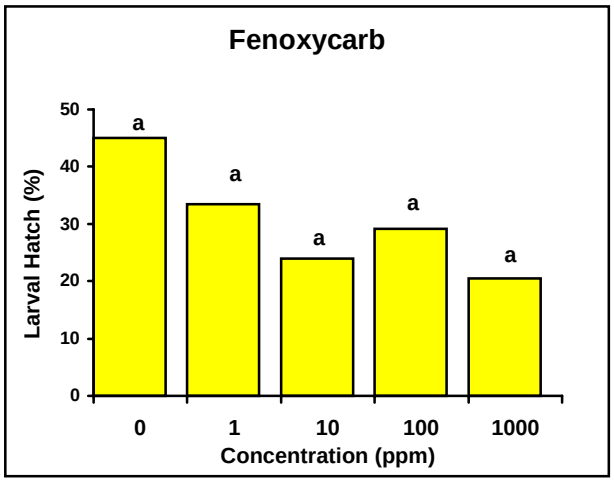

Figure 17. Percent of larval hatch from eggs oviposited by laboratory-reared bollworm females fed Fenoxycarb in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

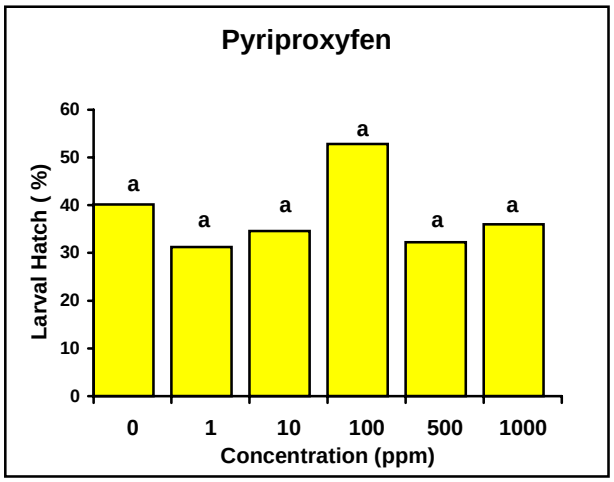

Figure 18. Percent of larval hatch from eggs oviposited by laboratory-reared bollworm females fed with Pyriproxyfen in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

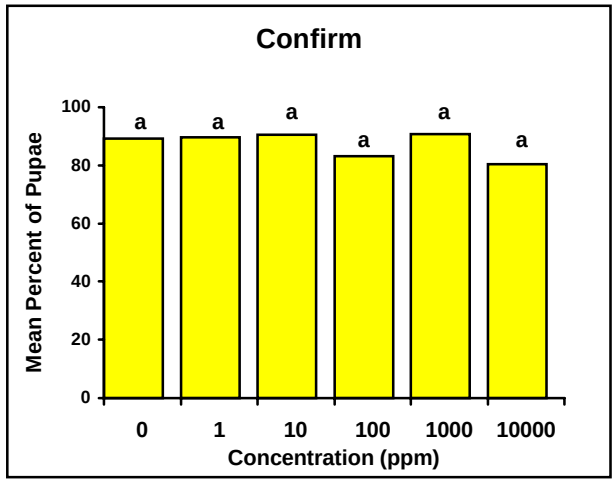

Figure 19. Survival of larvae to pupal stage from eggs oviposited by laboratory-reared bollworm females fed Confirm 2F in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

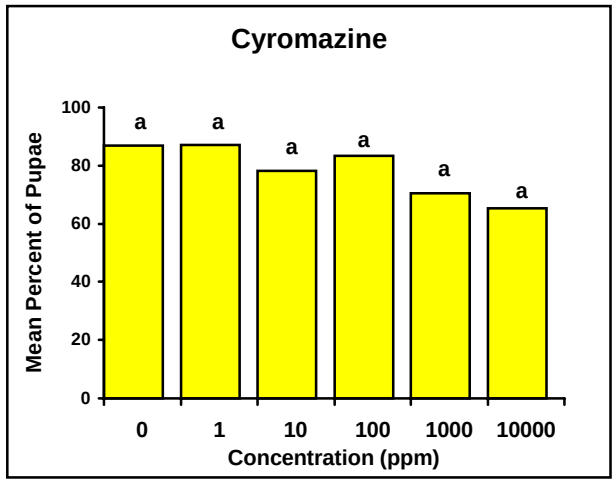

Figure 20. Survival of larvae to pupal stage from eggs oviposited by laboratory-reared bollworm females fed Cyromazine in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).

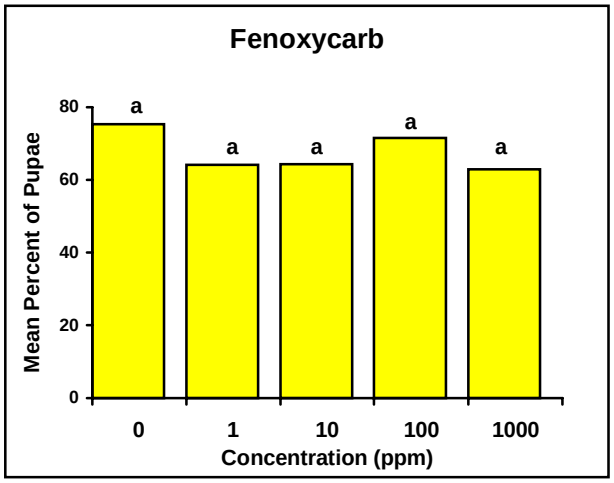

Figure 21. Survival of larvae to pupal stage from eggs oviposited by laboratory-reared bollworm females fed Fenoxycarb in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%)

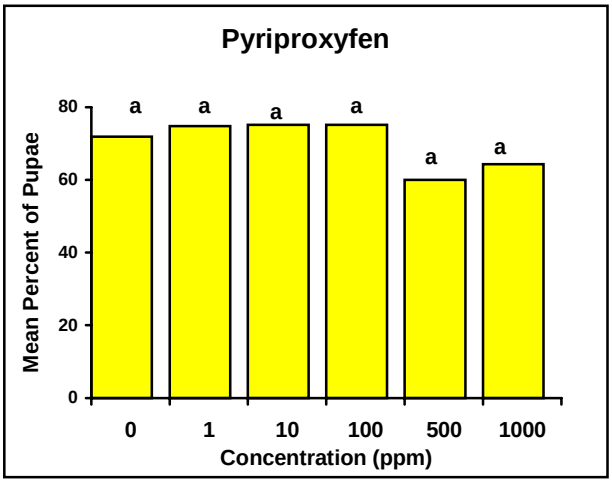

Figure 22. Survival of larvae to pupal stage from eggs oviposited by laboratory-reared bollworm females fed Pyriproxyfen in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (*P*=5%).