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TOXICITIES OF AZINPHOSMETHYL AND OTHER
APPLE ORCHARD PESTICIDES TO THE APHID
`REDATOR, Aphidoletes aphidimyza (RONDANI)
(DIPTERA: CECIDOMYIIDAE)

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Leslie A. Warner

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TOXICITIES OF AZINPHOSMETHYL AND OTHER APPLE ORCHARD PESTICIDES TO THE APHID PREDATOR, *Aphidoletes aphidimyza* (RONDANI) (DIPTERA: CECIDOMYIIDAE)

By

Leslie A. Warner

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ABSTRACT

TOXICITIES OF AZINPHOSMETHYL
AND OTHER APPLE ORCHARD PESTICIDES
TO THE APHID PREDATOR, *Aphidoletes aphidimyza* (RONDANI)
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Aphidoletes aphidimyza, a cecidomyiid predator of apple aphids, was tested for toxicities to azinphosmethyl and several registered and experimental pesticides. Mortalities from azinphosmethyl in eggs collected from 14 field sites differing in previous pesticide exposure revealed significantly higher LC50 values in populations taken from commercial orchard sites; the largest resistance ratio was 14. Among the life stages, LC50 ratios for azinphosmethyl ranged from 1 to 6-fold, with first instars the most susceptible and eggs the least. Egg mortality was greatest in embryos exposed just prior to eclosion. Egg and third instar mortalities were evaluated for 28 pesticides at concentrations equivalent to recommended field rates, and pesticides were grouped into three classes: those causing high mortality (>50%) in both stages (diazinon, methomyl, carbaryl, demeton, dimethoate, azinphosmethyl); those causing high mortality in one stage only (oxythioquinox, phosmet, permethrin, fenvalerate, oxamyl); and those causing low mortality (<30%) in both stages (phosalone, phosphamidon, carbophenthion, pirimicarb, plus several fungicides and miticides).

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INTRODUCTION

Since the commercial development of synthetic organic pesticides, agriculture has relied heavily on chemicals to reduce populations of arthropod pests and prevent excessive damage to crops. More recently integrated pest management (IPM) has been applied in several crop systems with some success (e.g. deciduous tree fruits), and expansion of these programs is likely (Blair and Edwards 1980). With IPM, all available pest control techniques are evaluated and consolidated into a program to manage pest populations so that economic damage is avoided and adverse side effects on the environment are minimized (NAS 1969). Future expansion of IPM programs will probably emphasize the integration of the complex interactions among species (Newsom 1980).

Pesticides are effective tools when utilized judiciously in IPM programs, but excessive application can produce undesirable effects, including the development of resistance and cross-resistance, problems which frequently necessitate further pesticide application and increase the costs of crop production. In commercial apple orchards none of the insect pests which directly attacks the fruit has developed resistance to the pesticides currently registered. With nearly zero tolerance of pest damage to the apples, and since no program for biological control of these direct pests is available, protection of the fruit is likely to continue to depend on insecticide applications.

Many secondary pests of apple (i.e. aphids and phytophagous mites) have acquired a degree of resistance to the compounds applied to control direct pests. Croft and Hoyt (1978) reviewed the current status of apple IPM, noting the adaptations of natural enemies to these pest complexes. In Michigan orchards azinphosmethyl is the principal broad-spectrum insecticide applied, and strains of the predatory mite, *Amblysieus fallacis* (Garman), have acquired resistance to this compound. Croft (1975) has developed an IPM program for mite control in Michigan apple orchards, relying on the maintenance of suitable predator:prey ratios through the use of selective insecticides and cultural practices. To maintain and possibly expand the benefits of this IPM program, potentially non-disruptive control techniques should be examined for management of other secondary apple pests.

Among the indirect pests of apple are two species of aphids (*Aphis pomi* De Geer, *Dysaphis plantaginea* (Passerini)) which can decrease yield and growth. To prevent or limit damage, growers typically apply systemic and broad-spectrum contact insecticides. Developing an integrated control program for aphids could reduce the amount of pesticides applied in the orchard while causing less disruption of existing natural enemy populations.

One of the first steps in developing an IPM program is identifying the predators of the pest species. Recently a cecidomyiid, *Aphidoletes aphidimyza*, has been found preying on apple aphids with increasing frequency (Adams and Prokopy 1977). Several characteristics of this species contribute to its potential as a biological control agent (Markkula et al. 1979a). This study was undertaken to assess the mortality rates in *A. aphidimyza* after exposure to those pesticides likely to be applied in Michigan apple orchards, with the results contributing to pesticide recommendations in an apple IPM program. Specifically the objectives of this work were:

1) To determine the susceptibilities of the life stages of *A. aphidimyza* to the lethal effects of azinphosmethyl.

2) To determine the levels of resistance of populations of *A. aphidimyza* in commercial apple orchards in Michigan.

3) To determine the susceptibility of eggs and third instar larvae of *A. aphidimyza* to pesticides commonly applied in Michigan apple orchards.

LITERATURE REVIEW

I. Tolerance and Resistance

Resistance of arthropods to pesticides includes 414 species (Georghiou 1979) of which 10 are natural enemies (FAO 1979). Quantitative assessments of resistant populations of a species can be obtained through dosage-mortality bioassays, using the standardized method of detection (FAO 1969). Georghiou and Taylor (1977a) have classified the factors affecting the development of resistance in pests, and several investigators have discussed the factors causing differential frequency in resistance development between pests and their natural enemies (Croft and Brown 1975, Morse 1978, Croft and Morse 1979).

Croft and Brown (1975) have reviewed the factors which influence the susceptibility of arthropod natural enemies to pesticides. Direct toxic effects of compounds can be influenced by environment and physiology, including developmental stage and levels of nourishment. Indirect effects of pesticides include the elimination of the food source for natural enemies, secondary poisoning following consumption of contaminated prey, and the effects of sublethal doses of pesticides on longevity, development, and reproductive rates of the natural enemy. Direct and indirect effects of pesticides interact with the genetic, biological, and operational factors outlined by Georghiou and Taylor (1977b) to determine the likelihood of resistance in a beneficial species.

Many arthropod species are inherently tolerant of the effects of pesticides; when tested for toxicity, populations with no previous exposure to a given compound or related chemicals exhibit little mortality. Developmental stages of a species may exhibit tolerance: in tests of the green lacewing, *Chrysopa carnea* Stephens (Bartlett 1964a), eggs were less susceptible to pesticides than adults, with larvae intermediate. Bartlett (1964b) generalized these results to include all holometabolous predators and parasites. Pupae are generally less susceptible to the effects of pesticides than larvae (Rettich 1980, Singh and Rawat 1980). Colburn and Asquith (1971) tested fourteen pesticides on all stages of the lady beetle, *Stethorus punctum* (LeConte), and pupae were tolerant of all but carbaryl. No trend in tolerances among other stages was evident, possibly indicating the importance of mode of pesticide action and uptake.

Mortality within a developmental stage may vary with the size, weight, sex, and physiological state of the subjects. Recently-molted *Heliothis* spp. were more susceptible than larvae with full cuticular development (Mullins and Pieters 1980). Exposing coccinellid eggs to chlordimeform when old (48-72 hrs) and young (<24 hrs)

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resulted in greater susceptibility in the more developed embryos (Streibert and Dittrich 1977). Elliot and Way (1968) tested the toxicities of systemic aphicides on eggs of two predatory anthocorid species. Unhatched eggs consistently contained embryos that had died just prior to hatch irrespective of egg age when treated, an effect of organophosphorous insecticides reported by Smith and Salkeld (1966).

The susceptibilities of A. aphidimyza eggs and third instar larvae were tested by Adams and Prokopy (1977); total egg mortality was determined by counting unhatched eggs and dead newly-hatched larvae. No consistent differences among stages is evident (Table 1), although certain compounds may be stage-selective (i.e. azinphosmethyl and demeton). Stage tolerance may depend on properties of the pesticide as much as on the physiology, development, and ecology of the species.

II. Apple Aphid Control

Rosy apple aphids (Dysaphis plantaginea (Passerini)) and green apple aphids (Aphis pomi DeGeer) are the most frequent and abundant aphid pests in Michigan apple orchards (Brunner and Howitt 1981). Detailed biologies of these pests have been reported by several investigators (Matheson 1919, Lathrop 1928, Blackman 1974). Many species of natural enemies attack these aphids, including members of the following insect families: Syrphidae, Coccinellidae,

Table 1. Laboratory toxicity of orchard pesticides to eggs and larvae of _A. aphidimyza_ (from Adams and Prokopy 1977).

Compound	Concentration (amt/100 gal)	Percent Mortality Egg	Early first instar	Late instar
Phosmet 50WP	1.50 lb	8	24	18
Azinphosmethyl 50WP	0.62 lb	86	14	18
Endosulfan 50WP	1.00 lb	6	29	46
Demeton 6EC	0.31 pt	8	57	32
Phosalone 3EC	1.50 pt	4	0	10
Carbaryl 50WP	1.00 lb	72	21	-
Phosphamidon 8EC	0.25 pt	34	27	16
Cyhexatin 50WP	0.31 lb	14	0	12
Propargite 30WP	1.50 lb	6	2	-
Thiram 50WP	2.00 lb	6	0	8
Captan 50WP	1.00 lb	8	2	6
Control (H_2O) -	-	4	0	8

Anthocoridae, Miridae, Cecidomyiidae, Ichneumonidae, Cynipidae, Chamaemyiidae, Ceraphronidae, and Chrysopidae (Evenhuis 1961, Oatman and Legner 1961, Westigard and Madsen 1965, Holdsworth 1970, Specht 1972, Adams and Prokopy 1977). Typically pesticides are applied when aphid populations approach unacceptable levels, and field studies have indicated which pesticides are aphicidal (Madsen and Bailey 1959, Pielou and Williams 1961 a,b, Madsen et al. 1961, Cessac 1963, Asquith 1967, 1970, Forsythe and Hall 1973, Forsythe 1976). Several of the recommended insecticides produce satisfactory knockdown, but reinfestation and resurgence can occur quickly. Other compounds produce good aphid control but disrupt predator:prey complexes, especially in mites. Another drawback to chemical control is the development of resistance to organophosphorous compounds in *A. pomi* and to cyclodienes in the wooly apple aphid, *Eriosoma lanigerum* (Hausmann) (Georghiou and Taylor 1976).

Several integrated approaches to apple aphid control have been attempted with varying degrees of success reported (Holdsworth 1970, Bonnemaison 1972, Madsen et al. 1975). Adams and Prokopy (1977) proposed an integrated control program for Massachusetts based on biological control by the predatory cecidomyiid midge, *A. aphidimyza*, recommending selective pesticide use for control of major pests. Expansion of this program has included monitoring of aphid and midge densities, using action thresholds, and implementing a predator:prey index to keep aphid populations below damaging thresholds (Prokopy et al. 1980).

III. *Aphidoletes aphidimyza*

Taxonomic confusion has surrounded the aphidophagous cecidomyiids, but several recent studies have helped clarify the species of this family (Harris 1966, 1973, Nijveldt 1969). Gagne (1971) found only three valid species of *Aphidoletes* described for North America, with *A. aphidimyza* by far the most abundant and widespread. The biology of *A. aphidimyza* has been reviewed extensively (Barnes 1929, Harris 1973, Markkula et al. 1979a, Adams and Prokopy 1980). Adults (2mm) are active at dusk and nocturnally; honeydew secreted by aphids is utilized as a food source. This species is monogenic (Sell 1976) and each female lays approximately one hundred eggs in several small clusters, usually on the underside of aphid-infested leaves. Females are able to locate aphid colonies even at very low densities (El Titi 1973).

Eggs are 0.3mm long, smooth, and orange. Larvae hatch in two or three days, growing to 2.5 or 3mm at maturity (7-14 days). Three instars are generally reported although Azab et al. (1965) found evidence for four. Over 60 species of aphids have been reported as food sources (Harris 1973). Larvae usually attack aphids by piercing their leg joints, paralyzing the aphid and dissolving its internal structures; the desiccated body remains attached to the leaves by the mouthparts.

Reports of average larval consumption of aphids have varied, depending on aphid species, age, and density. Humidity, temperature, sex of larvae, and intra-specific competition also affect consumption (Markkula et al. 1979a). In an apple terminal caging study, Adams and Prokopy (1980) found the consumption of A. pomi per cecidomyiid larva varied between 4 and 65, with mean consumption of 27.9.

Larvae of this midge usually pupate in the soil, forming cocoons at a depth of 3cm, although cocoons may be found occasionally on the host plant. Adults usually emerge after 7 to 14 days. Diapause begins in September after several generations have been completed. Larvae overwinter in cocoons and pupate in spring, emerging in Michigan within the first two weeks of June (Morse, unpublished data).

Several investigators have tested the effects of some pesticides on A. aphidimyza. Markkula et al. (1979b) assessed the toxic effects of two fungicides and four insecticides when applied to the pupation medium. The fungicides were not toxic to the midge but the insecticides caused 80% or greater mortality, and their use is not recommended for soil applications. Several acaricides are considered safe for foliage applications in greenhouses (Markkula and Tiittanen 1976). The ovicidal activity of methomyl was tested by David et al. (1980). Their results indicate high toxicity to midge eggs, even at one-fourth the recommended rate of field application for Michigan (Jones et al. 1980).

Adams and Prokopy (1977) completed an evaluation of mortalities caused by several apple orchard pesticides in two life stages of A. aphidimyza. Eggs and late instar larvae were collected from a research apple orchard which had received no insecticide or miticide treatment for six years. These were exposed to ten pesticides at concentrations equivalent to recommended field rates. Mortalities were calculated for the egg stage, early first instars, and late instars (Table 1, p. 7). Endosulfan and phosmet were only moderately toxic to the stages tested, and since these compounds are of low toxicity to predatory mites, their use was suggested in control programs for both aphids and mites.

Evidence of resistance in the midges to azinphosmethyl was also reported. Eggs and larvae were collected from two sources, a commercial orchard and the untreated research orchard. Mortalities observed in the two samples may indicate resistance to the insecticide (Table 2). In their toxicity tests only fifty individuals were exposed to each pesticide, and the results may be complicated by starvation effects.

Table 2. Laboratory toxicity of azinphosmethyl (0.62 lb/100 gal) to eggs and larvae of two populations of *A. aphidimyza* (from Adams and Prokopy 1977).

	Percent Mortality			
Type of Orchard	Egg	Early first instar	Egg and early first instar	Late instar
Abandoned	86	14	88	18
Commercial	6	38	42	6

MATERIALS AND METHODS

I. Overview

Studies were designed to determine the physiological toxicities of pesticides to the life stages of *A. aphidimyza* and to detect resistance in orchard populations of this predator. Susceptibilities to azinphosmethyl were compared among the life stages of a single strain and among eggs of laboratory colonies of different origins. To detect resistance, LC50 values were estimated for eggs collected from 14 sites differing in pesticide exposure. To assess differential susceptibility in life stages among strains, first instar LC50 values for 4 populations were compared with corresponding egg susceptibilities. Toxicities of registered and experimental pesticides were evaluated for eggs and third instars of a laboratory colony.

Each developmental stage was exposed to compounds in a manner reflecting pesticide uptake in the field, although complete coverage of eggs and larvae was ensured through immersion to reduce variation attributable to differential exposure. LC50 values were estimated with probit analysis (Finney 1970), utilizing either the M.S.U. computer program BNPGPROBITANALYSIS or a package developed by this author for use with a programmable calculator

(Hewlett-Packard 25).

II. Collection and Rearing

Cecidomyiids were collected from the field using one of two methods: 1) gathering apple leaves infested with aphids and midge larvae, or 2) placing aphid-infested trap plants at the collection site to attract ovipositing females. With the first method, second and third instar larvae were transferred to fava bean plants (*Vicia* spp.) which were heavily infested with pea aphids (*Macrosiphum pisi* Harris). Plants were placed in screened cages (60 x 75 x 45cm) with sand and/or Vermiculite sprinkled on the cage floor; larvae dropped to the cage floor or soil surface to pupate. After one week aphid-infested bean plants were placed in the cage for oviposition by emerging adults. Rearing continued by placing plants with eggs in new cages where larvae developed. After pupation plant stems were cut to soil level and new plants were added after adult emergence.

In the second method aphid-infested bean plants were placed at the field collection site for one to three nights. To ensure egg collection, each pot of 5-6 plants was placed 25m from all other pots. Plants were retrieved and placed in rearing cages where the rearing process proceeded as described above. Approximately one hundred larvae were needed to establish a viable colony. Samples of males collected after rearing in 1979 were identified to species, and only A. aphidimyza was found among the collected specimens.

III. Comparison of Life Stage Susceptibilities to Azinphosmethyl

Most of the following experiments were conducted with individuals collected from a laboratory colony which originated from the Graham Research Station of Michigan State University, near Grand Rapids, Michigan. The original sample was collected from orchards which were treated with azinphosmethyl several times per season for many years. In the egg development study, the source of eggs was a colony which originated from a commercial orchard near Grand Rapids, Michigan (i.e. Anderson), and had received similar azinphosmethyl treatments.

A. Susceptibility to Azinphosmethyl - Eggs

A modified slide dip method (Nakashima and Croft 1974) was used to assess the LC50 for the egg stage. Eggs were collected from the laboratory colony on bean plants and transferred to double-stick tape (13x13mm) affixed to one end of a microscope slide. Twenty to thirty eggs were placed on each slide in rows of five or six. Eggs are usually laid in clusters of 3-20. To increase genetic variability per slide and minimize bias, no more than four eggs from each cluster were placed on each slide.

Slides with mounted eggs were held in a high humidity chamber consisting of a damp sponge in a clear plastic box while pesticide solutions were prepared. Eggs on each slide were inspected for damage; those injured during transfer appear shriveled, and the number was recorded on a tag attached to each slide, along with total eggs present. Subtraction of damaged from total eggs yielded the number of viable eggs considered in each treatment. Overall control mortality was assessed with a water dip for each experiment.

Each slide was randomly assigned to a dose and dipped (5 sec), drained, and allowed to dry for ten minutes, then placed in the humidity chamber at room temperature (21-25°C) under 16 hours of fluorescent light. Newly-hatched larvae can crawl across the tape; to prevent larval starvation, aphids were added to each slide prior to hatch. Unhatched eggs and dead larvae found on the tape were counted 72 hours after immersion, and egg mortality was determined for each dose. Larval mortality was nearly zero and was ignored in the mortality calculations. Mortalities were corrected for control mortality using Abbott's formula (1925), then probit analysis was applied to estimate the population LC50. Two sources of azinphosmethyl were used, both formulations being wettable powders with 50% active ingredient (a.i.) but differing in the year of production: 1976 and 1980. The tests were replicated five times using the 1976 azinphosmethyl, once with the 1980 source.

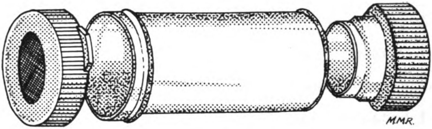
To determine the susceptibility of <u>A. aphidimyza</u> eggs as a function of embryological development, eggs were immersed in a .02% a.i. solution (1976 source) at different time intervals. Eggs were collected from the Anderson colony at two time periods, from 7 to 10 p.m. and 4 to 7 a.m., on two different nights. Eggs within each group (p.m. and a.m., respectively) were considered to be at the same stage of development ± 1.5 hours, with a nine hour lag in the a.m. group. Eggs were mounted on slides and randomly assigned to a time for immersion in the previously determined LC50 solution. The times for dipping were spread over two days as follows: Day I = 9 a.m., 1 p.m., 6 p.m.; Day II = 9 a.m., 1 p.m., 6 p.m. Eggs were placed in a humidity chamber until time of immersion, then dipped and returned to the chamber until hatch, with aphids added to each slide as previously described. Mortality was calculated for each time of immersion, and contingency tables were used to analyze the results, comparing day of dip, time of dip, and age of eggs when dipped to determine whether a period of greater susceptibility exists.

B. Susceptibility to Azinphosmethyl - Larvae

All instars were immersed in solutions of azinphosmethyl, and they also contacted residues during their movements after immersion. Field exposure is reflected by this method since larvae may contact the spray and residue during movement across the leaves. Estimates of LC50 values were obtained by exposing groups of larvae to different concentrations of azinphosmethyl, with water as the control, using corrected mortalities in probit analysis.

Two types of larval test chambers were constructed. Type I (Figure 1) consisted of a transparent plastic medicine vial (4.5 x 2.5 cm) with snap cap top. The bottom of each vial was cut off, the edge flamed, and another cap with extended sides was inserted. A section of fine mesh screen was attached to the snap cap by melting the edges into the plastic. Type II test chambers (Figure 1) were made from translucent nalgene vials (5.6 x 2.5 cm) with snap cap tops. Bottoms were removed and the top section of a 2.4 cm diameter vial was inserted to seal the chamber; fine mesh screening was attached to the snap cap. The major difference between chamber types was the fit of the bottom caps. Type I caps contacted the chamber tube at the flamed edge, leaving a gap between the tube and extended side of the cap where larvae were sometimes trapped. Type II caps contacted the chamber tube at the rim of the cap, about 1 cm into the tube.

The general larval test method consisted of removing the bottom cap and placing larvae on the sides of the chamber near the snap cap. The chamber was immersed in the pesticide solution for five seconds, then drained and blotted dry for 15 seconds. Aphids were added to the chamber (approximately 1.5 aphids per larva) and the bottom cap was inserted. The chamber was allowed to dry for one hour, then it was placed in a humidity chamber for the duration of the experiment. Specific details for each instar follow.



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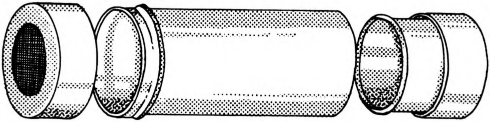

TYPE II

Figure 1.- Types of test chambers used to assess toxicities of pesticides to larvae of A. aphidimyza.

Twenty first instars (.6 mm long) were transferred to each Type II test chamber; to increase genetic variability, no more than five larvae from each leaf were placed in each chamber. After immersion and addition of aphids, the chambers were placed on their sides to dry, then placed in the humidity chamber for 24 hours. Numbers of dead larvae were recorded, with death defined as the inability to withdraw from the touch of a brush. Some surviving larvae crawled through the screening thus accurate counts of survivors could not be made. Mortality was calculated by dividing the number dead by the total number tested for each dose. Three replications of the LC50 estimate were made for the 1976 source of azinphosmethyl, one for the 1980 source.

Second instar larvae selected for testing were approximately 1.2 mm long. Mortality was calculated by dividing the number dead by the total number remaining in the chamber after 24 hours. This test was replicated four times with the 1976 azinphosmethyl and once with the 1980 source.

Third instars were approximately 2.3 mm long and were much more active than first and second instars; when handled they attempted to crawl away from the disturbance. Twenty larvae were placed in each Type I chamber which was immediately dipped and drained. Aphids were then added, and the bottom cap, containing 5cc of moistened sand, was inserted; the chamber remained upright throughout the test.

Larvae crawled down the sides, feeding on aphids, and survivors pupated in the sand. Aphids were added for three consecutive days, and dead larvae were counted on the fourth day. There were three replicates of this test using the 1976 source of azinphosmethyl and one test with the 1980 source.

C. Susceptibility to Azinphosmethyl - Adults

Adults cling to groundcover vegetation during the day, thus their primary exposure to azinphosmethyl is probably from residue contacted as they explore leaf surfaces. To estimate LC50 values for adults, residual toxicity was tested. Mason jars were thoroughly rinsed with the solution of azinphosmethyl (or water for controls) then drained and allowed to dry for one hour. Adults require a nutrient source for prolonged survival (Uygen 1971); a 1% honey and water solution was made available in the jars by soaking a small cellulose sponge with the solution and placing it in a small plastic cup. Males and females which had emerged within the previous 24 hours were collected from the rearing cage with an aspirator and were introduced to the treated jars. The rims were covered with fine-mesh cloth and rubber bands secured these. The number of dead adults in each jar was counted after one hour to determine mortality caused by handling, then the jars were placed in a tray of water at room temperature under 16 hours of light. Prolonged contact with the insecticide was likely since flies preferred clinging to the sides of the jars rather than the screening. Dead and live flies were counted after 24 hours, with death defined as immobility and usually coinciding with a prone position at the bottom of the jar. Mortality was calculated after deducting the first hour deaths, and probit analysis was applied to the corrected mortalities. The test was replicated three times using the 1976 source of azinphosmethyl.

IV. Susceptibilities of Field Populations to Azinphosmethyl

A. Eggs

In August 1980 cecidomyiid eggs were collected from fourteen separate sites in the southern half of the lower peninsula of Michigan. Each site can be classified in one of four categories:

1) N = no known pesticide exposure; nature preserves or wildlife experiment stations.
2) L = low probability of pesticide exposure; recently abandoned apple orchards or sites where pesticides may have been used but only infrequently and inconsistently.
3) C = commercial apple orchards where pesticide use is frequent and consistent, and azinphosmethyl is applied several times each season.
4) R = research orchards at fruit stations of Michigan State University, where pesticide

use is frequent and azinphosmethyl is applied several times each season.

Eggs were collected by placing aphid-infested trap plants around the collection site late in the afternoon, with eight to twelve pots per site. Early the following morning, all pots were retrieved and returned to the laboratory where eggs were promptly mounted on slides and dipped in solutions of the 1980 azinphosmethyl source. The ubiquitous presence of *A. aphidimyza* was evidenced by the effectiveness of this method; wherever plants were placed, eggs were found. Probit analysis was completed for each data set; to compare LC50 values, a t-test was used for two groups, low vs. high probability of azinphosmethyl exposure (N+L,C+R, respectively).

Colonies of *A. aphidimyza* established from field-collected samples were also tested for egg susceptibilities to the 1976 azinphosmethyl source. Probit analysis of the resulting mortalities provided estimated LC50 values for each population after colonization under laboratory conditions. The names assigned to the colonies tested are: Anderson, Graham, MSU, Klein, Warren, Rose Lake. Population locations and pesticide exposure histories are listed in Table 14, p. 44.

B. First Instar Larvae

When a sufficient number of eggs remained after completing the egg susceptibility tests, the first instars hatching on the bean plants were also treated, using the methods previously described. LC50 values were estimated for each population, and ratios of egg LC50 to first instar LC50 were compared to determine the constancy of the magnitude of the difference between stages among the different exposure histories.

V. Toxicities of Orchard Pesticides When Applied at Recommended Field Rates

A wide range of pesticides is currently registered for use on apples in Michigan, and several others are likely to be approved in the near future. Those compounds frequently applied by growers and some approved for experimental purposes were tested for mortalities produced in the two life stages of *A. aphidimyza*. Concentrations applied were those equivalent to maximum recommended field rates in the 1980 Fruit Pesticide Handbook (Jones et al. 1980), and are listed in Table 3. Eggs and third instar larvae were tested using methods similar to those described in Section II (A,B) with the following exceptions: 1) Mortalities for the first instar larvae which died on the tape were recorded and used in assessing the total mortality for the egg stage; 2) Third instar mortality was calculated after counting the number of emerged adults, usually two weeks after the immersion. Mortalities for eggs, early first instars, and third instars plus pupae are presented for comparison for each pesticide. Egg tests were replicated three or four times, with resulting mortalities averaged, while larval tests were replicated twice.

Table 3. List of pesticides tested for toxicity to *A. aphidimyza*.

Compound	Formulation	Field Rate (/100 gal)	Concentration (lbs ai/100 gal)
		ORGANOPHOSPHATES	
Dimethoate	4EC	1 pt	0.50
Diazinon	4EC	1 pt	0.50
Azinphosmethyl	50WP	.5 lb	0.25
Phosmet	50WP	1 lb	0.50
Phosphamidon	8EC	.25 pt	0.25
Demeton	6EC	.33 pt	0.25
Carbophenthion	8EC	.25 pt	0.25
Phosalone	3EC	1 pt	0.38
		CARBAMATES	
Methomyl	1.8L	2 pt	0.45
Pirimicarb	50WP	.25 lb	0.06
Carbaryl	80S	1.25 lb	1.00
Oxamyl	2L	1 pt	0.25
		SYNTHETIC PYRETHROIDS	
Permethrin[a]	2EC	.4 pt	0.10
Permethrin[b]	3.2EC	.25 pt	0.10
Fenvalerate	2.4EC	.33 pt	0.10
		CHLORINATED HYDROCARBONS	
Dicofol	35WP	1.33 lb	0.47
Endosulfan	3EC	1.33 pt	0.50

Table 3. Continued

Compound	Formulation	Field Rate (/100 gal)	Concentration (lbs ai/100 gal)
		MISCELLANEOUS	
Oxythioquinox	25WP	.5 lb	0.12
Propargite	6EC	.5 pt	0.38
Cyhexatin	50WP	.38 lb	0.19
Fenbutatin-oxide	50WP	.5 lb	0.25
		FUNGICIDES	
Bitertanol	50WP	.5 lb	0.25
Benomyl	50WP	.38 lb	0.19
Captan	50WP	2 lb	1.00
CGA 64251	10WP	.016 lb	0.002
Dodine	65WP	.5 lb	0.32
Manzeb+dinocap	80WP	2 lb	1.60
Metiram	80WP	2 lb	1.60

[a] ICI Formulation

[b] FMC Formulation

RESULTS AND DISCUSSION

I. Life Stage Susceptibility to Azinphosmethyl

A. Egg Susceptibility

After assessing the mortality caused by azinphosmethyl during *A. aphidimyza* embryonic development, a period of differential susceptibility was found which corresponds to the latter few hours of egg development. The data collected in this experiment are presented in Figures 2 and 3. In Figure 2, percent mortality is plotted against the time of day the eggs were immersed in the azinphosmethyl solution. Immersion time is confounded with embryonic age, and Figure 3 is the same data plotted against age of the embryo, with age zero corresponding to time of oviposition. The points are scattered, but average mortalities show a peak in susceptibility between 34 and 44 hours.

Contingency table analysis of total percent mortality for each cohort (PM and AM) for each date indicates independence of cohort effects and mortality (Table 4), both within each date and for the pooled data. However, comparison of mortalities from DAY I and DAY II shows a significant difference between days, with DAY II mortality 15.1% greater (Table 5). The effects of time of immersion were tested with the Kruskal-Wallis test (Zar 1974). No

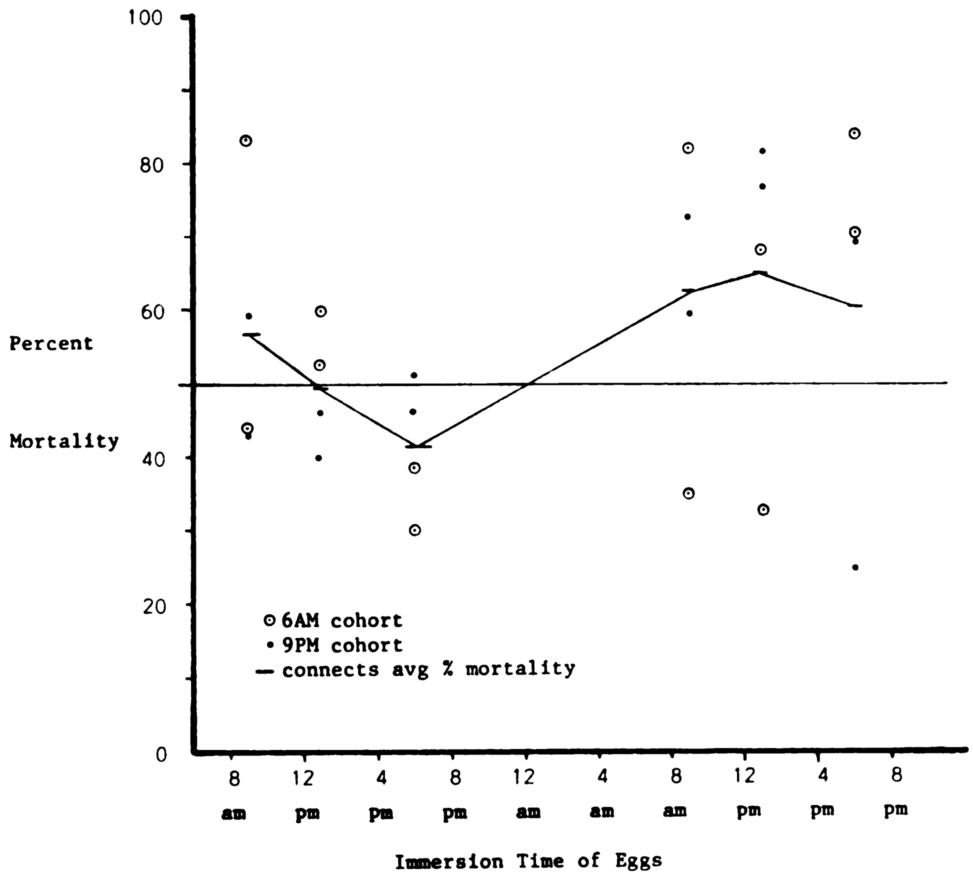

Figure 2. Mortality of A. aphidimyza eggs after immersion in azinphosmethyl (.02% a.i.) at various times during two consecutive days.

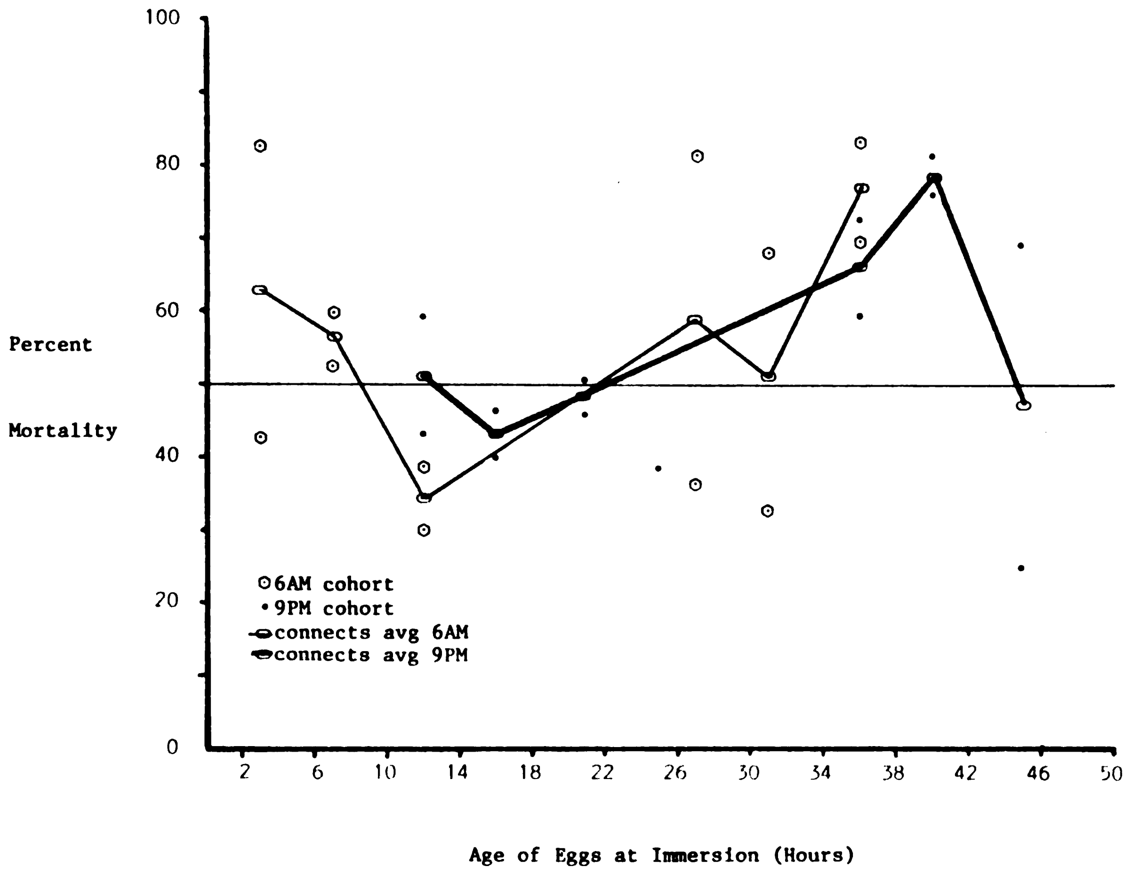

Figure 3. Mortality of A. aphidimyza eggs after immersion in azinphosmethyl (.02% a.i.) at various ages.

Table 4. Contingency table analysis of *A. aphidimyza* egg mortality: azinphosmethyl and cohort effects (after Zar 1974).

	11-1			11-17			Combined		
Cohort	Dead	Alive	% Mortality	Dead	Alive	% Mortality	Dead	Alive	% Mortality
9 pm	172	182	48.6	224	128	63.6	396	310	56.1
6 am	54	62	48.1	58	26	69.0	112	88	56.0
	$\chi^2 = 0.15$			$\chi^2 = 0.87$			$\chi^2 = 0.0005$		

$\chi^2_{0.05,1} = 3.84$; No significant differences.

Table 5. Contingency table analysis of *A. aphidimyza* egg mortality: azinphosmethyl and day of immersion.

Immersion	Dead	Alive	% Mortality
Day I	212	227	48.3
Day II	296	171	63.4

$\chi^2 = 20.92$, $\chi^2_{0.05,1} = 3.84$; Mortality is not independent of day of immersion.

significant differences ($P > 0.05$) were found in mortalities among the 3 dip times (Table 6), indicating time of immersion does not affect egg mortality.

Since time of dip has no effect on mortality, the hypothesis that age of the egg (corresponding to degree of embryological development) affects mortality was tested. Two ages are common to each of the four data sets: 12 and 36 hours. Homogeneity is accepted ($P > 0.10$) for these results (Table 7), and the contingency table analysis of the pooled data with subsequent large N produces the conclusion that mortality caused by exposure to the azinphos-methyl LC50 is dependent on the age of the embryo when immersed. Further tests of egg susceptibility in this study were conducted with eggs less than 28 hours in age.

Death of A. aphidimyza embryos occurred at or near the time of eclosion irrespective of age when treated, an observation consistent with the generalized response of embryos to organophosphates (OP's) reported by Smith and Salkeld (1966). In their review of ovicidal activities of pesticides, these authors hypothesized that the mode of action of OP's involved the delayed action of cholinesterase inhibition. During normal development acetyl choline and cholinesterase levels increase as the embryo matures. The presence of OP's inhibits cholinesterase but acetyl choline levels do not reach lethal levels until maturation, when neuromuscular activity increases. Death of less mature insect embryos is associated with much greater LC50

Table 6. Kruskal-Wallis test for effects of time of immersion in azinphosmethyl on egg mortality in *A. aphidimyza*.

		Percent Mortality (Rank)		
			Time of Immersion	
	Cohort	9 am	1 pm	6 pm
11-1	AM	40.5 (11)	42.5 (10)	56.4 (7)
	PM	52.8 (9)	58.7 (6)	35.4 (12)
11-17	AM	82.9 (1)	65.4 (3)	56.2 (8)
	PM	66.4 (2)	63.7 (4)	61.1 (5)

$H = 1.038$, $H_{0.05,4,4,4} = 5.692$; No significant difference.

Table 7. Contingency table analysis of *A. aphidimyza* egg mortality: azinphosmethyl and age of egg.

Age	Dead	Alive	% Mortality
12	62	69	47.3
36	103	63	62.0
		$\chi^2 = 6.43$	

$\chi^2_{0.05,1} = 3.84$; Mortality is not independent of age of egg.

values. The mode of action in the early stages probably differs from that in later stages which have more advanced development of metabolic and physiological systems; Smith and Salkeld suggest esterases as the target site in early embryos.

Embryonic retention of toxin after exposure to pesticides may obscure the relationship between physiological development and time of exposure. Assessing mortality after exposing eggs of differing ages to OP's could show which of the developing systems is most vulnerable to the toxin, but Smith and Salkeld (1966) found no reports of differential mortality associated with stages of embryogenesis. The results of this study show a period of greater susceptibility in _A. aphidimyza_ eggs corresponding to the completion of 70-90% of development. This period is probably associated with the development of the central nervous system in _A. aphidimyza_ embryos, evidence which supports the hypothesis of Smith and Salkeld.

B. Comparison of Life Stage Susceptibilities

Mortalities for each life stage were assessed with the 1976 source of azinphosmethyl, and results are listed in Table 8. The mean LC50 values for each stage are presented in Table 9 with significant differences found between the first instars and the eggs, second, and third instars ($p < 0.10$). Comparison of first instar and adult LC50 values showed no substantial difference; a high degree of variation exists in the adult data sets which may be due

Table 8. Probit analysis of susceptibilities of _A. aphidimyza_ life stages to azinphos-methyl (1976 source).

Stage	LC50	95% Fiducial Limits (Lower, Upper)	Regression Equation	χ^2	df	N
First Instar	.00682	.00458, .01513	Y = 1.39x + 8.01	0.7	1	160
	.00561	.00389, .00758	Y = 1.94x + 9.38	0.4	2	200
	.00508	.00421, .00613	Y = 3.01x + 11.91	4.6	2	200
Second Instar	.0086	.0024, .0154*	Y = 0.78x + 6.61	5.8	3	239
	.0127	.0096, .0176*	Y = 1.94x + 8.68	7.6[a]	2	196
	.0124	.0095, .0166	Y = 3.47x + 11.62	0.5	2	91
	.0165	.0123, .0220	Y = 1.76x + 8.14	4.8	3	214
Third Instar	.0364	.0251, .0589	Y = 1.52x + 7.18	1.7	3	180
	.0204	.0138, .0348	Y = 1.13x + 6.91	1.9	3	230
	.0152	.0049, .0262	Y = 1.01x + 6.84	5.3	2	150

Table 8. Continued.

Stage	LC50	95% Fiducial Limits (Lower, Upper)	Regression Equation	χ^2	df	N
Egg	.0481	.0256, .0704	Y = 3.55x + 9.68	0.9	2	97
	.0340	.0213, .0443	Y = 1.96x + 7.87	1.1	1	222
	.0135	.0085, .0168	Y = 3.97x + 12.42	1.8	3	463
	.0327	.0148, 2.014	Y = 1.31x + 6.94	0.1	1	634
	.0436	.0355, .0504	Y = 4.36x + 10.94	0.5	3	417
Adult	.0060	.0024, .0101	Y = 0.83x + 6.85	2.6	2	288
	.0043	.0017, .0071	Y = 0.90x + 7.14	1.8	1	271
	.0358	.0087, .3960	Y = 0.46x + 5.67	1.4	2	194

* 90% Fiducial Limits

[a] Value exceeds $\chi^2_{.05,2}$

Table 9. Mean LC50 values for susceptibilities of *A. aphidimyza* life stages to azinphos-methyl (1976 source).

Stage	Mean LC50	Replicates	Std. Dev.	Range	Test Method
Egg	.0344*	5	.0133	.0135, .0481	slide dip
First	.0058	3	.0009	.0051, .0068	dip + residue
Second	.0126*	4	.0032	.0086, .0165	dip + residue
Third	.0240*	3	.0110	.0152, .0364	dip + residue
Adult	.0154	3	.0177	.0043, .0358	residue

* Differs significantly ($\alpha = 0.10$) from first instar mean LC50 [Dunnett-type test (Gill 1978)].

to the difficulty of handling the fragile flies.

To further compare the three instars, the average weights of the larval sizes subjected to treatment were determined by weighing two groups of larvae for each instar. The ratios of LC50 values and mean weights appear to be geometric progressions (Table 10), each with a different mean (5.2 vs. 2). As larval weight increases, the LC50 increases but not at the same rate, probably reflecting the change in surface area and a corresponding change in the uptake of toxin per unit of weight, or actual dose.

As testing continued from 1979 to 1980 the activity of the 1976 source of azinphosmethyl seemed to diminish, and the 1980 source was obtained. Egg and larval stages were each tested once with the 1980 azinphosmethyl, and the results (Table 11) show consistent differences when compared with 1976 means (Table 12). Linear regression on the two sources explains 91% of the variation in the 1980 source; in subsequent comparisons the 1976 values are corrected, using the regression equation to approximate the 1980 susceptibility levels.

The ratios of the LC50 value for each stage to that of the first instar are listed in Table 12; ratios for each of the azinphosmethyl sources are approximately equal. Slopes from the regression lines for each stage have the same rank for both azinphosmethyl sources, although the slopes for the 1980 azinphosmethyl are consistently less. The lines derived from 1980 data are presented in Figure 4.

Table 10. Comparison of *A. aphidimyza* larval weights and corresponding LC50 values.

Instar	$\bar{x}$ weight (mg)	N	$\bar{x}$ LC50	Weight Ratio	LC50 Ratio
First	.021	140	.0058		
				5.2	2.2
Second	.109	76	.0126		
				5.2	1.9
Third	.572	40	.0240		

Table 11. Probit analysis of susceptibilities of *A. aphidimyza* life stages to azinphos-methyl (1980 source).

Stage	LC50	95% Fiducial Limits (Lower, Upper)	Regression Equation	χ^2	df	N
Egg	.0103	.0078, .0126	Y = 2.08x + 9.13	6.9	2	360
First	.0021	.0015, .0028	Y = 1.61x + 9.31	1.4	4	239
Second	.0053	.0040, .0079	Y = 1.56x + 8.55	1.4	3	238
Third	.0097	.0015, .0198	Y = 0.85x + 6.71	1.2	3	100

Table 12. Comparison of azinphosmethyl sources (1976 vs. 1980) for life stages of *A. aphidimyza*.

	LC50		LC50 Ratio (stage i/first instar)		Slopes	
Stage	1976	1980	1976	1980	1976	1980
Egg	.0344	.0103	5.9	4.9	3.0	1.6
First	.0058	.0021	1.0	1.0	2.1	1.6
Second	.0126	.0053	2.2	2.5	2.0	0.8
Third	.0240	.0097	4.1	4.6	1.2	2.1

Linear Regression of 1980 on 1976 Azinphosmethyl:

$$Y = .293x + .001$$

$$r^2 = .91, \ r = .96$$

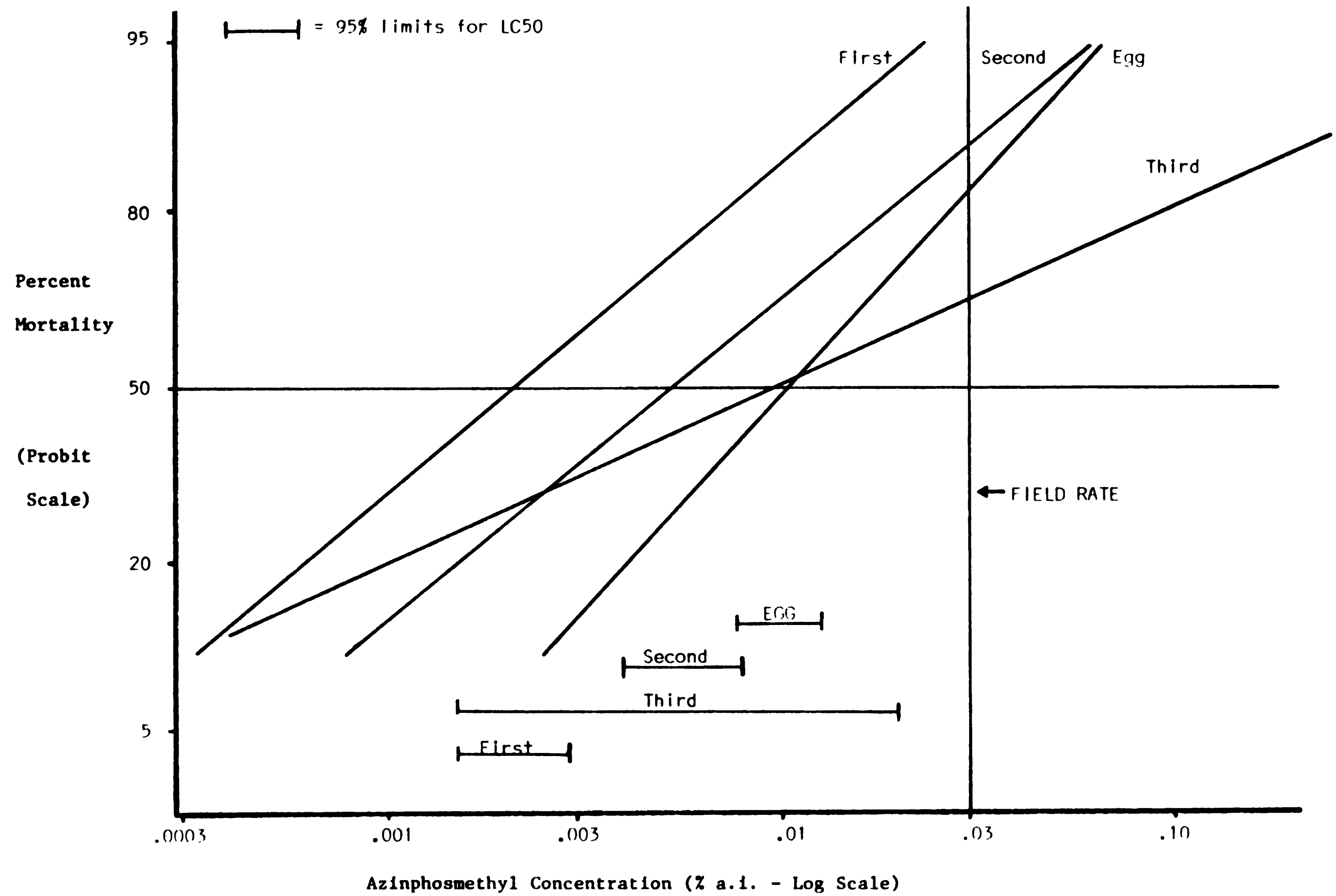

Figure 4. Susceptibility to azinphosmethyl of life stages of a laboratory colony of _A. aphidimyza_.

Mortalities expected in each stage when exposed to the maximum recommended field rate are predicted by observing the intersection of each regression line with .03%. First instar mortality will be greatest, approaching 97%, and third instar mortality least, about 66%.

These predictions are indicative of the direct toxicities expected after contact with the solution of azinphosmethyl and its residue, given an adequate food supply, temperatures between 21-24°C, and high humidity. The conditions of relatively high humidity and constant food supply can be expected in Michigan apple orchards throughout most of the season. Azinphosmethyl causes little mortality in _A. pomi_, although it has some knockdown effect (Pielou and Williams 1961b). Sublethal effects and the indirect toxicity in midge larvae after consuming contaminated aphids are not known. Research which addresses these topics is needed, as are field studies which will more precisely test the effects of field application of azinphosmethyl on life stages of _A. aphidimyza_.

II. Susceptibilities of Field Populations to Azinphosmethyl

A. Egg Stage

The locations of egg collection sites, their classifications, and the mortalities recorded for each dose tested are listed in Table 13. Results of probit analysis of this data are presented in Table 14, and none of the data

Table 13. Percent mortalities from azinphosmethyl in field-collected eggs of *A. aphidimyza*.

Population	County of Origin	Exposure History	Dose (% a.i.) .0005	.001	.002	.005	.010	.025	.050	.100	.200	.300	N
VerEllen	Macomb	C	-	-	-	4.2	7.8	15.0	57.8	54.1	95.7	-	290
Beck	Clinton	C	-	-	-	-	16.2	21.2	58.3	84.9	100.0	-	229
Erwin	Oakland	C	-	-	-	-	13.3	37.7	60.1	94.6	100.0	-	195
Royal	Kent	C	-	-	-	-	15.1	28.3	73.7	88.1	90.8	100.0	327
Klein	Kent	C	-	-	-	0.1	4.4	48.9	51.8	90.6	97.4	94.9	314
Anderson	Kent	C	-	-	-	2.7	20.8	42.2	69.2	87.0	97.2	90.0	279
Fennville	Allegan	R	-	-	-	0.0	15.9	20.2	45.0	91.7	-	-	426
Graham	Kent	R	-	-	-	12.0	22.6	41.8	60.5	95.7	100.0	100.0	381
Warren	Macomb	L	0.0	6.2	-	7.4	15.1	30.2	68.0	-	-	-	308
MSU	Ingham	L	-	-	0.0	2.4	8.7	48.0	92.6	-	-	-	215
John	Clinton	L	-	-	-	-	14.7	51.5	70.4	98.1	100.0	-	330
Heffron	Kent	L	-	-	-	7.8	27.7	69.0	83.0	95.0	100.0	100.0	291
Dansville	Ingham	N	-	-	-	0.0	5.3	49.1	94.6	-	-	-	344
Rose Lake	Clinton	N	-	-	0.2	5.8	20.9	44.0	80.8	-	-	-	334

C = Commercial orchard
R = Research orchard
L = Low probability
N = No known exposure

Table 14. Probit analysis of mortalities from azinphosmethyl in field-collected eggs of *A. aphidimyza*.

Population	Exposure History	LC50	95% Fiducial Limit (Lower, Upper)	Regression Equation	χ^2*	df	LC95
VerEllen	C	.061	.042, .081	Y = 2.12x + 7.58	9.4	4	.362
Fennville	R	.055	.045, .064	Y = 4.86x + 11.12	3.5	3	.120
Warren	L	.039	.029, .067	Y = 2.26x + 8.18	2.4	4	.103
Beck	C	.038	.030, .047	Y = 2.32x + 8.30	5.9	3	.194
Erwin	C	.034	.024, .044	Y = 2.65x + 8.90	2.2	3	.142
Royal	C	.034	.021, .046	Y = 2.17x + 8.19	5.6	4	.136
Klein	C	.033	.023, .044	Y = 2.29x + 8.38	7.4	5	.175
Graham	R	.031	.023, .039	Y = 2.59x + 8.89	9.5	5	.135
MSU	L	.030	.024, .035	Y = 6.26x + 14.56	0.7	3	.054
Anderson	C	.029	.019, .039	Y = 1.86x + 7.87	4.8	5	.145
John	L	.026	.021, .031	Y = 2.69x + 9.27	4.1	3	.106
Dansville	N	.026	.021, .030	Y = 5.55x + 13.83	0.1	3	.051
Rose Lake	N	.024	.019, .030	Y = 2.36x + 8.82	1.6	3	.120
Heffron	L	.018	.013, .022	Y = 2.36x + 9.15	1.1	3	.087

* No value exceeds $\chi^2_{.05,df}$; no significant deviation from regression equation.

sets deviates significantly from the regression line ($p > 0.05$). The populations are arranged in descending order of LC50 values. The sites with no or low probability of pesticide exposure are nearly separated from the commercial and research apple orchards. A t-test on the difference between means of the two groups (C+R, L+N) shows the means to be significantly different at the .025 level (Table 15). Ranges of LC95 values overlap at one point only (.12% a.i.); the group means differ significantly ($p \leq 0.01$, Table 15).

Resistance is often recognized as the ability of a population to survive field rates of the pesticide which normally kill the majority of susceptible populations. Additionally, a resistance ratio of 10 or more may indicate development of resistance in a population. The results of these tests support the hypothesis that resistance to azinphosmethyl has developed in some populations of _A. aphidimyza_. Although the ratio of maximum to minimum LC50 values is only 3.4 (VerEllen/Heffron), the data collectively represent two groups of populations with different pesticide exposure histories and differing mean values of lethal concentrations. Graphical presentation of the regression lines (Figures 5 and 6) provides further evidence of a low level of resistance development. Expected mortalities at the recommended field rate in the C+R populations are all less than 53% and as low as 10% in the Fennville population, while expected mortalities in the

Table 15. Comparison of population means for *A. aphidimyza* egg susceptibilities to azinphosmethyl.

Population type	Mean	Standard deviation	Replicates	t
		LC50		
C+R	.0394	.0119	8	2.23[a]
N+L	.0272	.0070	6	
		LC95		
C+R	.1761	.0788	8	2.96[b]
N+L	.0868	.0286	6	

[a] Significant at α = 0.025.

[b] Significant at α = 0.010.

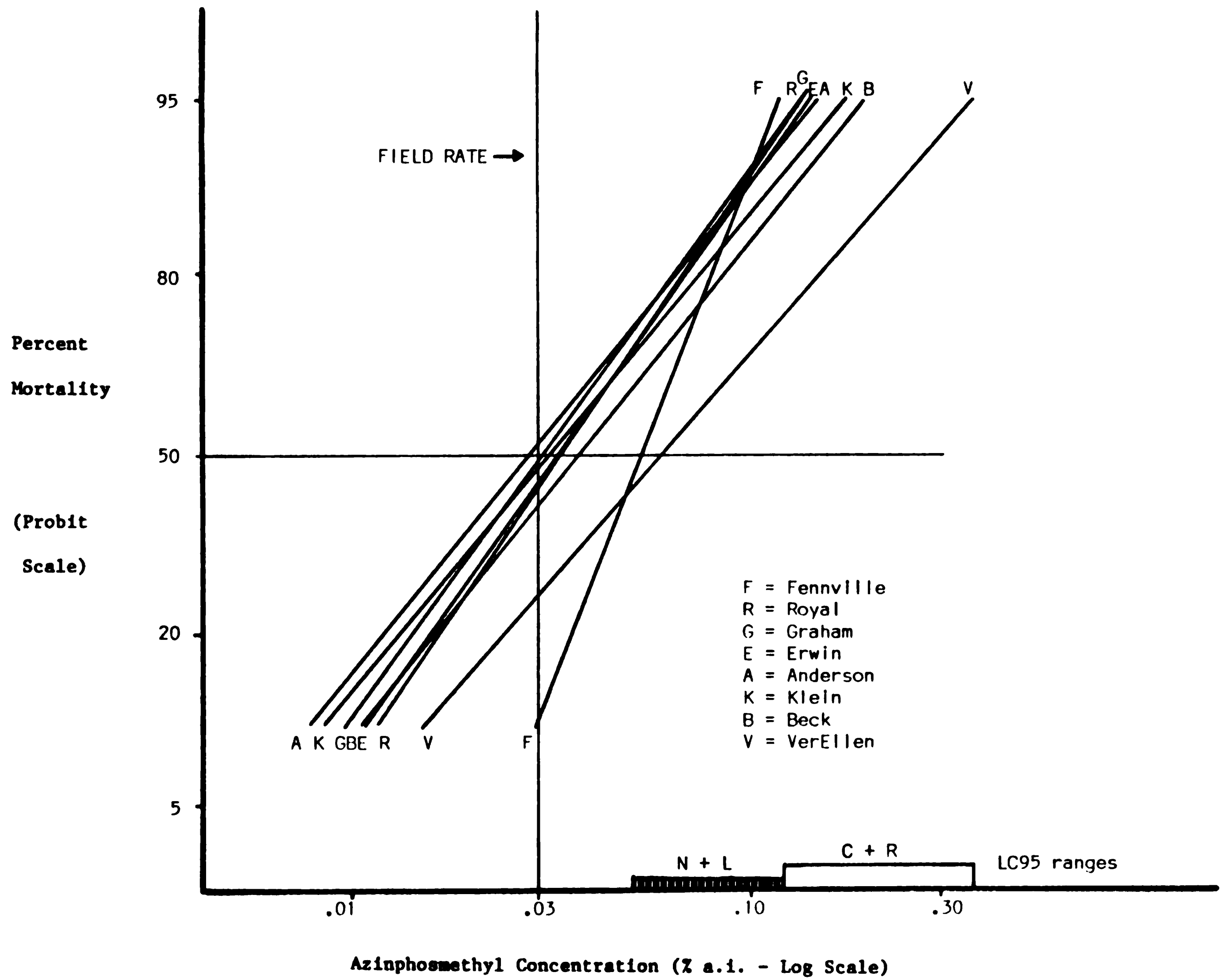

Figure 5. Susceptibility to azinphosmethyl of A. aphidimyza eggs collected from commercial and research apple orchards (C+R).

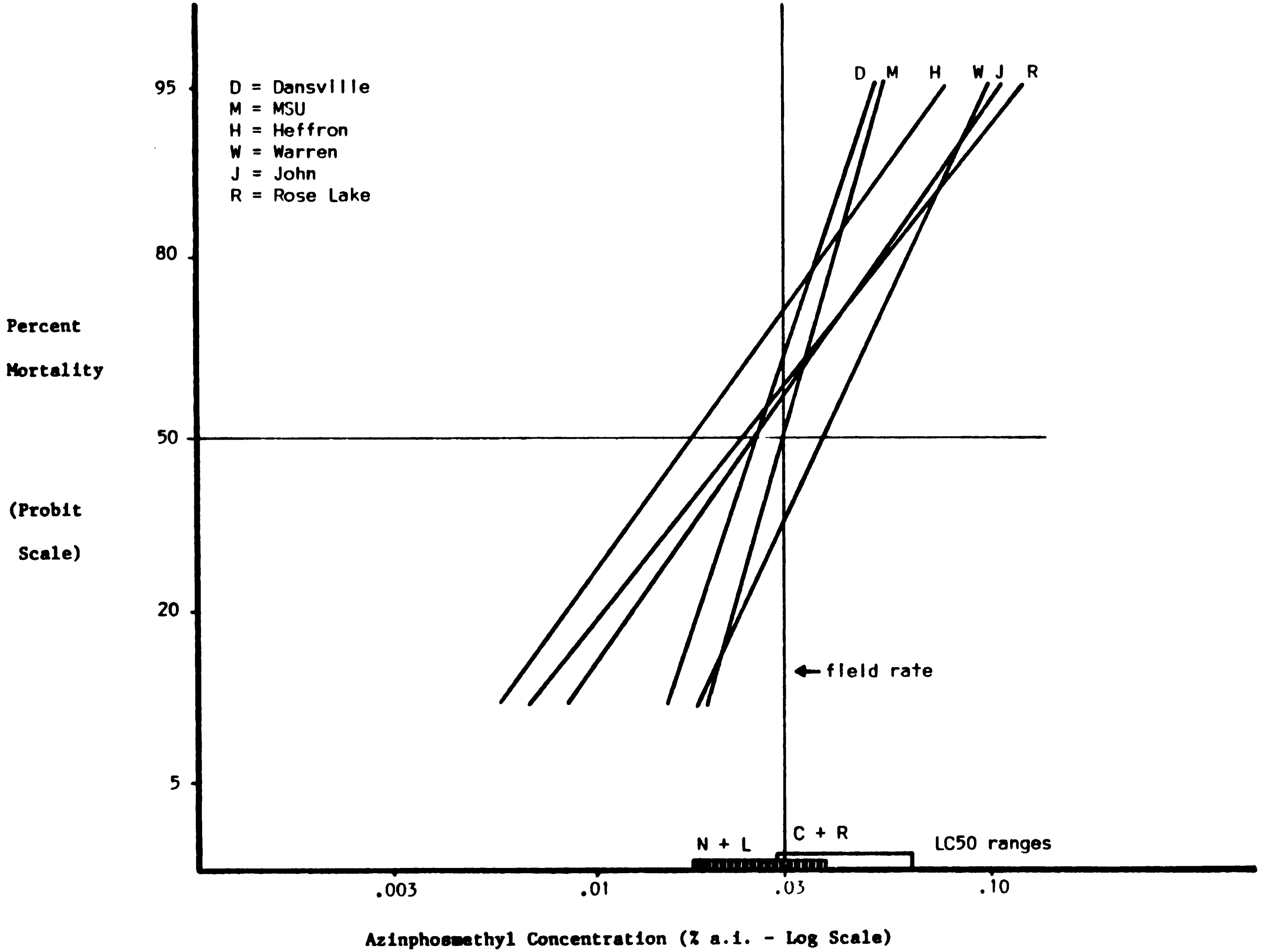

Figure 6. Susceptibility to azinphosmethyl of A. aphidimyza eggs collected from areas of little or no pesticide exposure (N+L).

N+L populations are generally above 50%, with the exception of Warren.

FAO (1969) recommends comparing susceptible LC50 values with those from field populations suspected of resistance development. The most susceptible populations found are those which have been colonized in the laboratory, with significant increases in susceptibility occurring after colonization (Table 16). Errors in correction of the 1976 source of azinphosmethyl could account for some of the differences, but 1980 source measurements for two of the colonies indicate the validity of the correction. Comparing the VerEllen LC50 with the lab LC50 for the Warren colony yields a 14-fold difference in susceptibility levels, a ratio indicative of low-level resistance development in the VerEllen population.

B. First Instar Larvae

The field survey of larval susceptibilities was not as extensive as that for the egg stage. One population from each type of exposure history was included, with results presented in Table 17. LC50 values for all but Rose Lake are considerably greater than that of the Graham lab colony; regression lines are plotted in Figure 7. Predicted mortalities for exposure to field rates of the pesticide indicate no survival for the Rose Lake population and 75-82% mortality for the other three populations.

To determine whether the magnitude of difference between egg and first instar stages is relatively constant,

Table 16. Comparison of *A. aphidimyza* egg LC50 values for laboratory colonies and field populations.

Population	1979* Lab Colony	Field Survey	1980 Lab Colony
MSU	.018	.030	-
Klein	.011	.033	-
Anderson	.007	.029	-
Graham	.011	.031	.010
Rose Lake	.009	.024	-
Warren	.004	.039	.005

Paired t-test:

$t = 15.64^a$

* Corrected to 1980 azinphosmethyl source.

[a] Significant at $\alpha = 0.01$.

Table 17. Azinphosmethyl susceptibility in first instar larvae of field-collected populations of _A. aphidimyza_.

Percent mortality

Population	Exposure History	Dose (% a.i.) .00075	.003	.010	.030	N
Royal	C	6.7	35.0	55.0	73.3	90
Graham	R	18.0	43.2	65.1	75.6	210
Rose Lake	N	7.4	62.5	92.6	100	124
Heffron	L	14.3	25.0	52.6	88.9	104

Probit analysis of data

Population	LC50	95% Fiducial Limits (Lower, Upper)	Regression Equation	χ^2*	df
Royal	.0080	.0043, .0182	Y = 1.23x + 7.58	0.5	2
Graham	.0050	.0030, .0080	Y = 1.00x + 7.32	0.5	2
Rose Lake	.0025	.0018, .0034	Y = 2.62x + 11.83	0.4	2
Heffron	.0068	.0040, .0125	Y = 1.39x + 8.02	2.1	2
Graham-Lab	.0021	.0015, .0028	Y = 1.61x + 9.31	1.4	4

* No value exceeds $\chi^2_{.10,df}$; no significant deviation from regression equation.

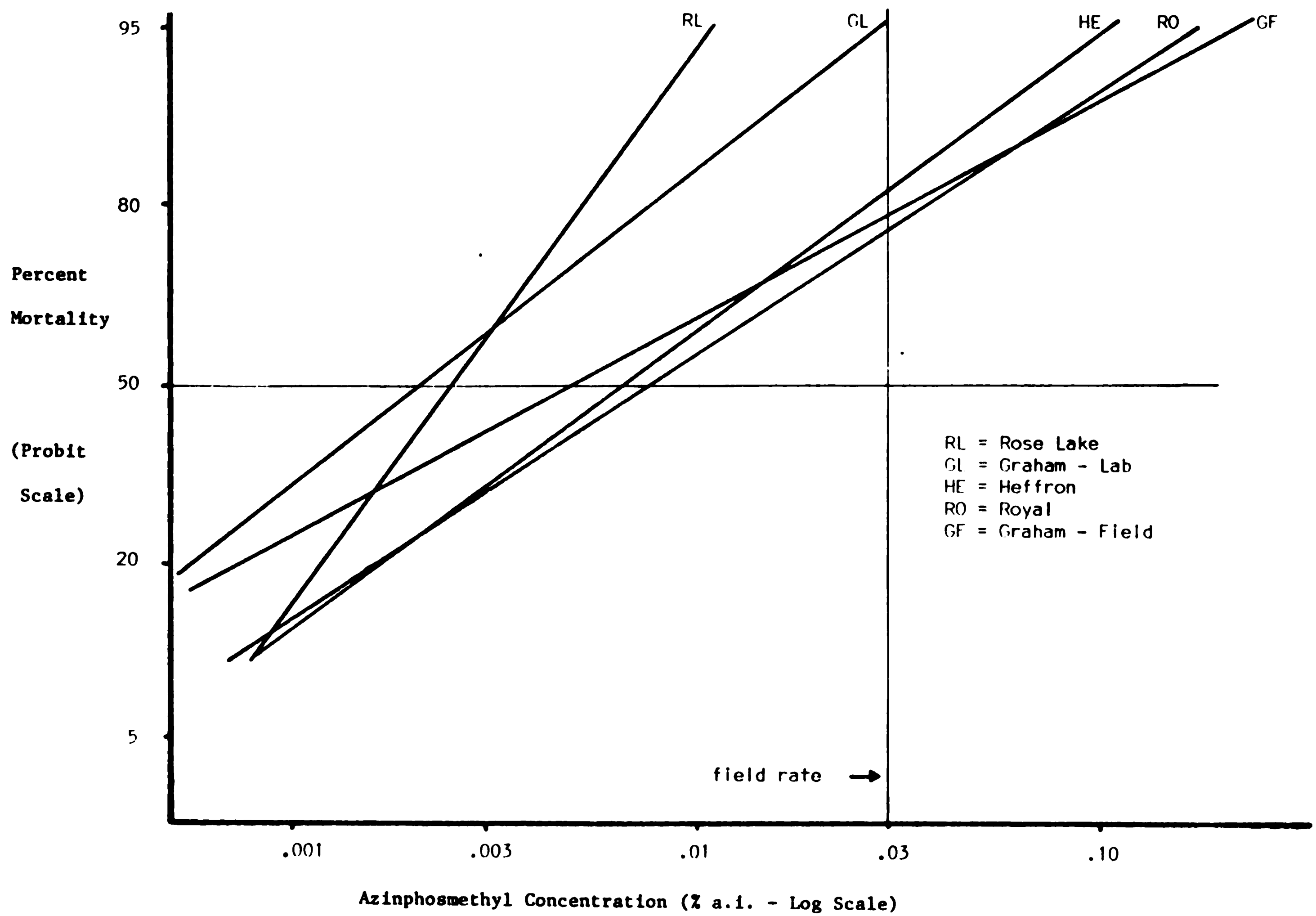

Figure 7. Susceptibility to azinphosmethyl of <u>A. aphidimyza</u> first instar larvae collected from laboratory and field populations.

the ratios of egg LC50 to larval LC50 for each population were compared (Table 18). The ratios vary considerably among the populations, but averaging the two high exposure and the two low exposure population values yields ratios which are of similar magnitude, and which are equivalent to the Graham lab colony ratio. This information supports the hypothesis but much more evidence is needed before generalizing the results.

III. Toxicities of Orchard Pesticides

Differential mortality occurred in the two types of test chambers used in these experiments. Eight of the pesticides tested had measures for both chamber types, and a t-test analysis of mortalities showed a significant difference between the two chambers (Table 19). Linear regression of Type II on Type I yielded an equation for correcting the Type II mortalities to equivalent mortalities for Type I chambers, with 82% of the total variation in the corrected values explained by the fitted regression. The following discussion uses these corrected values and they are indicated by the superscript "*".

The pesticides tested are classified into three groups: those causing high mortality (>50%) in both stages, those causing high mortality (>48%) in one stage only, and those causing low mortality (<30%) in both stages (Tables 20-22). In the high mortality group, azinphosmethyl appears to be the least toxic compound, but the

Table 18. Comparison of azinphosmethyl LC50 values for eggs and first instars of field-collected *A. aphidimyza*.

Population	Egg LC50	First Instar LC50	Ratio (Egg/First)
Royal	.034	.0080	4.2
Graham	.031	.0050	6.2
Rose Lake	.024	.0025	9.6
Heffron	.018	.0068	2.6
Graham-Lab	.010	.0021	4.8
		Average Values	
C + R	.0325	.00650	5.0
L + N	.0210	.00465	4.5

Table 19. Comparison of *A. aphidimyza* third instar mortalities from azinphosmethyl for two types of test chambers.

	Corrected Percent Mortality		
Compound	Type I	Type II	
Azinphosmethyl	60.3	25.0	
Fenvalerate	18.9	13.3	
Oxamyl	9.5	23.3	
Phosmet	21.4	15.9	Paired t-test:
Dimethoate	86.7	57.4	$t = 2.55$[a]
Demeton	78.2	66.7	
Endosulfan	83.3	53.3	
Morestan	6.7	6.7	

Linear Regression: Type I Mortality =

1.38 (Type II Mortality) + .56

$r^2 = .82$, $r = .91$

[a] Significant at $\alpha = .05$.

Table 20. Pesticides causing high mortality in eggs and larvae of *A. aphidimyza*.

	Percent Mortality			
Compound	Egg	Early first instar	Egg + first instar	Third instar + pupa
Diazinon	78	100	100	100
Methomyl	99	100	100	99
Carbaryl	95	44	97	75
Demeton	69	59	84	78
Dimethoate	38	33	60	87
Azinphosmethyl	55	4	56	60

Table 21. Pesticides causing stage-selective mortality in eggs and larvae of *A. aphidimyza*.

	Percent Mortality			
Compound	Egg	Early first instar	Egg + first instar	Third instar + pupa
Oxythioquinox	80	38	87	7
Phosmet	68	26	71	21
Permethrin[a]	68	6	69	11
Fenvalerate	45	30	56	19
Oxamyl	39	30	48	10
Endosulfan	7	6	13	83

[a] ICI formulation (2EC).

Table 22. Pesticides causing low mortality in eggs and larvae of *A. aphidimyza*.

Compounds	Egg	Early first instar	Percent Mortality Egg + first instar	Third instar + pupa
Permethrin[b]	24	2	25	4
Phosalone	9	8	16	8*
Phosphamidon	20	2	21	18*
Carbophenthion	9	17	25	15*
Pirimicarb	0	2	1	0
Dicofol	0	1	2	12*
Fenbutan-oxide	2	2	4	10*
Propargite	0	0	1	14*
Cyhexatin	4	0	4	3*
Captan	3	0	3	1*
Benomyl	4	0	4	2*
Dodine	2	0	2	1*
Manzeb+Dinocap	2	0	2	10*
Metiram	2	0	2	27*
Bitertanol	2	0	2	1*
CGA 64251	4	0	4	4*

* % mortality corrected for test chamber differences.

[b] FMC formulation (3.2EC).

data are from the 1976 source; mortalities caused by the 1980 source will be greater. Of the stage-selective compounds, five are more ovicidal than larvicidal; only endosulfan is less toxic to eggs than larvae. Stage selectivity is significant when a pesticide is applied to control apple pests; the majority of A. aphidimyza in the favored stage will survive the treatment, allowing for continued biological control of aphids.

Most of the low mortality pesticides are fungicides and acaricides but several insecticides could be useful in an IPM program for aphids. Pirimcarb is an aphicide not yet registered for use on apples which appears to have no direct toxic effects on A. aphidimyza and which could possibly be applied to reduce aphid populations to levels more favorable for midge control. Carbophenthion provides good control of San Jose scale, rosy apple aphid, woolly apple aphid, and white apple leafhopper (Jones, et al. 1980). Phosphamidon provides excellent control of rosy and green apple aphids. Phosalone is recommended for leafroller and codling moth control while also providing good control of apple maggot, spotted tentiform leafminer, pest mites, and aphids.

Direct comparison of these results with those of Adams and Prokopy (1977) is hampered by differences in dosage and/or formulation. Table 23 lists corrected mortalities for both data sets. Third instar mortalities for Massachusetts are generally less than the larval plus

Table 23. Mortalities caused by orchard pesticides in life stages of _A. aphidimyza_, from two separate studies.

	Adams and Prokopy 1977			Warner 1981		
Compound	Egg + first instar	lbs a.i.	Third instar	Egg + first instar	lbs a.i.	Third instar + pupa
Phosmet	22	.75	11	71	.50	21
Azinphosmethyl	87	.31	11	56[a]	.25	60[a]
Azinphosmethyl (Fitchburg)	39	.31	3	-	-	-
Endosulfan**	27	.50	41	13	.50	83
Demeton	56	.23	26	84	.25	78
Phosalone	0	.56	2	16	.38	8*
Carbaryl**	76	.50	-	97	1.00	75
Phosphamidon	47	.25	9	21	.25	18
Cyhexatin	4	.16	4	4	.19	3*
Propargite	0	.50	-	1	.38	14*
Captan	0	.50	0	3	1.00	1*

[a] 1976 azinphosmethyl source

* corrected for chamber differences

** formulations differed

pupal mortalities found in this study, but no trend among egg mortalities is apparent. Compounds which caused little egg plus first instar mortality in this study produced similar low mortalities in the study by Adams and Prokopy.

Low survival of eggs and first instars appears in both data sets for carbaryl, demeton, and azinphosmethyl. The Fitchburg population may represent a resistant strain, especially since the majority of the mortality is from early instar death, a factor virtually eliminated with the addition of aphids to test conditions in the present study. Phosmet and phosphamidon are exceptions to egg mortality classification. Phosmet results in the present study varied, with one replicate showing 11% mortality while the other three had greater than 75% mortality. Phosphamidon mortality primarily differs by the amount of early larval mortality in Adams' and Prokopy's work; again, starvation may be confounding their results.

The results of this study are consistent with other findings. Markkula et al. (1979b) reported emergence of adults from treated pupae was unaffected by the fungicides benomyl and thiram, while diazinon and malathion caused 100% mortality, and mevinphos and pyrethrin caused approximately 80% mortality each. Markkula and Tiittanen (1976) treated second and third instars with acaracides, finding all six pesticides produced less than 10% mortality, including oxythioquinox and dicofol, consistent with results in Table 22. Ovicidal activity of methomyl reported by David et al. (1980) is consistent with the 100% mortality found in this study.

Laboratory reports on the direct toxicities of pesticides against particular life stages of arthropods are useful for identification of highly toxic and practically non-toxic compounds. Results reported here are based on a single strain reared in the laboratory, and test methods may not approximate actual exposure in the field. Larvae on vegetation tend to congregate under aphids and may avoid direct contact with pesticide spray and with the residue if aphid colonies are dense. Indirect effects of pesticides have not been considered here, and consumption of contaminated aphids may have lethal or sublethal effects. In addition, food source disruption can occur if pesticides are applied which are toxic to aphids or are effective knockdown agents. Although larvae are mobile, they are restricted to crawling over plant surfaces to find aphids, and they seem to have limited powers of prey location. Interspecific and intraspecific competition is generally not considered in tests of pesticide effects. In this screening study most of the variables were controlled in order to assess the direct toxic effects of pesticides on *A. aphidimyza*, resulting in information useful in initial preparation of integrated pest management recommendations.

CONCLUSION

Susceptibilities of *A. aphidimyza* life stages to azinphosmethyl follow the generalization proposed by Bartlett (1964a), that of adult susceptibility being greatest, eggs least, and larvae intermediate, if adult survival after direct exposure to spray is assumed to be much less than survival after residue exposure. During embryogenesis maximum susceptibility occurs after 70% of development is complete, a finding which may shed light on the mode of action of azinphosmethyl.

Low levels of resistance were found in selected populations of *A. aphidimyza*. This might be expected considering the already high levels of tolerance to azinphosmethyl, with field mortality not exceeding 90% for any stage except first instar larvae (Figure 4). Resistance in this species could be diluted easily considering the dispersal potential of adults. The polyphagous habits and ubiquitous presence in habitats surrounding orchards (Harris 1973, Morse unpublished) could produce a constant influx of susceptible individuals, diluting the resistance genes and maintaining low levels of resistance in orchard populations. Furthermore, azinphosmethyl resistance may be unstable as manifested by the increased susceptibility of populations after 2 or more generations of laboratory colonization (Table 16). Many authors (e.g. Keiding 1967) have noted unstable resistance to organophosphorous compounds in laboratory strains of arthropods. If an unstable, low level of resistance is present in A. aphidimyza, elimination of migration of susceptible wild types during laboratory selection experiments would possibly stabilize resistance and increase levels substantially.

If the primary mode of action of azinphosmethyl is inhibition of cholinesterase, as suggested by Smith and Salkeld (1966), similar levels of resistance might appear in each life stage; weak evidence for this was found (Table 17). Resistance to the mode of action of azinphosmethyl may be present in the embryo, even if selection pressures are more intense on other stages. After selecting larvae of the housefly Musca domestica with diflubenzuron, Grosscurt (1980) found both larvicidal and ovicidal resistance had developed, though he suggests they are not linked. The presence and functioning of resistance mechanisms among life stages could provide a basis for stabilization of resistance in insect species.

Several pesticides with little direct toxicity to A. aphidimyza have been identified in the survey of pesticide mortalities. All fungicides and most acaricides are placed in this category, as are several insecticides. Phosphamidon and carbophenthion are currently registered on apple as is phosalone, a compound highly toxic to predatory mites and not recommended for use in integrated mite control in Michigan. Pirimicarb has potential for reducing aphid populations to levels more favorable for control by midges, but it is not registered for apples. The permethrin formulations differed in toxicity to *A. aphidimyza* and could be applied without severely disrupting midge populations, but introduction of synthetic pyrethroids in apple has been discouraged by Croft and Hoyt (1978). Predatory mites are highly susceptible to these compounds, and pest mite outbreaks may occur.

Applications of stage-selective compounds would ensure survival of most individuals in the favored stage, allowing continuation of biological control within the orchard. Since egg toxicities are much greater than larval plus pupal toxicities (except endosulfan), these insecticides do not follow Bartlett's generalization of stage tolerance in natural enemies. Other factors may determine life stage susceptibilities, such as pesticide mode of action, development of detoxification systems, and penetration of toxin. Application of compounds from the high mortality group should not be recommended unless a large proportion of the population is pupating, escaping direct contact with the pesticide.

Adults of *A. aphidimyza* do not emerge until June, preventing biological control of early season aphid populations by this species. Pesticide applications prior to *A. aphidimyza* emergence can include compounds highly toxic to this midge. Further research which assesses field toxicities of orchard pesticides to midges and aphids and combines toxicity information with effective monitoring for predator:prey ratios will assist in the implementation of IPM for aphids in apples.

LIST OF REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Adams, R. G. Jr., and R. J. Prokopy. 1977. Apple aphid control through natural enemies. In: Lord, W. J. and W. J. Bramlage, eds. Fruit Notes 42: Cooperative Extension Service, Univ. Mass., 6-10.

__________. 1980. Aphidoletes aphidimyza (Rondani) (Diptera: Cecidomyiidae): an effective predator of the apple aphid (Homoptera: Aphididae) in Massachusetts. Protection Ecology 2: 27-39.

Asquith, D. 1967. Mite and apple aphid control on apple trees following soil applications of Temik. J. Econ. Entomol. 60: 817-819.

__________. 1970. Codling moth, red-banded leaf roller, apple aphid, European red mite, and two-spotted spider mite control on apple trees. J. Econ. Entomol. 63: 181-186.

Azab, A. K., M. F. S. Tawfik, and I. I. Ismail. 1965. Morphology and biology of the aphidophagous midge, Phenobremia aphidivora Rubsaamen. Bull. Soc. Entomol. Egypte 49: 25-45.

Barnes, H. F. 1929. Gall midges (Diptera: Cecidomyiidae) as enemies of aphids. Bull. Entomol. Res. 20: 433-442.

Bartlett, B. R. 1964a. Toxicity of some pesticides to eggs, larvae, and adults of the green lacewing, Chrysopa carnea. J. Econ. Entomol. 57: 366-369.

__________. 1964b. Integration of chemical and biological control. In: P. DeBach, ed. Biological Control of Insect Pests and Weeds. New York: Reinhold, 844 pp.

Blackman, R. L. 1974. Aphids. Ginn. B. Co. Ltd: London, 175 pp.

Blair, B. D. and C. R. Edwards. 1980. Development and status of extension integrated pest management programs in the United States. Bull. Entomol. Soc. Amer. 26: 363-368.

Bonnemaison, L. 1972. Integrated control of apple orchard aphids. Abst., Revue de Zoologie Agricole: 48-64.

Brunner, J. F. and A. J. Howitt. 1981. Tree Fruit Insects. Cooperative Extension Service, Michigan State University: 96 pp.

Cessac, M. 1963. [Efficacite en plein champ d'un acaricide nouveau: L'ethion (8.167 R. P.)]. Abstr., Phytiat. Phytopharm. 9: 87-94.

Colburn, R. and D. Asquith. 1971. Tolerance of the stages of Stethorus punctum to selected insecticides and miticides. J. Econ. Entomol. 64: 1072-1074.

Croft, B. A. 1975. Integrated Control of Apple Mites. Coop. Ext. Service, Michigan State Univ. Bulletin E-825.

__________, and A. W. A. Brown. 1975. Responses of arthropod natural enemies to insecticides. Ann. Rev. Entomol. 20: 285-335.

__________, and S. C. Hoyt. 1978. Considerations for the use of pyrethroid insecticides for deciduous fruit pest control in the U.S.A. Environmental Entomol. 7: 627-630.

__________, and J. G. Morse. 1979. Recent advances on pesticide resistance in natural enemies. Entomophaga 24: 3-11.

David, P. J., R. L. Horsburgh, J. P. McCaffrey, and L. F. Ponton. 1980. Apple: the ovicidal effects of methomyl (Lannate-L) on beneficial insects. Pesticide Research Experiments, Shenandoah Valley Res. Sta., VA Polytech. Inst. and State Univ.

Elliot, W. M. and M. J. Way. 1968. The action of some systemic aphicides on the eggs of Anthocoris nemorum (L.) and A. confusus Reut. Ann. Appl. Biol. 62: 215-226.

El Titi, A. 1973. [Influences of prey density and morphology of the host-plant on the egg distribution of the aphidophagous gall midge Aphidoletes aphidimyza (Rond.) (Diptera: Itonididae)]. Abst., Z. Ang. Entomol. 72: 400-415.

Evenhuis, H. H. 1961. Some notes on the dipterous enemies of aphids harmful for apple growing in Nova Scotia. Canadian Entomol. 43: 1020-1021.

Food and Agriculture Organization (FAO). 1969. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. FAO Plant Protection Bulletin 17: 76-82.

__________. 1979. Pest resistance to pesticides and crop loss assessment - 2. Report of the Second Session of the FAO Panel of Experts, Plant Production and Protection; Paper 6/2, Rome.

Forsythe, H. Y. Jr. 1976. Control of the Rosy Apple Aphid in Ohio. Ohio Agric. Res. and Dev. Center no. 214, Wooster, Ohio.

__________, and F. R. Hall. 1973. Summer Control of the Apple Aphid in Ohio. Research Circular 196, Ohio Agric. Res. and Dev. Ctr., Wooster, Ohio.

Finney, D. J. 1970. Probit Analysis. New York: Cambridge Univ. Press, 333 pp.

Gagne, R. J. 1971. The genus _Aphidoletes_ Kieffer (Diptera: Cecidomyiidae) in North America. Entomol. News 82: 177-181.

Georghiou, G. P. 1979. The management of insecticide resistance. Symposium on Pesticide Resistance, International Congr. of Plant Protection, Washington D.C.

__________, and C. E. Taylor. 1976. Pesticide resistance as an evolutionary phenomenon. Proceedings of XV International Congress of Entomol., Washington D.C. 759-785.

__________. 1977a. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 319-323.

__________. 1977b. Operational influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 653-658.

Gill, J. L. 1978. Design and Analysis of Experiments, vol. 1. Iowa State University Press: Ames, 409 pp.

Grosscurt, A. C. 1980. Larvicidal and ovicidal resistance to diflubenzuron in the house fly (_Musca domestica_). Proc. K. Akad. Wet. Ser. C Biol. Med. Sci. 83: 127-142.

Harris, K. M. 1966. On gall midge genera (Diptera: Cecidomyiidae). R. Entomol. Soc. Lond. 118: 313-358.

__________. 1973. Aphidophagous Cecidomyiidae (Diptera): taxonomy, biology, and assessments of field populations. Bull. Entomol. Res. 63: 305-325.

* Holdsworth, R. P. Jr. 1970. Aphids and aphid enemies: effect of integrated control in an Ohio apple orchard. J. Econ. Entomol. 63: 530-535.

Jones, A. L., M. E. Whalon, and J. A. Flore, eds. 1980. The 1980 Fruit Pesticide Handbook. Coop. Ext. Ser., Mich. State Univ. Ext. Bull. E-154, 95 pp.

Keiding, J. 1967. Persistence of resistant populations after the relaxation of the selection pressure. World Review of Pest Control 4: 115-130.

Lathrop, F. H. 1928. The biology of apple aphids. The Ohio Journal of Science 28: 177-204.

Madsen, H. F. and J. B. Bailey. 1959. Control of the apple aphid and the rosy apple aphid with new spray chemicals. J. Econ. Entomol. 52: 493-496.

Madsen, H. F., P. H. Westigard, and L. A. Falcon. 1961. Evaluation of insecticides and sampling methods against the apple aphid, *Aphis pomi*. J. Econ. Entomol. 54: 892-894.

Madsen, H. F., H. F. Peters, and J. M. Vakenti. 1975. Pest management: Experience in six British Columbia apple orchards. Canadian Entomol. 107: 873-877.

Markkula, M., and K. Tiittanen. 1976. Mortality of *Aphidoletes aphidimyza* larvae treated with acaricides. Annales Agric. Fenniae 15: 86-87.

__________, M. Hamalainen, and A. Forsberg. 1979a. The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. Ann. Ent. Fenn. 45: 89-98.

Markkula, M., M. Rimpilainen, and K. Tiittanen. 1979b. Harmfulness of soil treatment with some fungicides and insecticides to the biological agent *Aphidoletes aphidimyza* (Rond.) (Dipt., Cecidomyiidae). Ann. Agric. Fenn. 18: 168-170.

Matheson, R. 1919. A study of plant lice injuring the foliage and fruit of the apple. Cornell Agric. Exp. Sta. Mem. 24: 683-762.

Morse, J. G. 1978. Comparative resistance to azinphos-methyl, in the predatory mite, Amblyseius fallacis, and its prey, Tetranychus urticae in greenhouse experiments. M.S. Thesis, Mich. State Univ., East Lansing, MI, 65 pp.

Mullins, W., and E. P. Pieters. 1980. Weight vs. Toxicity: A Need for Revision of the Standard Method for Testing Heliothis Resistance of Insecticides. Paper presented at Ent. Soc. Amer., Dec. 1980, Atlanta GA.

Nakashima, M. J., and B. A. Croft. 1976. Toxicity of benomyl to the life stages of Amblyseius fallacis. J. Econ. Entomol. 67: 675-7.

National Academy of Sciences (NAS). 1969. Insect Pest Management and Control. Publ. 1695, Washington D.C., 508 pp.

Newsom, L. 1980. The next rung up the integrated pest management ladder. Bull. Entomol. Soc. Amer. 26: 369-374.

Nijveldt, W. 1969. Gall Midges of Economic Importance. London: Crosby Lockwood & Son, Ltd, 191 pp.

Oatman, E. R., and E. F. Legner. 1961. Bionomics of the apple aphid, Aphis pomi, on young, non-bearing apple trees. J. Econ. Entomol. 54: 1034-1037.

Pielou, D. P. and K. Williams. 1961a. The effectiveness of residues of insecticides in preventing reinfestation of apple leaves by apple aphid, Aphis pomi DeG. I. Diazinon, Trithion, and Sevin. Canadian Entomol. 93: 93-101.

__________. 1961b. The effectiveness of residues of insecticides in preventing reinfestation of apple leaves by apple aphid, Aphis pomi DeG. II. Thiodan and Guthion. Canadian Entomol. 93: 1036-1040.

Prokopy, R. J., W. M. Coli, and R. G. Hislop. 1980. Sampling methods and provisional economic threshold levels for major apple insect and mite pests in Massachusetts. In: W. J. Lord and W. J. Bramlage, eds. Fruit Notes 45: 15-18.

Rettich, F. 1980. Field evaluation of permethrin and decamethrin against mosquito larvae and pupae (Aedes cantans). Acta Entomol. Bohemslov 77: 89-96.

Sell, P. 1976. [Monogeny in Aphidoletes aphidimyza (Rond.)]. A. Ang. Entomol. 82: 58-61.

Singh, O. P., and R. R. Rawat. 1980. Toxicity of some insecticides against pupae and adults of Dicladispa armigera (Coleoptera: Chrysomelidae) on rice (Oryza sativa). Indian J. Agric. Sci. 50: 271-272.

Smith, E. H. and E. H. Salkeld. 1966. The use and action of ovicides. Ann. Rev. Entomol. 11: 331-356.

Specht, H. B. 1972. The apple aphid Aphis pomi (Homoptera: Aphididae) on apple under summer conditions in a controlled environment cabinet. Canadian Entomol. 104: 105-111.

Streibert, H. P. and V. Dittrich. 1977. Toxicological response of insect eggs and larvae to a saturated atmosphere of chlordimeform. J. Econ. Entomol. 70: 57-59.

Westigard, P. H. and H. F. Madsen. 1965. Studies on the bionomics of summer generations in California of the apple aphid, Aphis pomi DeGeer (Homoptera: Aphididae). Canadian Entomol. 97: 1107-1114.

Zar, J. H. 1974. Biostatistical Analysis. New Jersey: Prentice-Hall, Inc., 620 pp.