THE EFFECT OF A NEEM (*AZIDIRACHTA INDICA*) -BASED INSECTICIDE ON OVIPOSITION DETERRENCE, SURVIVAL, BEHAVIOUR, REPRODUCTION AND DEVELOPMENT OF WESTERN CHERRY FRUIT FLY (*RHAGOLETIS INDIFFERENS*) (DIPTERA : TEPHRITIDAE).

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Ed. J. vanRanden

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The effect of a neem (*Azidirachta indica*)-based insecticide on oviposition deterrence, survival, behaviour, reproduction and development of Western Cherry Fruit Fly (*Rhagoletis indifferens*) (Diptera: Tephritidae).

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SEPT 24, 1996
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ABSTRACT

In the laboratory and in a cherry orchard, a proprietary, neem-based insecticide formulation (NBI) was evaluated as a potential pest management tool for the Western Cherry Fruit Fly, *Rhagoletis indifferens* Curran. NBI was tested for its potential effects on oviposition, survival, behaviour, reproduction and development. Using adult *R. indifferens*, I found evidence for slight oviposition deterrence in the field, but this effect was not duplicated in the laboratory. No behavioural changes were observed in adult flies exposed to the NBI under field conditions. A strong negative effect on the development of eggs and decreased survival was seen when flies ingested the NBI extract with food and water immediately after eclosion. Total suppression of egg development was obtained in adults continuously exposured to concentrations > 0.5 % NBI. By delaying the onset of exposure to the NBI, egg development was increased. Larvae of *R. indifferens* were very sensitive to the effects of NBI. Incorporation of NBI into an artificial larval-diet resulted in a decrease in the formation of pupae, and subsequent adult emergence. Late third instar larvae exposed to NBI in sand formed puparia; however, numbers of adults and pupae developing inside the puparia were decreased at concentrations as low as 0.05 % NBI. Applied as a foliar spray at the appropriate time within a cherry orchard, NBI may prevent adult female *R. indifferens* from maturing viable eggs, and decrease adult survival. Well timed root drenches with a NBI could control *R. indifferens* by disrupting pupation. Applied in either manner, NBI may provide cherry orchardists with an effective, new control tactic.

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1.0. INTRODUCTION

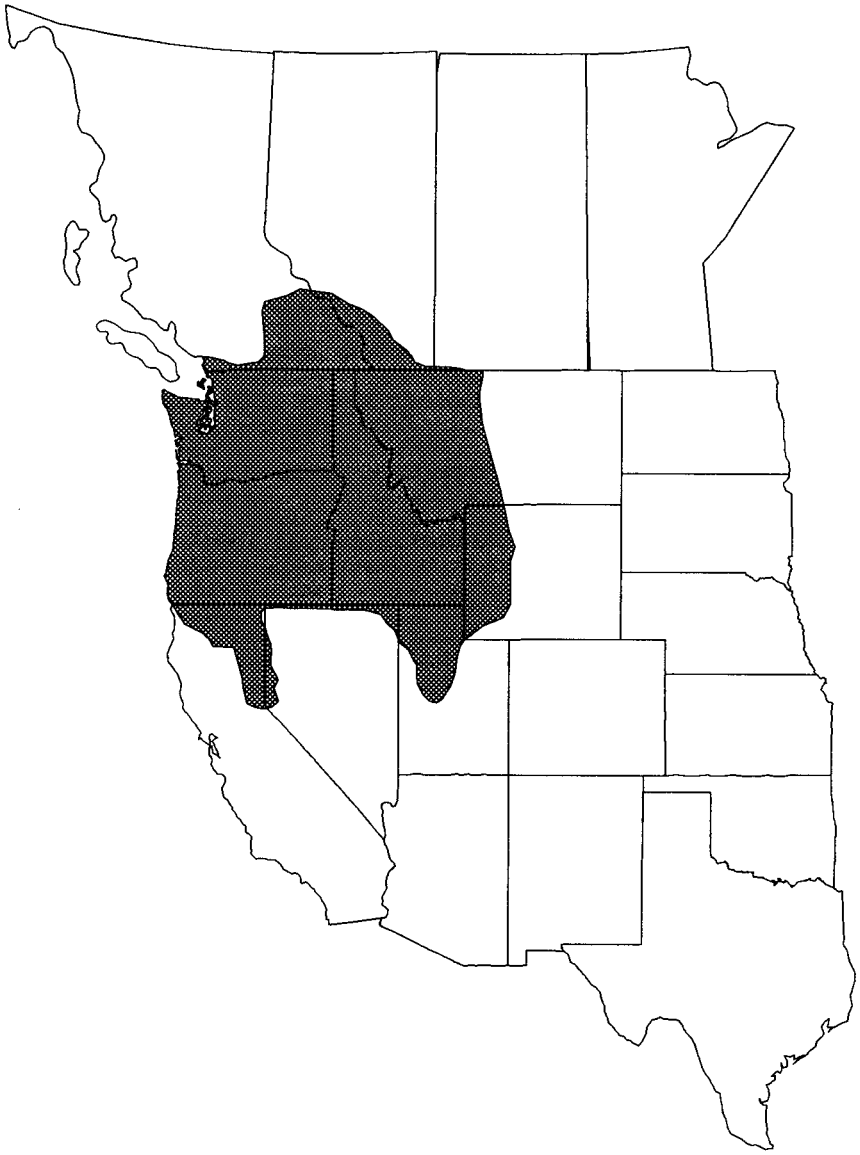
1.1 Significance of the Western Cherry Fruit Fly and Cherry Cropping

The Western Cherry Fruit Fly (WCFF), *Rhagoletis indifferens* Curran , is a key pest of cultivated cherries, *Prunus avium* L. and *P. cerasus* L., in British Columbia, Washington, Oregon, northern California and other regions of the U.S. Pacific Northwest (Fig. 1) (AliNiazee 1973, 1974, 1986; Harris 1989; Roitberg and Angerilli 1989). The species is indigenous to western North America, and wild populations are maintained on the pin cherry, *P. emarginata* L.

Growers in B.C. produce an average of 5.5 million kg of sweet cherries / year, which represents almost 80% of Canada's sweet cherry production (Lustzig 1990), and 2.6% of the crop produced in the U.S.A. (Childers 1983; Lustzig 1990). Sweet cherries are a high-value crop, netting the grower $ 6863 / ha as compared to a return of $ 3528 / ha for apple (5-year average, all packing and processing charges deducted) (Lustzig 1990).

In unmanaged cherry orchards, infestation levels of WCFF may approach 100%, resulting in a total loss of the crop (Fischer-Colbrie and Bush-Petersen 1989; pers. obs.). Chronic infestations can cause a grower to be expelled from the local marketing agency (Lusztig 1990). As a result, orchardists must maintain an intensive management program, primarily involving regular applications of chemical insecticides. WCFF dictates both the timing and type of control measures undertaken within the orchard environment (Tree Fruit Production Guide 1994)

Figure 1. Approximate distribution of Western Cherry Fruit Fly, *Rhagoletis indifferens*, in North America (adapted from AliNiazee 1986; Harris 1989)



1.2 WCFF Biology and Host Selection

Adult WCFFs eclose over a period of about 11 weeks, beginning in late May and peaking in the 3rd week of June. Females mature for about 1 week, during which they mate, feed on surface leachates, fruit exudes and insect honeydews, and actively search for exogenous protein, which facilitates egg production (AliNiazee and Brown 1977; Hendrichs et al. 1993). Females typically develop 35 - 45 eggs during this maturation period, but may develop up to 65 eggs (Banham and Arrand 1978).

Mature females begin to search for suitable oviposition sites. The preferred host is sweet cherry but WCFF is also found in sour cherry, chokecherry, *P. virginiana* L., Japanese plum, *P. salicina* L., Klamath plum, *P. subcordata* L., and its native host wild pin cherry (White and Elson-Harris 1992). Selection of an oviposition site by female *R. indifferens* is a complex, hierarchical process. Host trees and fruit clusters within the tree, are located primarily by vision (Prokopy 1968a). After arrival on the fruit, females assess fruit chemistry, surface structure, shape, size, and colour (Prokopy 1977, 1983; Prokopy and Roitberg 1989). If the fruit is acceptable, the female fly will deposit a single egg under the skin of the cherry, and then drag its ovipositor across the fruit surface, releasing a host marking pheromone (HMP). Conspecifics are highly deterred from ovipositing into HMP-marked fruit in nature (Prokopy et al. 1976).

Most eggs hatch within 3 - 9 days. Larvae migrate to the interior of the fruit where they feed on fruit pulp, and introduced microflora (bacteria and yeasts) surrounding the pit. Larvae pass through 3 instars over 14 - 21 days. Third instars cut one or more holes through the skin of the cherry, and about four days later will exit through a hole, drop to the ground, and form puparia 2.5 - 10 cm deep in the soil (AliNiazee 1974).

WCFF is univoltine and pupae spend the winter in obligatory diapause beneath host trees (Banham and Arrand 1978). Host fruits are normally available every year in the immediate vicinity of the pupation site. All adult activities, such as feeding, mating and

sheltering, take place on the host tree. As a result, the dispersal of adults is very limited, and is typically confined within an orchard (Jones and Wallace 1955; Fletcher 1989). Movement between non-adjacent orchards is believed to be low, but may increase when flies have wind assistance (Brunner 1995).

1.3 WCFF Management : The Need for Alternative Tactics

Direct damage to cherries is caused by larval feeding, and introduction of bacteria that facilitate fruit rot and increase post-harvest disorders. Due to export restrictions and quality-control objectives, packinghouses in Canada and the U.S.A. sample all incoming fruit for evidence of WCFF. Packinghouses reserve the right to degrade infested cherries and to cancel a grower's contract if quality is consistently poor (Lustzig 1990; Hawkes 1995; Jones 1995). Tolerance limits set by packinghouses vary from 0 - 4% infestation, depending on the country of export, and whether the fruit is destined for the processed or fresh fruit market (Burditt and Hungate 1988; Fischer-Colbrie and Bush-Petersen 1989; Lusztig 1990; Hawkes 1995; Jones 1995). As the culturing and harvesting costs for cherries are high, price reductions due to infestation rates that exceed the set tolerance limits may result in severe financial loss to the grower (Fischer-Colbrie and Bush-Petersen 1989). Protection of commercial crops is therefore obligatory.

It is difficult and impractical to detect and cull fruit infested by WCFF at harvest in conventional cherry orchards. Therefore, pest management strategies always target adult WCFF, before oviposition begins. In the 1950's, the insecticide of choice was DDT, which eventually gave way in the 1960's and 1970's to the organophosphates diazinon and malathion, and more recently to dimethoate; all but DDT are still in regular use today (Tree Fruit Production Guide 1994). Dimethoate is used primarily in B.C. It controls adults as a contact toxicant, and controls larvae and eggs within the fruit due to its systemic action. In Washington and Oregon, dimethoate is not used due to export regulations; instead WCFF is controlled by malathion, applied every 7 - 10 days for the entire season (Long 1995). Heavy

use of synthetic chemical pesticides in conventional orchards may have a negative impact on pollinators (Crane and Walker 1983) and natural-enemy complexes (DeBach and Rosen 1991; Prokopy and Powers 1995), result in ground and surface-water contamination, leading to accidental human and livestock poisonings, and the decline of local plant and animal populations (Pimental et al. 1980, 1992; Ascher 1993), and may accelerate the development of pesticide resistance in the target insect (Pimental et al. 1980).

In organic orchards, control is achieved primarily by the removal of all cherries at harvest, which eliminates breeding material and reduces WCFF numbers for the subsequent season. In addition, cherries are hand sorted at harvest to cull WCFF-infested fruit. Growers can achieve high levels of control with this technique, but it is labour intensive and can only be justified by the high price that organic cherries demand in the market place.

1.4 Potential for Neem as an Alternative Control Tactic

In recent years there has been an increased interest in natural plant-derived materials as alternative pesticides for conventional, broad-spectrum toxicants. Extracts of seeds from the neem tree, *Azidirachta indica* A. Juss, have attracted the most attention (Jacobson 1989; Schmutterer 1990, 1995; Ascher 1993).

Neem extracts have a wide range of effects against insect pests, including repellence, feeding and oviposition deterrence, toxicity, sterility and growth-regulatory-activity (Jacobson 1989; Schmutterer 1990, 1995; Ascher 1993). The desirable properties of neem include low toxicity to birds, fish and mammals (Larson 1989; Isman et al. 1991; Ascher 1993; Jacobson 1995) minimal persistence in the environment (Larson 1989; Stark and Walter 1996), and comparatively little impact on natural enemies and beneficial insects (Hoelmer et al. 1990; Stark et al. 1990, 1992; Mordue and Blackwell 1993; Lowery and Isman 1994; Schmutterer 1995), including the European Honey Bee, *Apis mellifera* (Naumann et al. 1994, Schmutterer 1995) than synthetic chemical pesticides. Resistance to

neem products may be possible, but the development of resistance is slow and unstable (Vollinger 1995).

The most important constituent of neem-based formulations is the triterpenoid azadirachtin , which is found primarily in the seed kernel of the neem tree. Azadirachtin is generally believed to affect the neurosecretory cells of the insect brain, disrupting both the synthesis and release of neurohormones that regulate the titre of ecdysteroids and juvenile hormones (Rembold 1989, 1995), thus interfering with endocrine-regulated life processes and development (Rembold 1989; Schmutterer 1995). Neem seed extracts contain a considerable number of additional bioactive compounds, many of which function as anti-feedants or oviposition deterrents, and contribute to hormonal disruption (Schmutterer 1990; Jacobson 1989; Ascher 1993).

Two neem insecticides are now commercially available in the United States as emulsifiable-concentrates (Margosan-O, W.R. Grace and Co., Columbia, MD, and Azatin, Agridyne Technologies Inc., Salt Lake City, UT). In 1985, neem-based insecticides were approved for use on non-food crops in the U.S.A.(Larson 1989) and by 1993 neem was also registered for conditional food crop use; recently they received an exemption from residue tolerance studies on food crops by the U.S. EPA (Stark and Walter 1996). Neem products remain unregistered in Canada, the research and development of a neem-based insecticide formulation is on-going.

1.5 Objectives

My objective was to explore the potential role of a proprietary, neem-based insecticide formulation (NBI) in the management of WCFF. Experiments were conducted to identify the effects of the NBI on adult and immature WCFFs and to characterize the response of foraging adult WCFFs to NBI in the laboratory and in the field.

2.0 THE EFFECT OF A NEEM-BASED INSECTICIDE ON ADULT WCFF

2.1 Introduction

Crude neem preparations can deter oviposition by insects (Naumann and Isman 1995); among the affected insects are tephritid fruit flies (Singh and Srivistava 1983; Prokopy and Powers 1995; Chen et al. 1996). Commercially developed, refined NBI, however, have not induced oviposition deterrence (Naumann and Isman 1995; Prokopy and Powers 1995), suggesting that deterrent compounds may be lost in processing.

Sterility and other effects on fitness , such as decreased survival and reduced mobility, occur after exposure to neem seed preparations (Jacobson 1989; Schmutterer 1990, 1995; Ascher 1993); again tephritid fruit flies are among the affected species (Steffens and Schmutterer 1982; Stark et al. 1990; Prokopy and Powers 1995). Although the antifeedant action of neem (Blaney and Simmonds 1995) can indirectly account for reductions in female reproductive rates, sterility appears to result primarily from changes in the ecdysteroid titre (Schmutterer and Rembold 1995). Depending on the timing of application, ovaries are improperly developed or degenerate, oocytes are abnormal and discoloured, and vitellogenesis is inhibited (Koul 1984; Shmutterer and Rembold 1995).

My objective was to evaluate a NBI against adult *R. indifferens*, testing for 1) oviposition deterrence, 2) changes in adult foraging behaviour, and 3) decreases in adult egg development.

2.2 Materials and Methods

2.2.1 General

For initial experiments, an emulsifiable concentrate NBI, containing 1.75% azadirachtin was obtained from Dr. M.B. Isman, University of British Columbia. Later, a

proprietary emulsifiable concentrate NBI containing 2.2% azadirachtin, but otherwise identical to the first formulation, was obtained from PheroTech Inc., Delta, B.C. For each experiment the NBI was mixed with distilled water, and the same formulation was used throughout the entire experiment. All application rates are expressed in percent concentration of the NBI, with the source of the formulation noted.

Cherries infested by *R. indifferens* were picked in 1994 from infested trees located at the Agriculture and Agri-Food Canada Research Station, Summerland, B. C. . The fruits were suspended above moist vermiculite on a wire mesh screen. Mature larvae dropped through the screen to the vermiculite and formed puparia, which were separated from the vermiculite, and stored in the dark at 4 ^{o} C for 8 months. Beginning in April 1995, puparia were removed and placed in 30 x 30 x 30 cm acrylic plastic, eclosion cages containing a 3:1 sugar:enzymatic yeast hydrolysate diet (Prokopy and Boller 1971) and distilled water in a small plastic bottle (Nalgene, 125 ml) fitted with a cotton dental wick. Cages were kept at 70 % RH, 27 ^{o} C, and a 16L:8D photo-regime. Eclosion occurred in 3 - 4 weeks. Uninfested fruits were obtained by covering unpicked, immature fruits with fine mesh bags, just after pollination in the early summer, before insect attack. These protected fruits were collected as needed.

2.2.2 Oviposition Deterrence - Laboratory Studies

Flies were collected every three days from eclosion cages and placed in groups of 5 - 50 (4 females:1 male) in holding cages containing diet and water. When flies were 14 - 17 days old, females in the holding cages were provided with ≈ 100 freshly-picked cherries for 24 h. This procedure gave females experience with oviposition substrates and host discrimination and reduced their eggload (Roitberg and Prokopy 1981, 1983; vanRanden and Roitberg 1996).

To examine the efficacy of the NBI as a deterrent to oviposition, no-choice bioassays of oviposition behaviour were conducted in 10 x 10 x 10 cm cages, containing a

single gravid female, under the same environmental conditions in which eclosing flies were held. Experiments were conducted in two steps separated by a 15 min break. The first step presented an uninfested, untreated, deep red, ripe, cherry of 17-22 mm diameter; the second step presented the same female with a similar control cherry or an experimental cherry treated with a 10.0 , 1.0, or 0.1 % dilution of the NBI obtained from Dr. M.B. Isman. Fruits were submerged for 20 sec in distilled water (controls) or the appropriate NBI solution, air dried, attached to a wire, and suspended from the top of the cage. Ten individual flies were tested for each treatment. The response of each fly to the experimental fruit was scored as accept or reject, based on whether or not the female oviposited in it. The time until first oviposition was also recorded. Flies which failed to oviposit when presented with the uninfested cherry in the first step were disqualified.

2.2.3 Oviposition Deterrence - Field Studies

Experiments were conducted at the Agriculture and Agri-Food Canada Research Station, Summerland, B. C., in the summer of 1995. The experimental orchard consisted of 54 sweet cherry trees with a mixture of early- and late-fruiting and deep red to blush fruit cultivars. The orchard was bounded on the north by a small sour-cherry plantation, on the west by peaches, on the south by a high density apple planting and on the east by an open field. The orchard had been insecticide free for at least 5 years.

Population levels of the WCFF were measured with Pherocon - AM (Trece Inc., Salinas, Calif.) factory baited traps, placed at a density of 20 traps/ha for the duration of the experiments. Experiments were conducted at two fly density levels : 1) early season - June 19 - 25 : 25.4 ± 3.7 (mean ± SE) flies/day/trap, and 2) late season - July 11 - 17 : 6.4 ± 1.6 flies/day/trap .

Branch ends located in mid-canopy (2 m above ground) in the northern aspect of the tree were covered (just after petal fall) by large, fine mesh bags approximately 1 m long by 30 cm wide. These bags excluded WCFF and other insects until the time of testing. A

single, bagged, branch section typically bore 50 - 100 cherries; 36 branches were located on separate trees to exclude potential systemic effects of the NBI treatment. For the early-season experiment, the mesh bags were rolled back, exposing the branch section, and 8 replicates were sprayed to the point of run off with either a 0.8 % dilution of the NBI obtained from Phero Tech Inc. or a distilled water control. Treatments were applied on June 19, and re-applied on June 22; on June 25 bags were rolled back into place and secured, with no WCFF trapped inside. Fourteen days later the cherries were picked, and examined for infestation. Rates of superparasitism (oviposition into a fruit already containing a conspecific larvae) were also recorded. Late-season experiments followed the same protocol, using 10 replicates; treatments were applied on July 11, re-applied July 14, and branches re-covered on July 17.

2.2.4 Adult Foraging Behaviour - Field Studies

Adult female WCFF were observed on treated and control-branch sections in the oviposition deterrence experiment. Twelve flies for each treatment were observed for 15 min, or until it left a branch section. The following behaviours were recorded: oviposition, marking, preening, walking, sitting, grazing, feeding at oviposition punctures, and superparasitism (AliNiazee and Brown 1977; Roitberg et al. 1982; Hendrichs et al. 1993). These behaviours were selected as a potential index of deterrence or irritation caused by the NBI, and all are easily observed and recorded. Oviposition, and depositing HMP (marking) are always accompanied by preening of the ovipositor (Roitberg et al. 1982). Feeding at oviposition punctures has been described previously (AliNiazee and Brown 1977). Grazing flies wander on the plant surface, extend and retract the proboscis, and apparently imbibe foliar leachates as a source of carbohydrates (Hendrichs et al. 1993).

2.2.5 Adult Fertility and Survival - Laboratory Studies

In the first experiment, to test the effect of NBI on adult survival and fertility, 4 flies (3 females:1male) were placed in 10 x 10 x 10 cm cages within 24 h of eclosion and the cage was randomly assigned to an experimental treatment. Protein-Sucrose diets (Prokopy and Boller 1971) were prepared by mixing 10 g of the diet with 2 ml of the NBI supplied by Dr. M.B. Isman at 10.0, 1.0 , 0.5, 0.2 and 0.1 % . Two control treatments, distilled water with regular diet and distilled water with sucrose, were used to provide an indication of the eggload and longevity that could be expected under ideal diet conditions (unlimited protein access) and poor diet conditions (carbohydrates only) (Hendrichs et al. 1993). Distilled water was provided by a wetted, cotton, dental wick. For the first 18 days immediately following eclosion, flies were given unlimited access to the treated food. At day 18, 10 cages per treatment were randomly selected, and had one female fly removed for dissection, the remaining flies had their experimental diet removed and replaced with the regular protein-sucrose diet.

As an additional measure of interest the remaining flies were checked every two or three days for survival, until all flies in the cages had died. The number of flies in each treatment group was 47, 43, 36, 47, and 40; these flies were tested against 1.0, 0.5 , 0.2, 0.1 % NBI and a distilled water control respectively.

To measure the effect of NBI treatments on eggload, the number of fully yolked, mature, terminal ovarioles in the abdomen (Minkenberg et al. 1992), flies were placed in a FAA solution (18:1:1 70% ethanol: glacial acetic acid: formalin) before dissection under 50x magnification. The ovaries and mature ovarioles were removed and counted, and abnormal appearance or discolouration was noted.

Five additional experiments varied the timing of exposure to the NBI treatments. These experiments were as follows: early exposure (Exp. 2 - 4) to the NBI for 2, 5 or 9 days immediately after eclosion; and late exposure (Exp. 5 and 6) to the NBI on days 7 and 8, or

9 - 18 after eclosion. Exp. 2 -6 tested diet with 10.0 , 1.0, and 0.2% dilutions of the NBI and a distilled water control. Flies were placed in FAA on day 18 for dissection to determine eggload.

2.2.6 Statistical Analyses

Time for acceptance of treated fruit in the laboratory, and eggload measures were analyzed by analysis of variance (ANOVA); means were separated with the Ryan-Einot-Gabriel-Welsch Multiple Q (REGWQ) Test (GLM procedure, REGWQ option, SAS 1994) (Day and Quinn 1989); zero means and variance were not included in the analysis. Differences in means for superparasitism and infestation rates in the field oviposition experiments, and behavioural observation data were analyzed with t-tests (T-TEST procedure, SAS 1994). In all cases $\alpha = 0.05$.

2.3 Results

2.3.1 Oviposition Deterrence - Laboratory Studies

Female flies accepted 100% of control fruits, and 90, 100 and 90% of fruits treated with dilutions of 0.1, 1.0 and 10.0% NBI respectively, indicating no evidence of oviposition deterrence. Time until first oviposition did not vary significantly between treatments (Fig. 2).

2.3.2 Oviposition Deterrence - Field Studies

In the early-season experiment, a slight but significant reduction in infestation rate was seen on branches treated with the NBI when compared to the control branches (Fig. 3). However, no difference in infestation rate occured in the late season experiment. Superparasitism rates did not significantly differ between the two treatments in either the early- or late-season experiments (Fig. 3).

Figure 2. Time elapsed before oviposition into cherries treated with different concentrations of a neem-based insecticide by female *Rhagoletis indifferens*. No significant differences between treatments, ANOVA, $P > 0.05$, n = 10.

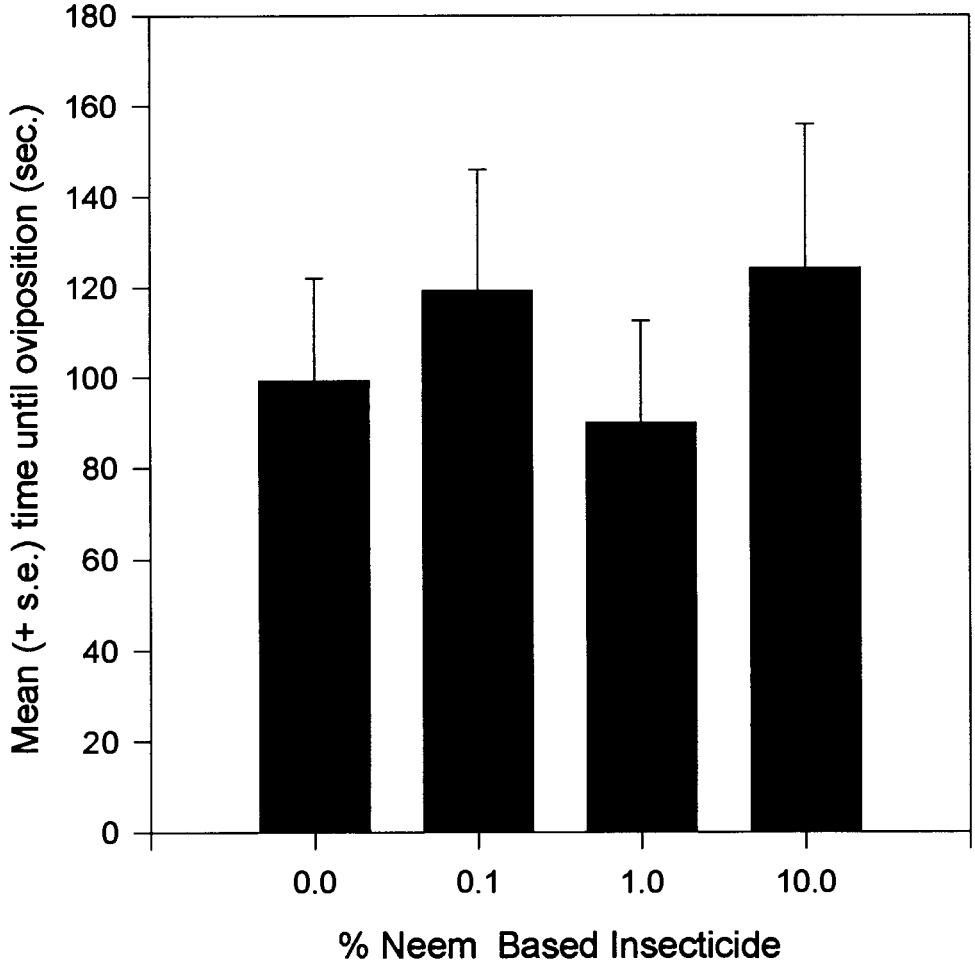
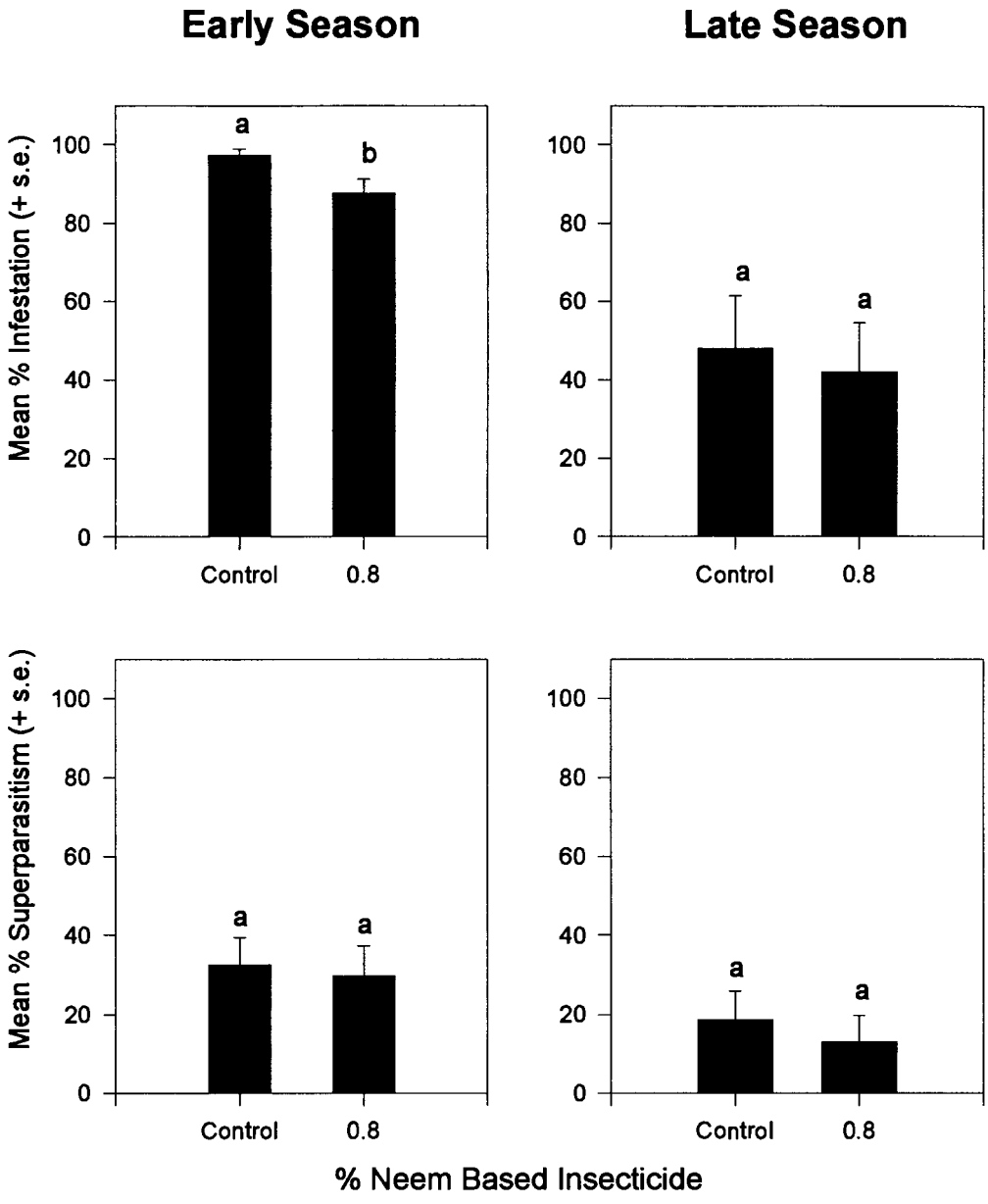

180
160
140
120
100
80
60
40
20
0
Mean (+ s.e.) time until oviposition (sec.)
0.0
0.1
1.0
10.0
% Neem Based Insecticide

Figure 3. Percentage of cherries infested or superparasitized by WCFF, *Rhagoletis indifferens,* on branches treated with 0.8% of a neem-based insecticide or a distilled water control during early season (n = 8), and late season conditions (n = 10). Paired bars sharing the same letter are not significantly different (t-test, $P > 0.05$).


Early Season
Late Season
Mean % Infestation (+ s.e.)
Mean % Superparasitism (+ s.e.)
100
80
60
40
20
0
a
b
a
a
a
a
a
a
Control
0.8
% Neem Based Insecticide

2.3.3 Adult Foraging Behaviour - Field Studies

I observed no significant differences in foraging behaviour between flies on NBI treated branches and controls (Tables 1, 2).

2.3.4 Adult Fertility and Survival - Laboratory Studies

Flies continuously exposed to 10.0, 1.0 and 0.5 % of a NBI developed no eggs (Table 3, Exp. 1), and had discoloured ovaries that were greatly reduced in size. The gut was distended and swollen with diet. After exposure to diet treated with 0.2 % NBI, egg development was comparable to that in sugar fed flies and typically contained a mixture of apparently healthy eggs and malformed, discoloured eggs within the ovaries. Females fed diet treated with a 0.1 % dilution of the NBI produced as many eggs as those fed the protein control diet, although the variation between flies was higher .

Survival was significantly decreased with increased concentration of NBI in flies with continuous access to treated diet (Fig. 4). Some flies exposed to the 1.0 and 0.5 % NBI did not fly; they remained relatively inactive at the bottom of the experimental cage, with their wings held in a down-turned position.

The results of Exp. 2 - 6 indicate that both the timing and length of NBI exposure can significantly affect the eggload of treated flies (Table 3, Exp. 2-4). Early exposure caused reduction of eggload in a dose-, and time- dependent manner. Late exposure had no effect (Table 3, Exp. 5 and 6).

Table 1. Analysis of proportional time spent in different behaviors, indexed during a 15 min observation period of female *Rhagoletis indifferens* under field conditions on cherry trees treated with a 0.8 % NBI dilution or a water control.

Behaviour	Percent of time spent in behaviour (mean ± s.e.)		t - statistic (left) and *P* value of $H_O : p_1 = p_2$
	Control	0.8 % NBI [1]	
Preening	20.6 ± 6.9	30.6 ± 6.8	0.96, 0.35
Grazing	7.1 ± 5.3	3.6 ± 1.2	- 0.66, 0.53
Walking	17.0 ± 4.8	17.0 ± 3.6	0.00, 1.00
Sitting	13.9 ± 8.4	8.6 ± 4.4	- 0.62, 0.54
Probing	23.9 ± 9.4	15.9 ± 5.4	- 0.81, 0.43
Feeding at Probing Puncture	3.3 ± 2.6	3.0 ± 2.2	- 0.08, 0.94

[1] NBI provided by Phero Tech Inc., Delta, B.C.

Table 2. Frequency analysis of different behaviors; indexed during a 15 minute observation period of female *Rhagoletis indifferens* under field conditions on cherry trees treated with a 0.8 % NBI dilution or a water control.

Behaviour	Frequency of behaviour (mean ± s.e.)		t - statistic (left) and *P* value of $H_0 : \mu_1 = \mu_2$
	Control	0.8 % NBI [1]	
No. of Probes	1.8 ± 0.7	2.25 ± 0.8	0.33, 0.75
No. of Ovipositions	1.43 ± 0.69	1.33 ± 0.56	- 0.11, 0.92
No. of Superparasitisms	0.29 ± 0.18	0.75 ± 0.35	1.17, 0.26

[1] NBI provided by Phero Tech Inc., Delta, B.C.

Table 3. Effect on eggload of continuous, truncated or delayed exposure of adult female WCFF, *Rhagoletis indifferens*, to diets containing various dilutions of a neem-based insecticide[1].

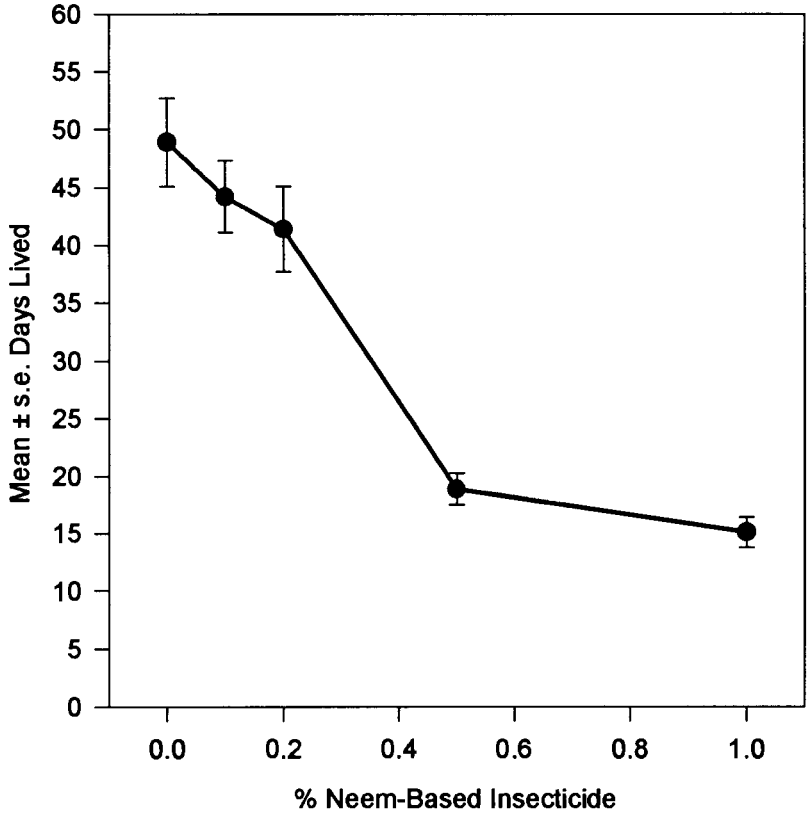
Exp. No.	Days of exposure to diet (eclosion = day 0)	% NBI[2]	Eggload n	Mean ± s.e. number of eggs	
1	1 - 18	10.0	10	no eggs developed	
		1.0	9	no eggs developed	
		0.5	10	no eggs developed	
		0.2	9	16.9 ± 4.4	a
		0.1	10	28.6 ± 1.7	b
		0.0	10	27.7 ± 0.6	b
		0.0(S)[3]	8	11.6 ± 0.9	a
2	1 - 2	10.0	9	12.1 ± 4.0	a
		1.0	8	8.6 ± 3.4	a
		0.2	13	31.0 ± 2.1	b
		0.0	10	35.0 ± 1.6	b
3	1 - 5	10.0	10	no eggs developed	
		1.0	10	no eggs developed	
		0.2	10	21.4 ± 4.5	a
		0.0	9	38.0 ± 2.2	b
4	1 - 9	10.0	10	no eggs developed	
		1.0	15	no eggs developed	
		0.2	9	15.7 ± 3.8	a
		0.0	11	40.4 ± 2.3	b

Table 3. (Continued)

Exp. No.	Days of exposure to diet (eclosion = day 0)	% NBI[2]	Eggload n	Mean ± s.e. number of eggs	
5	7 - 8	10.0	10	24.5 ± 4.5	a
		1.0	9	25.8 ± 5.3	a
		0.2	10	31.9 ± 2.7	a
		0.0	8	35.4 ± 1.6	a
6	9 - 18	10.0	13	35.7 ± 3.5	a
		1.0	16	32.8 ± 4.1	a
		0.2	14	41.1 ± 2.4	a
		0.0	10	45.9 ± 3.1	a

[1] Means within an experiment followed by the same letter are not significantly different, ANOVA - means separated by REGWQ, $P > 0.05$.
[2] NBI provided by Dr. M.B. Isman, UBC, Vancouver, BC
[3] Sugar only diet, see text

Figure 4. Effect of a neem-based insecticide incorporated into diet on survival of adult WCFF, *Rhagoletis indifferens* .


60
55
50
45
40
35
30
25
20
15
10
5
0
Mean ± s.e. Days Lived
0.0
0.2
0.4
0.6
0.8
1.0
% Neem-Based Insecticide

2.4 Discussion

2.4.1 Oviposition Deterrence and Foraging Behaviour

I had hypothesized that the within the complex process of host selection behaviours employed by *R.indifferens*, oviposition might be the most affected by exposure of adult females to a NBI. However, there was evidence of only slight oviposition deterrence in the field (Fig. 3) and none in laboratory bioassays. By contrast, in a similar laboratory bioassay WCFF was strongly deterred from ovipositing in the presence of HMP (Prokopy et al. 1976). The observed early season reduction in oviposition was weak ($\approx$ 10%), and other factors that could influence oviposition, such as availability of host fruit, demography of fly population, weather, and time of year, make it difficult to conclude that the effect was caused solely by the presence of NBI.

In studies demonstrating oviposition deterrence in the tephritids, Singh and Srivastava (1983) and Chen et al. (1996) prepared extracts directly from neem seeds. Extracts prepared in this manner vary widely in the levels of the bioactive compounds such as azadirachtin (Isman et al. 1990), but neither Singh and Srivastava (1983) nor Chen et al. (1996) report the levels of azadirachtin, or other components, of their extracts. The current study is best compared to the results of Prokopy and Powers (1995) who compared the neem-based insecticide Margosan-O to an untreated control and did not observe oviposition deterrence in the apple maggot, *Rhagoletis pomonella* Walsh, which has a similar ecology and method of host assessment as the WCFF. Apparently whatever is responsible for the observed oviposition deterrence in crude neem extracts is lost in the process of refining and enhancing azadirachtin content of commercially available NBI (Naumann and Isman 1995) . It is possible that capture of volatiles from crude neem extracts followed by coupled gas chromatographic-electroantennographic detection analysis (Arn et al. 1975) may reveal

potentially deterrent compounds (J.H. Borden, G. Gries, R. Gries, H.D. Pierce Jr., and M. Duthie pers. comm.[1]). Alternatively, bioassays may disclose oviposition deterrence of compounds that are relatively non-volatile and not antennally perceived.

2.4.2 Fertility and Survival

The NBI tested here proved to be a strong inhibitor of egg development and decreased survival of WCFF when ingested with diet. Previous studies on tephritid flies have documented similar effects (Steffens and Schmutterer 1982; Stark et al. 1990) and propose azadirachtin as the active agent responsible. The NBI used in my study, however, contains several components which are known to be bio-active against insects and which may potentially contribute to the observed sterility and decreased survival, including azadirachtin (Rembold 1989; Schmutterer 1995), neem oil (Rembold 1989; Naumann and Isman 1995; Stark and Walter 1996), and the emulsifier, which has recently been shown to have a toxic effect on at least two insect species (J. H. Borden, M.Greenwood and M.Duthie, pers. comm. of unpublished data [1]).

According to Schmutterer and Rembold (1995), azadirachtin is the primary sterilizing agent of neem-based products, and inhibits oogenesis and vitellogenesis by inducing a temporal shift in ecdysteroid and juvenile hormone titres. Pure azadirachtin is known as an effective sterilent of female insects in several orders (Rembold and Sieber 1981a, b; Koul 1984; Schmutterer 1987; Dorn et al. 1987; Schmutterer and Rembold 1995). Lowery and Isman (1996) recently completed a study in which the different components of a neem formulation were tested separately for sterility effects on a homopteran, and demonstrated that sterility is due primarily to azadirachtin, and not to the neem oil or the emulsifying agent. Unfortunately Lowery and Isman (1996) did not use a distilled water or

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[1] Centre for Pest Management, Simon Fraser University, Burnaby, B.C.

an untreated control, making it impossible to totally eliminate the emulsifier as a contributing agent of sterility. Because the sterilizing effect of azadirachtin is due to temporal disruptions of hormonal processes (Schmutterer and Rembold 1995), application after the initial phases of the gonadotropic are passed should be ineffective (Rembold and Sieber 1981a,b). The lack of any reduction of eggload in WCFF when exposure to the NBI was delayed (Table 3, Exp. 5 and 6) and supports this hypothesis for WCFF.

Sterility results can be compared to those of Stark et. al (1990) who report a decrease in the number of eggs laid by the tephritid, *Bactrocera cucurbitae* Coquillet, after topical treatment of 3rd instar larvae and pupae with a refined neem seed extract containing 1.85 ppm azadirachtin and additional substances. Because propensity to mate, and mating behaviour was not affected, the effect was "probably the result of physiological changes induced by azadirachtin" (Stark et al. 1990), but ovarian development and eggload were not assessed and the dose at which complete sterility occured was not determined. In addition Stark et al. (1990) did not separate out the additional components of the neem seed extract to determine that the effect was due solely to azadirachtin. For WCFF, the effective concentration of NBI (for continuous exposure), resulting in 50% inhibition of egg development (EC_{50}), appears to fall somewhere between a 0.2 and 0.5 % dilution (Table 3). Direct comparisons of the EC_{50} with the results of Stark et al. (1990) is difficult, due to differences in the route of application (topical vs. oral), the lifestage treated, and the neem seed formulation tested. However, if azadirachtin content alone is used for the comparison it appears that egg development in *B. cucurbitae* can be disrupted at lower levels than is required for adult WCFF.

The reduced survival of WCFF when exposed to diet containing NBI (Fig. 4) contrasts with the results of Prokopy and Powers (1995) who observed no increase in mortality of apple maggots when exposed to the neem formulation Margosan-O as adults. However, Prokopy and Powers (1995) only collected mortality data for 72 h after application of Margosan-O, making it unlikely that they would have observed mortality

effects even if they did exist. Reduced survival of adult tephritid flies has beeen reported previously (Steffens and Schmutterer 1982; Stark et al. 1990). However, in both cases the neem-based compound tested was applied to immature stages, and subsequent adult survival was monitored. No mechanisms were proposed in either study for the observed decreases in survival. Although a slow-acting toxic effect of the emulsifier, or of other NBI components, cannot be excluded, the distended guts full of ingested diet observed in dissected WCFF suggest that interference of peristalsis or uptake from the gut may have influenced the early death of WCFF exposed to NBI in their diet.

Steffens and Schmutterer (1982) observed that adult Mediterranean fruit flies which developed from larvae exposed to a crude methanolic neem seed extract had significantly reduced flight ability, startle activity, female chemotactic response and mating propensity. Similarly in this study, some adult WCFF were apparently unable to fly after ingesting treatments above 0.2% NBI in diet, and remained inactive on the cage floor, with their wings held in a down-turned position. These flies walked, rather than flew, to sources of food and water; such sub-lethal effects of NBIs need to be explored further.

2.4.3 Implications for Pest Management

If a NBI is applied at the appropriate time within a cherry orchard, at a sufficient dosage, female WCFF might be prevented from maturing viable eggs, while living for a short period of time. Because adult female WCFF shelter, mate, and forage within the cherry tree and rarely immigrate or emigrate (Jones and Wallace 1955; Brunner 1995), they would ingest the NBI in the course of feeding on foliar surface leachates, insect honeydews and proteinaceous food sources. Normal foraging behaviour would be expected (Table 1), and the ready feeding on treated diet suggests that uptake of the NBI in the field could occur.

The key question is whether sufficient NBI can be ingested under field conditions to reproduce the results seen in the laboratory (Table 3; Fig. 4). A solution to this question might be to apply NBI in a proteinaceous bait spray. The combination of bait and insecticide

has been used to control various species of tephritids, most notably the Mediterranean fruit fly (Roessler 1989). Typically such protein bait sprays contain 19 - 23.4 % malathion. Recent studies have judged bait sprays to be as effective as insecticides in controlling tephritid fruit flies (Haniotakis et al. 1989; Mohammed and AliNiazee 1989). If the attractive constituents in bird droppings and other naturally occurring proteinaceous food sources were known, they might be used to increase the attractiveness of baits to protein-seeking female fruit flies (Prokopy et al. 1992, 1993; Hendrichs et al. 1993). By substituting NBI for malathion in bait spray, one would produce a less-toxic alternative, with a reduced potential impact on natural enemies, which could be used in both conventional and organic cherry orchards.

3.0 THE EFFECT OF A NEEM-BASED INSECTICIDE ON IMMATURE WCFF

3.1 Introduction

Neem-based formulations are potent disrupters of insect development, working at the hormonal level to disrupt ecdysteroid, and juvenile hormone titres (Schmutterer 1988, Rembold 1995). Such shifts in hormone titre are attributed primarily to azadirachtin, and can lead to aberrant metamorphosis and molting behaviour, resulting in reduced survival of the insect. Neem-based formulations exert their strongest effects on the immature stages of insect species (Schmutterer 1995). In most studies, no discernible effect on adult predators, parasitoids and pollinators, e.g. honey bees, has been observed (Stark et al. 1990, 1992; Hoelmer et al. 1990; Naumann et al. 1994; Schmutterer 1995) .

Conventional control of the WCFF targets adults prior to oviposition. Systemic insecticides, however, could kill eggs and larvae in fruit if the active ingredient could be translocated by the tree. The systemic action of neem-based products has been demonstrated by either direct or indirect evidence for movement within treated plants leading to control of insect pests (Larew et al. 1985; Abdul Kareem et al. 1989; Osman and Port 1990; Marion et al. 1990; Sundaram et al. 1995). Systemic control of WCFF might be possible if eggs or larvae were sensitive to NBI .

Another opportunity for control occurs when WCFF larvae drop to the soil to pupate. Late third instars and early pupal stages can be targeted for control by treating the soil at the base of the tree with an insecticide (Saul et al. 1983). Root drenches of diazanon are currently used in California as one component of Mediterranean fruit fly control (Stark et al. 1990). Experimental applications of neem products as a root drench, or a mulch, have provided positive results against numerous insects (Larew et al. 1985; Abdul Kareem et al. 1989; Stark et al. 1990; Osman and Port 1990).

My objectives were: 1) to test the hypothesis that including NBI in larval-diets of the WCFF would decrease the proportion of larvae which successfully pupate and inhibit subsequent eclosion of adults from larvae which do pupate, and 2) to determine if including NBI in a pupation medium could lead to reduced or abnormal pupation, decreases in the weight of puparia, and inhibit adult development and eclosion.

3.2 Materials and Methods

3.2.1 General

The NBI containing 2.2% azadirachtin, obtained from PheroTech Inc., Delta, B.C. was used. Fruit infested by *R. indifferens* were hand picked as above from infested cherry trees at the Agriculture and Agri-Food Canada Research Station, Summerland. All experiments were conducted within an environmental chamber set at 70 % RH., 27 ^{o} C, and a 16L:8D photoregime.

3.2.2 Larval-diet Experiment

Infested cherries were opened with a knife, and larvae were removed with a fine brush. Only 1st and 2nd instar larvae were used. Larval-diet was prepared according to AliNiazee and Brown (1977), with minor modifications. Ten ml of NBI, at an appropriate dilution, was added at 65^{o} C, during the "cool-down period", just before the addition of the final diet ingredients, when the mixture was still thin enough to ensure even distribution. Control diets were prepared by adding 10 ml of distilled water. Approximately 50 g of diet was poured into disposable, plastic petri dishes (8.5 cm diameter, 2.5 cm deep) and allowed to cool for 24 h in a refrigerator . Each of four replicates / treatment consisted of 30 larvae added to each petri dish, for a density of 0.60 larvae / g diet. The surface of the diet was indented with the blunt end of a small brush to facilitate larval entrance. Five treatments

consisted of NBI dilutions of 0.4, 0.2, 0.1 and 0.05 % and a distilled-water control. Every 2 -3 days dishes were checked, and the numbers of puparia were counted and the presence of live larvae was noted. After 4 weeks puparia were collected from the dishes, counted and washed and placed by replicate groups into individual 62.5 ml condiment cups.

3.2.3 Pupal Experiment

For pupation, 100 g lots of unsifted beach sand were placed in 15 cm diam. plastic flower-pot saucers (Stark et al. 1990). Treatments mixed into the sand with a glass rod were: 10 ml of distilled water (control) or NBI in distilled water to make up 10 ml of 0. 5 , 0. 25, 0.13 and 0.06 % dilutions. Each treatment was replicated six times.

Infested cherries were suspended above a collection plate into which emergent third instars dropped in search of pupation sites. Larvae were collected daily and 30 - 50 were placed on the sand in each saucer. Fourteen days after the larvae had been placed on the sand all puparia were removed, washed, and counted and placed by replicate groups into individual 62.5 ml condiment cups..

3.2.4 Overwintering, Additional measures, Statistical Analyses

Condiment cups containing the puparia from both experiments were held in the dark at 4 ^{o} C for 5 months to simulate winter conditions. Beginning in January 1996, puparia were removed to monitor for adult eclosion and placed by replicate group into 10 x 10 x 10 cm cages containing distilled water and diet. Each puparium was weighed 3 weeks after removal from cold storage using a Cahn21 automatic electrobalance. The number of deformed puparia (deflated, discoloured, abnormally shaped) were counted at this time, and later dissected for internal examination. Eclosion, and survival data were collected daily for 5 weeks following the eclosion of the first fly. All puparia, and surviving adults were examined by dissection at this time.

Mean numbers of live and dead pupae within treated puparia were analyzed by a two-tailed t-test (T-TEST procedure, SAS 1994). All other means were subjected to ANOVA followed by REGWQ (GLM procedure, REGWQ option, SAS 1994) (Day and Quinn 1989); zero means and variances were not included in analysis. Percentage data were transformed to arcsine square-root before ANOVA to satisfy the criteria for normality and homoscedasticity.

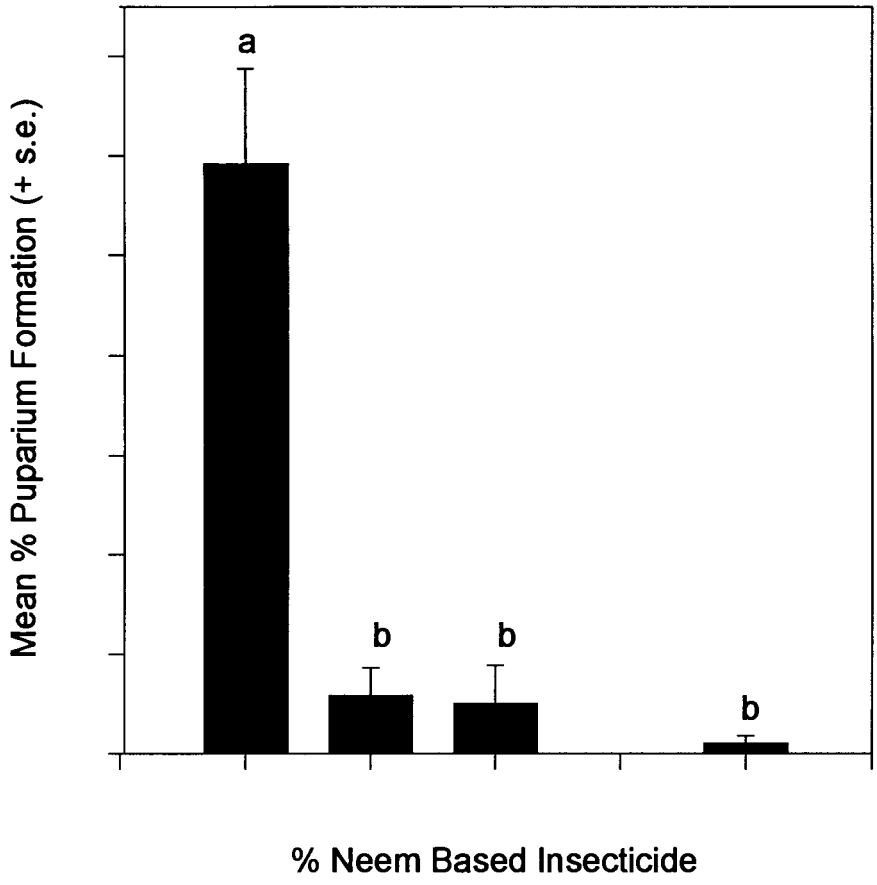
3.3. Results

3.3.1. Larval-diet Experiment

The percent of successful pupation was significantly decreased in all NBI diets as compared to that in the control (Fig. 5). Average time to pupation was 6 days in the controls, no different from that for any NBI diets (ANOVA, $P > 0.05$, n = 4). Control larvae appeared vigorous, and responded briskly when touched. The control diet showed obvious signs of larval mining. Larvae in NBI diets were lethargic, responded only slightly when touched and left few signs of feeding in the diets. Puparia from control diets were $\approx$ 2.4 x heavier on average than those from NBI diets; there was no significant difference among the NBI diets (REGWQ test, $P < 0.05$, n = 4).

No puparium from any NBI diet produced an adult fly, compared to an average eclosion rate of 33% from puparia in the control diet. When puparia were dissected those from NBI diets were either empty, or contained a dark fluid, presumably the remains of undeveloped larvae. Adults that emerged from the control treatment puparia appeared morphologically normal; they mated and fed readily, and females developed eggs.

Figure 5. Comparison of the percentage pupation of WCFF, *Rhagoletis indifferens*, which developed in larval diets containing different concentrations of a neem-based insecticide diluted in distilled water. Bars sharing the same letter are not significantly different, REGWQ test, $P > 0.05$, n = 4 replicates / treatment. Zero mean and variance not included in analysis.


Mean % Puparium Formation (+ s.e.)
a
b
b
b
% Neem Based Insecticide

3.3.2. Pupal Experiment

Exposure of third instar larvae to NBI had no effect on the percentage of individuals forming puparia nor on development to pupae within the puparium (Table 4). Significantly more deformed puparia were obtained in the 0.5% dilution of NBI than in the controls; treatments of 0.25, 0.13 and 0.06 % resulted in percentages that were intermediate between the control and 0.5% treatment. Adult eclosion was significantly reduced by all experimental treatments.

Analysis of dissected puparia after 5 weeks revealed that the control group contained significantly more individuals (pupae and adult) which had developed normally within the puparium, but remained unemerged (Table 5). There was no difference between treatments in percentages of pupae dead within the puparia, but there were significantly more live pupae in the control group than in groups which had been exposed to an NBI dilution at any concentration.

Although flies were held for 5 weeks to allow eclosion to occur, too few adults eclosed to perform a statistical analysis. Of the eight adult flies (3 males and 5 females) that eclosed after NBI treatment (2 in 0.06 %, 2 in 0.13 %, 4 in 0.25 % and 0 in 0.5 %), six died within the 5 week holding period. Dissections revealed 30 and 21 eggs within the females exposed to 0.13 and 0.25 % NBI respectively. By contrast, the control groups produced 12 adult flies (5 male and 7 female) of which 7 died within the 5-week holding period. The four remaining females, when dissected, contained 65, 34, 52 and 46 eggs, twice as many on average than in the flies subjected to NBI treatments.

Table 4. Effect of a neem-based insecticide applied in a sand pupation medium on the development and maturation of WCFF, *Rhagoletis indifferens* [1].

% dilution NBI	Rep-licates	% puparium formation	Puparium weight, mg (mean ± se)		% deformed puparia (mean ± se)		% adult eclosion (mean ± se)	
0.0	6	100	3.6 ± 0.2	a	0.3 ± 0.3	a	4.9 ± 1.3	a
0.06	6	100	3.6 ± 0.1	a	1.6 ± 1.0	ab	1.0 ± 1.0	b
0.13	6	100	3.5 ± 0.1	a	2.6 ± 1.3	ab	0.8 ± 0.5	b
0.25	6	100	3.7 ± 0.2	a	3.8 ± 1.4	ab	1.9 ± 0.9	b
0.50	6	100	3.6 ± 0.1	a	6.4 ± 1.4	b	no eclosion	

[1] Means within a column, followed by the same letter are not significantly different, REGWQ test, $P > 0.05$. Zero means and variances were not included in analysis.

Table 5. Effect of a neem-based insecticide applied in a sand pupation medium on the inhibition of development of WCFF, *Rhagoletis* indifferens within puparia [1].

% dilution NBI	Rep-licates	Percent normally developed pupae and adults within puparia (mean ± se)							
		Live pupae		Dead pupae		Total pupae		Adults	
0.00	6	8.8 ± 2.2	a	14.6 ± 3.1	a	23.3 ± 4.5	a	8.0 ± 1.6	a
0.06	6	0.4 ± 0.4	b	5.8 ± 1.9	a	6.2 ± 1.7	b	0.9 ± 0.6	b
0.13	6	1.6 ± 1.2	b	4.9 ± 0.8	a	6.5 ± 0.9	b	1.4 ± 1.1	b
0.25	6	0.5 ± 0.5	b	5.9 ± 2.3	a	6.4 ± 2.3	b	0.6 ± 0.6	b
0.50	6	1.8 ± 1.0	b	7.1 ± 1.2	a	8.9 ± 2.1	b	1.8 ± 0.6	b

[1] Means followed by the same letter are not significantly different, ANOVA - means separated by REGWQ test, $P > 0.05$.

3.4 Discussion

3.4.1 Larval-diet Experiment

NBI proved to be a powerful developmental inhibitor of larval WCFF. Concentrations of NBI as low as 0.05 % produced complete inhibition of adult eclosion, and no evidence of development of any kind was revealed in dissected pupae. These results are similar to those obtained by Steffens and Schmutterer (1982), who found reduced adult eclosion in larval Mediterranean fruit fly exposed to a crude methanolic NBI in artificial diet, but did not perform dissections of puparia. In their study, the LC_{50} for inhibition of adult eclosion was between 10 - 15 ppm of a crude methanolic neem seed extract. No resolution was obtained between the NBI treatments tested; the LC_{50} for WCFF is apparently lower than 0.05 % NBI.

All NBI treatments (Fig. 5), decreased pupation and reduced larval vigour. In contrast to Steffens and Schmutterer (1982), no increase in the time to pupation was recorded, although live larvae were noted in the NBI treatments up to 7 days after all pupation had stopped in the controls. None of these larvae formed a puparium during the 4-week duration of the experiment, and none was alive at the time of collection.

The absence of larval mining, and the reduction in larval movement suggest that the NBI may be acting as an antifeedant or a toxin which stops or slows feeding and movement of developing larvae. While azadirachtin is known to have both toxic and antifeedant effects (Jacobson 1989; Mordue and Blackwell 1993; Schmutterer 1995), the emulsifier has also demonstrated toxic effects in similar larval-diet experiments on both a coleopteran and dipteran (J. H. Borden, M. Duthie and M. Greenwood pers.comm. unpublished data [2]). In

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[2] Centre for Pest Management, Simon Fraser University, Burnaby, B.C.

larvae which did form puparia, it appears that metamorphosis to pupae and adults was disrupted, or else they were not of sufficient size to survive the overwintering period.

3.4.2 Pupal Experiment

The results of the pupal experiment can be compared to those of Stark et. al (1990), who treated late 3rd-instar larvae and pupae of 3 species of tephritid flies in a similar manner, and reported that a refined neem-ethanol formulation functioned as an effective inhibitor of adult eclosion. The mechanisms of inhibition, however, appear to be different. In my experiments, decreased adult eclosion was due to fewer flies developing to adulthood, and heavy pre-emergence mortality in those that did. In contrast, Stark et al. (1990) discovered that 95% of all neem treated flies developed to adulthood, but failed to emerge due to an inability to expand the ptilina and general paralysis. The increase in the number of deformed puparia obtained after exposure of third instars to 0.5 % NBI, contrasts with the results of Stark et al. (1990) who did not see any outwardly visible signs of deformation at the doses they tested. Among the few flies that did emerge from NBI treatments there was $\approx$ 50% decrease in eggload, and an increased proportion of flies that died before the end of the 5-week holding period. These results are similar to those reported by Stark et al. (1990) for eclosed adults. While Stark et al. (1990) attribute their results primarily to the effect of azadirachtin, subsequent work has shown that other components of neem formulations, including neem oils and the emulsifier, can contribute to insecticidal effects (Rembold 1989; Naumann and Isman 1995; Stark and Walter 1996; Lowery and Isman 1996; J. H. Borden, M.Greenwood and M.Duthie, pers. comm. of unpublished data [3]). A natural next step would be to separate out the effect of the other components of the NBI tested here, and to determine the LC_{50} of these compounds.

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Despite the large number of puparia (1204) included in the study, few adults eclosed. In the control group, for example, only 4.7 % of all puparia emerged as adults, compared to the usual 30 % - 40 % (Burditt and Hungate 1988). A large proportion of puparia were empty, containing only a thin waxy lining, all that remained of the diapausing fly. High mortality of pupae may have been due to the early removal of puparia from overwintering conditions and increased handling of puparia at the time of collection . In addition, when the puparia were in cold storage, they were infected by a fungus observed growing on the surface and in some cases penetrating the puparium. However, only 3% of all pupae died from apparent fungal infection.

3.4.3 Implications for Pest Management

The results of this study demonstrate that NBI containing azadirachtin is a powerful disrupter of normal WCFF development, resulting in reduced adult eclosion and decreasing the percent pupation. Root drenches at the time third instars drop to the ground coupled with an additional post-harvest application, targeting the residual population in unpicked cherries, could reduce overwintering populations of WCFF, save substantial labour costs on organic farms, and eliminate at least one spray of dimethoate on conventional farms.

To duplicate the strong effect of NBI on 1st and 2nd instars of WCFF (Fig. 5) in the field, the bio-active agent(s) must be taken up and translocated within the vascular system of the tree. While azadirachtin has been successfully tested for systemic action in aspen (Sundaram et al. 1995), the uptake, persistence and fate of all NBI components in cherry trees would have to be tested.

4.0 CONCLUSIONS

NBI demonstrated a variety of effects on WCFF, at all life stages. Larvae were the most strongly affected, and showed strong disruptions of normal development at very low experimental doses. However, the inability to resolve the active agent within the NBI makes application to pest management of the WCFF difficult. One of the attractions of azadirachtin-containing formulations is the minimal impact on beneficial insects, and the evidence that other components of the formulation have toxic effects on larvae of at least some insect species, may eliminate this benefit. If an alternative neem-based insecticide is developed, post-harvest root drenches targeting late third instar and pupal WCFF would be the most feasible tactic.

Experiments on adult WCFF showed that NBI has promise as a potential sterilent and mortality agent. The doses needed to achieve 100% sterility are relatively high, and would need to persist for a lengthy period on the foliage to ensure exposure to adult females. Again, an alternative formulation would need to be developed to eliminate the potential toxic effects of the emulsifier. Phytotoxic effects of NBI on cherry foliage, and the ability of female flies to acquire sufficient NBI from the plant surfaces under field conditions are key questions that must be answered if NBI is to be used as a foliar application for control of adult WCFF in cherry orchards.

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