Techniques to Measure Azinphosmethyl Resistance in *Platynota idaeusalis* (Lepidoptera: Tortricidae)

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ABSTRACT Two techniques were studied to survey for azinphosmethyl resistance in the tufted apple bud moth, *Platynota idaeusalis* (Walker). Third instars were tested using a dry film contact method and topical applications were made to adult males attracted by pheromones. The dry film contact method was time-consuming and involved handling of larvae; for these reasons, the method was deemed to be unacceptable. The pheromone trap method using adult males had the greatest potential, but it presented problems with high control mortality. Although this experiment was designed to study different resistance-measuring techniques, a population from a Pennsylvania State University-owned experimental orchard was shown to be more tolerant than populations from a commercial orchard and a laboratory colony.

KEY WORDS *Platynota idaeusalis*, insecticide, resistance, Tortricidae, leafroller, azinphosmethyl

THE ORGANOPHOSPHATE insecticide azinphosmethyl has been used in apple orchards for the past 20–30 years. A recent survey of pesticide use in selected apple-producing counties in Pennsylvania revealed that azinphosmethyl was the most frequently used insecticide (Hull et al. 1983). Resistance to this material has developed among secondary pests (i.e., mites, aphids, and leafhoppers), but not among key pests (i.e., plum curculio, *Conotrachelus nenuphar* [Herbst]; codling moth, *Cydia pomonella* [L.]; apple maggot, *Rhagoletis pomonella* [Walsh]; and redbanded leafroller, *Argyrotaenia velutinana* [Walker]) (Croft & Bode 1983). Several leafrollers (Tortricidae) have exhibited tolerance or resistance to azinphosmethyl or related organophosphates. Azinphosmethyl-tolerant strains of the fruittree leafroller, *Archips argyrospilus* (Walker), have been found in apple orchards in the Okanagan Valley region of British Columbia (Vakenti et al. 1984). The LC_{50} of phosalone for a laboratory colony of obliquebanded leafroller, *Choristoneura rosaceana* (Harris), larvae was 150-fold less than that for a field-collected colony (Reissig 1978). Suckling et al. (1984) confirmed azinphosmethyl resistance in the light brown apple moth, *Austrotortrix postvittana* (Walker), using four different testing methods in New Zealand.

Tufted apple bud moth (TABM), *Platynota idaeusalis* (Walker), is a key pest of apple in south-central Pennsylvania orchards (Adams, Franklin, and York counties). Hull et al. (1983) found an average of 2.9%, with a high of 12.4%, apples injured by larval feeding in a survey of 16 orchards in these counties. TABM is present but not a serious problem in other fruit-growing regions of Pennsylvania. One possible explanation for this phenomenon is the development of resistance to commonly used insecticides in these south-central counties. The objective of this study was to develop techniques to survey for insecticide resistance in *P. idaeusalis*. Two techniques were investigated using third instars and adults.

Materials and Methods

Dry Film Contact Test. This laboratory test was conducted during 1984 and 1985 on four populations of larvae obtained from three sources. Two populations (NC-2 [second generation] and NC-3 [third generation]) originated from a stock colony obtained from G. Rock at North Carolina State University. Another population (C-6 [sixth generation]) originated from field-collected larvae located in a commercial orchard in Bendersville, Pa., and the final population (E-4 [fourth generation]) originated from larvae collected from an experimental orchard of The Pennsylvania State University in Arendtsville, Pa. The field-collected larvae and larvae from succeeding generations were reared on an artificial diet (Shorey & Hale 1965) modified by replacing agar with carrageenan and by adding a vitamin mix. Larvae were held in cups (29.6 ml) at 27°C with a 16:8 (L:D) photoperiod. Third instars were 9 days old; this age was chosen to correspond to the middle of the larval period based on development rates reported by Berkett et al. (1976). These larvae were tested with a method similar to that of Wells et al. (1983).

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Glass culture tubes (9.3 by 2.4 cm; surface area, 76.4 cm^2) were evenly coated by rotating acetone solutions of technical grade (91%) azinphosmethyl (Mobay Chemical, Kansas City, Mo.) while the acetone was allowed to evaporate. Ten larvae of approximately the same size were placed in each tube and the tubes were capped with diSPo plugs (American Scientific, McGaw Park, Ill.). Larvae were not weighed to prevent possible injury during handling. After 4 h, larvae were transferred (with a small camel's-hair brush) to clean culture tubes without food and placed in the incubator at 27°C with a 16:8 photoperiod. Transfer was necessary because the larvae web themselves above contact with the tube and, thus, with the insecticide. Five concentrations and a control (acetone only) were used. The experiment was replicated between two and six times, depending on the strain used. In all, 1,083 larvae were tested. Mortality was determined 48 h after treatment. A larva was considered dead when no movement was discernible.

Topical Application Test. This test was conducted during the first and second adult flights during summer 1984. Two commercial orchards located in Bendersville (B) and Biglerville (Bi), and one peach orchard (P) owned by The Pennsylvania State University were used. Adult males were caught and tested using the pheromone trap method of Riedl et al. (1985). First-flight adults were tested during late June, second-flight adults were tested during late August and early September. Scentry traps (Scentry, Phoenix, Ariz.) and Pherocon 1C traps (Zoecon, Palo Alto, Calif.) were placed in the orchards nightly. The adhesive in the bottom of the trap was spread out to assure a thin, even covering (adult control mortality was found to be higher in traps that had thicker adhesives). Traps, with between 1 and 49 captured adults, were collected the next morning and transported to the laboratory. Technical azinphosmethyl was applied to the thoracic dorsum of moths still trapped on the adhesive in 1-μl acetone solutions using a microdispenser (Drummond Scientific, Broomall, Pa.). Three and five concentrations plus controls (acetone only) were used against first- and second-flight adults, respectively. Individual traps were used as replicates, and there were between 3 and 19 replicates per dose, depending on the strain. In all, 294 first-flight adult males were tested; 819 second-flight adult males were tested. The trap bottoms with the treated adults were held at ca. 20°C. Mortality was determined 24 h after treatment. A moth was considered dead when its body or appendages did not move when touched.

Data Analysis. Data from both tests were subjected to the probit procedure of the Statistical Analysis System (SAS Institute 1982, 287–292). Control mortality was corrected for using the optimization of control (OPTC) option of this program. This option allows the procedure to give the initial estimate of control response as the reciprocal of the total number of subjects (the sum of the values of the subjects variable) in the experiment. The 95% FL were calculated for the LC or LD_{50} values, and the LC or LD values were considered significantly different by the criterion of nonoverlap of the 95% FL.

Results and Discussion

Dry Film Contact Test. Third instars from different generations of the NC strain showed significantly different LC_{50}'s; the later generation (NC-3) was more susceptible (Table 1). The C-6 strain exhibited susceptibility similar to that of the NC-2 strain, although the C-6 strain had been colonized for six generations. The E-4 strain was not significantly different from the NC-2 strain, but was 123- and 20-fold more tolerant than the NC-3 and C-6 strains, respectively. Wells el al. (1983) calculated the dose (mg/g insect) (LC_{50} value divided by the gram weight of a larva) for larvae in the dry film contact test. The LC_{50}'s for NC-2 (0.5 mg/g), NC-3 (0.04 mg/g), and C-6 (0.24 mg/g) strains were lower than the value Wells et al. (1983) obtained (4.8 mg/g), but that for the E-4 (4.7 mg/g) strain was similar.

During the past few years, two of the three strains were exposed to different levels of azinphosmethyl. The laboratory strain originated from several commercial orchards near Hendersonville, N.C. The strain was undoubtedly exposed to organophosphates in the field, but because of mixing of the colony, the effects of this exposure are unclear. The strain was reared in colony for 10–12 generations (equivalent to 5–6 seasons in the field) in North Carolina without exposure to azinphosmethyl. Thus, without selection pressure from the chemical, it was unlikely that this strain would show any tolerance to azinphosmethyl. In the C-6 orchard, azinphosmethyl was used in combination with methylparathion in orchard sprays for the past 5 years. The E-4 block was used for various chemical and biological experiments, including tests with pyrethroids from 1978 to 1981, and sampling studies on the TABM from 1982 to 1984. Azinphosmethyl was the only organophosphate insecticide used, and it was used only on selected plots during the biological studies. This block had comparatively high TABM populations and apple injury the last few years, and control with azinphosmethyl was not always successful.

The difference in LC_{50} values between the E-4 and C-6 strains may be explained by the mobility of the moth. Using computer simulations, Georghiou & Taylor (1977) found that immigration was an important factor in slowing the evolution of resistance. TABM has many hosts (Bode 1975) and can survive outside an apple orchard. The E-4 block was bordered by younger Pennsylvania State University-owned orchards and by a commercial orchard; the C-6 orchard in Bendersville was bordered by woods. Possibly, susceptible individuals

Table 1. Responses of third-instar and adult male TABM to azinphosmethyl tested by the dry film contact method and the topical method, respectively, Adams County, Pa., 1984

Life stage	Strain	*n*	NC	C ± SE	LC_{50} or LD_{50}	95% FL	Slope ± SE
3rd instar	NC-3	330	60	0.01 ± 0.01	0.00013	NR–0.0003	1.03 ± 0.05
	C-6	247	50	0.02 ± 0.02	0.0008	0.0004–0.0013	0.82 ± 0.08
	NC-2	210	40	0.10 ± 0	0.0017	0.0005–0.0045	0.64 ± 0.11
	E-4	296	60	0.08 ± 0.03	0.016	0.0038–0.41	0.44 ± 0.13
Adult	B-2	321	57	0.23 ± 0.04	35.2	12.0–68.0	1.0 ± 0.06
	Bi-2	498	104	0.25 ± 0.04	50.8	22.2–81.0	1.2 ± 0.04
	P-1	220	54	0.13 ± 0.07	173.9	68.0–NR	0.7 ± 0.10
	Bi-1	74	18	0.05 ± 0.05	293.9	96.0–NR	1.0 ± 0.12

NC, number of controls; C ± SE, estimated control mortality ± standard error; LC_{50}, mg/cm^2; LD_{50}, $\mu g/g$; NR, not a realistic value.

immigrated into the C-6 orchard from the woods and retarded resistance development in the strain.

As a technique to detect resistance, the dry film method presented several problems. Because small numbers of larvae were collected from the field, several generations of TABM had to be produced in the laboratory to provide sufficient numbers to test. Laboratory rearing could have affected the field-collected strains by influencing the frequency of resistant alleles, resulting in false conclusions about these strains. Keiding (1967) discussed the difficulty in maintaining field resistance in the laboratory to several organophosphates for the housefly, *Musca domestica* L. Although labor-intensive, this problem could be solved by collecting large numbers of egg masses or larvae in the field. In addition, each larva had to be transferred to a clean culture tube because of its webbing behavior, which allowed it to avoid contact with the insecticide. This transfer was not only time-consuming, but also increased the risk of injury. Although this indirect exposure method more closely paralleled the field situation, it cannot be recommended as a testing procedure for TABM.

Topical Application Test. First-flight adults were generally less susceptible than second-flight adults (e.g., the Bi-1 [first flight] strain was 5.8-fold more tolerant than the Bi-2 [second flight] strain [Table 1]). The P-1 (first flight) strain had an LD_{50} similar to that of the Bi-1 strain. The LD_{50} for first-flight adults had wide fiducial limits because ≤50% mortality was produced in the test. This problem was corrected in the tests with second-flight adults by increasing the concentration. Second-flight adults from the B-2 (second flight) and Bi-2 strains had similar LD_{50}'s.

Riedl et al. (1985) found a slight increase in susceptibility from the first to the second flight of *C. pomonella* adults. Genetic factors such as selective overwintering mortality and seasonal immigration could have removed or added individuals to the population that would have increased or slowed the onset of resistance for the first and second flights, respectively. Nongenetic factors such as the age of the moth also could have contributed to the susceptibility difference. If the age of TABM males attracted to a pheromone trap changes over the season (younger and more uniform-aged males during first flight), more susceptible moths might be found during the second flight. Riedl et al. (1985) found that susceptibility to azinphosmethyl increases with age for *C. pomonella*.

Control mortality was a concern in this study, especially during second flight (ca. 24%). Riedl et al. (1985) reported an average control mortality of 5% for *C. pomonella* with no significant effect attributed to the thin layer of adhesive. Perhaps older and weaker moths were captured during the second flight, and the combined factors of age and stress during capture contributed to the high control mortality.

Topical application of adults has been widely used as a standard method for detecting resistance (Barnes & Moffitt 1963). Use of the pheromone trap method for topical application with TABM has several advantages, the most important of which is that laboratory rearing is unnecessary. Laboratory rearing is time-consuming, costly, and experimental results may not be obtained for several months. This method permits quick assessment of resistance and identification of the specific location of resistant individuals within or outside an orchard (Suckling et al. 1985). Important disadvantages of the method include the unknown age of the moths tested, the testing of males only, and the sometimes low number of moths to test.

This study was designed to develop techniques to survey for insecticide resistance in TABM populations. Thus, the results describing differences between the various strains are preliminary in nature. It appears, however, that after many years of use, azinphosmethyl-tolerant strains may have developed. The extent of tolerance geographically and its overall importance are unknown.

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