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DEVELOPMENT OF A LABORATORY STRAIN OF THE TOBACCO BUDWORM RESISTANT TO DENIM® AND A FIELD KIT FOR RESISTANCE MONITORING

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Abstract

A laboratory strain of the tobacco budworm, *Heliothis virescens,* was selected every generation by larval dip using Denim® (emamectin benzoate). A moderate level of resistance was detected after selection for seven successive generations. The LC_{50} for the parental strain by dip was 0.8 PPM while that for the selected strain (generation 8) was 6.1 PPM, a resistance ratio of 7.6-fold. Using this resistant strain, we developed a feeding disruption assay for the diagnosis of Denim® resistance in neonates of the tobacco budworm. The presence of blue feces on a white background is a marker for resistance. The kit is in the commercialization phase of development.

Introduction

Emamectin benzoate is a semisynthetic derivative of avermectin that has potent acaricidal, insecticidal, and nematicidal activity (Putter et al., 1981). Avermectins are produced in the fermentation process of the actinomycete, *Streptomyces avermitilis*, which was first isolated from a soil sample collected from Japan (Burg et al., 1979). Avermectins act on different types of chloride-gated channels regulating skeletal muscles in insects and other invertebrates (Arena, 1994). Avermectins have little or no cross resistance with other chemical classes (Clark et al., 1994), have very little effect on non target organisms and beneficials (Lasota and Dybas, 1991), neither persist nor accumulate in the environment and are used at very low rates in the field (Jansson and Dybas, 1998). Emamectin benzoate was developed to act specifically on lepidopteran pests and has LC_{50}s less than 1 PPM (Jansson et al., 1996). Our goal was to evaluate the potential for insect resistance development to Denim® using an important economic pest of cotton and other crops, the tobacco budworm. We also introduce in this paper a new diagnostic kit for monitoring larval resistance to Denim® that is based on the combination of three technologies--feeding disruption as measured by fecal production, hydrateable meal pads and a new device (Bailey et al, 1998; Roe et al., 1999; Roe et al., 2000a,b; Roe et al, 2002).

Materials and Methods

Insects

Tobacco budworm larvae were reared individually in 30 ml plastic cups (Solo Cup Co., Urbana, IL) on standard artificial diet (Burton 1970) at 27±1ºC with a 14:10 (light:dark) cycle and 50-60% relative humidity. Adults were fed a 20% sucrose solution and kept at the same conditions.

Selection

Late third instars (30±5 mg) of the tobacco budworm were used for selection. Treatment was carried out by larval dip in the appropriate Denim® solution (0.16% EC) made in distilled water. Larvae were dipped in the solution using soft forceps, placed on a paper towel to dry at room temperature (1 min), and then returned to the rearing cup with diet where they were allowed to develop to the pupal stage under standard rearing conditions. The pupae were removed from the diet and allowed to mate in mass and lay eggs. We treated 1000-1200 larvae per generation and obtained 200-500 pupae from these larvae. The treatment concentration was increased each generation to keep mortality between 60-70%.

LC_{50}s

LC_{50}s for both the susceptible and 8th generation of selection were obtained by larval dip (the same technique used for selection). The insects used to estimate the LC_{50} were not selected in the 8th generation. Larval mortality was recorded 12 days after treatment. Two replicates from the susceptible strain and three replicates from the 8th generation selected strain were used to estimate the LC_{50}. Five doses and 25 larvae (third instars) per dose were used for each replicate. Abbot's correction (Abbott, 1925) was applied to all data. Median lethal doses were estimated by plots of probit mortality versus log dose (Finney, 1971) using the method of least squares and inverse predictions of 95% fiducial ranges (Sokal and Rohlf, 1995). Calculations were made in Microsoft Excel spreadsheets (Microsoft, 1997).

Feeding Disruption Assay

Feeding disruption assays were conducted on parental (Denim® susceptible) and selected (8th generation) budworms using the new Agdia® resistance assay plates (Figure 1). Each plate contains 16 wells in a 4x4 format. The dark circle is a hydrateable meal pad with a blue indicator dye used to monitor feeding. The meal pads extend below the well and are open below the well to the outside. Hydration is maintained via a wetted surface below the wells. For a detail protocol on the use of these plates, refer to Roe et al. (2002). The dry meal pads were rehydrated from the top using 100 µl of distilled water (control) or the appropriate concentration of Denim® in distilled water for 30 min before adding insects to the well. Excess liquid was removed using Q-tip cotton swaps. Neonates (one larva for each well) were transferred to the diet using a camel hair brush, wells were sealed using a semipermeable, transparent tape, and the plates placed on the surface of a wet paper towel in a sealed plastic container. Plates were incubated at standard rearing conditions. The number of blue fecal pellets produced per well was recorded 24h after the addition of the insects. The pellets were counted using a dissecting microscope. Fecal production is a measure of the susceptibility of the insect to Denim® in the meal pad. Larval mortality was also determined. Insects were considered dead if they did not respond to touch by a blunt probe.

Results and Discussion

Selection History

The selection history is summarized in Figure 2. The first round of selection was 1 PPM of Denim® and produced 67% mortality. At this selection dose in the second generation, mortality was reduced to 47.5%. For generations 3-8, we had to increase the selection dose every generation in order to maintain greater than 60% mortality. In the 8th generation, 7.5 PPM of Denim® produced 64.8% mortality, indicating that the insects had developed resistance to the insecticide. Resistance occurred rapidly in these selection studies but the overall resistance level in the 8th generation based on the change in our selection dose is only 7.5-fold.

LC_{50}s

LC_{50}s for two replicates of the susceptible strain and three replicates of the 8th generation selected strain are shown in Figure 3 (upper). For Rep 1 and Rep 2 of the parental (susceptible) strain, the LC_{50}s were 0.60 (0.21-1.28 95% confidence interval) and 0.99 (0.57-1.74) PPM, respectively. For the selected strain the LC_{50}s for Reps 1-3 were 6.37 (3.01-15.65), 6.6 (4.51-10.47) and 5.42 (1.36-31.35) PPM, respectively. The lower graph in Figure 3 is the combined results for all replicates for each treatment where the LC_{50}s were 0.8 (0.46-1.34) and 6.1 (4.15-9.33) PPM for the parental and selected strains, respectively. This is a resistance ratio of 7.6-fold. It is clear from these results that selection in the laboratory with Denim® by dipping results in the rapid development of larval resistance. The slope of the dose-mortality line for the selected strain suggests that greater resistance levels may be possible by additional selection. For example, 24% of the budworms survived the highest dose tested (12 PPM).

Feeding Disruption Assay for Denim® Resistance in the Tobacco Budworm

This laboratory has been developing novel feeding disruption assays for monitoring resistance in Lepidoptera including the tobacco budworm (Bailey et al., 1998; Roe et al., 1999; Roe et al., 2000). The bioassay kit is now in the commercialization phase. The kit consists of a specially designed white plastic 16-well plate with recessed, hydrateable meal pads containing a diagnostic dose of insecticide and a blue indicator dye to monitor larval feeding (Figure 1). The appearance of blue feces easily seen on the background of the white plate is a measure of feeding rate. Neonates that produce blue feces (at a diagnostic dose of insecticide) at a greater rate than a known susceptible population are diagnosed as being resistant. For fast acting insecticides like Denim®, the end point can simply be mortality.

The relationship between the concentration of Denim® in the meal pad hydration solution and fecal production by parental budworms is shown in Figure 4. As expected, as the Denim® concentration increases, fecal production decreases. In the concentration range of 0.0025 to 0.01 PPM, fecal production on the average decreased from 48 to 4 pellets per neonate, respectively. This concentration range was used as a diagnostic dose range to examine the use of the feeding disruption assay to monitor budworm resistance to Denim®. A comparison of the number of fecal pellets produced on average by neonates of parental (susceptible) and selected budworms is shown in Figure 5. At all concentrations tested, the selected (resistant) budworms produced a greater number of fecal pellets than the parental strain as would be expected. The difference was greatest at the 0.0075 PPM dose; this dose appears to be an optimum dose for resistance monitoring using feeding disruption as a marker for resistance. However, Denim® is fast acting and mortality was also a good end point for resistance detection. The advantage of the Feeding Disruption Assay plates in general, is that both endpoints can be used to measure resistance. Mortality at 0.0025, 0.005, and 0.0075 PPM of Denim® was 16, 48, and 54%, respectively, for the parental (susceptible) strain while no mortality occurred for the selected strain (Figure 6). Resistance was easily detected both by feeding disruption and mortality for Denim® resistance levels of 7.6-fold, which demonstrates the sensitivity of this assay method for resistance detection.

Acknowledgments

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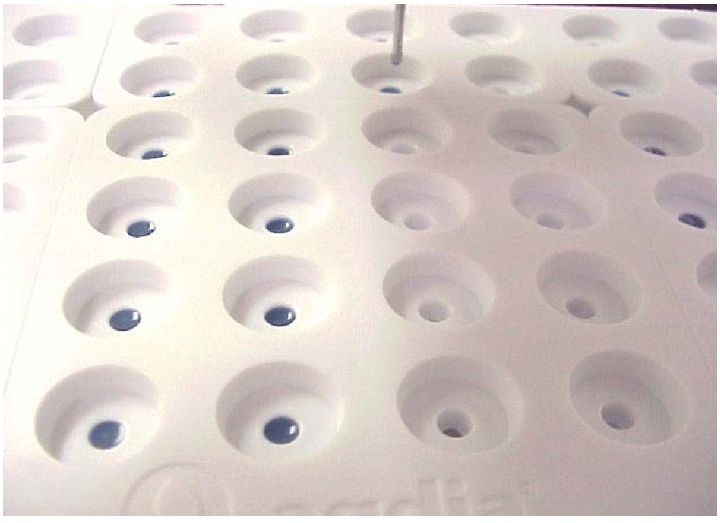


Figure 1. Agdia® plate used for feeding disruption assay showing the diet with a blue indicator (Used by permission from Norma Hoffman, Agdia® Inc.).

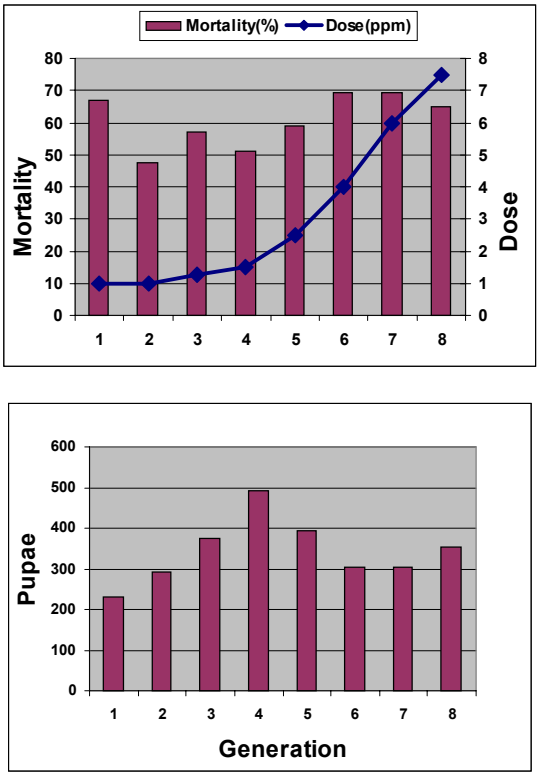
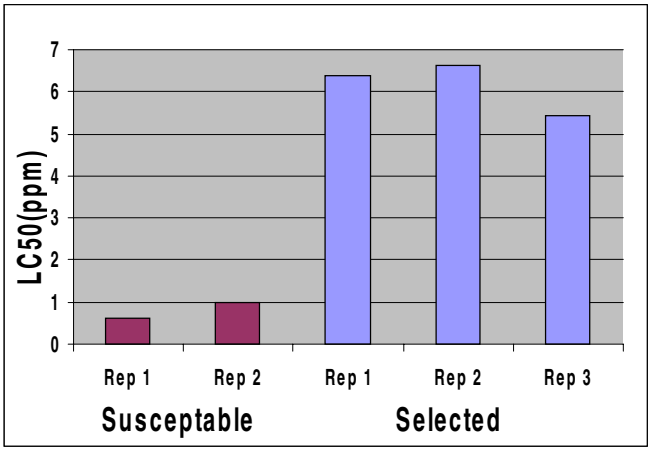


Figure 2. History of selection. Upper: Dose (PPM) and uncorrected mortality (%); lower: number of pupae per each generation of selection by larval dip using Denim®.



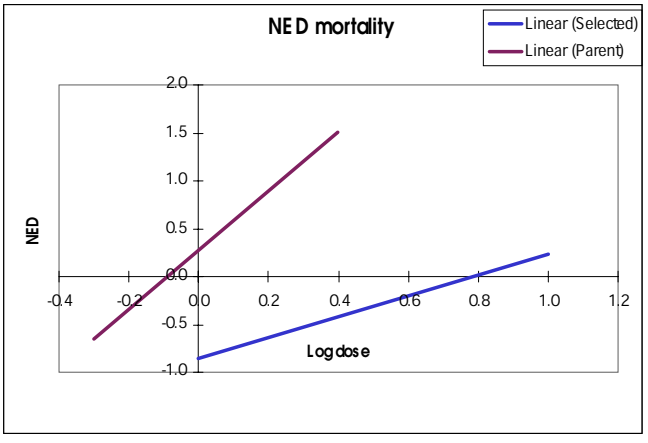


Figure 3. Upper: LC_{50}s for two replicates of the susceptible strain and three replicates of the 8th generation of the selected strain; lower: Loge-dose probit plot of toxicity of Denim® using larval dip. NED (Normal Equivalent Deviate) is probit minus 5.

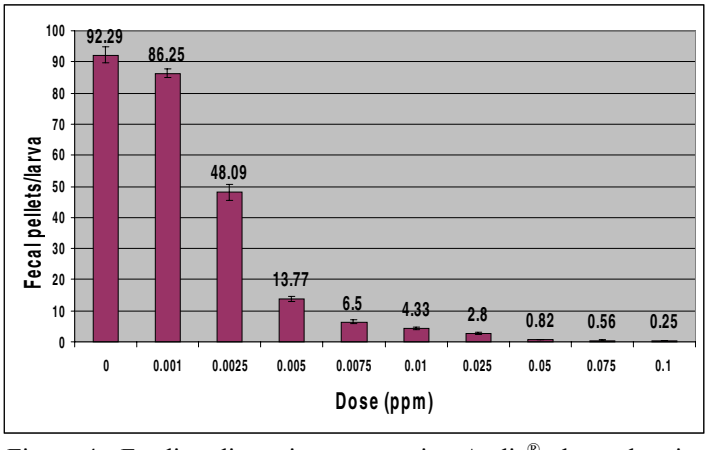


Figure 4. Feeding disruption assay using Agdia® plates showing the average number of fecal pellets produced per parental (susceptible) tobacco budworm neonate. Error bars represent ±1standard error of the mean.

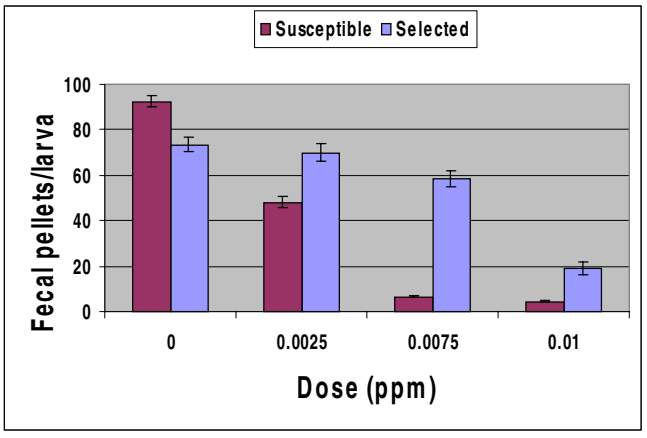


Figure 5. Comparison of number of fecal pellets produced per neonate for parental (susceptible) and selected tobacco budworms at different doses of Denim®. Error bars represent ±1 standard error of the mean.

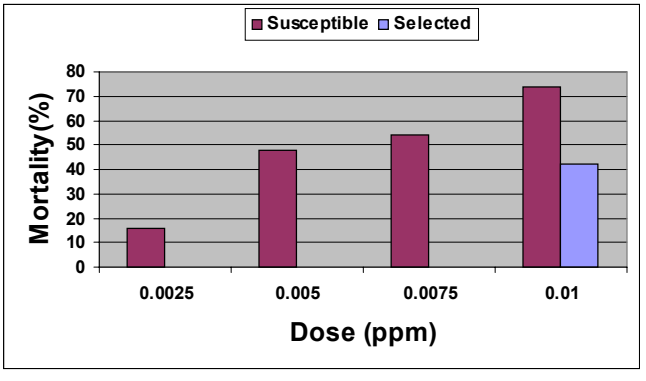


Figure 6. Neonate mortality for parental (susceptible) and selected budworms at different doses of Denim®. Thirty insects were used for each dose.