AN ABSTRACT OF THE THESIS OF

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Title: Monitoring Insecticide Resistance of the Western Tentiform
Leafminer Phyllonorycter elmaella (Doganlar and Mutuura) (Lepidoptera:
Gracillariidae) in Northern Oregon

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Abstract approved: _____

Studies were conducted to evaluate two modified pheromone-trap
assays for monitoring the susceptibility of adult Phyllonorycter
elmaella (Doganlar and Mutuura) males to four insecticides:
azinphosmethyl (organophosphorus compound), oxamyl (carbamate),
endosulfan (chlorinated hydrocarbon), and esfenvalerate (pyrethroid).
Pheromone-baited sticky traps were used in both assays to lure,
capture, and hold moths for testing. The topical bioassay involved
treating each moth with 0.2 microliters of insecticide dissolved in
acetone dispensed from a microapplicator. The insecticide-laced
adhesive method eliminated treating each moth individually because the
insecticide was incorporated directly into the trap adhesive. Only

the topical assay was useful for measuring high levels of azinphosmethyl resistance. Concentration-response lines for esfenvalerate could not be established because moth mortality did not stabilize during the bioassay. Both assays were useful for determining the base-line susceptibility to oxamyl.

Several factors which affect bioassay results were studied. These included: 1) trap adhesive brands and their effect on moth survival, 2) environmental conditions during the bioassays, 3) environmental conditions (temperature and relative humidity) experienced by moths in the field before testing, 4) sub-lethal exposure of moths to insecticides in the field prior to collecting, and 5) the timing of bioassays in relation to adult P. elmaella flights.

Tanglefoot[®] trap adhesive was the least toxic of three adhesive brands used to capture and hold the moths during bioassays. High temperature and low humidity during the bioassay increased mortality. Therefore, environmental conditions were critical for reliable assay results and were standardized at 15.6 ^{o}C, 60% RH, and a light-dark cycle of 16:8 h. Mortality assessments were made after 24 h to minimize check mortality. High temperatures at the collection site up to 48 h prior to collection increased bioassay mortality. The application of a low rate of oxamyl and a field rate of azinphosmethyl to an orchard up to a week before moths were collected increased bioassay mortality. Check mortality was low when moths were trapped in cool weather during the early flight period of the first and second flights. Male moths were approximately 1.7 times more susceptible

than females after 24 h to topical treatments with oxamyl.

A survey was conducted to determine susceptibility of P. elmaella from commercial orchards and an unsprayed abandoned orchard to azinphosmethyl and oxamyl. Topical and insecticide-laced bioassay results indicate that all populations tested were still susceptible to oxamyl. Topical assays indicate that high levels of azinphosmethyl resistance had developed in WTLM populations from commercial apple and sweet cherry orchards in northern Oregon.

Monitoring Insecticide Resistance of

the Western Tentiform Leafminer

<u>Phyllonorycter</u> <u>elmaella</u> (Doganlar and Mutuura)

(Lepidoptera: Gracillariidae) in Northern Oregon

by

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Introduction

The western tentiform leafminer (WTLM), Phyllonorycter elmaella,

has been a pest in apple and cherry orchards in the Pacific Northwest

since 1980. The WTLM closely resembles two other species, P.

blancardella (F.) and P. crataegella (Clements), both of which are

widely distributed in the central and eastern United States. The life

history and damage of these three Phyllonorycter species are similar.

Studies of P. blancardella and P. crataegella have shown that large

infestations of larvae feeding inside leaves can cause premature

defoliation, fruit drop, and reduced yields.

Few effective insecticides are presently available for leafminer

control on tree fruits. Field and laboratory studies suggest that

resistance development to several insecticides has occurred and that

resistance is partially responsible for recent leafminer outbreaks.

Azinphosmethyl resistance appears to be widespread in P. blancardella

populations in eastern North America. Pyrethroid and low level

methomyl resistance has also developed in some populations.

Residual as well as topical bioassay methods have been employed

for documenting insecticide resistance in P. blancardella. Problems

associated with the use of previous resistance monitoring methods

indicated a need for an improved test procedure which was better

suited for gracillariid leafminer species. A new bioassay method which has proven successful in resistance surveys of several lepidopterous species is the pheromone-trap assay. Feral insects are collected in the field with adhesive-coated pheromone traps and exposed to various insecticide concentrations. The insecticide is applied either topically to each insect or it is picked up through contact with the insecticide-laced adhesive.

The main objective of this research was to evaluate whether pheromone trap assays can be adapted for monitoring susceptibility levels to insecticides in P. elmaella. A second objective was to investigate how environmental conditions (temperature and humidity) during the bioassay and certain stresses experienced by test insects in the field prior to collection (high temperatures, sublethal exposure to insecticides) influence their mortality response.

Literature review

<u>Tentiform leafminer bionomics.</u>

In North America, three species of leafminer (Lepidoptera: Gracillaridae) are common tree fruit pests: the spotted tentiform leafminer (STLM), <u>Phyllonorycter</u> <u>blancardella</u> (F.); the apple blotch leafminer (ABLM), <u>P.</u> <u>crataegella</u> (Clements); and the western tentiform leafminer (WTLM), <u>P.</u> <u>elmaella</u> (Doganlar and Mutuura). The STLM is an introduced European species while the ABLM and the WTLM are native species. Their appearance and life history are similar (Beckam et al. 1950, Pottinger and LeRoux 1971, Doganlar and Mutuura 1980).

The <u>Phyllonorycter</u> species on tree fruits in the western US is commonly known as the western tentiform leafminer although this name is not officially recognized by the Entomological Society of America. This leafminer was first identified as <u>P.</u> <u>sorba</u> (Pottinger and LeRoux 1971) but was later renamed <u>P.</u> <u>elmaella</u> (Doganlar and Mutuura 1980). However, Wagner (personal communication) believes that the WTLM involved in western USA infestations is still incorrectly identified and is actually an unnamed <u>Phyllonorycter</u> species.

The biology of the WTLM is described by Hoyt (1983). It is similar to that of the STLM (Pottinger and LeRoux 1971) and the ABLM (Beckam et al. 1950). These leafminers overwinter on the ground as pupae inside mines in fallen leaves. Adults emerge from their pupation sites in early spring. Eggs are laid individually on lower leaf surfaces. Larvae emerge and immediately enter the leaf where they begin to mine by separating the upper and lower leaf epidermis

during feeding. They have five instars. The first three are called sap feeders and their mines can only be seen from the lower leaf surface. The fourth and fifth instars are referred to as tissue feeders. These latter instars feed on leaf tissue below the upper epidermis producing tent-shaped mines visible from the upper side of the leaf. Pupation occurs inside the mine. The WTLM has three generations per year in Washington (Hoyt 1983), but there can be a fourth or partial fourth in warm years (Beers et al. 1987). The number of WTLM generations in northern Oregon is similar to that in Washington.

The STLM attacks apple, pear, and cherry in addition to wild hosts such as hawthorn, wild plum and wild cherry (Swan and Papp 1972). Borden et al. (1953) indicate that apples and pears are primary hosts for ABLM while plums, peaches and quince are secondary hosts and not as susceptible to attack. The host list given by Hoyt (1983) indicates similar feeding preferences for the WTLM.

Leafminers are indirect pests since they do not utilize the fruit as food. The damage they cause is due to feeding by the larval stages within the leaves. The degree of injury is related to the number of mines per leaf. Larval feeding reduces the leaf surface area resulting in a decrease in photosynthesis. Twenty mines per leaf can reduce the net photosynthetic rate by up to 25% (Bodner and Procter 1981). Consequently, high STLM infestations have been reported to cause premature leaf drop, a reduction in shoot growth, fruit size, and return fruit set. In addition, premature fruit ripening and fruit drop have been observed (Pottinger and LeRoux

1971). Leafminer feeding also increases the susceptibility of leaves to spray phytotoxicity (Coli and Prokopy 1982). Fruit can be sunburned if heavy defoliation occurs (unpublished data).

Pest status.

Leafminers were not considered economic pests in the past although they had been present in orchard systems for many years. Slingerland and Crosby (1915) described the STLM as a common but not serious pest. Until P. blancardella outbreaks occurred in the early 1960's, infestations of this insect were infrequent. In 1961, major infestations of P. blancardella occurred in Quebec and Novia Scotia. It caused severe damage to apple foliage and was labeled a "major pest" in Southwestern Quebec in 1964 (Johnson et al. 1976). The STLM also developed high infestations in Michigan apple orchards in the mid-70's despite regular spray programs (Dutcher and Howitt 1978).

P. crataegella had a similar history regarding it's occurrence in an area long before it became a pest. Outbreaks were uncommon until Weires (1977) reported large infestations of the ABLM in New York's Hudson Valley during 1974-76. These infestations originated in a small area and within three years, most of Columbia County reported problems with this leafminer. P. crataegella was also heavily infesting commercial orchards in Connecticut during this time (Maier 1981).

There was a recorded outbreak of the ABLM in northern California in 1951-52. According to Borden et al. (1953), the damage was most serious in pear orchards in the Sierra foothills and in apple orchards in the coastal counties. This leafminer was tentatively identified as

Lithocolletis (= Phyllonorycter) crataegella but the possibility
exists that this was caused by the unidentified species currently
named P. elmaella (Wagner, personal communication).

Confirmed outbreaks of the WTLM have developed more recently in
the Pacific Northwest (Brunner 1981, Hoyt 1983, Hathaway et al. 1985).
These outbreaks have been scattered in their occurrence, flaring up in
different areas and years in an erratic pattern. The general trend
has been for populations to increase dramatically over a few years and
then crash (Hoyt personal comm.).

Chemical control and insecticide resistance.

Melander (1914) was the first to report insecticide resistance
when he observed reduced efficacy of lime-sulfur sprays directed
against San Jose Scale, Quadraspidiotus perniciosus(Comstock)
(Homoptera: Diaspididae). Since then, insecticide resistance problems
have risen dramatically (Georghiou and Mellon 1983). There are now at
least 447 species of insects and mites which have become resistant to
one or more pesticides (Georghiou 1986).

At one time, leafminers were controlled through the use of broad-
spectrum insecticides directed towards major pests such as codling
moth and apple maggot. However, leafminer outbreaks have become more
frequent in recent years due to the development of resistance (Weires
1977, Pree et al. 1980, and Maier 1981). Subsequently, growers in
many fruit growing areas were forced to apply control measures
specifically for leafminer.

Insecticide resistance of gracillariid leafminers on tree fruits

has become a worldwide problem. Organophosphorus (OP) resistance in
P. blancardella was first reported from Italy in the early 1960's
(Kremer 1971). In North America, successive leafminer outbreaks in
various fruit-growing areas have stimulated studies on the chemical
control and resistance of these insects. During the P. crataegella
outbreak in the Hudson Valley, Weires (1981) determined that this
leafminer species had become resistant to the OP compounds
azinphosmethyl, phosmet, phosalone, dimethoate, and the carbamate
carbaryl. However, the ABLM was still susceptible to the systemic
carbamates methomyl and oxamyl.

Pree et al. (1980) tested the resistance of P. blancardella to
azinphosmethyl during the severe outbreaks of 1976-77 in Ontario.
Using a diagnostic dose of azinphosmethyl, they determined that the
STLM was highly resistant in 8 of 10 orchards tested. These orchards
had a history of heavy OP insecticide use. Further studies showed
that the moths had also developed resistance to phosmet and diazinon.
The carbamate methomyl was still effective at that time. Recent
reports indicated that the STLM in Ontario had developed resistance to
the pyrethroids after only 4-5 years of use (Pree et al. 1986). Pree
et al. (1990) indicated that STLM populations in southern Ontario had
become resistant to methomyl.

Orchard insecticide studies conducted in Washington showed that
the WTLM had developed resistance to a number of OP insecticides
(Hoyt, personal communication). It was found that oxamyl was the only
registered insecticide which was still effective. Laboratory
bioassays conducted by McClain (1985) indicated high levels of

azinphosmethyl resistance in WTLM populations from Utah. Most populations were still susceptible to oxamyl and methomyl, but localized tolerance was starting to develop.

Resistance monitoring.

Insecticides may fail to control pest arthropods after relatively short periods of use due to a population's increase in levels of resistance (Georghiou and Taylor 1977, Tabashnik and Croft 1982). This potential for control failure indicates that early detection of resistance in a population is important for certain resistance-management strategies to be effective (Metcalf 1980). Bioassays should be sensitive enough to detect resistant individuals at a frequency of 1% or less (Rousch and Miller 1986).

"Resistance" is a heritable decrease in sensitivity to a chemical within a pest population. The term resistance can be used interchangeably with "tolerance" and "insensitivity" (Brent 1986). Toxicological assays have been the main methods for monitoring resistance. Specific assays are employed to determine whether changes in a population's susceptibility have occurred.

The first step in a resistance monitoring program is to establish the baseline response of susceptible populations to a pesticide. This is critical since it provides a reference point for comparing resistant populations (Brown 1958). Generally, a population is tested with a range of pesticide concentrations to determine its degree of resistance (Anonomyous 1974). The response of the population is then quantified by calculating various parameters such as the LC_{50}, LC_{90}, and the slope of the concentration-response line (Finney 1971).

Concentration-response line parameters are adequate for documenting high levels of resistance but may not be appropriate for detecting small changes in resistance frequency if the method is not sensitive enough (Roush and Miller 1986). Producing concentration-response lines is also more time consuming because the test individuals are distributed among a range of treatments versus being treated at on rate (Halliday et al. 1990).

The use of a single discriminating or diagnostic dose is an alternative to generating concentration-response lines. The procedure involves treating test subjects with a selected rate of insecticide which discriminates between susceptible and resistant populations. This method is more efficient when the numbers of available test individuals are limited, for detecting small changes in resistance frequencies (Roush and Miller 1986, Halliday et al. 1990) and for large scale resistance surveys (Brent 1986). However, it is difficult to determine which dose to use (Dennehey et al. 1983). The response of a susceptible population to an insecticide needs to be charactorized first before a discriminating dose can be selected. The precision of toxicological assays is greatly influenced by the standardization of bioassay procedures and the sample size (Robertson et al. 1984). Large sample size is important for resistance detection programs (Roush and Miller 1986). It has been suggested that at least 100 individuals should be tested from a population (Anonymous. 1974, and Miyata 1983). Robertson et al. (1984) recommend that a minimum total sample size of 120 insects (240 are better) are necessary for the reliable estimation of a concentration-response line.

It is important to recognize that variables in the laboratory such as post-treatment temperature and humidity conditions can significantly affect the response to a pesticide during a test (Robertson and Worner 1990). Temperature (Sparks et al. 1982) and relative humidity (Reichenbach and Collins 1984) can significantly affect an insect's response to an insecticide by altering its uptake, absorbtion, metabolism and excretion of the chemical.

Field-collected insects used in bioassays often vary in age, size and physiological conditions which introduces variability in the bioassay. Riedl et al. (1985) have shown that the susceptibility of the codling moth, Cydia pomonella (L) (Lepidoptera: Tortricidae) varies with the age of the individual. They suggested testing field-collected moths before peak flight, e.g. early in the emergence curve, when the age distribution is more uniform. Knight and Hull (1989) reported that sublethal exposure of tufted apple bud moth males, Platynota idaeusalis (Walker) (Lepidoptera: Tortricidae) to insecticide residues on foliage prior to collection adversely influenced bioassay results through increased mortality.

Leafminer bioassay methodology.

As stated earlier, gracillariid leafminer outbreaks in North America have frequently been attributed to insecticide resistance. Residual as well as topical bioassay procedures have been employed in resistance studies with these leafminers to determine whether control failures were due to resistance. However, certain drawbacks limited their application. Previous bioassay methods have often been

cumbersome, were not suitable for testing certain insecticides, or required costly equipment not readily available. For instance, the glass vial residue methods used by Weires (1977), Weires et al. (1982), Van Driesche et al. (1985), and McClain (1985) were not able to measure high levels of resistance to certain insecticides. At high concentrations, the carrier in the insecticide solution does not evaporate completely leaving a sticky residue of supersaturated droplets on the glass surface which increases mortality as the small moths adhere to it. Pree et al. (1980) was able to produce meaningful concentration-response lines for P. blancardella by treating moths topically with insecticide administered through a Potter spray tower. However, this piece of equipment is expensive and not widely available thus restricting the applicability of this bioassay.

The drawbacks of the above bioassays indicate a need for standard test methods for monitoring resistance levels of arthropod populations which are reliable, quick, and easy to use. The assays should detect changes in resistance levels prior to field failure of an insecticide. The validation of a bioassay's ability to detect incipient resistance is necessary for reliability. An insecticide failure in the field is a good indication that a resistance detection program is not working. Resistance detection programs initiated in Australia to monitor increases in pyrethroid resistance in Heliocoverpa (= Heliothis) armigera (Hubner) (Lepidoptera: Noctuidae) failed to predict a resistance related outbreak (Gunning et al. 1984) although the assays were able to document the 12-fold increase in fenvalerate resistance after the outbreak occurred. Citing the failure to predict this

outbreak, Roush and Miller (1986) suggest that the modification of existing resistance monitoring methodology may be necessary to make them useful for resistance management programs, especially if the field validation of a bioassay is a failure. New assay methods can also be tried.

Two bioassay methods have recently been developed which allow for collecting and testing large numbers of insects. Both of these methods use baited traps to lure and capture feral insects for testing purposes. The topical method described by Riedl et al. (1985) for codling moth, Cydia pomonella (L.) was validated against existing methodology (Barnes and Moffitt 1963) for this insect. It utilizes a micro-applicator to apply a small dose of insecticide dissolved in acetone to male moths collected with a pheromone trap. The insecticide-laced adhesive method developed by Haynes et al. (1986) incorporates the insecticide directly into the trap adhesive which eliminates the need to treat each insect individually. This method was first used to assay Liriomyza trifolii (Burgess) (Diptera: Agromyzidae) attracted to yellow cards coated with the poison-adhesive mixture (Haynes et al. 1986). Later it was adapted to test pink boll worm Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) susceptibility to insecticides when lured to the poisoned substrate with pheromones (Haynes et al. 1987). Knight and Hull (1989) tested both pheromone trap assays with the tufted apple bud moth. They showed that both methods were reliable for obtaining concentration-response lines and for discriminating between susceptible and resistant populations.

Materials and Methods

Study orchards.

The moths for this research were collected in sweet cherry orchards near The Dalles, Oreg., in apple blocks at the Oregon State University Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, Oreg., and in an abandoned homestead (St. Cloud) near Skamania, Wash. The cherry orchards were commercially managed and consisted of mature trees of Bing, Lambert, Royal Anne, and Van varieties. These orchards generally receive a ground application of parathion followed by 5-6 aerial applications of malathion for cherry fruit fly (Rhagoletis indifferens (Diptera: Tephritidae) control during late May through mid-July. These sites had moderate to high WTLM infestations as evidenced by seasonal pheromone trap catches and leaf infestations. The apple blocks consisted of mature Red Delicious, Golden Delicious and Rome trees. These were usually treated with 4 azinphosmethyl cover sprays each season for codling moth control. There was no history of WTLM outbreaks at the MCAREC. Seasonal monitoring with pheromone traps revealed a low level population. Both the cherry and apple collection sites were located in major fruit growing districts while the abandoned site, which consisted mostly of seedling apple trees, was at least 30 miles from any commercial orchard. The abandoned site has not received pesticide applications for at least 30 years (probably more). Temperature and % relative humidity (% RH) were monitored during the season with recording hygrothermographs at one orchard in The Dalles and at the

MCAREC. Daily maximum and minimum temperatures were used to calculate degree days (Baskerville and Emin 1969) using a lower threshold temperature of 5 °C (Barrett 1988). Degree days were accumulated from March 1 and plotted against trap catches to provide a visual representation of adult WTLM phenology.

Bioassays.

Two pheromone trap assays were evaluated for monitoring insecticide susceptibility of male P. elmaella moths. One was the topical assay described by Riedl et al. (1985) and the other the insecticide-laced adhesive bioassay described by Haynes et al. (1987). Both assay methods had to be modified for the WTLM because of its small size. Pherocon 1C traps baited with TLM pheromone dispensers (Trece Inc., Salinas, Calif.) were used for both assays. Moths were usually collected by placing traps in infested orchards during evening hours until the next morning. When flight activity was heavy, enough moths could be collected during a 1/2 hour period in the morning. Traps with moths were placed in a cooler and brought to the laboratory. All bioassays were conducted with technical grade insecticides with the following purity: azinphosmethyl 86.9% (Mobay Chemical Co., Kansas City, Mo.), oxamyl 97% (DuPont Chemical Co., Wilimington, Del.), endosulfan 96% (FMC Corp., Middleport, N.Y.), and esfenvalerate 84% (DuPont Chemical Co.).

Topical assay.

Approximately 0.3-0.4 g of the polybutene adhesive Tangletrap® (Tanglefoot Co., Grand Rapids, Mich.) was uniformly applied with a

spatula as a thin coat to a 12.5x23 cm area on a 1C trap bottom. Only moths attatched to the adhesive on their ventral surface were used in the tests. These were treated topically on their dorsum with a 0.2 microliter droplet of insecticide dissolved in acetone or acetone alone (check). The insecticide was administered to each test insect with a microsyringe mounted in a repeating dispenser (Hamilton Co., Reno, Nev.). Ranges of concentrations were prepared by serial dilutions. Treated moths were held in a growth chamber for 24 h at 15.6 $^\circ$C, 60% RH, and a 16:8 photoperiod. Mortality readings were taken with the moths on the trap 24 h after a topical application. Insects were prodded with a soft brush under a stereo dissection microscopescope. Moths were scored as dead when there was no movement at all. Moths were classified as moribund if they displayed one or more of the following: rapid and continuous fluttering of wings in response to prodding, rapid uncontrolled twitching of abdomen, wings, or antennae; extension of claspers with little movement; movement of body scales only. A moth was scored as alive if it was able to move its antennae, legs, wings, or head every time it was prodded. Moths whose legs and wings were immobilized by the trap adhesive were also scored as alive if they exibited some movement at the base of each wing. Categories of dead and moribund were combined for mortality analysis.

Insecticide-laced adhesive assay.

The insecticide was directly incorporated in the trap adhesive with this method and moths were exposed to different concentrations of

insecticide as soon as they were caught in the traps. Insecticide-laced adhesive was prepared by mixing 0.1 ml of different insecticide solutions with 1 g of adhesive in a small glass jar with a stirring rod for 5 min. Azinphosmethyl solutions were prepared with methyl chloride since this was a better solvent for this compound than acetone. Methanol was used as the solvent for oxamyl. Approximately 0.08-0.12 g of an insecticide-laced adhesive concentration was spread uniformly with a spatula onto a 12X14 cm insert cut from a 1C trap liner. The treated area measured 9x11 cm. Two coated inserts were placed inside a non-sticky Pherocon 1C trap and held down with one paper clip each. After the moths were collected in the field, the trap inserts were immediately placed in a growth chamber set to the same conditions described for the topical assay. Mortality was assessed 24 h from the mid-point of the trapping period using the same criteria as for the topical assay.

<u>Comparison of topical and insecticide-laced pheromone trap assays</u>.

Traps were prepared for both techniques and placed in a commercial sweet cherry orchard during early morning on three different days. Oxamyl was used for this experiment. Three replicates per rate, method and day were made using the first 50 moths encountered on the trap as an observation. Concentration-response lines were generated for both methods each day the test was conducted. Trap catches were recorded to determine whether oxamyl mixed into the trap adhesive was repellent to WTLM . Data were transformed to log(X+1) to normalize data (Steel and Torrie 1980) and were analyzed by analysis of variance with the rate of oxamyl as the main effect.

Effect of adhesive on moth survival.

Three different adhesives, Tanglefoot[®], Tangletrap[®] (Tanglefoot
Co., Grand Rapids, Mich.), and Stikem Special[®] (Seabright Ltd.,
Emeryville, Calif.) were investigated for possible adverse mortality
effects on the moths. Four traps and liners per adhesive brand were
prepared following the procedure described for the topical bioassay
and placed in the field overnight to collect moths for testing. Moths
were not treated with insecticide or acetone. Traps with moths were
held at 18.3 °C, 60% RH, and a 16:8 photoperiod. Mortality was
assessed every 12 to 24 h until all moths had died. Only moths
adhered to the glue on their ventral surfaces at observation were
counted. Over time some individuals fell over and became stuck on
their sides. A two-way analysis of variance was conducted using the
arcsine transformation of percent mortality with the adhesive type as
the main effect.

Time-mortality studies.

Tests were conducted to determine time-mortality curves of WTLM
male moths treated with either azinphosmethyl or esfenvalerate. One
study was designed to eliminate adhesive-related mortality. Moths
were reared from cherry leaves containing WTLM pupae collected from
The Dalles at the onset of a third flight period. Emerged moths were
aspirated from their plastic rearing containers, anesthetized with
CO_2, sexed under a stereo dissection microscope, treated topically
with different rates of either insecticide and then placed in 25-dram
glass vials plugged with moist cotton dental wicks. Ten male 3-4 day

old moths were placed in each vial. Moths were held at 15.6 $^{\circ}$C, 60%
RH and a light-dark cycle of 16:8. Moth mortality was observed at 24
h intervals for azinphosmethyl. A proposed observation interval of 24
h for esfenvalerate was changed to 6-12 h intervals at the start of
this test because knockdown of WTLM male moths at the high rate
occurred almost immediatly after treatment. Both procedures were
repeated 2 more times for a total of 30 moths per treatment. Equal
numbers served as controls. Treatment means were calculated and
plotted over time.

In one insecticide-laced adhesive assay using oxamyl, moth
mortality was evaluated at 12 h intervals for up to 30 h. This was
done to observe time-mortality relationships with respect to the
insecticide rate for this method.

Sex-related bioassay response to oxamyl.

This test was conducted to determine if male moths responded
differently than females when topically treated with 0.008 ug/moth of
oxamyl. Moths were collected, reared and handled in the same way as
those used in the time-mortality vial studies. After sexing the
moths, 10 males were treated and then placed into a glass vial. An
equal number of males were treated with acetone only as a control.
The same procedure was used for female moths. Mortality assessment
was made at 24 h intervals until all moths died. Treatment means were
calculated and plotted over time.

Environmental conditions during the bioassay.

Initial tests led to standard post-treatment holding conditions

of 15.6 °C, 60% RH and light-dark cycle of 16:8 to minimize control
mortality. Additional tests using the topical application method were
performed to study how temperature and % RH affect mortality during
bioassays. To investigate temperature effects, concentration-response
lines were generated at 60% RH and temperatures of 12.8, 15.6, and
18.3 °C for azinphosmethyl. To observe the effects of different
humidity levels on moths, trapped moths were first treated with 0.2
microliters of the LC_{50} equivalent of oxamyl. Check moths were
treated with acetone. Following treatment, the moths were held
elevated inside covered 11x25x31 cm plastic containers in a growth
chamber at 15.6 °C. To regulate humidity, saturated salt solutions
were prepared (Winston and Bates 1960) and poured into the crispers
below the trap bottoms with the treated moths. Humidity levels were
monitored with narrow-range lithium chloride humidity sensors
(American Instrument Co., Silver Springs, Md.). The % RH readings for
the different salt solutions were: magnesium chloride, 35%; dextrose,
72%; and potassium sulfate, 96%. In addition, the standard 60% RH in
the growth chamber was included as a treatment in this experiment.
The experiment was repeated three times using 50 moths per replicate.
Equal numbers served as checks. Mortality was assessed after 24 h.

Effect of weather conditions prior to collection.

Small fragile insects such as the leafminers in this study may be
particularly susceptible to stresses from adverse weather conditions.
This may increase their sensitivity to insecticides and affect their
bioassay response. To determine if environmental conditions
experienced by the test insects in the field influenced bioassay

results, temperatures and % RH levels at various time periods prior to collection were correlated with check mortality. The high and low temperatures and % RH levels which occurred at the time of trap placement (0 h), and during the 24 h and 48 h periods preceding trap placement, were used in this analysis.

Timing of bioassays in relation to adult WTLM flights.

The timing of the bioassays was related to both adult generations and flight curves within a generation. Seasonal trap catches were plotted to discriminate between the 3-4 adult generations. Additionally, each flight curve for a generation was broken into 3 components and assigned values which were: pre-peak flight (1), peak flight (2), and post-peak flight (3). To evaluate variability of mortality response between generations (1-4) and within a flight curve (1-3), these values were correlated against check mortality values obtained from topical bioassays.

Field exposure to insecticides prior to collection.

Three 1-acre blocks in a mature sweet-cherry orchard in The Dalles were used for this study. Before this orchard was sprayed with insecticides, concentration-response lines were generated for the leafminer population at the study site using the topical assay method for both oxamyl and azinphosmethyl. A low rate of oxamyl 2L (146.2 ml/hectare) was then applied with a speed-sprayer in a spray volume of 1870 l/hectare to one of the blocks. A low label rate of azinphosmethyl 35 WP (59.3 g/hectare) was applied to another block while the third block served as an untreated control. On days 1, 3

and 7 after spray application, 6 to 10 traps prepared for topical
assays were placed in each of the blocks during early morning and left
for about 1/2 h until a sufficient number of moths was trapped. Half
of the moths on each trap from the three blocks were topically treated
with 0.2 ul of acetone as controls while the remaining moths were
treated topically at the LC_{50} level. Moths trapped in the oxamyl 2L
block received the oxamyl LC_{50} rate. The azinphosmethyl LC_{50} rate was
applied to the moths collected from the azinphosmethyl block. One
half of the trapped moths from the control block were treated with
either oxamyl or azinphosmethyl. Moths were held for 24 h at 15.6 $^\circ$C,
60% RH, and a 16:8 photoperiod. For evaluation, mortality was
determined for the first 90 to 150 upright moths for each treatment.
Equal numbers served as controls. Mortality means and their
associated standard errors were calculated.

Resistance survey of WTLM populations.

The topical pheromone trap assay was the primary technique used
to determine the response of populations from the study orchards to a
representative of four classes of insecticides: azinphosmethyl (OP),
oxamyl (carbamate), endosulfan (chlorinated hydrocarbon), and
esfenvalerate (pyrethroid). Moths from the MCAREC and the abandoned
Skamania site were also tested with the insecticide-laced adhesive
method to determine their response to either azinphosmethyl or oxamyl.

Data analysis.

LC_{50}'s, LC_{90}'s, corresponding confidence limits (CL) and
coefficients of the concentration-response regression lines were

estimated by the probit analysis program POLO (LeOra Software 1987). LC_{50} values were considered significantly different if their 95% CLs did not overlap. Abbott's formula (1925) was used to correct for control mortality. Analysis of variance and correlations were performed with Number Cruncher Statistical System (1987).

Results

Bioassay comparison.

Responses of P. elmaella moths to oxamyl in the topical and insecticide-laced adhesive assays are summarized in Table 1. Both bioassay methods indicate an increase in oxamyl susceptibility in the populations from Sep 5 to 12. The LC_{50} and slope values decreased over the three sampling dates for both methods. As indicated by the confidence limits, the LC_{50} values obtained on Sep 12 were significantly lower than those on the first two (topical assay) or first (insecticide-laced assay) sampling dates. The LC_{50} values, width of the confidence limits, and slopes of the concentration-response lines generated from data obtained with these two assay methods indicate similar levels of variability. However, the width of the LC_{90} confidence limits were greater with the insecticide-laced assay. Check mortality increased dramatically from the 1st to the 3rd sampling dates. It increased 10.2- and 15.3-fold in the topical and insecticide-laced assay, respectively, over a one-week period. The adhesive assay had consistently lower control mortality than the topical assay on all three dates. There was no difference in the number of WTLM male moths captured in traps laced with oxamyl (P=0.05) compared to check traps indicating that this insecticide did not inhibit moth collection (Table 2).

In a side-by-side comparison of the two assay methods with the OP azinphosmethyl, male P. elmaella moths showed a definite concentration-response in the topical assay while mortality in the

Table 1. LC_{50} and LC_{90} values and coefficients of the concentration-response lines for male P. elmaella moths in pheromone traps treated topically with oxamyl or insecticide-laced adhesive.

Method Date	n	Check mortality	LC_{50} (95% CL)	LC_{90} (95% CL)	Slope ($\pm$SE)
Topical			ug/moth	ug/moth	
5 Sep	90	3.0	0.007 (0.006-0.009)	0.022 (0.018-0.028)	2.711 ($\pm$0.194)
7 Sep	150	11.3	0.005 (0.004-0.006)	0.02 (0.017-0.025)	2.116 ($\pm$0.169)
12 Sep	150	30.7	0.002 (0.001-0.003)	0.013 (0.01-0.021)	1.522 ($\pm$0.186)
Insecticide-laced			ug/g adhesive	ug/g adhesive	
5 Sep	90	0.7	0.201 (0.159-0.237)	0.495 (0.397-0.745)	3.275 ($\pm$0.381)
7 Sep	150	2.0	0.158 (0.120-0.233)	0.826 (0.460-2.729)	1.787 ($\pm$0.187)
12 Sep	150	10.7	0.102 (0.07-0.143)	0.743 (0.409-2.620)	1.486 ($\pm$0.199)

Table 2.   Average number of male P.

elmaella moths captured in pheromone

traps with different rates of oxamyl-

laced adhesive.

Oxamyl (mg) /g adhesive	Avg. no. male moths /trap
0.0	106.8 ns
0.013	112.7
0.063	101.3
0.125	100.8
0.25	101.4
0.5	107.0

ns = not significant (p=0.01).

insecticide-laced assay plateaued at 30% and did not increase further even at high concentrations (Fig. 1).

Effect of adhesive on moth survival.

When the adhesive layer on the trap bottom is too thick, mortality increases as some moths acquire an oil-soaked appearance and die from becoming immersed in the glue. In addition to physical effects on moth survival, some commercial polybutene adhesives may be inherently more toxic than others. Fig. 2 shows how untreated moths survive capture and storage on different commercially available trap adhesives. Stikem Special was significantly more toxic than the other two brands throughout most of the test. Tanglefoot and Tangletrap produced time-mortality curves which were similar. After 24 h there was no statistical difference (P=0.05) in moth mortality on trap liners with these two adhesives. Moth mortality was approximately 40% after 24 h for Stikem Special while Tanglefoot and Tangletrap produced mortality levels of 7.7 and 1.3% respectively. Variability in mortality was greater with Stikem Special than with either of the other two adhesives as indicated by the large error mean bars in Fig. 2. The decrease in mortality in the test with Tanglefoot between 72 and 84 h is a result of moths being removed from the test after falling over onto their sides and changing the live/dead ratio.

Time-mortality studies.

Male WTLM moths treated topically with azinphosmethyl and placed inside glass vials showed rate-related responses over time (Fig.3). Mortality increased the fastest at the highest rate and stabilized

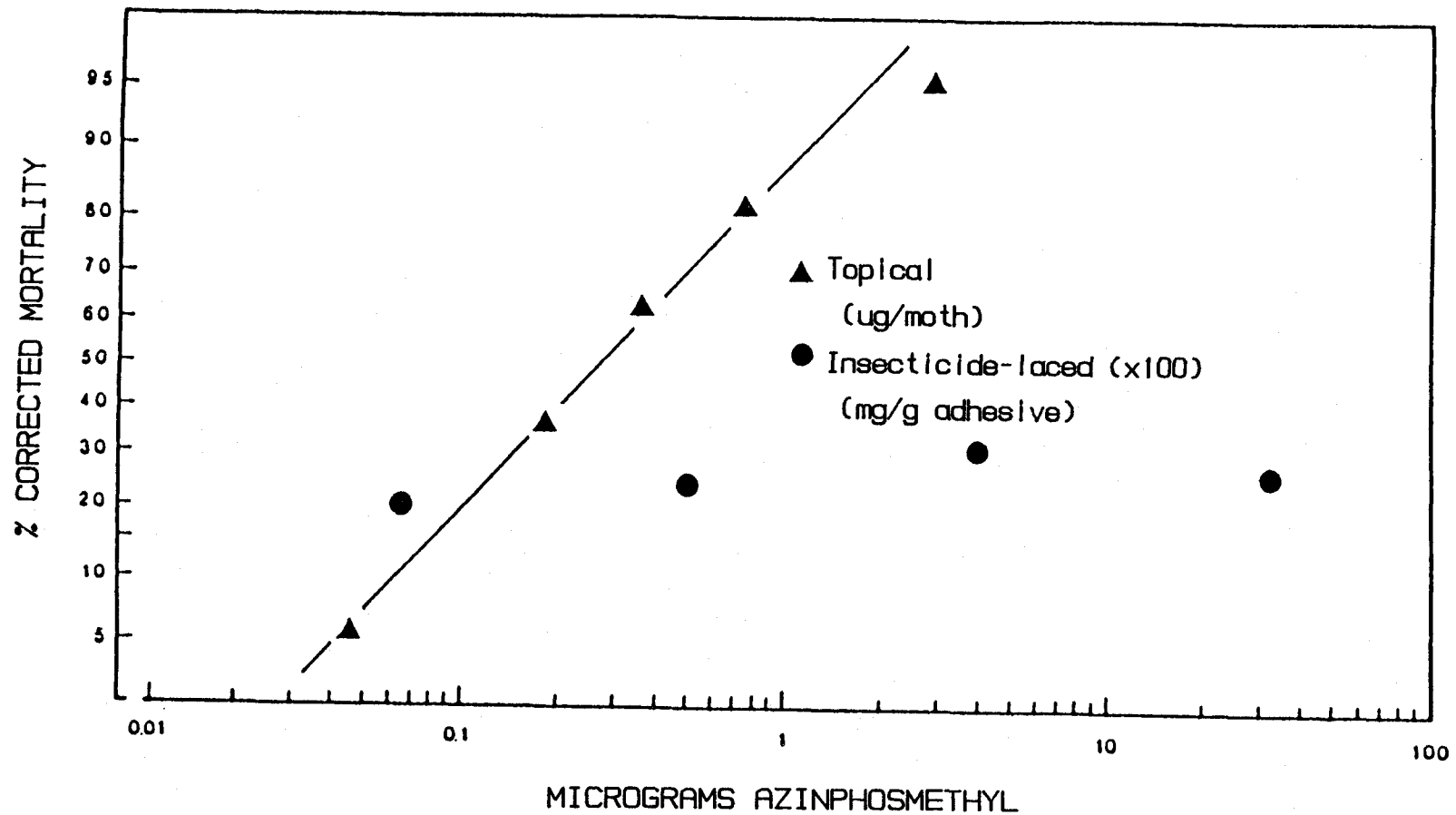Fig. 1. Concentration-mortality response of male P. elmaella moths in pheromone traps treated topically with azinphosmethyl or exposed to azinphosmethyl-laced trap adhesive.
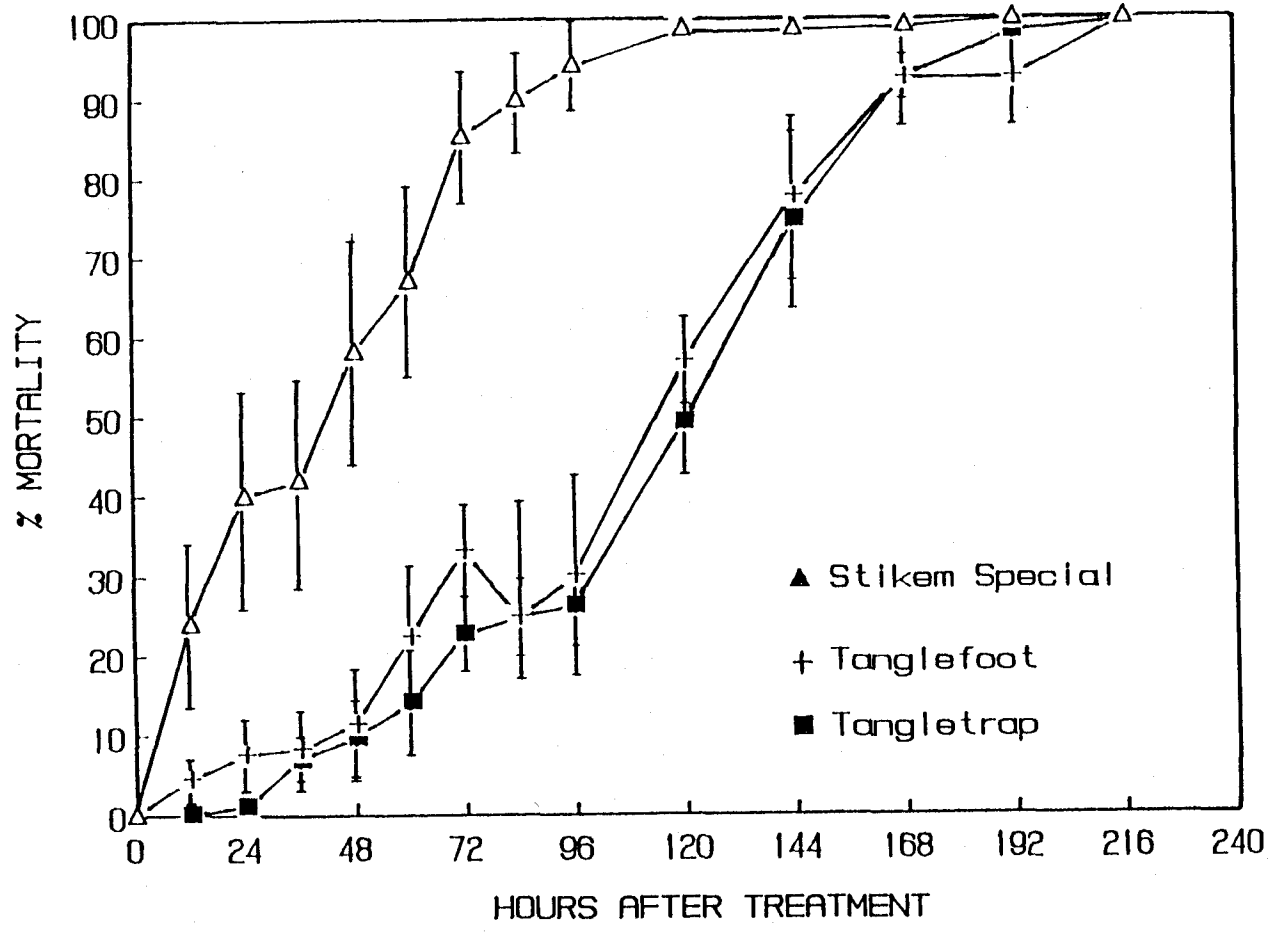
Fig. 2. Effect of adhesive brand on mortality of untreated male
P. elmaella moths in pheromone traps held at 15.6 °C and 60% RH.

Fig. 3. Time-mortality response of male P. elmaella moths held
in glass vials after topical application of azinphosmethyl.

after 48 h. Mortality of acetone-treated check moths was still low after 48 h (3.3%) and increased to 33% after 120 h.

Esfenvalerate treated moths also produced rate-related responses (Fig. 4). Moths treated with the highest rate (0.01 ug/moth) showed knockdown within 10 min after the application of esfenvalerate. "Apparent" mortality rose quickly to 80% within 12 h. However, many moths previously scored as dead soon recovered. The response of check mortality over time was similar to the check mortality values obtained from the azinphosmethyl vial study. The time-mortality response of WTLM male moths in an oxamyl-laced adhesive assay was also rate-related (Fig. 5). Check mortality increased rapidly after 24 h.

Sex-related bioassay response to oxamyl.

Male moths were more susceptible than females to topically applied oxamyl (Fig. 6). Treated male moths were approximately 1.7- and 2.1-fold more susceptible than treated female moths after 24 and 48 h respectively. Male check mortality rose rapidly and was approximately 3-fold higher than untreated female moths after 24 and 48 h. From 48 h on, untreated male moths had higher mortality than either treated or untreated female moths.

Effect of environmental conditions during bioassays.

Mortality of P. elmaella males in the bioassays was affected by the temperature and humidity conditions during the 24 h post-treatment period. Mortality of oxamyl- and acetone-treated moths decreased as humidity rose in the post-treatment holding chambers (Fig. 7). Increasing the relative humidity level from 35 to 96% reduced
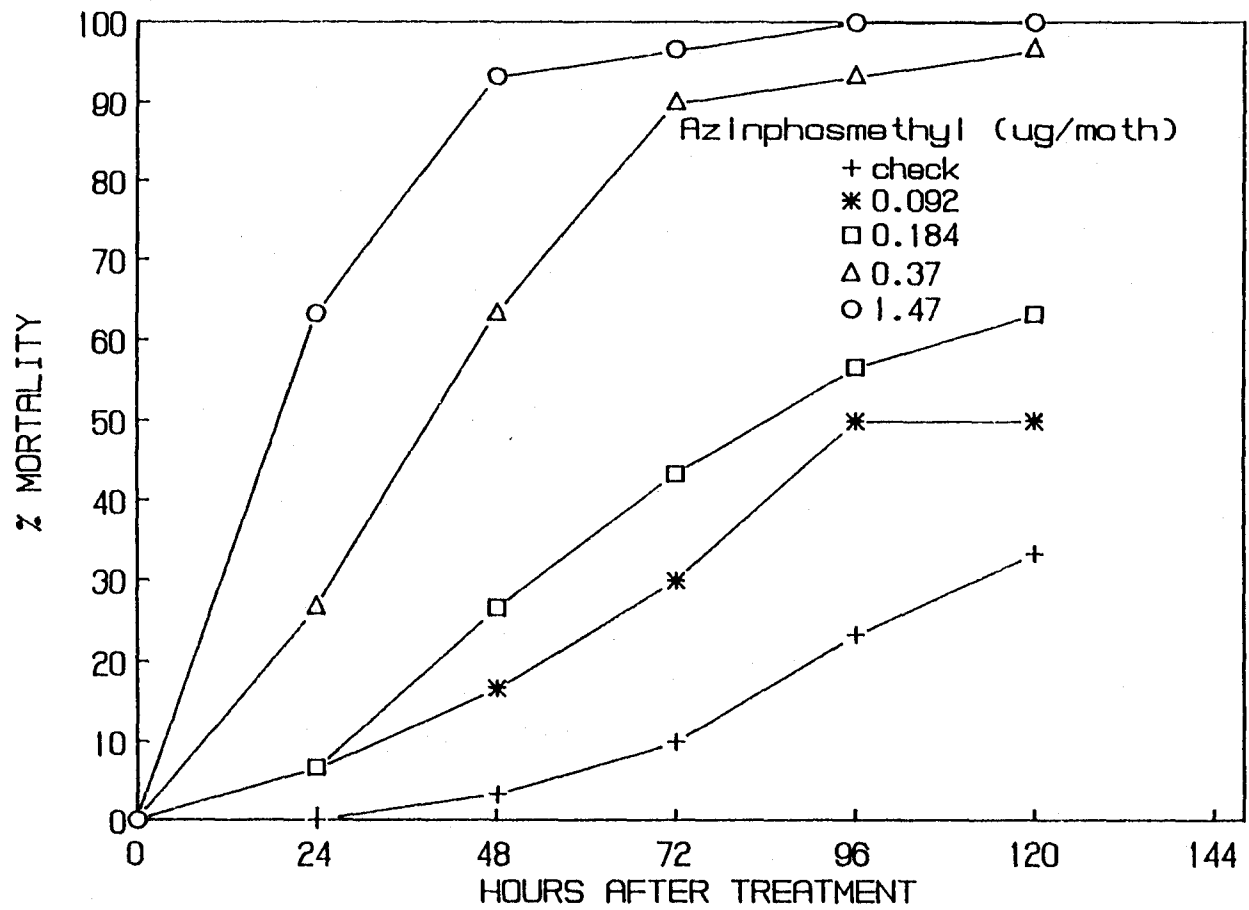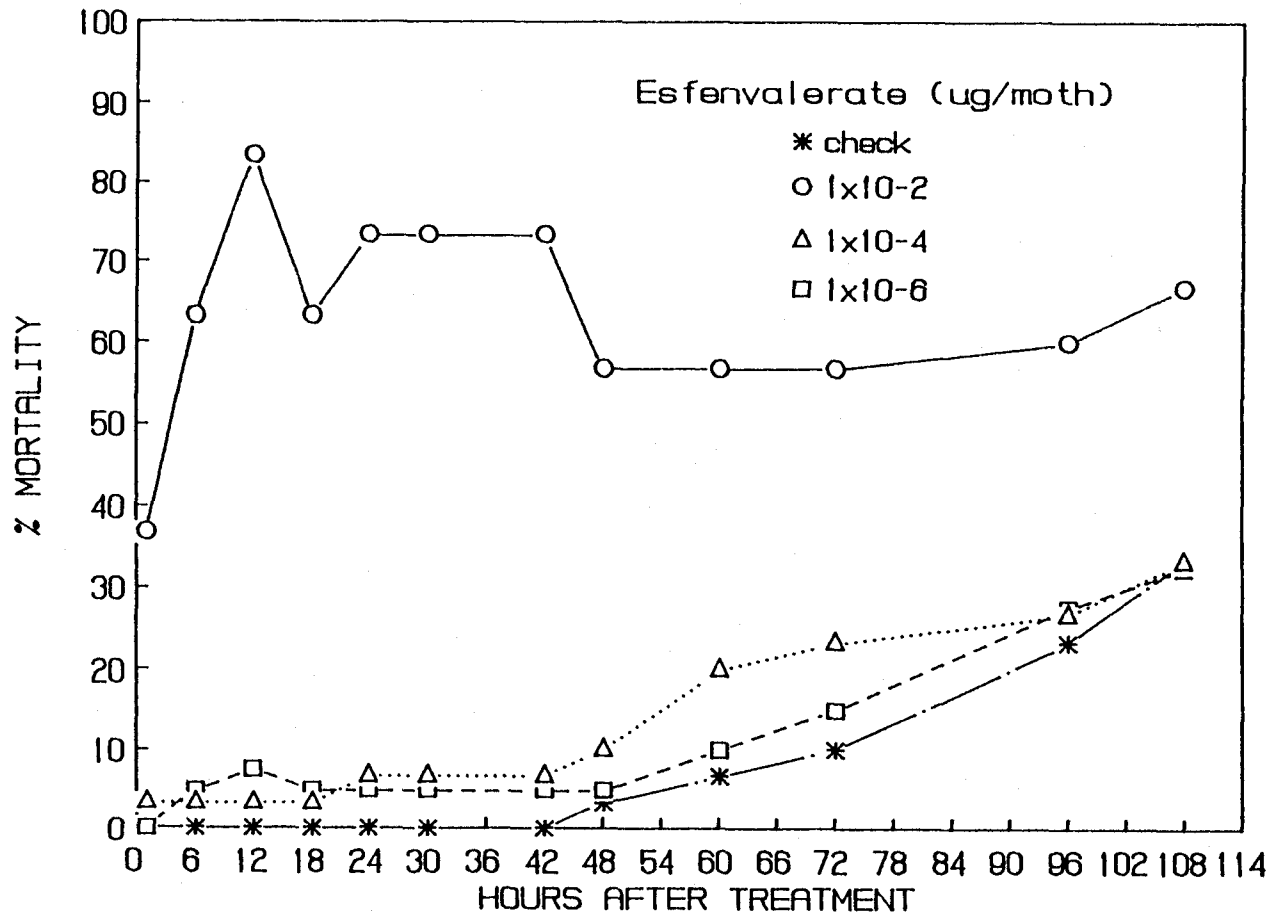
Fig. 4. Time-mortality response of male P. elmaella moths held in glass vials after topical application of esfenvalerate.
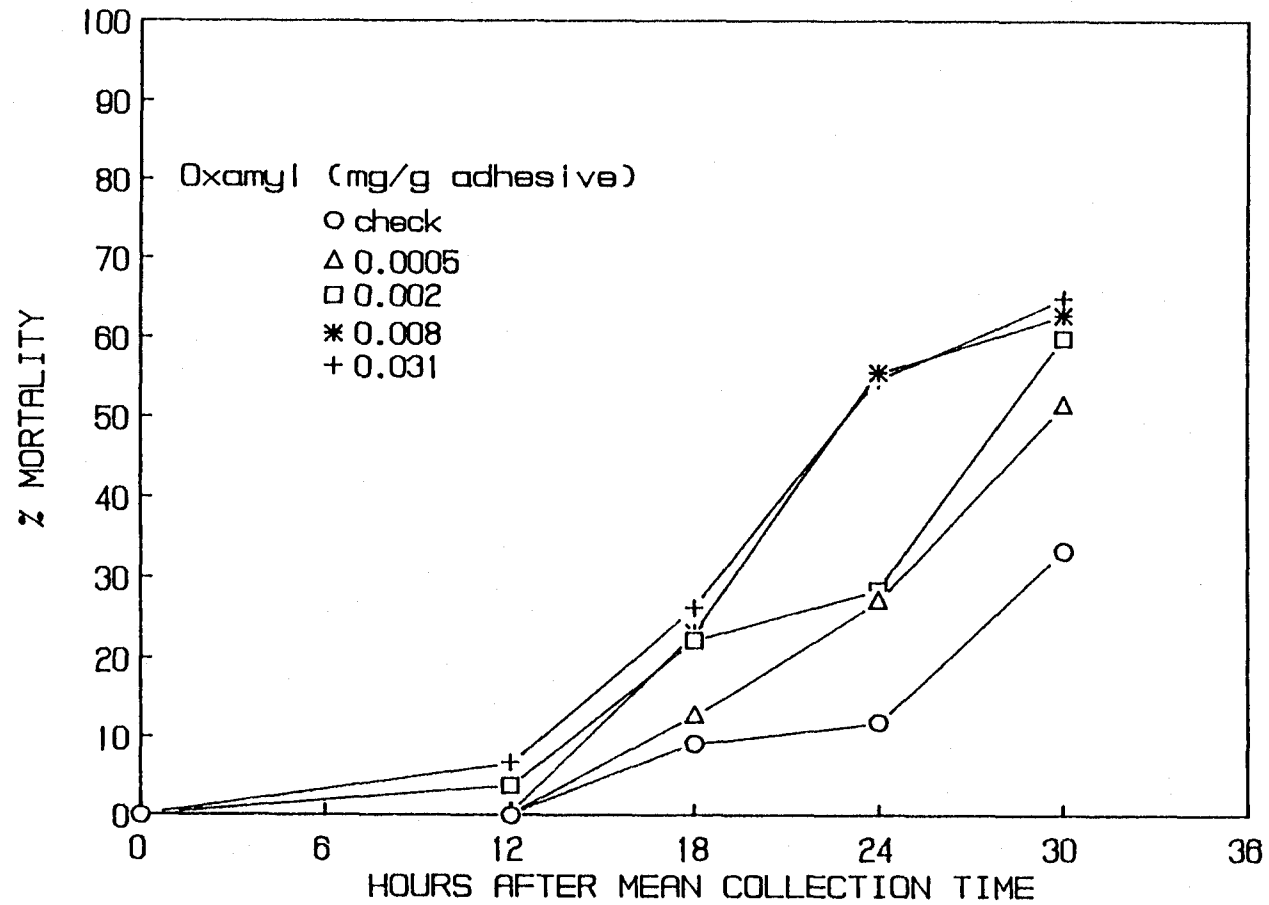
Fig. 5. Time-mortality response of male P. elmaella moths to oxamyl using the insecticide-laced assay method.
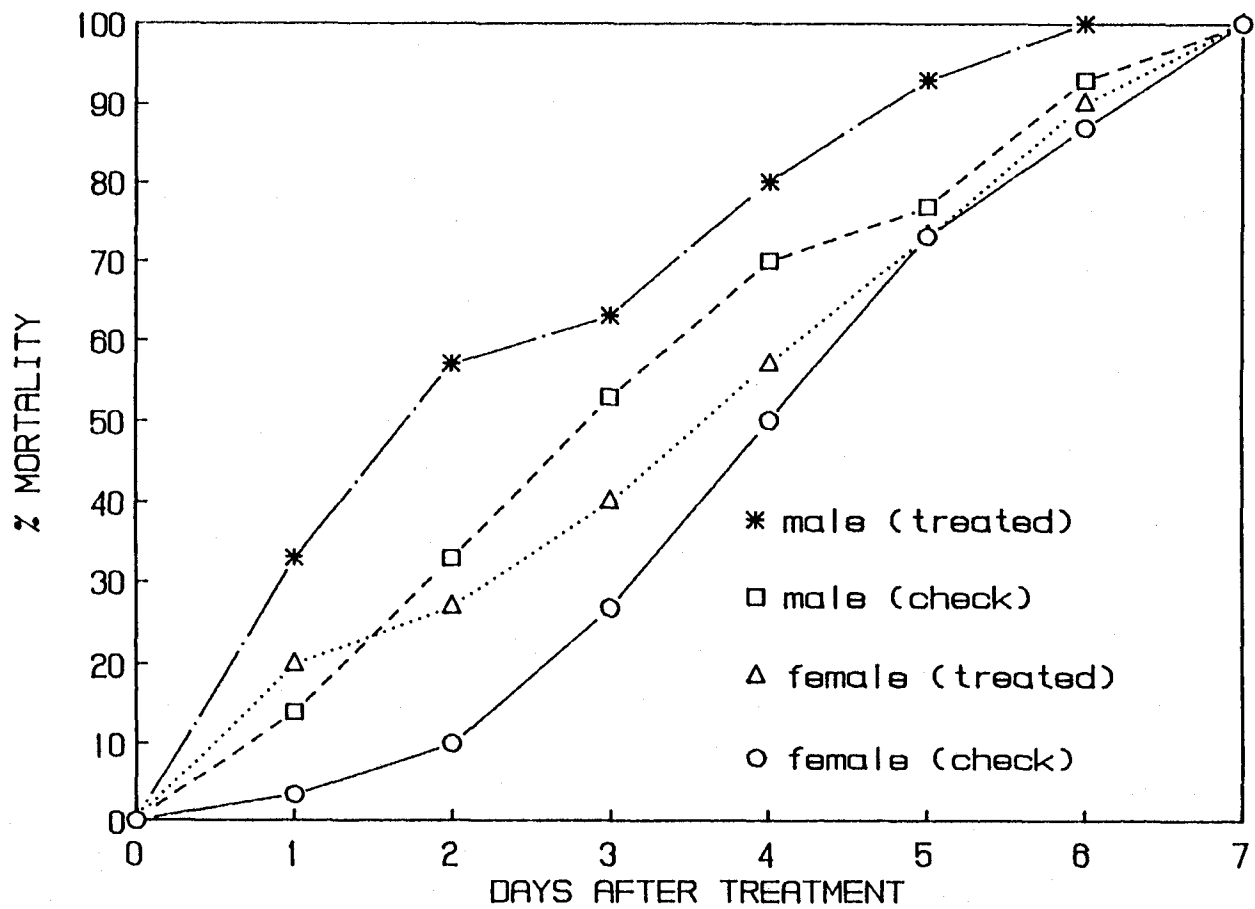
Fig. 6. Time-mortality response of male and female P. elmaella
moths held in glass vials after topical treatment with oxamyl (0.008 ug/moth).
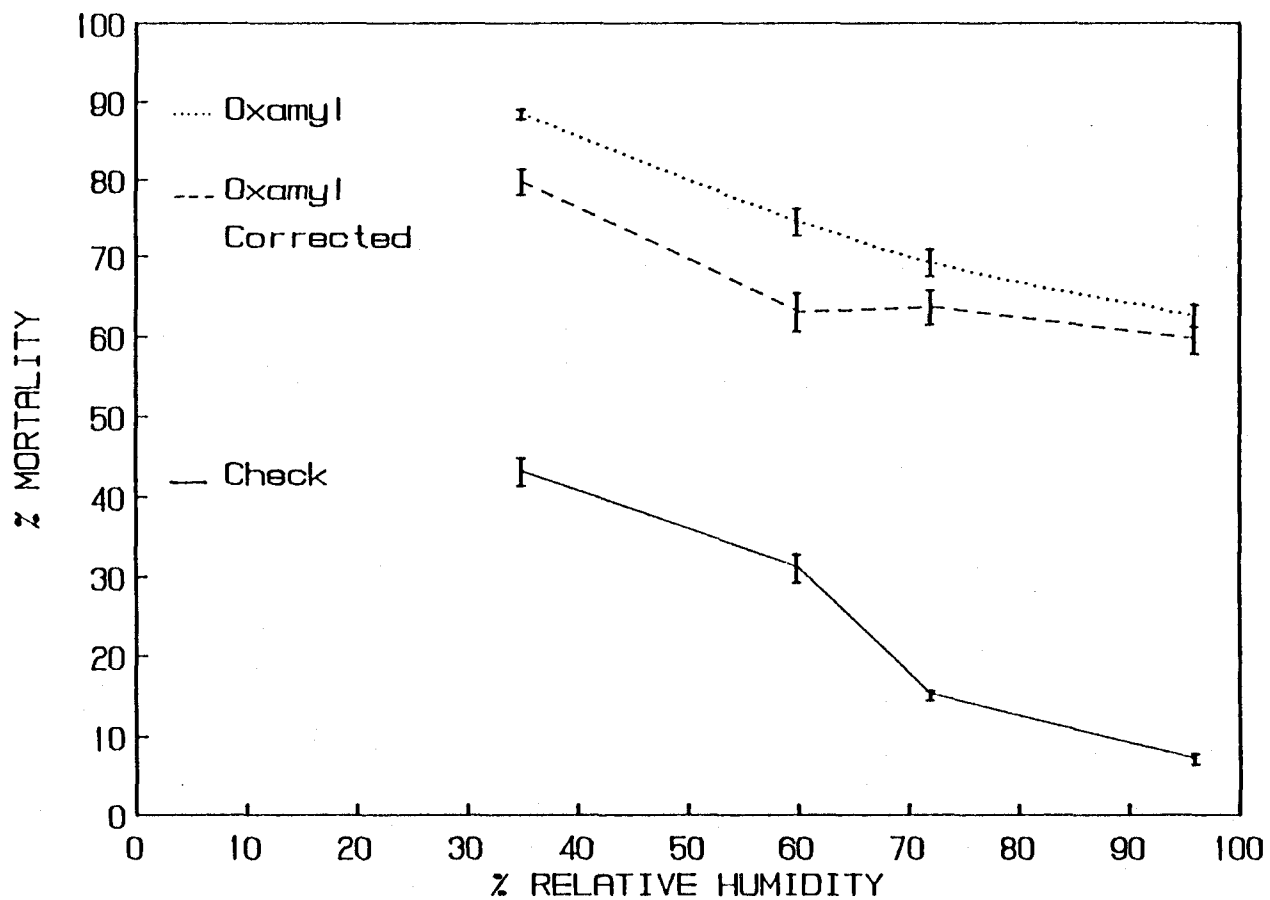
Fig. 7. Response of oxamyl treated (0.008 ug/moth) and untreated field collected male P. elmaella moths held on pheromone traps and exposed to different humidity levels at 15.6 °C (topical assay).

mortality of oxamyl-treated moths by 26%. Check mortality declined as well over the same humidity range. Adjusting for natural mortality partially removed the effects of low humidity on the mortality of oxamyl-treated moths (Fig. 7). Mortality at the 60% RH level was higher than what was normally expected at this humidity. Different post-treatment temperature conditions also affected moth mortality in the bioassays. Concentration-response lines were generated for azinphosmethyl-treated moths held at three different temperatures for 24 h (Fig. 8). The LC_{50} approximately doubled when the temperature was lowered from 18.3 to 15.6 ^{o}C and from 15.6 to 12.8 ^{o}C. Conversely, the slopes of the response lines became steeper as the temperature was increased. Check mortality was 4, 16, and 17% at temperatures of 12.8, 15.6, and 18.3 ^{o}C, respectively.

Effect of weather conditions prior to collection.

Table 3 shows the correlation coefficients, r, when % mortality of control moths in various topical bioassay was correlated with temperatures or % RH at the time of trap placement (0 h) or with the minima or maxima during the preceding 24 h or 48 h periods prior to moth collection. High temperatures between 0 and 24 h and 0 and 48 h prior to moth collection had significant r-values (P=0.05) indicating a positive correlation between temperature and check mortality. There was also a significant negative correlation between mortality and the minimum humidity levels during the 24 h period preceding trap placement.
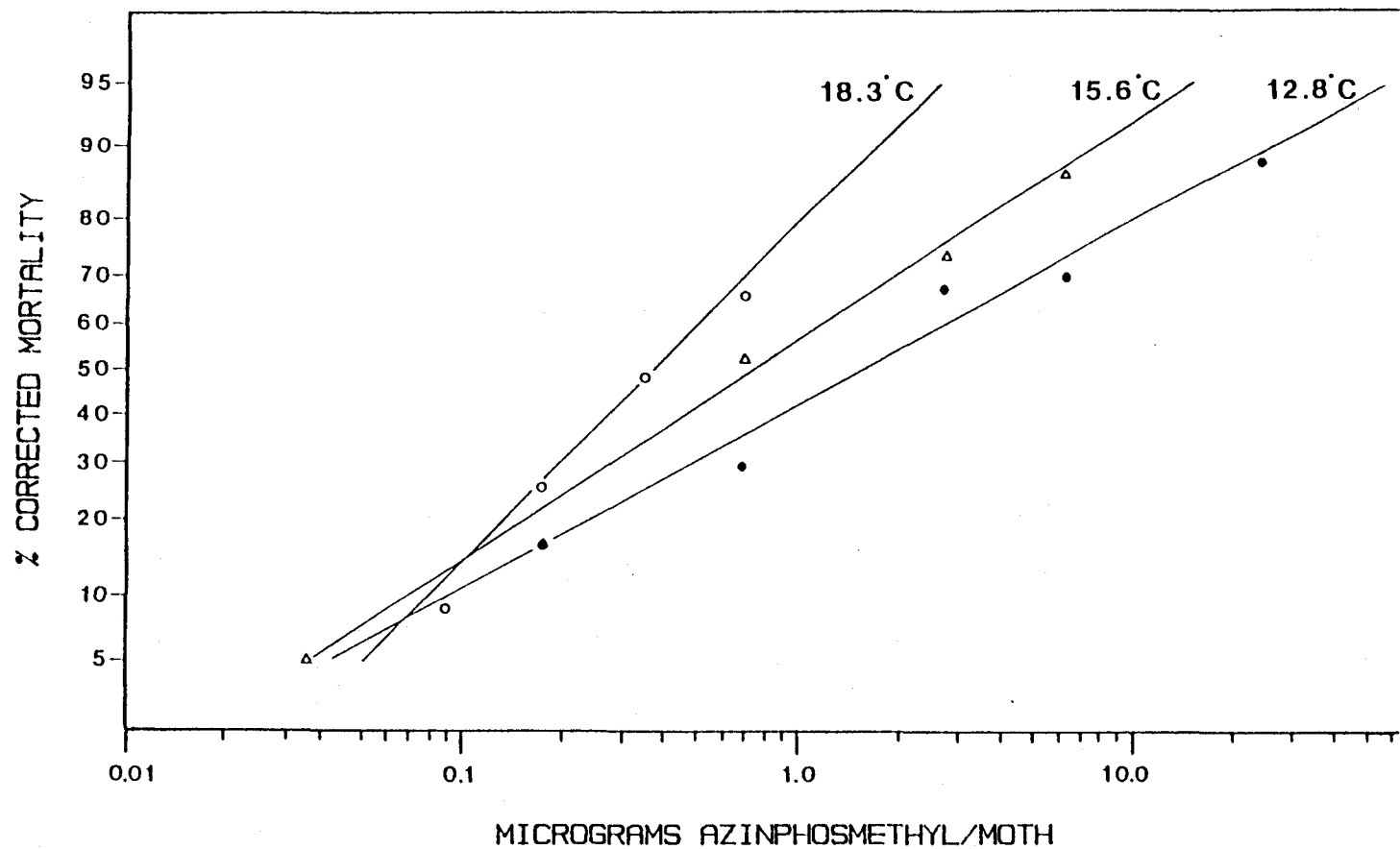
Fig. 8. Concentration-mortality response lines for field collected male P. elmaella male moths on pheromone traps assayed topically with azinphosmethyl and held under different temperature conditions and constant relative humidity (60% RH).

Table 3. Correlation analysis of % check mortality and (a) timing of bioassays and (b) environmental conditions in the field.

Factor	n [z]	r-value [y]
(a)		
Generation	39	0.461 **
Flight period	39	0.409 *
(b)		
Temp (T) at collection	37	-0.198
T, pre-24 h hi	37	0.383 *
T, pre-48 h hi	37	0.406 *
T, pre-24 h low	37	0.012
T, pre-48 h low	37	0.011
% RH at collection	35	0.193
% RH, pre-24 h hi	35	0.301
% RH, pre-48 h hi	35	0.260
% RH, pre-24 h low	35	-0.489 **
% RH, pre-48 h low	35	-0.257

[z] n = number of data pairs used in analysis.

[y] * significant at P = 0.05 and ** at P = 0.01.

<u>Timing of bioassays in relation to adult WTLM flights</u>.

Adult WTLM trap catches from two years were plotted by calender date and on a degree-day scale (base 5°). In 1988, trap catches from the MCAREC (Fig. 9) and The Dalles (Fig. 10) show three complete flights and a partial fourth. 1989 was a cooler year and only 3 adult flights were observed at the MCAREC (Fig. 11). Check mortality values obtained from topical bioassays were positively correlated with adult WTLM generations (Table 3) indicating that check mortality increased with each adult flight. Additionally, check mortality was positively correlated with the timing of bioassays during a flight period. Check mortality increased as flights progressed from early (pre-peak) to late flight (post-peak).

<u>Effect of field exposure to insecticides prior to collection</u>.

Oxamyl caused a substantial reduction in the adult WTLM population as indicated by the lower pheromone trap catches in the treated area compared to the catches in the unsprayed plot (Table 4). The azinphosmethyl spray caused little adult mortality in the field. However, both orchard-applied insecticides affected moth response in the bioassays. Topically treated moths collected from the oxamyl- and azinphosmethyl-sprayed plots showed an increase in mortality when compared to moths from the untreated plot (Fig. 12). The increase in bioassay mortality because of prior insecticide exposure in the field was no longer apparent 7 days after the application.

<u>Resistance survey</u>.

Moths collected from sweet cherry orchards in The Dalles and
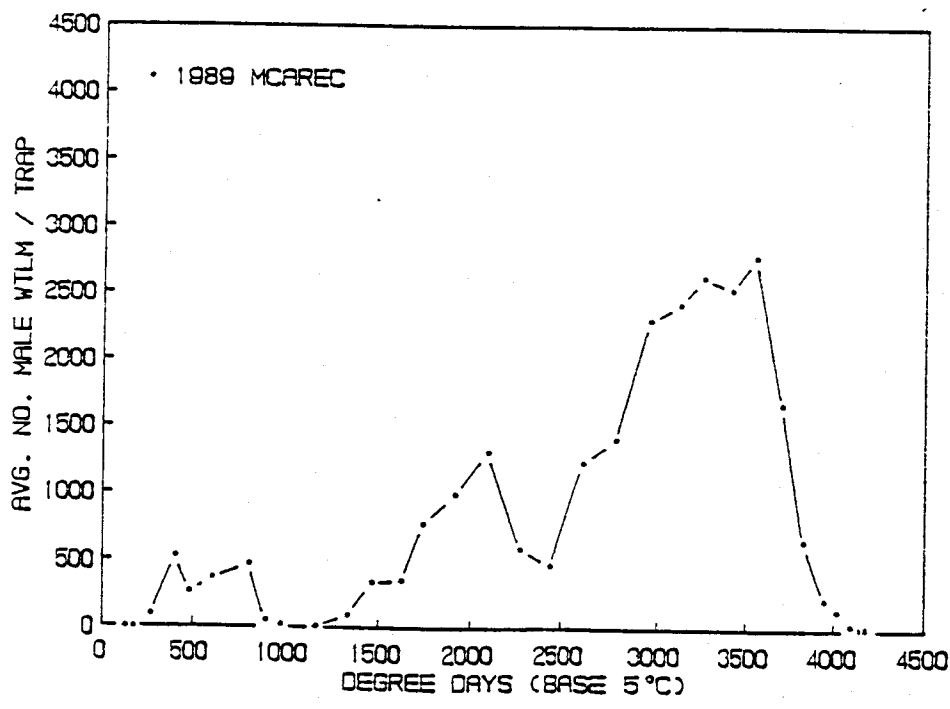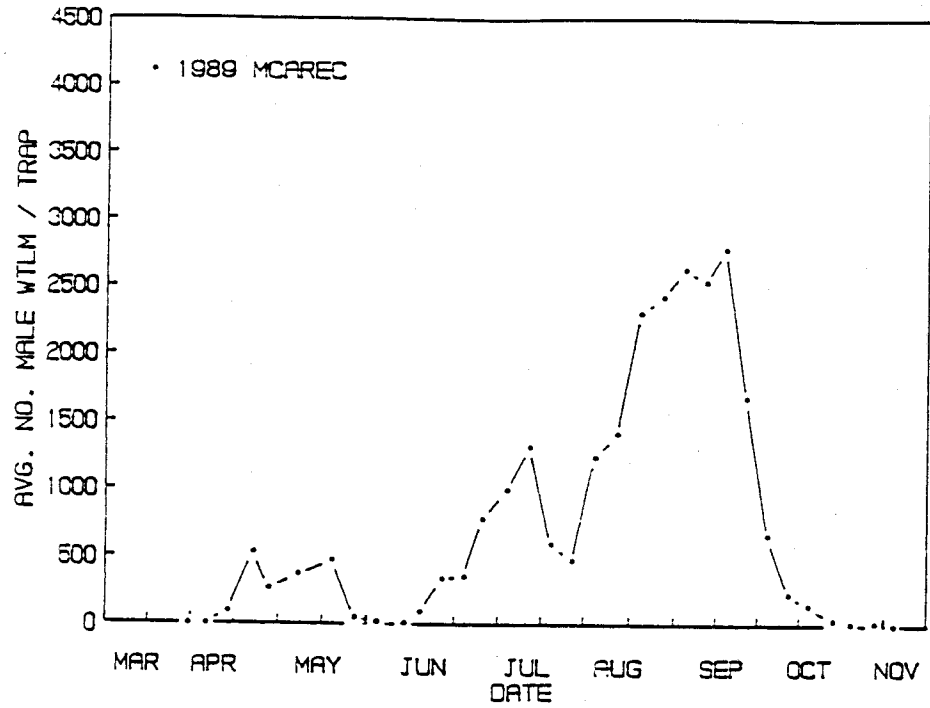
Fig. 9. 1989 seasonal WTLM flight curves from the
MCAREC (apple) plotted by (a) calender date or
(b) degree-days (base 5 °C).
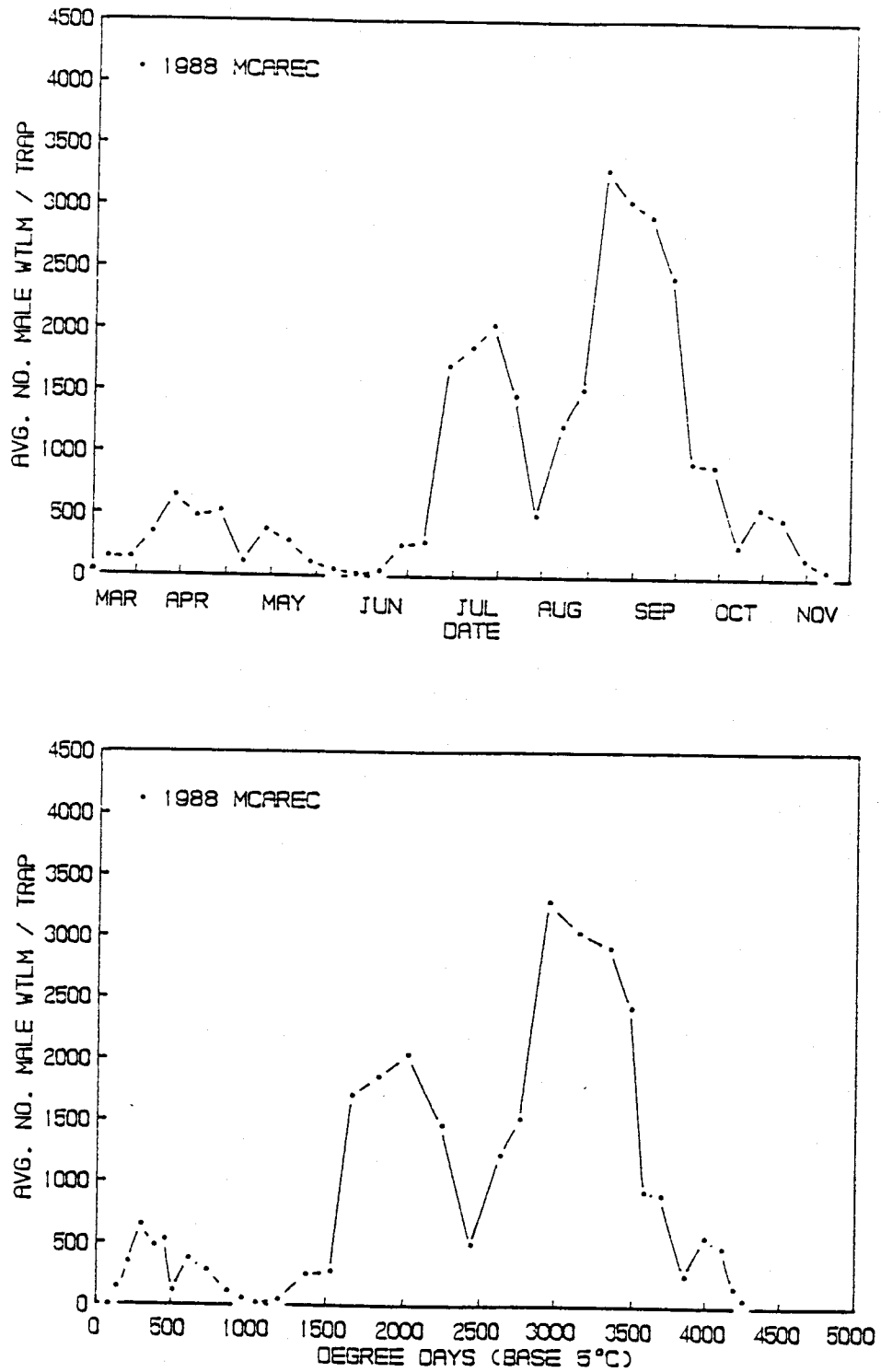
Fig. 10. 1988 seasonal WTLM flight curves from the
MCAREC (apple) plotted by (a) calender date or
(b) degree-days (base 5 °C).
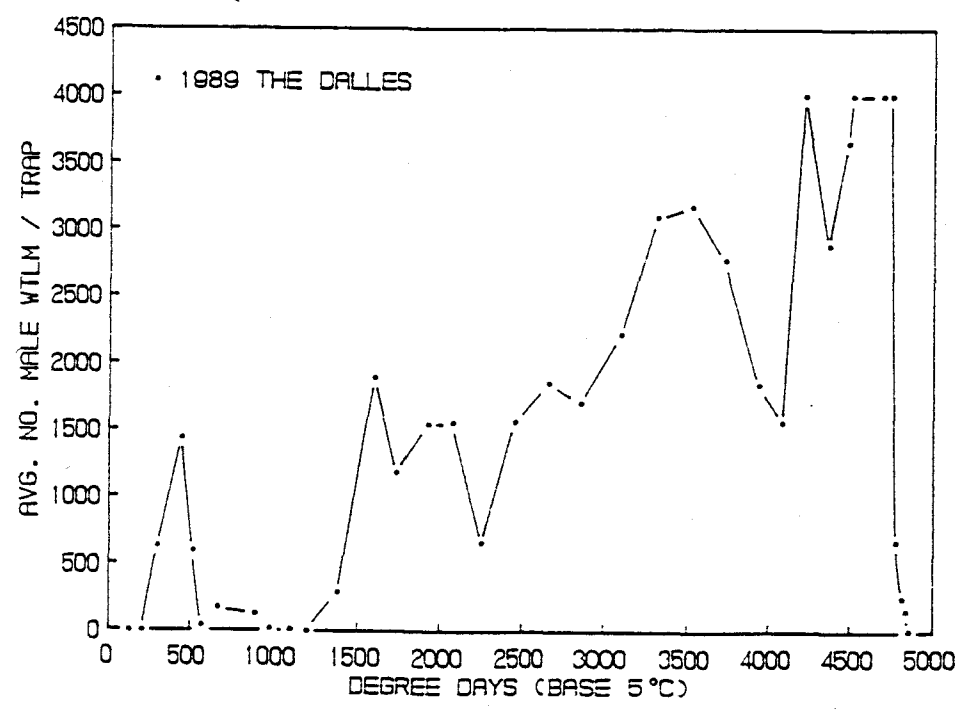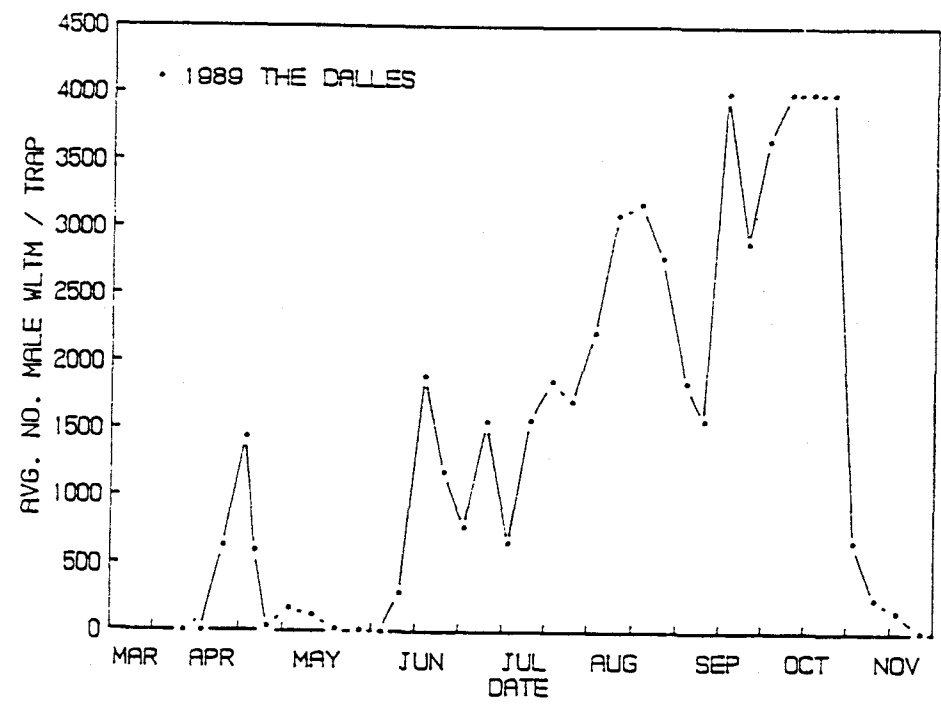
Fig. 11. 1989 seasonal WTLM flight curves from The Dalles (cherry) plotted by (a) calender date or (b) degree-days (base 5 °C).

Table 4. Average number of male P. elmaella moths
captured in pheromone traps after field applications of
oxamyl and azinphosmethyl.

| | No. male WTLM moths/trap/day | | |
| | Days after application | | |
Insecticide	1	3	7
Oxamyl	82	174	95
Azinphosmethyl	241	240	301
Untreated	343	210	233
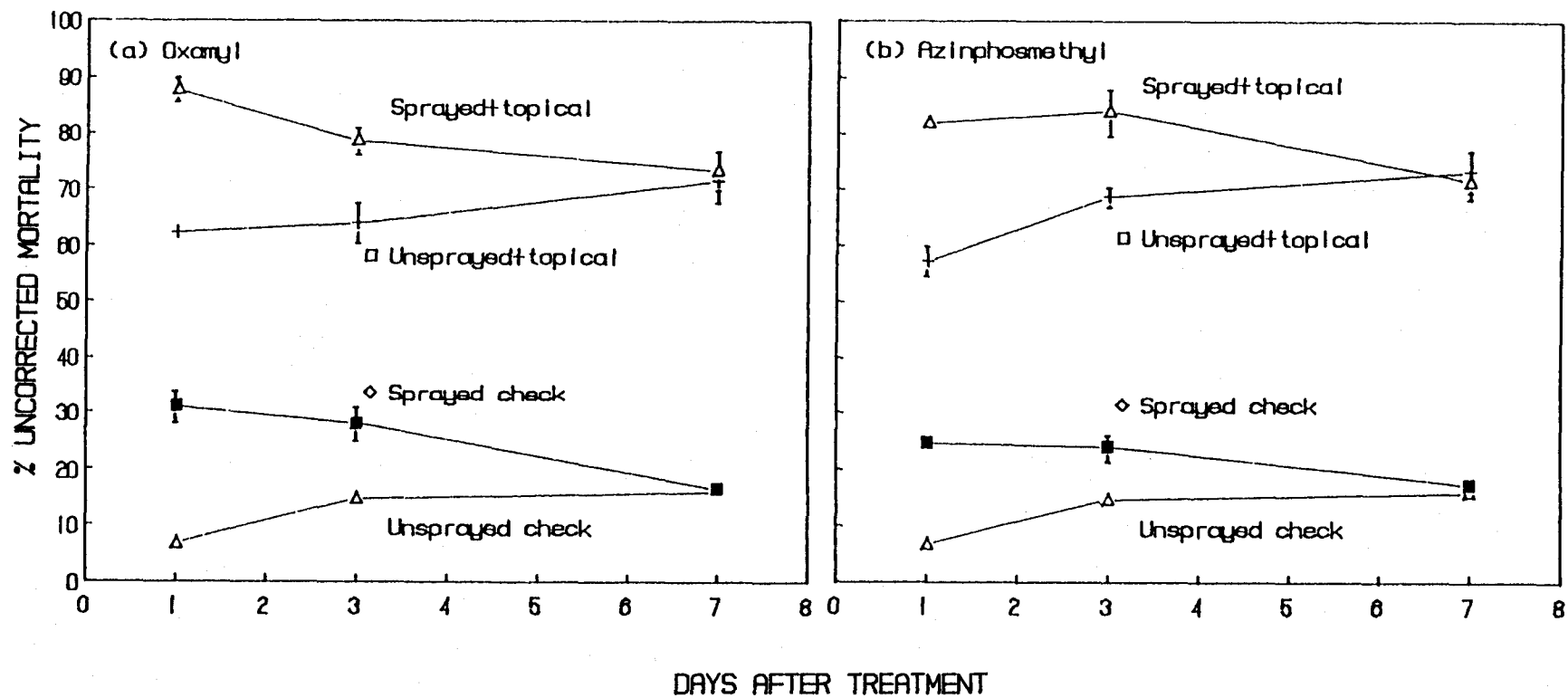
Fig. 12. Mortality response of field-collected male P. elmaella moths on pheromone traps in the laboratory following exposure to a field application and topical application of (a) oxamyl and (b) azinphosmethyl.

apple blocks at the MCAREC produced similar concentration-response lines when they were treated topically with azinphosmethyl (Table 5). Moths from the abandoned Skamania site were 45 to 87 times more susceptible at the LC_{50} level and 71 to 285 times more susceptible at the LC_{90} level then moth populations from the other collection areas. The slope of the abandoned site's azinphosmethyl concentration-response line was steeper than lines generated from moths collected from commercial orchards. Figure 13 shows representative concentration-response lines obtained from testing WTLM populations from the MCAREC and the abandoned site with topically applied azinphosmethyl. About 5 to 7% of the abandoned site's population survived high rates of azinphosmethyl.

When oxamyl was administered topically to WTLM male moths from commercial orchards in The Dalles and apple blocks at the MCAREC, the resulting concentration-response lines were similar to the response of the population from the abandoned Skamania site (Table 6). This indicated that moths in these commercial orchards were still susceptible to oxamyl. All tests except the pre-peak, 3rd flight from MCAREC had overlapping confidence limits (95%) at the LC_{50} level. The LC_{90} confidence limits all overlapped. When WTLM male moths, collected from the abandoned site and a commercial orchard in The Dalles, were exposed to oxamyl administered with the insecticide-laced assay, similar concentration-response lines were produced (Fig. 14). This was additional evidence that WTLM from commercial orchards were still susceptible to oxamyl.

Treating WTLM moths with esfenvalerate yielded inconsistent

Table 5. LC_{50}, LC_{90} values and coefficients of the concentration-response lines for topically applied azinphosmethyl of male P. elmaella moths collected with pheromone traps in study orchards.

Site (Host)	Timing	Moths tested (n)	LC_{50} (ug/moth) (95% CL)	LC_{90} (ug/moth) (95% CL)	Slope (+SE)
The Dalles - O (cherry)	Prepeak, 1st flight	480	0.137 (0.087-0.196)	1.195 (0.799-2.098)	1.364 (0.166)
The Dalles - M (cherry)	Prepeak, 1st flight	474	0.325 (0.160-0.582)	3.999 (1.956-13.057)	1.175 (0.108)
MCAREC (apple)	Prepeak 2nd flight	614	0.282 (0.224-0.350)	1.241 (0.940-1.790)	1.992 (0.188)
The Dalles - O (cherry)	Prepeak, 3rd flight	395	0.345 (0.199-0.509)	0.910 (0.595-3.137)	3.046 (0.521)
The Dalles - M (cherry)	Prepeak, 3rd flight	267	0.224 (0.134-0.340)	1.341 (0.769-3.976)	1.648 (0.325)
The Dalles - McC (cherry)	Prepeak, 3rd flight	611	0.313 (0.111-0.687)	2.649 (1.083-27.232)	1.381 (.155)
St. Cloud (abandoned)	Prepeak, 3rd flight	478	0.004 (0.003-0.005)	0.014 (0.010-0.024)	2.267 (0.338)
MCAREC (apple)	Prepeak, 3rd flight	883	0.769 (0.369-1.277)	8.033 (4.420-23.58)	1.258 (0.266)
MCAREC (apple)	Prepeak, 4th flight	295	0.179 (0.063-0.319)	1.602 (0.795-9.140)	1.348 (0.349)
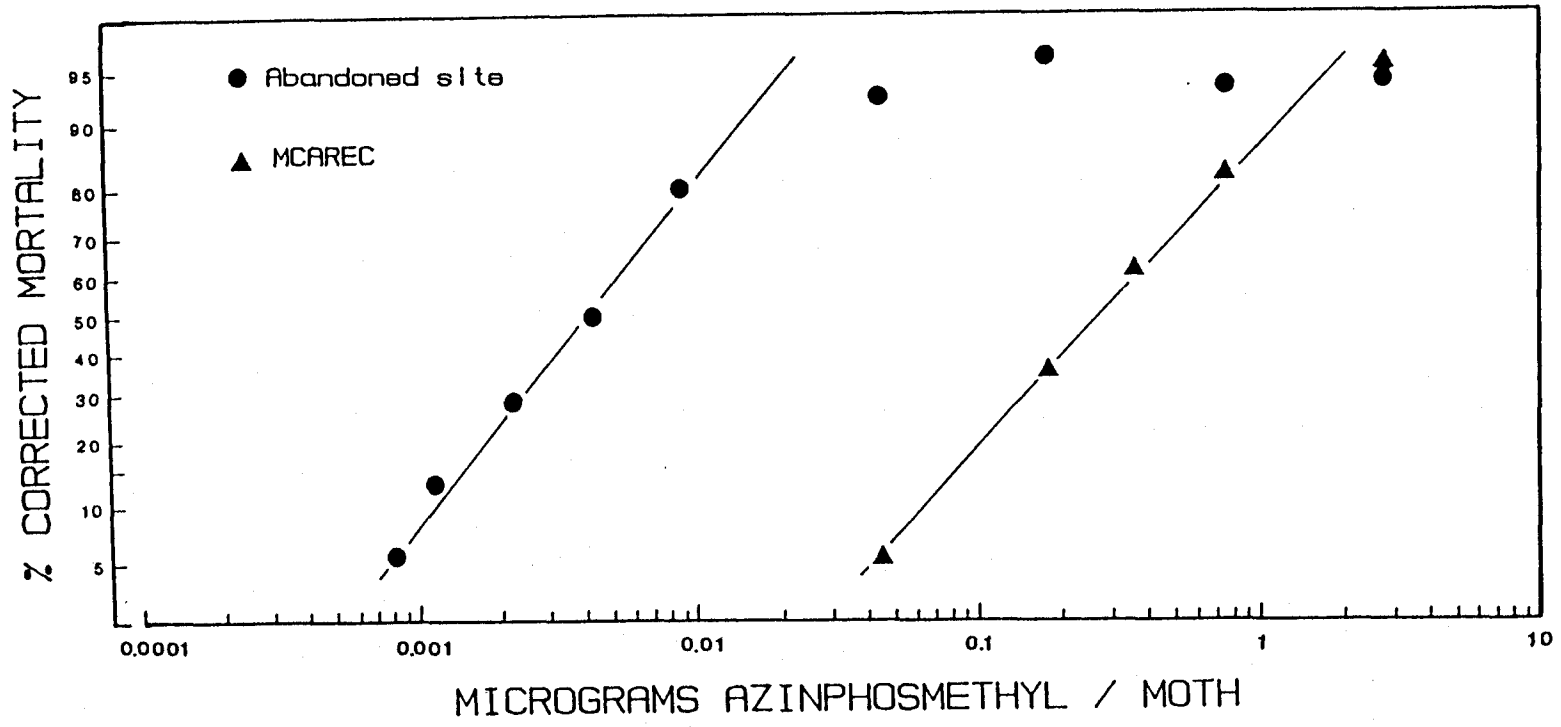
Fig. 13. Concentration-response lines for field-collected male P. elmaella moths from the MCAREC and abandoned Skamania site assayed topically with azinphosmethyl.

Table 6. LC_{50}, LC_{90} values and coefficients of the concentration-response lines for topically applied oxamyl for male P. elmaella moths collected with pheromone traps in study orchards.

Site (Host)	Timing	Moths tested (n)	LC_{50} (ug/moth) (95% CL)	LC_{90} (ug/moth) (95% CL)	Slope (+SE)
MCAREC (apple)	Prepeak, 2nd flight	760	0.0018 (0.0011-0.0026)	0.0210 (0.0138-0.0400)	1.207 (0.165)
St. Cloud (abandoned)	Prepeak, 2nd flight	402	0.0025 (0.0019-0.0030)	0.0110 (0.0083-0.0163)	1.970 (0.227)
The Dalles - O (cherry)	Peak, 2nd flight	455	0.0026 (0.0008-0.0050)	0.0172 (0.0086-0.0863)	1.567 (0.234)
The Dalles - O (cherry)	Prepeak, 3rd flight	515	0.0029 (0.0021-0.0037)	0.0152 (0.0118-0.0214)	1.778 (0.193)
MCAREC (apple)	Prepeak, 3rd flight	259	0.0059 (0.0045-0.0085)	0.0220 (0.0133-0.0607)	2.280 (.455)
The Dalles - M (cherry)	Prepeak, 4th flight	518	0.0027 (0.0019-0.0035)	0.0090 (0.0068-0.0149)	2.446 (0.424)
MCAREC (apple)	Prepeak, 4th flight	325	0.0041 (0.0018-0.0063)	0.0274 (0.0160-0.0966)	1.556 (0.377)
St. Cloud (abandoned)	Prepeak 4th flight	168	0.0025 (0.0017-0.0033)	0.0068 (0.0050-0.0120)	2.990 (0.605)
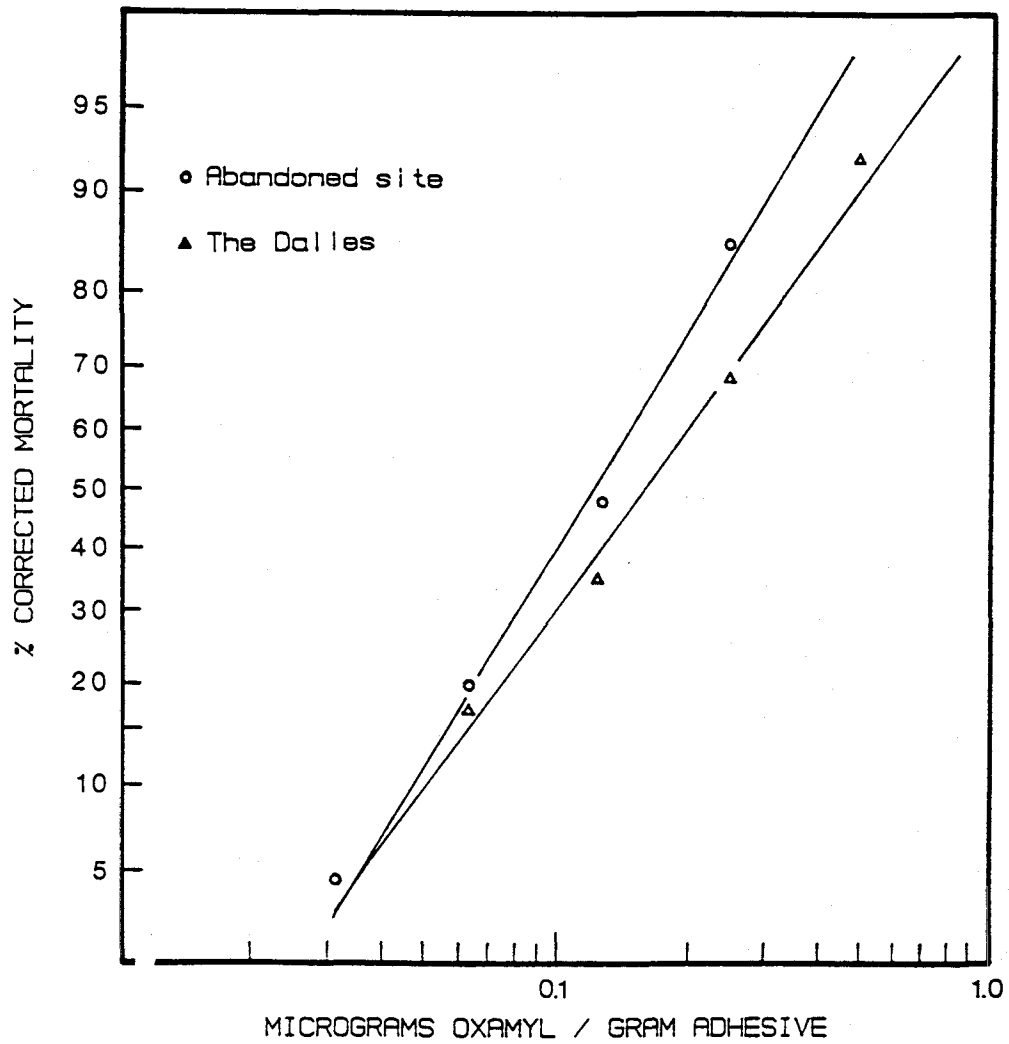
Fig. 14. Concentration-mortality response lines for field-collected male P. elmaella moths from The Dalles and the abandoned Skamania site assayed with oxamyl-laced adhesive.

results. Because some of the treated moths initially scored as dead recovered after 24 h, useful dose-response lines could not be obtained. Table 7 shows the line parameters for endosulfan applied topically to WTLM male moths collected from apple blocks at the MCAREC and sweet cherry orchards in The Dalles. The confidence limits at the LC_{50} level overlapped indicating the response from these 2 sites were similar.

Table 7. LV_{50}, LC_{90} values and coefficients of the concentration-response lines for topically applied endosulfan for male _P. elmaella_ moths collected with pheromone traps in study orchards.

Site (Host)	Timing	Moths tested (n)	LC_{50} (ug/moth) (95% CL)	LC_{90} (ug/moth) (95% CL)	Slope (+SE)
MCAREC (apple)	Prepeak, 2nd flight	384	0.032 (0.017-0.058)	0.307 (0.139-1.555)	1.300 (0.155)
The Dalles - 0 (cherry)	Peak, 2nd flight	455	0.019 (0.009-0.029)	0.107 (0.062-0.382)	1.682 (0.206)

Discussion

This study has shown that the topical application and the insecticide-laced adhesive pheromone trap assays can be adapted for for detecting and monitoring insecticide resistance in WTLM populations. However, modifications of both assay methods are required because of the small size of the WTLM moth. Suggested modifications include a smaller droplet size for the topical application of an insecticide and a reduction in the amount of adhesive on the trap to minimize adhesive-induced mortality. An advantage of these two pheromone-trap assays is the ability to easily trap and treat large numbers of test insects. Large sample sizes are needed for resistance detection studies (Robertson et al. 1984, Roush and Miller 1986). Pheromone-trap collection also is more efficient at low population density, eliminates laboratory rearing of test subjects and reduces mortality associated with the handling of moths.

Certain limitations in the application of the adhesive assay for monitoring insecticide resistance in the WTLM were noted during the course of this study. It was not possible to obtain meaningful concentration-response lines for azinphosmethyl-resistant WTLM populations with the adhesive assay since mortality never exceeded 30% in spite of high concentrations of insecticide in the adhesive. This problem may be related to insecticide uptake from the adhesive. It is possible that the adhesive interferes with uptake in such a way that only a limited amount of insecticide can be absorbed which is not sufficient to produce mortality in highly resistant moths. In

addition, the adhesive assay produced concentration-response lines with greater variability than the topical assay for oxamyl. Again, this may be related to uptake since the dose of the insecticide absorbed by each moth is dependent on how much of the body is in contact with the adhesive. The topical assay, on the other hand, delivers precise amounts of insecticide to each individual moth which reduces variability due to differential uptake.

Because of the noted drawbacks of the adhesive assay, the topical method appears to be better suited for characterizing the toxicological response of resistant WTLM populations. However, the insecticide-laced assay is easier to use and requires no special application equipment making it a useful test method for diagnostic-dose surveys and for resistance detection programs where early discovery of low-frequency resistance is desirable (Metcalf 1980).

Although the pheromone trap assays appear to be reliable and are easy to conduct, there is a concern about their usefulness as resistance monitoring tools. Robertson and Worner (1990) criticize test protocols where only one developmental stage is tested because results may not reflect a field population's true response to an insecticide. Physiological and morphological differences between males and females may affect an insect's response to an insecticide. A sex-related differential response to an insecticide was observed during this study. Results here indicated that male WTLM moths were more susceptible than females to oxamyl. Both Riedl et al. (1985) and Knight and Hull (1989) showed that males were more susceptible to topically applied insecticides than females. It is possible,

therefore, that test results derived only from males moths may not be indicative of resistance in other life stages of WTLM. Differences in response to an insecticide can also occur between different developmental stages of insects. Companhola and Plapp (1989) reported that adult Heliothis virescens (F.) (Lepidoptera: Noctuidae) were more resistant to insecticides than larvae. Differences in larval instar susceptibility were also noted. Therefore, differences in insecticide susceptibility between adults and larvae should be studied (Haynes et al. 1987).

Biossay mortality was shown to be affected by several factors including the brand of adhesive used to capture and hold the moths, environmental conditions in the field prior to moth collection and during the bioassays, prior field exposure to insecticides, and the timing of the bioassays in relationship to WTLM flights. The investigation of these variables was necessary to standardize the bioassay procedures so results can be compared (Robertson and Worner 1990).

Mortality due to the polybutene trap adhesive is a concern when it is used as a bioassay substrate. If the adhesive layer is too thick, it will increase mortality (Riedl et al. 1985, Sanderson et al. 1989). On the other hand, if not enough adhesive is applied, trapping efficiency may be reduced. There is also the possibility that the adhesive may emit toxic volatiles. However, when prescribed amounts of Tangletrap, the preferred adhesive in this study, are properly applied, mortality of WTLM check moths can be kept low.

Another concern is that a behavioral response of an insect

attracted to pheromone traps laced with an insecticide may alter test results if the insecticide repels the insect. If this avoidance behavior is correlated with physiological resistance, then bioassay results will not reflect the true response of the population. The possibility exists that insecticide volatiles escaping from the adhesive in insecticide-laced assays may limit catches by interfering with an insect's attraction to artificial sex pheromones (Sower and Shorb 1985) or be repellent in itself (Haynes et al. 1987). This was not apparent with WTLM.

Tests suggest that the duration of the bioassay should not exceed 24 h to avoid unacceptably high control mortality. Ideally, choosing the time when the bioassay is terminated should be based upon when the mortality of treated insects starts to stabilize. However, in this study, check mortality often rose to unacceptable levels before insecticide-induced mortality at high rates stabilized.

Moth mortality in the bioassays was very sensitive to temperature and humidity. Therefore, the environmental conditions for conducting bioassays with the WTLM need to be defined and standardized. Studies suggest that bioassays should be conducted at 15.6 $^{\circ}$C. This is a compromise between trying to achieve steeper concentration-response lines at warmer temperatures and maintaining low check mortality at cooler temperatures. Humidity levels should be 60 %RH or greater to minimize check mortality. The requirements for precise holding conditions during the bioassay may limit the usefulness of both pheromone-trap assay methods since controlled environments are not always readily available. High humidity can be maintained with moist

paper-towels (Follett 1985) but temperature control may be more difficult to achieve. If bioassays are conducted under different environmental conditions, it is difficult to compare susceptibility levels between populations. However, it may be possible to make the necessary corrections using the temperature, humidity and moth mortality relationships developed for azinphosmethyl and oxamyl in this study.

In addition to environmental conditions during the bioassay, the weather extremes experienced by male P. elmaella moths in the field prior to collection also affected their mortality response in the bioassays. Heat stress from high temperatures coupled with low humidity during the 24 and 48 h periods before collection increased moth mortality in the bioassays. Since the physiological condition of the moths cannot be easily determined, it is important to avoid periods of hot weather when trapping WTLM moths for bioassays.

Insecticide applications to orchard trees prior to moth collection represents another complicating factor when assaying field-collected insects. Riedl et al. (1985) speculated and Knight and Hull (1989) demonstrated that previous exposure to insecticides can increase bioassay mortality of moths collected from sprayed orchards. This can confound test results and make a population appear more susceptible than it actually is. Tests here show that WTLM mortality is higher when moths are collected from orchards recently sprayed with either oxamyl or azinphosmethyl than from an unsprayed adjacent block. This sublethal effect lasted for a week in this study. Knight and Hull (1989) recommended a 48 h waiting period before trapping P.

idaeusalis moths from insecticide-treated orchards in order to avoid this added mortality. The interval following the application of an insecticide to an orchard before WTLM are collected for bioassays should be based on the persistance and rate of the insecticide applied.

The age and vigor of the test insects can also influence test results. Riedl et al. (1985) showed that mortality of C. pomonella moths increases with their age. Keil et al. (1985) and Sanderson et al. (1989) suggest standardizing the age of reared L. trifolii at 0 to 3 d to reduce control mortality. However, Haynes et al. (1987) demonstrated that the age of P. gossypiella moths did not adversely affect test results, nor did age affect the mortality response of P. idaeusalis (Knight and Hull 1989).

The age structure of a WTLM population changes throughout the season. As hot weather advances the physiological age of moths, age variability is greater during the summer and early fall than in the spring. The first flight is discrete. However, during the summer flights overlap. At the onset of the third and fourth generations, the population is a mixture of old moths from the previous generation and young moths from the current generation. In order to capture moths of a more uniform age, Riedl et al. (1985) and Knight and Hull (1987) placed traps in the field during early spring flight.

There is indirect evidence that the age distribution of field-collected WTLM males affected their response in bioassays. Tests conducted during the ascending part of the flight curve, when the population consisted primarily of young individuals had consistently

lower check mortality than later on when the flight curve was
descending. Check mortality increased with each generation. It is
suggested, therefore, to collect moths for bioassays during times when
the age structure of the population is more uniform (ascending flight
curves, early generations). For timing bioassays it is helpful to
monitor the flight periods with pheromone traps. In addition, it may
be helpful to time bioassays based upon cumulative degree-days (DD)
(Robertson and Worner 1990). This was not attempted here but it
appears to be a practical approach to timing bioassays on a yearly or
seasonal basis with insect populations whose mortality response
increases within each flight and from generation to generation. If
this degree-day approach is considered for timing of pheromone-trap
bioassays, moths should be collected between first emergence and a
cumulative total of 400 DD (from March 1; base 5 $^{\circ}$C) during the first
flight and between 1250 and 1600 DD during the second flight (see
Figs. 9b, 10b, and 11b).

For a number of years now, OP insecticides have not been
effective in suppressing WTLM populations in commercial apple and
cherry orchards in northern Oregon. Lack of control under
azinphosmethyl cover spray programs has also been observed in recent
field trials at the MCAREC (Riedl and Shearer 1988, 1989a, 1990).
Bioassays with field-collected male WTLM moths confirmed that the
cause for control failure in local orchards is the development of OP
resistance. Thus the WTLM, like the other two gracillariid leafminer
species on tree fruits, has become resistant to azinphosmethyl. It is
likely that this resistance extends to other compounds of OP chemistry

since OP insecticides as a group have lost effectiveness in the field for control of WTLM. According to the survey of WTLM populations in commercial cherry and apple orchards, azinphosmethyl resistance appears to be wide-spread and equally high throughout the area. Resistance levels were as high as 87-fold in some orchards. By comparison, Pree et al. (1980) found 160-fold azinphosmethyl resistance in P. blancardella on apple in Ontario. It should be noted that a small proportion (5-7%) of the susceptible reference population from the isolated abandoned St. Cloud site survived high rates of azinphosmethyl. Since leafminers are very dispersive (Whalon and Croft 1985) it is possible that resistant moths from commercial fruit producing areas have colonized this site. It is also possible that this low level of OP resistance is an inherent feature of the abandoned St. Cloud population.

Sweet cherry orchards in The Dalles have experienced several severe WTLM infestations since 1981 while Hood River has not been affected. In both areas azinphosmethyl resistance levels are very high in WTLM populations. Therefore, factors other than resistance appear to be responsible for the recent outbreaks on cherries in The Dalles. One explanation is the disruptive effect of insecticide sprays on the biological control of WTLM. High levels of parasitization of WTLM can occur in Washington (Barrett 1988), Utah (Barrett and Jorgenson 1986) and Oregon (unpublished data). Studies have shown that insecticides can be very disruptive to the natural enemy complex of leafminers (Dutcher and Howitt 1978, Weires et al. 1982, Van Driesche et al. 1985, Shearer 1986). The timing of

insecticide sprays is important to avoid disruption of natural enemies (Johnson et al. 1976, Dutcher and Howitt 1978) yet sprays should also be timed to sensitive leafminer life stages to help maximize control (Pree et al. 1990b). It is possible that the 5-7 weekly malathion sprays applied to sweet cherry orchards in The Dalles for cherry fruit fly control are more disruptive to the WTLM natural enemy complex than monthly cover sprays of azinphosmethyl in Hood River apple orchards.

Although P. blancardella has developed resistance to the carbamate methomyl in southern Ontario (Pree et al. 1990), oxamyl is still effective against WTLM (Hathaway et al. 1982, Riedl and Shearer 1989b). Because pheromone-trap assays indicated that susceptibility levels from The Dalles, MCAREC and the abandoned Skamania site are similar, it is concluded that moths from these areas are still susceptible to oxamyl. Field tests in Oregon (Riedl and Shearer 1989b) and Washington (Hathaway et al. 1985) indicate that some suppression can be achieved with endosulfan. The mortality response data for topically applied endosulfan can be used for reference in future resistance studies. It is likely that they do not represent the baseline response of a susceptible WTLM population since the moths for the tests were collected in commercial orchards.

Summary

This study demonstrated that the topical as well as the insecticide-laced pheromone trap assays can be useful tools for monitoring insecticide susceptibility in field populations of P. elmaella. Several factors which can influence results obtained with these assays were investigated in order to develop recommendations for the standardized use of pheromone trap assays in resistance studies of P. elmaella. The main results of this research are summarized below:

1. The topical bioassay was successfully used to monitor the insecticide susceptibility of P. elmaella to three of four insecticides: azinphosmethyl, oxamyl and endosulfan. The exception was esfenvalerate which produced variable bioassay responses since test insects recovered during the bioassays and mortality did not stabilize. The topical assay may be better suited for measuring elevated levels of resistance and characterizing them with concentration-response lines because high concentrations of insecticide can be delivered directly to the test insects. The insecticide-laced adhesive assay may be more useful for resistance detection surveys because it is easier to use.

2. The mortality response of P. elmaella moths in the bioassays was very sensitive to changes in the environmental conditions. Mortality of moths increased with temperature but decreased with rising humidity. Therefore, it is important to maintain precise temperature and humidity levels during the bioassays to achieve reliable and reproducible results. It is recommended to conduct

bioassays of WTLM under the following conditions: 15.6 $^{\circ}$C, 60% RH and 16:8 photoperiod.

3. The time when pheromone traps should be placed in the field for collecting moths for testing is also important. Bioassay results are influenced by extreme heat and orchard applied insecticides prior to moth collection. Additionally, moth mortality increases with each succeeding generation and as each flight period progresses. It is recommended, therefore, that moths be collected early in the season during cool weather and at the onset of a flight period. If an insecticide was applied to a survey orchard, allow time for deleterious residues to wear off prior to collecting moths for bioassays.

4. Surveys were conducted to evaluate the susceptibility of P. elmaella from commercial apple and sweet cherry orchards to three different insecticides. Insecticide resistance of WTLM in commercial orchards was defined in relationship to a susceptible population from an isolated abandoned site. High levels of resistance to the OP azinphosmethyl were found in moths collected from all sprayed survey sites. P. elmaella moths in The Dalles and Hood River, Oreg. are still susceptible to the carbamate oxamyl. Concentration-response lines for endosulfan from commercial sites were also generated. It still needs to be determined why outbreaks of P. elmaella have occured in The Dalles and not in Hood River even though WTLM populations in both areas have similar levels of OP resistance.

5. These bioassays can be used to answer additional questions pertaining to cross-resistance, synergism and the level and the

distribution of insecticide resistance in WTLM populations.

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