EFFECT OF OVASYN® ON FEEDING RESPONSE, MORTALITY AND REPRODUCTION OF ADULT BOLLWORM

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Abstract

Ovasyn® (amitraz) was evaluated for its potential use as a toxicant and as an insect reproduction inhibitor (IRI) in an adult control program for bollworm, *Helicoverpa zea* (Boddie) using feeding attractants/stimulants. Ovasyn (1.5 EC) mixed with 1.0 M sucrose on a ppm AI weight:volume basis was fed to sex pheromone trap-captured males to determine toxicity. The 24 h LC_{90} was 583 ppm (95% CL of 468 to 916). Evaluation of 583 (1X LC_{90}), 1,166 (2X), 2,915 (5X) and 10,000 ppm for effect on proboscis extension and gustatory responses of sex pheromone trap-captured males indicated that when compared with 1.0 M sucrose alone, there was a significant reduction at concentrations above 1,166 ppm for proboscis extension and at all concentrations for gustation. Mean lethal times were 319 min for 583 ppm, 160 for 1,166, 57 for 2,915 and 67 for 10,000; mean lethal time at 583 ppm was significantly slower from that at 2,915 and 10,000 ppm. At sublethal doses of 10, 25, 50, 75 and 100 ppm fed to laboratory-reared females within 24 h of emergence, there were no significant differences compared with control females fed only 1.0 M sucrose in larval hatch (%), mating frequency (mean no. of spermatophores per female), and survival of the hatched larvae to the pupal stage. At concentrations above 25 ppm, there was a significant reduction in gustatory response of laboratory-reared females compared with that of females fed only 1.0 M sucrose. Although Ovasyn was toxic to adult bollworm, its relatively low toxicity compared with other insecticides, its inhibition of proboscis extension and gustatory response and lack of significant IRI effects indicate limited potential for use of Ovasyn as a toxicant in adult control of bollworm with feeding attractants/stimulants.

Introduction

Ovasyn® (amitraz) belongs to the formamidine group of insecticides and possesses ovicidal and residual toxicities to the neonate larvae of Heliothine insects (Leonard et. al. 1990; Durant 1991; Kirk et al. 1993). Treacy et al. (1987) reported that the formamidene chlordimeform increased locomotion and spin-down of tobacco budworm larvae on cotton and resulted in larvae abandoning the plants. Also, as a contact poison, the formamidine insecticides are characterized by their ability to cause mortality and disruption of behavioral patterns essential to mating and reproduction in noctuid adults (Giles and Rothwell 1983; Hassall 1990; Salvisberg et al. 1980). Latheef (1992) reported that tobacco budworm moths exposed to Ovasyn-treated cotton plants showed increased number of spermatophores per female compared to profenofos-, thiodicarb-, and methyl parathion-treated cotton. Clarke and Haynes (1992) reported that chlordimeform caused symptoms of hyperexcitation, abnormal patterns of egg laying, and reduced number of eggs in cabbage looper moths treated topically with the compound. Salvisberg et al. (1980) reported that topical applications of chlordimeform were toxic to *Spodoptera littoralis* moths and at sublethal doses caused symptoms of hyperexcitation in the majority of adults that resulted in abnormal patterns of egg laying, and reduced number of eggs and fertility. Wolfenbarger et al. (1974) reported that 0.25% chlordimeform hydrochloride reduced fecundity and hatching of larvae from eggs deposited by tobacco budworm adults reared from larvae treated with the compound. Although chlordimeform has been withdrawn from the market (Sparks et al. 1990), Ovasyn, which has been reported to be less toxic than chlordimeform, is still commercially available and is being used for bollworm/budworm control. No study has been reported on the effect of Ovasyn on feeding response, mortality, and reproduction of adult bollworm (BW), *Helicoverpa zea* (Boddie), when provided for ingestion in a feeding stimulant solution to newly-emerged females or sex pheromone trap-captured males. These effects are of special interest in the development of adult control technology using feeding attractants/stimulants (Lingren et al. 1993).

In the study reported here, we evaluated the effect of Ovasyn on sex pheromone trap-captured males relative to lethal concentration, mean lethal time and proboscis extension and gustatory responses. We also wanted to characterize the effect of Ovasyn at sublethal doses on reproduction (insect reproduction inhibition - IRI), larval hatch and larval development of the insect when fed to laboratory-reared BW females. The objective of this study was to determine whether or not Ovasyn when ingested by female bollworm will induce ovicidal and eclosion mortalities similar to those caused by the compound as a contact or volatile poison. We chose to use natural populations of the insect to study lethal concentration, lethal time, and proboscis extension and gustatory responses because wild males were readily available during the test period, and there was no significant difference in responses relative to these variables between wild males and females (Lopez, unpublished data). This information is required as part of an overall evaluation of toxicants for compatibility and efficacy in the development of adult control technology for bollworm using attracticides.

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Materials and Methods

Chemical

We formulated a 1.0% dilution (10,000 ppm AI, weight:volume) of Ovasyn (1.5 EC ≈19.8% AI by weight); (AgrEvo, Wilmington, DE) in 1.0 M sucrose (grade II, Sigma Chemical Co., St. Louis, MO). All test solutions were then prepared from this formulation by appropriate dilution with sugar solution. The 1.0 M sucrose was prepared with deionized water. Test solutions were placed in 100 ml amber medicine glass bottles and stored in a refrigerator. Before each use, solutions were warmed to ambient temperature with tap water.

Insects

Both sex pheromone trap-captured males and laboratory-reared females and males were used in the studies. Sex pheromone trap-captured males were captured using 75-50 Texas pheromone traps (Hartstack et al. 1979; Hartstack and Witz 1981) baited with laminated plastic Zealure (Hercon Environmental, Emigsville, PA). The traps were operated in an agricultural area in the Brazos River Valley close to College Station, TX. Only males trapped the previous night which had been given deionized water *ad libitum* before testing were used in the evaluations. Laboratory-reared bollworm moths were obtained from a culture maintained as described by Lopez and Lingren (1994). Pupae were sexed and placed separately in one gallon glass jars without any food for emergence.

Determination of Lethal Concentration and Lethal Time

The lethal concentration (LC) values were determined for sex pheromone trap-captured males to optimize toxicant concentrations in the feeding stimulant formulation to use in the various test solutions. Determination of LCs was based upon preliminary feeding studies with ppm concentrations which were either increased or decreased by trial and error. The ppm concentrations used were 0, 100, 200, 300, 400, 600, 800, and 1,000. At least 10 males were fed for ½ h on each concentration in each replication with five replicates with some exceptions. At 300 and 10,000 ppm, there were 4 and 1 replicates, respectively. The moths in each replication were placed inside quart jars without food and examined for mortality after 24 h. Mortality was based on the inability of the moths to right themselves after being forced on their backs. LCs were calculated using POLO-PC (LeOra Software 1994) To determine lethal time, 10 males for each concentration were fed test solutions at 1x, 2x, and 5x the LC_{90} concentration and 10,000 ppm, placed individually in 1 oz plastic cups and checked for mortality at 15, 30, and 45 min and every hour until 6 h when checking was increased to 12, 18, and 24 h. The midpoints of the intervals at the end of which moths were observed to be dead were used to calculate LT.

Determination of Proboscis Extension Response

Proboscis extension is essential for feeding of adult BW; therefore, determining the effect of Ovasyn on the proboscis extension response is important if it is to be used as a toxicant in a feeding stimulant/attracticide formulation for adult control of BW moths. The methods used to determine the effect of Ovasyn on proboscis extension response were similar to those described by Lopez et al. (1995). Proboscis extension response of sex pheromone trap-captured males was evaluated at night in an insectary under red light. Test solutions were the same ones used for lethal time determination. Test solutions were placed in the wells of porcelain spot plates and males were allowed to contact the test solution with the front tarsi. If the proboscis was extended and contacted the solution for feeding initiation, a positive response was recorded. If the proboscis was extended partially and did not touch the test solution or there was no proboscis extension, a negative response was recorded. Test solutions were evaluated consecutively by concentration and evaluation of 10 males with each concentration was a replication. Males were only tested once and discarded. Ten replications were made.

Determination of Gustatory Response

All tests were conducted during the day 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). The conduct of gustatory response studies and the feeding apparatus used were similar to those described by Lopez and Lingren (1994) and Clemens (1996). To determine the gustatory response of sex pheromone trap-captured bollworms, males were mounted individually in the feeding apparatus and were offered the test solutions in a disposable polystyrene microcentrifuge tube (0.5 ml). Test solutions were the same as used for lethal time and proboscis extension response determinations. Gustatory response was determined by pre- and post-feeding weights of test solutions corrected for evaporation weight loss using containers on which the moths did not feed. A total of 10 moths was tested with each treatment that included a check of 1.0 M sucrose alone.

Determination of IRI Effects

Only laboratory-reared females which emerged during the previous 24 h period were used. The sublethal concentrations used were 0, 10, 25, 50, 75, and 100 ppm. Each female was fed for ½ h and the amount ingested was determined as previously described for gustatory response. Each female was placed in a 1 quart glass jar and paired with a male of the same age. The mouths of the jars were closed with a paper towel. A strip of paper towel was suspended from the mouth of each jar which provided ovipositional substrate for the moths to climb and oviposit. Moths were fed 10% sucrose in a 25 ml plastic soufflé cup with a lid through which a cotton dental wick was inserted. Dead males were removed and replaced. Beginning the 2nd day after pairing and for three consecutive days, a sample of up to ca. 30 eggs from each treatment in each replication was collected by cutting a piece of paper toweling containing the eggs and placed in 25 ml cups with lids to determine larval hatch. Larval hatch was determined by checking each egg sample starting on the 2nd day after

collection and for three consecutive days. Hatched larvae from each cup were counted and removed. A maximum of 10 larvae were randomly collected from each consecutive day of egg sampling and reared individually on a soybean-wheat germ insect diet (Arthro Feeds "*Manduca* Premix-*Heliothis* Premix," Stonefly Industries, Bryan, TX). The number of larvae surviving to the pupal stage was determined ca. 22 days after the larvae were placed on diet. A total of 7 replicates were done.

At the end of each test, female moths were dissected under a 30X stereozoom microscope to determine mating frequency by counting the number of spermatophores in the bursa copulatrix. Males transfer a spermtatophore to the female at each successful mating (Callahan 1958). Data from females that did not mate were not included in determining IRI effects except for mating frequency. Females which died or were locked *in copula* at any time during the tests were dissected to determine mating frequency and were not evaluated on subsequent days.

Data Analyses

Analyses of variance of the data were conducted using the GLM procedure of SAS (1988). When F values for treatment were significant at the 5% level, means that were significantly different at the 5% level were separated using Tukey's studentized range test (HSD).

Results and Discussion

Toxicity values of Ovasyn to sex pheromone trap-captured BW males are presented in Table 1. The dosage mortality equation indicated good fit of the mortality data with P^2 = 9.39 and df = 5. The LC_{50} and LC_{90} (95% CL) in ppm were 315 (249-380), and 583 (468-916), respectively. The LC_{50} value for Ovasyn is considerably higher than that reported for numerous insecticides by Clemens (1996) and Lopez et al. (1997) which generally had LC_{50} values $\leq$ 50 ppm. Although Ovasyn is toxic to adult BW males when mixed with a feeding stimulant and fed to the moths, many insecticides which are presently commercially available are much more toxic and would most likely be preferable to Ovasyn unless it has some very unique toxicological and environmental characteristics. Mean lethal time (LT) in minutes was 319 at 583 ppm (1x LC_{90}), 160 at 1,166 (2x), 57 at 2,915 (5x) and 67 at 10,000 ppm (Fig. 1). The LT at the highest two concentrations were significantly faster than at 583 ppm. These LT values are similar to those reported for organophosphates (acephate, malathion, and profenofos), and carbamates (carbaryl, methomyl, and thiodicarb) by Clemens (1966).

Evaluation of the feeding response of sex pheromone trap-captured BW males to the same concentrations used for LT determinations indicated significant inhibition at some of the higher concentrations compared to 1.0 m sucrose alone (Figs. 2 and 3). More than 70% of the males extended their proboscis on contact with 1.0 M sucrose alone. At the highest two concentrations evaluated, (2,915 and 10,000 ppm), there were significant reductions in proboscis extension response. For the gustatory response, there was significant inhibition in the amount ingested at all concentrations of Ovasyn compared to the control. The proboscis extension response results indicate that field BW males will respond to toxic concentrations of Ovasyn at least up to 2x the LC_{90}, but higher concentrations will inhibit response. The inhibition of gustatory response of field BW males may reflect the toxic effects of Ovasyn to the males, but these results do show that the field males will ingest toxic amounts of the feeding stimulant and Ovasyn mixture. It is possible that some of the inhibitory effects, especially inhibition of gustatory response are due to the hyperexcitation response, which has been reported in cabbage looper (Clark and Haynes 1992) and *S. littoralis* adults (Salvisberg et al. 1980) for topical applications of chlordimeform which has similar effects to Ovasyn.

Evaluations of the gustatory response to sublethal concentrations of Ovasyn by laboratory-reared BW females indicated that ingestion of test solutions was significantly inhibited at concentrations above 50 ppm when compared to the control; however, there was a trend for reduced gustatory response at all concentrations (Fig. 4). The IRI evaluations of Ovasyn at sublethal dosages showed that there were no significant reductions in % larval hatch of eggs (Fig. 5), mean number of spermatophores per female (Fig. 6) or survival to the pupal stage of larvae hatching from eggs (Fig. 7) when compared to the untreated controls. There was a trend for a reduction in larval hatch and mating frequency at concentrations above 10 ppm; however, there was no consistent concentration effect. This lack of significant IRI effects may reflect the significant inhibition of the gustatory response of laboratory-reared females at Ovasyn concentrations above 50 ppm, which limited the amount of Ovasyn ingested at the higher concentrations. Other possible reasons for the lack of IRI effects on BW females similar to those reported for cabbage looper and *S. littoralis* adults are differences in the enhanced effects of chlordimeform compared to Ovasyn, differential effects on various noctuid species, or differences in the routes of uptake by topical application as compared to ingestion. Whatever the reason for the lack of IRI effects of ingested Ovasyn when mixed with a feeding stimulant, the results obtained indicate that Ovasyn has limited potential use at sublethal rates for use in an attracticidal formulation for adult control of BW.

Conclusion

Although Ovasyn was toxic to adult BW, the relatively low toxicity compared to other commercially available insecticides, the inhibitory effects on proboscis extension and gustatory responses at both lethal and sublethal concentrations, and the lack of significant IRI effects at sublethal concentrations indicate limited potential for use of

Ovasyn as a toxicant in adult control of BW with feeding attractants/stimulants.

Disclaimer

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References

Callahan, P. S. 1958. Serial morphology as a technique for determination of reproductive patterns in *Heliothis zea* (Boddie). Ann. Entomol. Soc. Amer. 51: 413-428.

Clarke, D. C., and K. F. Haynes. 1992. Sublethal effects of chlordimeform on chemical communication and other reproductive behaviors in the female cabbage looper moth (Lepidoptera: Noctuidae). Arch. Insect Biochem. Physiol. 19: 105-117.

Clemens, C. G. 1996. Effects of feeding stimulant and insecticide mixtures on feeding response and mortality of adult male corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). 109 pp. M. S. Thesis, Texas A&M University, College Station, TX.

Durant, J. A. 1991. Effectiveness of selected ovicides against the bollworm-tobacco budworm complex on cotton. Proc. Beltwide Cotton Res. Conf. 748-749.

Giles, D. P., and D. N. Rothwell. 1983. The sublethal activity of amidines on insects and acarids. Pestic. Sci. 14: 303-312.

Hartstack, A. W., and J. A. Witz. 1981. Estimating field populations of tobacco budworm moths from pheromone trap catches. J. Econ. Entomol. 10: 901-914.

Hartstack, A. W., Jr., J. A. Witz, and D. R. Buck. 1979. Moth traps for the tobacco budworm. J. Econ. Entomol. 72: 519-522.

Hassall, K. A. 1990. The biochemistry and uses of pesticides. Structure, metabolism, mode of action and uses in crop protection. 2nd ed. VCH, Weinheim.

Kirk, I. W., V. S. House, and L. F. Bouse. 1993. Ovicide formulation and aerial application parameters influence control of tobacco budworm on cotton, pp 319-339. *In*: B. N. Devisetty, D. G. Chasin and P. D. Berger [eds.], Pesticide Formulations and Application Systems, 12th vol., ASTM STP 1146, Amer. Soc. Testing and Materials, Philadelphia, PA.

Latheef, M. A. 1992. Mortality and mating frequency of tobacco budworm (Lepidoptera: Noctuidae) adults as influenced by insecticides sprayed on cotton. Proc. Beltwide Cotton Prod. Res. Conf. 864-866.

Leonard, B. R., J. B. Graves, S. Micinski, and G. Burris. 1990. Ovicidal properties of selected insecticides against *Heliothis* spp. in cotton. In: Proc. Beltwide Cotton Prod. Res. Conf. 267-273.

LeOra Software. 1994. POLO-PC. A user's guide to logit or probit analysis. LeOra Software, Berkeley, CA.

Lingren, P. D., V. M. Bryant, Jr., J. R. Raulston, M. Pendleton, J. Westbrook, and G. D. Jones. 1993. Adult feeding host range and migratory activities of corn earworm, cabbage looper, and celery looper (Lepidoptera: Noctuidae) moths as evidenced by attached pollen. J. Econ. Entomol. 86: 1429-1439.

Lopez, J. D., Jr., and P. D. Lingren. 1994. Feeding response of adult *Helicoverpa zea* (Lepidoptera: Noctuidae) to commercial phagostimulants. J. Econ. Entomol. 87: 1653-1658.

Lopez, J. D., Jr., C. G. Clemens, and R. W. Meola. 1997. Evaluations of some insecticides mixed with a feeding stimulant for adult bollworm. Proc. Beltwide Cotton Prod. Res. Conf. 940-941.

Lopez, J. D., Jr., P. D. Lingren, and D. L. Bull. 1995. Proboscis extension response of adult *Helicoverpa zea* (Lepidoptera: Noctuidae) to dry sugars. J. Econ. Entomol. 88: 1271-1278.

Salvisberg, W., R. Neuman, and G. Voss. 1980. Chlordimeform: Mode of toxic action in various developmental stages of *Spodoptera littoralis*. J. Econ. Entomol. 73: 193-196.

SAS Inst. 1988. SAS/STAT™ Guide for Personal Computers. Version 6.03, Cary, NC.

Sparks, T. C., R. B. Leonard, F. Schneider, and J. B. Graves. 1990. Effects of formamidines on octopamine titers in eggs of the tobacco budworm. Proc. Beltwide Cotton Prod. Res. Conf. 235-239.

Treacy, M. F., J. H. Benedict, K. M. Schmidt, R. M. Anderson, and T. L. Wagner. 1987. Behavior and spatial distribution patterns of tobacco budworm (Lepidoptera: Noctuidae) larvae on chlordimeform-treated cotton plants. J. Econ. Entomol. 80: 1149-1151.

Wolfenbarger, D. A., E. Cantu, P. D. Lingren, and A. A. Guerra. 1974. Activity of chlordimeform-HCl and chlordimeform against arthropods attacking cotton. J. Econ. Entomol. 67: 445-446.

Table 1. Toxicity of Ovasyn to sex pheromone trap-captured male bollworms when fed in 1.0 M sucrose. LCs are based on ppm AI (weight:volume).

N	slope ± SE	χ^2(df)	LC_{50}	95% CL	LC_{90}	95% CL	LC_{99}	95% CL
350	8.24±1.01	9.39(5)	315	249-380	583	468-916	1,139	773-2,883

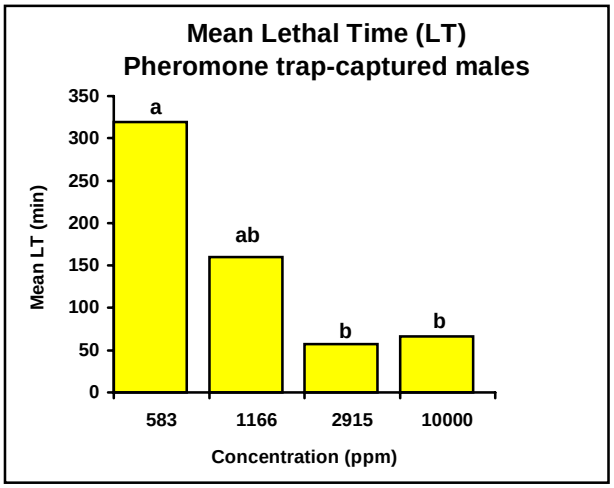


Figure 1. Mean Lethal Time (min) for male bollworm moths fed with Ovasyn in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (P=5%).

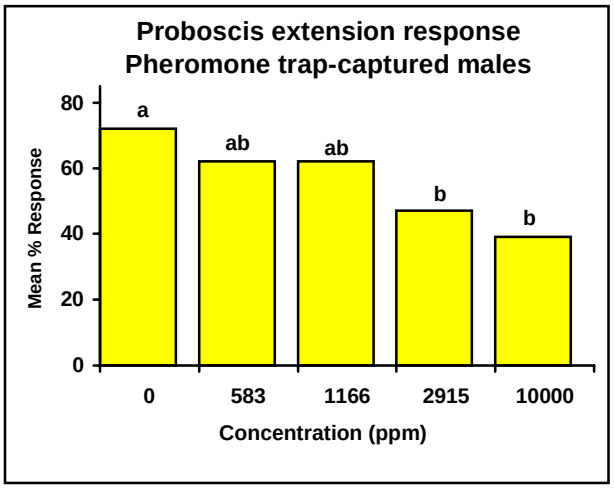
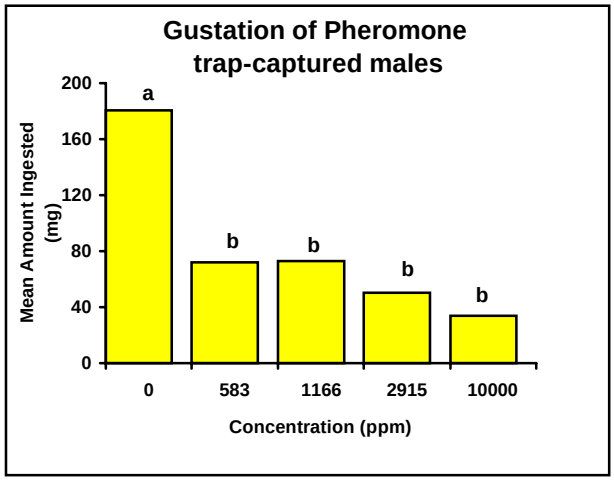


Figure 2. Percent of moths extending their proboscis to Ovasyn in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (P=5%).



igure 3. Gustatory response of male bollworm moths to Ovasyn in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (P=5%).

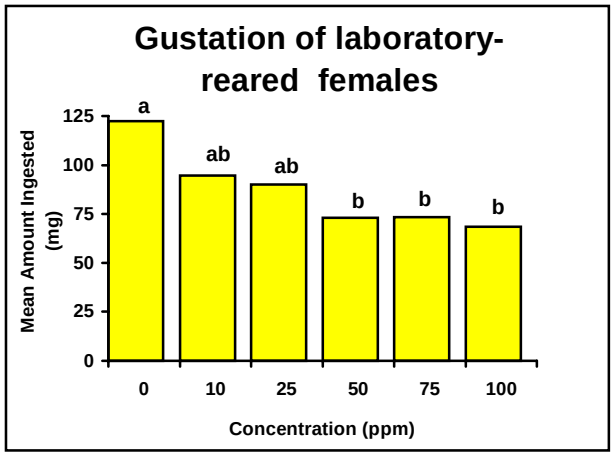


Figure 4. Gustatory response of laboratory-reared female bollworm to Ovasyn mixed with 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (P=5%).

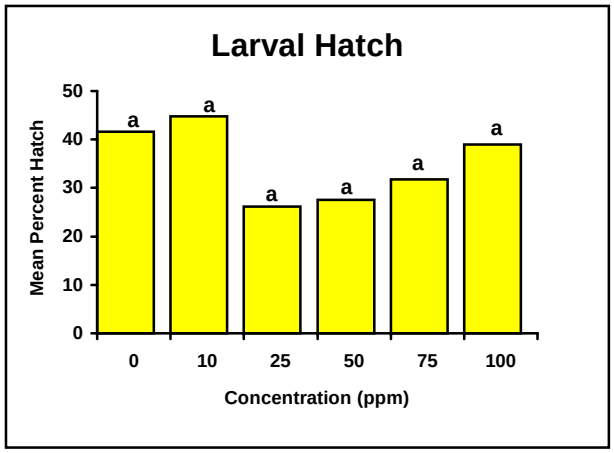


Figure 5. Percent hatch of larvae from eggs deposited by laboratory-reared female bollworm when fed with Ovasyn in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (P=5%).

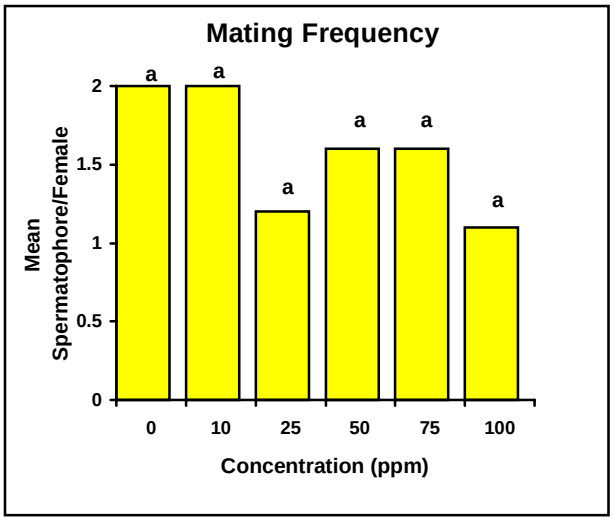


Figure 6. Mating frequency of laboratory-reared females fed with Ovasyn in 1.0 M sucrose and paired with a male. Means followed by the same lower case letter are not significantly different (P=5%).

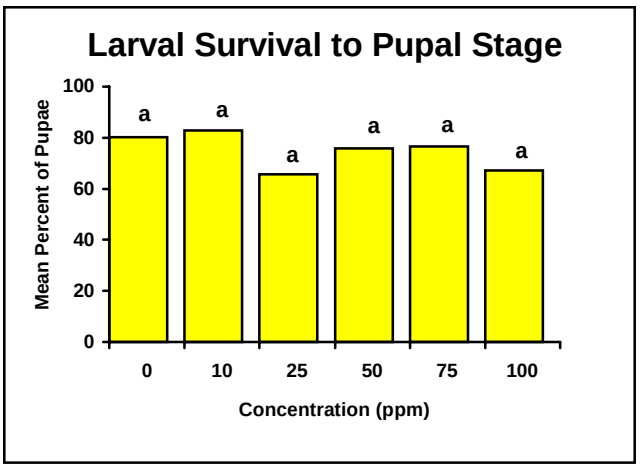


Figure 7. Survival of larvae to pupal stage from eggs deposited by laboratory-reared female bollworm fed with Ovasyn in 1.0 M sucrose. Means followed by the same lower case letter are not significantly different (P=5%).