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## Role of neonicotinyl insecticides in Washington apple integrated pest management. Part I. Control of lepidopteran pests

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### Abstract

Three neonicotinyl insecticides, acetamiprid, thiacloprid and clothianidin, were evaluated for their impact on four species of lepidopteran pests of apple in Washington, the codling moth, *Cydia pomonella* (L.), the Pandemis leafroller, *Pandemis pyrusana* Kearfott, and the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and *Lacanobia subjuncta* (Grote & Robinson). None of the neonicotinyl insecticides demonstrated sufficient activity against *P. pyrusana*, *C. rosaceana*, or *L. subjuncta* to warrant field trials. Conversely, all had some activity against one or more stages of *C. pomonella*. Acetamiprid was highly toxic to larvae in laboratory bioassays, and had relatively long activity of field-aged residues (21 days). It also showed some toxicity to *C. pomonella* eggs (via topical exposure) and adults. Acetamiprid provided the highest level of fruit protection from *C. pomonella* attack in field trials conducted over five years in experimental orchards with extremely high codling moth pressure. Thiacloprid performed similarly in bioassays, but fruit protection in field trials was slightly lower than acetamiprid. Clothianidin showed moderate to high toxicity in bioassays, depending on the *C. pomonella* stage tested, but poor fruit protection from attack in field trials. None of the neonicotinyl insecticides were as toxic to larvae or effective in protecting fruit as the current standard organophosphate insecticide used for *C. pomonella* control, azinphosmethyl. However, both acetamiprid and thiacloprid should provide acceptable levels of *C. pomonella* control in commercial orchards where densities are much lower than in the experimental orchards used for our trials. The advantages and disadvantages of the neonicotinyl insecticides as replacements for the organophosphate insecticides and their role in a pest management system for Washington apple orchards are discussed.

**Keywords:** acetamiprid, *Choristoneura rosaceana*, clothianidin, *Cydia pomonella*, insecticide resistance, *Lacanobia subjuncta*, *Pandemis pyrusana*, thiacloprid

### Abbreviation:

MFR Maximum field rate

### Introduction

Since the introduction of the organophosphate insecticides after World War II, this group of compounds has been the core of pest control programs in Washington apple. While organophosphate insecticides initially controlled almost the entire spectrum of orchard pests, uses were dropped for mite, aphid, and leafhopper pests as resistance evolved. Lepidopteran pests, primarily tortricids, remain the principal target of organophosphate insecticides. Azinphosmethyl, an organophosphate insecticide, has been the main control tactic for codling moth, *Cydia pomonella* (L.), since the 1960s.

A number of issues have been raised recently regarding the use of organophosphate insecticides. Resistance in *C. pomonella* has been documented in the western United States since the early 1990s (Knight 1992; Knight *et al.* 1994; Varela *et al.* 1993), in the midwest (Chapman 1997), and the southeast (Bush *et al.* 1993).

Resistance, and potential cross-resistance (Dunley and Welter 2000) to new pesticides, has been a major factor in shaping pest management programs in tree fruits. Worker safety has also become a more prominent issue with organophosphate insecticides, sometimes substantially altering producer's ability to use the material. While organophosphate insecticides vary widely in mammalian toxicity, several of the materials commonly used (past and present) in orchards are acutely toxic. Increasing concerns over worker exposure have led to increasing restrictions on use rates, numbers of applications, re-entry intervals, and personal protective equipment requirements. Some of these restrictions make scheduling of orchard operations, particularly high contact activities such as hand thinning of crop load, more difficult than in the past. Furthermore, environmental contamination, especially in relation to surface waters containing endangered species, is a concern with several organophosphate insecticides. Setbacks from bodies of water have

been required by court action to provide greater protection for salmon in selected rivers of the western US. Taken in sum, these factors have increased the need for alternative control tactics and non-organophosphate insecticides in orchard integrated pest management.

The use of mating disruption for *C. pomonella*, the key pest of western US apples, has increased dramatically since its registration in the early 1990s (Brunner *et al.* 2002; Thomson *et al.* 2001). Successful use of this technique, particularly when used on an areawide basis, has been the foundation in re-shaping pest management programs. There is consensus, however, that mating disruption is not a stand-alone tactic for *C. pomonella*, and insecticides are needed to supplement control in certain circumstances (Brunner *et al.* 2002; Thomson *et al.* 2001). Two newer classes of insecticides have been successfully adopted in mating disruption-based programs. The insect growth regulators have, in general, a very favorable profile regarding environmental effects, worker safety, and natural enemy toxicities. However, they have a relatively narrow pest spectrum, targeting primarily lepidopteran pests. A second group, the neonicotinyl insecticides, collectively has a wider spectrum of activity covering a range of pests in different orders, including Lepidoptera. They also have favorable environmental and worker safety profiles (Schmuck 2001; Tomiazawa and Casida 2003).

This paper presents part of an ongoing effort to characterize the efficacy of several neonicotinyl insecticides for tortricid pests of pome fruit: the codling moth, *Cydia pomonella*, the Pandemis leafroller *Pandemis pyrusana* Kearfott, the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and a noctuid, the speckled cutworm, *Lacanobia subjuncta* (Grote & Robinson). Initial investigations were designed to establish toxicity and longevity of neonicotinyl insecticides using bioassays and to evaluate their efficacy in field tests.

## Materials and Methods

### *Insecticides*

The neonicotinyl insecticides evaluated were acetamiprid (Assail, Cerexagri, Inc., [www.cerexagri.com](http://www.cerexagri.com)), thiacloprid (Calypso, Bayer CropScience, [www.bayercropscience.com](http://www.bayercropscience.com)) and clothianidin (Clutch, Arvesta Corp., [www.arvesta.com](http://www.arvesta.com)). Other insecticides were included in bioassays and field trials as standards; these are products typically used for pest control by apple growers in Washington or are new products that have a particular mode of activity that was of interest in this study. These included spinosad (Success, Dow AgroSciences LLC, [www.dowagro.com](http://www.dowagro.com)), methoxyfenozide (Intrepid, Dow AgroSciences LLC.), difluorobenzamide (Rimon, Crompton Corp., [www.cromptoncorp.com](http://www.cromptoncorp.com)), azinphosmethyl (Guthion, Bayer CropScience), and phosmet (Imidan, Gowan Company, [www.gowanco.com](http://www.gowanco.com)).

### *Rearing methods*

Laboratory colonies of leafrollers were established from field populations collected from apple. The *P. pyrusana* colony was started from larvae collected in a commercial apple orchard near Yakima, Washington, in 1985, and the *C. rosaceana* colony from larvae collected in a commercial orchard near Mattawa, Washington, in 1990. Larvae were reared on an artificial pinto-bean diet following

the methods of Shorey and Hale (1965). Colonies were maintained at constant temperature (23° C, L:D 16:8). The *L. subjuncta* colony originated from larvae collected in an apple orchard near Quincy, Washington, in 1999. Larvae were reared in the laboratory on a combination of artificial cutworm diet (Bio-Serv, #F9170, [www.bio-serv.com](http://www.bio-serv.com)) and untreated apple leaves (*Malus domestica* Borkhausen, 'Delicious') following the methods described by Doerr *et al.* (2002). *C. pomonella* were obtained as eggs or pupae from a colony maintained at the USDA-ARS laboratory in Wapato, Washington, since 1960.

### *Dose-response bioassays (C. rosaceana, P. pyrusana and L. subjuncta neonates, apple)*

A leaf disk bioassay was used to assess toxicity of the insecticides to neonate larvae. Treatments were prepared from a stock solution using formulated insecticide in 0.5 liter water. Four to eight concentrations were made by serial dilution of the stock solution. Two µl of a wetting agent (Latron B-1956, Dow AgroSciences), were added to each concentration. Each bioassay also included a check (water plus wetting agent). 'Delicious' apple leaves were collected from an orchard that had received no pesticide applications. Leaves were dipped in an insecticide solution three times to ensure adequate wetting and then allowed to air dry. A 2.3 cm diameter leaf disk was cut from each treated leaf, and four leaf disks treated with the same concentration of insecticide were placed in a small covered petri dish (Falcon 1006, 50 × 9 mm, Becton-Dickinson Labware, [www.bdbiosciences.com](http://www.bdbiosciences.com)). Five 1- to 2-day-old larvae per petri dish were placed directly on the leaf disks. Ten dishes (50 larvae) were used for each insecticide concentration. Petri dishes were placed inside a plastic container and kept at constant temperature (23° C, L:D 16:8). Mortality was evaluated after 7 days. Failure to move one body length in response to probing with a fine camel's-hair brush was scored as dead.

### *Dose-response bioassay (C. pomonella neonates, apples)*

An apple-dip bioassay was used to assess toxicity of the insecticides to neonate *C. pomonella* larvae. Treatments were prepared by diluting formulated insecticide in 1 liter water containing 0.32 ml/liter of an organosilicone surfactant (Sylwet L-77, Helena Chem. Co., [www.helenachemical.com](http://www.helenachemical.com)). Seven concentrations were made by serial dilution of the stock solution, with each bioassay having a check (water plus surfactant). Mature 'Delicious' apples were collected from an unsprayed orchard. Ten apples were dipped in each concentration and allowed to air dry. A section of waxed paper (2 × 2 cm) containing 10 *C. pomonella* eggs was placed on the stem end of the treated apples. The apples with eggs were placed individually in clear plastic containers (Anchor Packaging #409CX, [www.anchorpackaging.com](http://www.anchorpackaging.com)) and kept in a growth chambers (22° C, L:D 16:8). The eggs hatched in approximately 4-5 days, and the number of successful larval entries was recorded at 14 days.

### *Dose-response bioassay (C. pomonella eggs, topical exposure)*

A modification of the apple-dip bioassay described above was used to assess toxicity of insecticides to *C. pomonella* eggs when applied topically. Preparation of the concentrations and fruit treatment were as described above, except only six concentrations were used. Organic 'Fuji' apples free from insecticide residues were

obtained from a packing house in Wenatchee, Washington. *C. pomonella* pupae were placed in a 60 × 30 × 36 cm wire mesh oviposition chamber. After *C. pomonella* adults emerged, 60 untreated apples were placed in the chamber, and females were allowed to lay eggs on them for 72 h. A carbohydrate and water source (5% honey dissolved in water) was introduced into the chamber using cotton wicking. Ten apples containing at least five eggs each were dipped into the same concentration of an insecticide, allowed to air dry, and then placed in clear plastic containers. The apples with *C. pomonella* eggs were held under constant conditions at 23° C, L:D 16:8. *C. pomonella* egg hatch was recorded after 10 days. Only eggs that showed no signs of larval development were recorded as dead; larvae that died while hatching were not.

#### *Dose-response bioassay (C. pomonella eggs, residual exposure)*

A modification of the apple-dip bioassay described above was used to assess toxicity of insecticide residues to *C. pomonella* eggs. Preparation of the concentrations, source of fruit, fruit treatment, and oviposition chambers were as described above. Treated apples ('Fuji'), were placed in an oviposition chamber with *C. pomonella* adults, and females were allowed to oviposit for 24 hours. Apples were removed and placed individually in clear plastic containers, and held in growth chambers at 23° C, L:D 16:8. *C. pomonella* egg hatch was recorded after 10 days. Egg mortality was assessed as described above for the topical ovicide bioassay.

#### *Dose-response bioassay (C. pomonella adults, residual exposure)*

Treated plastic containers were used to evaluate the residual toxicity of insecticides to *C. pomonella* adults. Treatments were prepared by diluting formulated insecticide in 1 liter water containing 0.32 ml/liter of the organosilicone surfactant. Six or seven concentrations were used for each insecticide. One ml of an insecticide/surfactant mixture was added to a 120 ml plastic portion cup (Prairie Packaging, Inc., #S400, [www.prairiepack.com](http://www.prairiepack.com)). The concentration in the cup was swirled for approximately 15 seconds, removed, and the residue allowed to air dry. Five cups (replicates) were treated per concentration. *C. pomonella* pupae were held in an untreated plastic emergence chamber, and adults allowed to emerge. Five 1- to 2-day-old unsexed *C. pomonella* adults were added to each of the treated cups. A carbohydrate and water source (5% honey dissolved in water) was introduced into the cup using cotton wicking. Cups were held at constant conditions of 23° C, L:D 16:8. Adult survival was assessed after 24 hours. A moth was considered dead if no response to probing with a camel's hair brush was observed.

#### *Field-aged residue bioassays (C. rosaceana, P. pyrusana, L. subjuncta, C. pomonella neonates)*

An evaluation of field-aged insecticide residues was conducted using modifications of the methods described above for dose-response bioassays. In each test, insecticides were applied to previously untreated 'Delicious' apple trees. Treatments were applied to the point of drip using a handgun sprayer at 300 psi to achieve thorough coverage. Plots with single trees, replicated three times, were used. An untreated check and, in most cases, an industry standard insecticide were included in each experiment. Ten leaves or 10 apples per replicate were collected from the interior canopy

of each tree at 1, 4, 7, 14, 21 and 28 days after treatment. For *C. rosaceana*, *P. pyrusana*, and *L. subjuncta*, five arenas were prepared from each treated or check tree (15 arenas/treatment), and for *C. pomonella* 10 arenas were prepared for each treated or check tree (30 arenas/treatment). The bioassays were prepared, stored and evaluated as described above in the dose-response protocol (leaf-disk or apple-dip bioassays).

#### *Data analysis of bioassays*

Probit regression lines and LC<sub>50</sub> values were estimated using the probit option of POLO-PC (LeOra Software 1987). Probit models from each bioassay were then used to calculate toxicity indices relative to field use rates. Expected mortality was calculated using each probit model at 1× and 0.1× MFR (maximum field rate, the highest labeled concentration for use on apples, assuming a dilute spray). For the field-aged residue studies, mean mortality data were corrected by Abbott's formula (Abbott 1925), then analyzed using a one-way analysis of variance (SAS Institute 1995). A *t* ratio test on the slope parameter was used to determine if the data of each probit analysis fit a linear model (Robertson and Preisler 1992).

#### *Field trials*

Small plots, one to three trees in a single row, were sprayed with a handgun to the point of drip to obtain thorough coverage, simulating a dilute spray. Buffer trees and rows were included in the plot layout to ensure treatments did not contaminate neighboring plots. Handgun applications were made with a multiple-tank sprayer (Parker Mfg., Wenatchee, Washington).

Airblast plot sizes ranged from three trees (single row) to 15 trees (5 trees × 3 rows), with appropriate buffer trees and rows. A dual-tank airblast sprayer (Rears Pak-Blast, Rears Mfg., Eugene, OR) was calibrated to deliver either 234 or 935 liters/ha (see tables). Experimental orchards for all field trials were located in the vicinity of Wenatchee, Washington; the cultivar sampled was 'Delicious'.

*C. pomonella* sprays targeted the first and second generations. The first application for each generation was based on a degree-day model (Beers *et al.* 1993) adapted from the principle described by Baskerville and Emin (1968). Subsequent applications for the generation were an interval of days based on the presumed length of residual control of the materials being evaluated. *C. pomonella* control was assessed by picking and examining 100 fruit per replicate just prior to commercial harvest (late August through mid-September), and the number of *C. pomonella*-injured fruits was recorded.

#### *Field experiment design and analysis*

Field experiments were randomized complete block designs, with 3-5 replications. *C. pomonella* fruit injury was expressed as a percentage reduction of injury from the untreated check [(1 - (proportion injured fruit in treatment/proportion in check)) × 100]. Thus, a higher value indicated better *C. pomonella* control. This result was then compared to the industry standard organophosphate insecticide (Fisher's Least Significant Difference Test, *P* = 0.05) (SAS Institute 1995). In most cases the standard was azinphosmethyl, in one case it was phosmet, and in eight cases no organophosphate standard was included. In addition, a metadata analysis was done on the percentage reduction of fruit injury for

acetamiprid and thiacloprid. A homogeneity of slopes model (PROC GLM, SAS institute 1995) was used to test the relationship between fruit injury and total g AI/ha applied, and whether or not the addition of oil affected the efficacy. *F*-tests were used to determine significance.

## Results and Discussion

### Larval bioassays

The LC<sub>50</sub> estimates for leafrollers to the neonicotinyl insecticides were high relative to that of spinosad, the most commonly used insecticide for leafroller control in the post-bloom period (NASS 2002) (Table 1). The toxicity indices for both *P. pyrusana* or *C. rosaceana* at 1× MFR were estimated to be less than 70%, while at 0.1 × MFR the indices for all three neonicotinyl insecticides were estimated to be equal to or less than 1%. For spinosad, the toxicity index at only 0.1 × MFR was estimated to be above 95% mortality for both *C. rosaceana* and *P. pyrusana*. The toxicity index is not a direct predictor of an insecticide's field efficacy, as other factors such as longevity of residues and interactions with plant surfaces can mediate performance. The toxicity index can be useful in comparing insecticides within a bioassay technique, using field rates for standardization. Further, if the bioassay method used reflects to a reasonable degree an insect stage's exposure to a pesticide in the field, the toxicity index of a candidate insecticide can be used

as an indicator of the inherent toxicity in comparison to a compound with known field performance characteristics, e.g., spinosad.

*L. subjuncta* results were similar to those for leafrollers. Although only two neonicotinyl insecticides were tested both had toxicity indices of less than 30% mortality for the 1 × MFR and less than 1% mortality for the 0.1 × MFR (Table 1). Relative to spinosad, none of the neonicotinyl insecticides have high inherent toxicity to leafrollers or *L. subjuncta*, and would therefore be less likely to provide control under field conditions.

The LC<sub>50</sub> estimates for the neonicotinyl insecticides against *C. pomonella* larvae were small relative to those for leafrollers or *L. subjuncta*, but were similar to the LC<sub>50</sub> for azinphosmethyl (Table 1). The toxicity indices at 1 × MFR were above 94% mortality for the neonicotinyl insecticides, while that of azinphosmethyl was essentially 100% (the probit model asymptotes as it approaches 100%). At 0.1 × MFR, the estimated *C. pomonella* larval mortality was between 60-70% for the neonicotinyl insecticides, while the toxicity index for azinphosmethyl at 0.1 × MFR was higher, nearly 98%. Data from these bioassays indicate that the neonicotinyl insecticides are not as toxic to *C. pomonella* larvae as the industry standard, azinphosmethyl.

### Ovicide bioassays (*C. pomonella*)

The method of exposure affected the toxicity of neonicotinyl insecticides to *C. pomonella* eggs. Acetamiprid and thiacloprid had

**Table 1.** Dose-mortality bioassays of neonate larvae of various lepidopteran species using neonicotinyl and standard insecticides, 1999-2003

Insecticide	Year	No. individuals	MFR mg AI/liter	Slope (SE) <sup>a</sup>	Toxicity Index			
					LC <sub>50</sub>		Estimated mortality (%) at proportion of maximum field rate	
					Estimated value mg AI/liter (95% CI)	1× MFR	0.1× MFR	
<b><i>Pandemis pyrusana</i> larvae</b>								
Acetamiprid 70WP	2001	400	45	1.4 (0.4)	410.5 (245.7-1627.4)	7.56	0.23	
Thiacloprid 480SC	2001	400	75	3.0 (0.9)	58.6 (23.9-81.0)	62.37	0.36	
Clothianidin 50WP	2003	350	56	2.9 (0.7)	186.0 (127.7-251.8)	7.07	0.00	
Spinosad 2SC	2001	400	46	3.0 (0.8)	0.4 (0.2-0.6)	99.98	96.76	
<b><i>Obliquebanded leafroller</i> larvae</b>								
Acetamiprid 70WP	2001	400	45	1.8 (0.4)	107.6 (67.2-154.6)	22.84	0.55	
Thiacloprid 480SC	2001	400	75	4.1 (0.7)	56.6 (43.0-70.2)	69.77	0.02	
Clothianidin 50WP	2003	350	56	2.1 (0.4)	75.0 (45.1-109.1)	42.12	1.08	
Spinosad 2SC	2001	400	46	4.8 (1.0)	0.5 (0.3-0.6)	100.00	100.00	
<b><i>Lacanobia subjuncta</i> larvae</b>								
Acetamiprid 70WP	2000	400	45	3.0 (0.6)	71.3 (46.8-100.3)	26.10	0.0136	
Thiacloprid 480SC	2000	400	75	3.9 (1.1)	110.3 (62.5-149.5)	28.53	0.0004	
Spinosad 2SC	2000	400	46	2.4 (0.6)	1.6 (0.9-2.5)	99.97	85.5568	
<b>Codling moth larvae (no. entries)</b>								
Acetamiprid 70WP	1999	600	45	2.0 (0.4)	3.1 (1.4-5.1)	99.0	62.42	
Thiacloprid 480SC	1999	600	75	1.2 (0.2)	3.5 (0.7-8.7)	94.3	64.81	
Clothianidin 50WP	2003	700	56	1.2 (0.2)	2.4 (0.2-6.7)	95.1	67.65	
Azinphosmethyl 50WP	1999	600	449	2.4 (0.5)	6.9 (3.0-10.1)	100.0	97.64	

<sup>a</sup> A 't' ratio test on the slope parameter of each probit line was conducted and in each case was greater than 1.96 indicating a linear fit for the data.

LC<sub>50</sub>s similar to the insect growth regulators when eggs were exposed topically (Table 2). The toxicity indices of acetamiprid and thiacloprid (topical exposure) at 1 × MFR were similar to the insect growth regulators (methoxyfenozide and difluorobenzamide), while clothianidin was lower. At 0.1 × MFR, acetamiprid and thiacloprid had values slightly lower than the insect growth regulators, with clothianidin substantially lower at 8% estimated egg mortality (Table 2). These data support the premise that two of the neonicotinyl insecticides, acetamiprid and thiacloprid, would have some topical activity against *C. pomonella* eggs, however, not to the same degree as the insect growth regulators.

The residual exposure method produced much higher LC<sub>50</sub>s for *C. pomonella* eggs for all three neonicotinyl insecticides compared to the topical exposure method (Table 2). The toxicity indices at 1 × MFR for the neonicotinyl insecticides were only 33-53% mortality, whereas the indices for the insect growth regulators were >97%. At 0.1 × MFR, the toxicity indices for the neonicotinyl insecticides were <25% mortality compared to 82% and 54% mortality for methoxyfenozide and difluorobenzamide, respectively

(Table 2).

The two insect growth regulators showed little differences in activity against *C. pomonella* eggs based on the route of exposure, and both would be expected to be effective in killing *C. pomonella* eggs, at least as fresh residues. Acetamiprid and thiacloprid would be expected to have some effect on *C. pomonella* eggs present at the time of an application, but less effect on eggs laid thereafter. Clothianidin would not be expected to have much effect on *C. pomonella* eggs. The combination of limited ovicidal activity and higher larvicidal activity may enhance field performance of acetamiprid and thiacloprid against *C. pomonella*.

#### Adult bioassays (*C. pomonella*)

The neonicotinyl insecticides had less effect on adult *C. pomonella* overall than azinphosmethyl, as estimated by the toxicity index (Table 3). Clothianidin and acetamiprid had high toxicity indices at 1 × MFR, while at 0.1 × MFR all of the neonicotinyl insecticides had low toxicity indices relative to azinphosmethyl. Adulticidal activity of the neonicotinyl insecticides would likely be expected to provide

**Table 2.** Dose-mortality bioassays of codling moth eggs using neonicotinyl and insect growth regulator insecticides, 2001-2004

Insecticide	Year	No. individuals	MFR mg AI/liter	Slope (SE) <sup>a</sup>	Toxicity Index		
					LC <sub>50</sub> Estimated value mg AI/liter (95% CI)	Estimated mortality (%) at proportion of maximum field rate	
					1× MFR	0.1× MFR	
<b>Codling moth eggs (topical exposure)</b>							
Acetamiprid 70WP	2004	1476	45	1.3 (0.1)	0.5 (0.3-0.7)	99.4	89.2
Thiacloprid 480 SC	2002	597	75	1.4 (0.1)	2.0 (0.6-4.0)	98.5	78.1
Clothianidin 50WP	2004	1457	56	2.0 (0.1)	27.1 (15.1-41.1)	72.1	7.9
Methoxyfenozide 2F	2002	596	75	1.6 (0.1)	0.4 (0.1-0.8)	100.0	97.9
Difluorobenzamide 0.83EC	2004	1547	78	1.0 (0.04)	0.2 (0.1-0.3)	99.6	94.8
<b>Codling moth eggs (residual exposure)</b>							
Acetamiprid 70WP	2001	758	45	0.8 (0.04)	34.9 (7.9-207.4)	53.3	23.7
Thiacloprid 480 SC	2004	1192	75	0.9 (0.1)	281.0 (145.2-3765.5)	33.3	9.1
Clothianidin 50WP	2004	1780	56	0.7 (0.03)	62.3 (31.2-185.5)	49.4	23.7
Methoxyfenozide 2F	2001	995	75	1.0 (0.04)	0.9 (0.1-2.2)	97.3	82.3
Difluorobenzamide 0.83EC	2004	1470	78	2.4 (0.1)	7.0 (5.2-8.8)	99.4	53.6

<sup>a</sup> A 't' ratio test on the slope parameter of each probit line was conducted and in each case was greater than 1.96 indicating a linear fit for the data.

**Table 3.** Dose-mortality bioassays of codling moth adults using neonicotinyl insecticides and azinphosmethyl, 2002-2004

Insecticide	Year	No. individuals	MFR mg AI/liter	Slope (SE) <sup>a</sup>	Toxicity Index		
					LC <sub>50</sub> Estimated value mg AI/liter (95% CI)	Estimated mortality (%) at proportion of maximum field rate	
					1× MFR	0.1× MFR	
<b>Codling moth adults (residual exposure)</b>							
Acetamiprid 70WP	2002	175	44	1.5 (0.2)	4.0 (2.2-6.6)	94.1	52.4
Thiacloprid 480SC	2002	175	57	1.5 (0.2)	19.8 (10.2-37.1)	80.3	25.9
Clothianidin 50WP	2004	300	57	2.8 (0.6)	10.2 (6.3-13.4)	98.0	22.5
Azinphosmethyl 50WP	2002	100	1121	1.9 (0.3)	7.2 (3.8-13.1)	100.0	93.4

<sup>a</sup> A 't' ratio test on the slope parameter of each probit line was conducted and in each case was greater than 1.96 indicating a linear fit for the data.

limited enhancement to overall *C. pomonella* control.

#### Field-aged residue bioassays

The residual activity of neonicotinyl insecticides against *P. pyrusana* and *C. rosaceana* reflected their low toxicity in dose-response bioassays. While acetamiprid had some activity against *P. pyrusana* and *C. rosaceana* as 1-day-old residues, its activity dropped off quickly thereafter (Table 4). At 7 days (*P. pyrusana*) or 21 days (*C. rosaceana*), mortality was not different from the untreated check. Thiacloprid and clothianidin had less activity than acetamiprid, with 1- and 4-day-old residues not causing higher mortality than the untreated check. None of the neonicotinyl insecticides demonstrated residual activity similar to that of the standard, spinosad, which caused high levels of mortality through 21 days. While these data cannot be directly compared because the tests were not conducted within the same year, it appears that the neonicotinyl insecticides would not provide sufficient efficacy against leafrollers under field conditions.

Acetamiprid was the only neonicotinyl insecticide tested against *L. subjuncta*. There was virtually no activity of this material against *L. subjuncta* larvae even on 1-day-old residues (Table 4).

All three neonicotinyl insecticides produced high levels of mortality of *C. pomonella* neonates for at least 14 d, and had substantial activity even after 28 days (Table 4). Clothianidin has a

shorter length of residual activity, based on the 21 day evaluation. The standard insecticide, azinphosmethyl, also showed high levels of activity through 21 days, with some residual activity even after 28 days. Twenty-one days has been a standard re-treatment interval for azinphosmethyl use for *C. pomonella* control for many years (Smith *et al.* 2004), and is inevitably the standard reapplication period against which new materials are compared.

Thiacloprid was the only neonicotinyl insecticide tested in the field-aged residue bioassay examining ovicidal activity against *C. pomonella* eggs. Residual activity was short and variable compared to the insect growth regulators (Table 4), which caused >85% egg mortality after 28 days. However, the level of egg mortality in the field-aged bioassay was higher than might have been expected based on laboratory bioassays of *C. pomonella* eggs laid on fresh residues of thiacloprid (Table 2). In the residual *C. pomonella* egg bioassay, thiacloprid had the least activity of the neonicotinyl insecticides and *C. pomonella* egg mortality of more than 80% as observed in the field-aged residue bioassay would not have been predicted. It is possible that thiacloprid residues in the apple-dip bioassay method used in the laboratory were much lower than those that resulted from the field applications. While the laboratory method of dipping apples in insecticide concentrations with a wetting agent ensured uniform coverage of fruit, the addition of the wetting agent could have resulted in lower levels of thiacloprid per unit of surface

**Table 4.** Field-aged residue bioassays of neonate larvae of various lepidopteran species using neonicotinyl and standard insecticides, 1998-2004

Insecticide	Rate (gm AI/ha) <sup>b</sup>	Year	Corrected % mortality <sup>a</sup>					
			1 DAT	4 DAT	7 DAT	14 DAT	21 DAT	28 DAT
<b><i>Pandemis pyrusana</i></b>								
Acetamiprid 70WP	167	2001	85.1	40.3	39.3 ns <sup>c</sup>	39.3 sn	4.7 ns	---
Thiacloprid 4F	211	2004	65.3	27.6 ns	0.0 ns	0.0 ns	---	---
Clothianidin 50WP	105	2003	15.7 ns	22.4	17.9 ns	8.7 ns	---	---
Spinosad 2SC	105	2004	100.0	100.0	100.0	100.0	89.8	59.1
<b><i>Obliquebanded leafroller</i></b>								
Acetamiprid 70WP	167	2001	84.2	54.9	72.4	52.1	10.0 ns	---
Thiacloprid 4F	211	2004	90.9	41.5 ns	24.7 ns	0.0 ns	---	---
Clothianidin 50WP	105	2003	36.8	13.1 ns	42.8 ns	15.2 ns	0.0 ns	---
Spinosad 2SC	105	1998	100.0	100.0	100.0	92.6	91.8	61.2
<b><i>Lacanobia subjuncta</i></b>								
Acetamiprid 70WP	167	2001	17.1 ns	---	---	---	---	---
<b>Codling moth neonates (entries)</b>								
Thiacloprid 480 SC	210	2002	98.1	96.8	96.3	95.4	91.0	93.3
Acetamiprid 70WP	167	2002	100.0	100.0	98.8	98.9	97.7	96.6
Acetamiprid 70WP	167	2003	100.0 a	100.0 a	100.0 a	100.0 a	97.2 a	89.5 a
Clothianidin 50WP	210	2003	100.0 a	100.0 a	100.0 a	92.8 a	69.4 b	71.1 a
Azinphosmethyl 50WP	1121	2003	100.0 a	100.0 a	100.0 a	92.9 a	100.0 a	78.9 a
<b>Codling moth ovicide (residual exposure)</b>								
Thiacloprid 480 SC	210	2002	81.4 a	89.3 a	61.2 b	44.9 b	70.0 b	50.1 b
Difluorobenzamide 7.5 WG	89	2002	76.9 a	90.1 a	93.5 a	96.6 a	98.8 a	93.0 a
Methoxyfenozide 2F	281	2002	87.9 a	82.5 a	90.4 a	93.6 a	96.2 a	86.6 a

Means within a bioassay in the same year followed by different letters are statistically different (Dunnnett's Comparison with Control,  $P=0.05$ ).

<sup>a</sup>DAT - Days after treatment application.

<sup>b</sup>Dilute applications simulating 3,741 liters/ha.

<sup>c</sup>ns, not significantly different than the untreated control (Dunnnett's Comparison with Control,  $P=0.05$ ).

<sup>d</sup> ---, test not continued because treatment mortality was not statistically different from untreated control on the previous assessment date.

**Table 5.** Control of codling moth with various neonicotinyl insecticides in replicated field trials, 1999-2004

Insecticide	Hort.		Year	Location / block no. <sup>a</sup>	Application method and liters/ha <sup>b</sup>	Applications per year	% fruit injury <sup>c</sup>	NN-Std
	Rate (g AI/ha)	Mineral oil rate (vol:vol)						
Acetamiprid 70WP	83		1999	TF 24	H dilute	4	83	ns
Acetamiprid 70WP	113		1999	TF 24	H dilute	4	86	ns
Acetamiprid 70WP	142		1999	TF 24	H dilute	4	92	ns
Acetamiprid 70WP	167		1999	TF 24	H dilute	4	94	ns
Acetamiprid 70WP	167		2000	CV 18	A 935	4	90	ns
Acetamiprid 70WP	167		2000	CV 19	A 935	4	91	ns
Acetamiprid 70WP	111		2000	TF 24	H dilute	4	69	*
Acetamiprid 70WP	167		2000	TF 24	H dilute	4	59	*
Acetamiprid 70WP	111	0.25%	2000	TF 24	H dilute	4	74	*
Acetamiprid 70WP	167	0.25%	2000	TF 24	H dilute	4	79	ns
Acetamiprid 70WP <sup>d</sup>	235		2001	CV 18	A 935	4	87	*
Acetamiprid 70WP	235		2001	CV 18	H dilute	4	95	ns
Acetamiprid 70WP	84	0.25%	2002	TF 24	H dilute	6	83	*
Acetamiprid 70WP	84	0.25%	2002	TF 24	H dilute	4	89	*
Acetamiprid 70WP	84	1.0%	2002	TF 24	H dilute	4	87	*
Acetamiprid 70WP	167		2002	TF 24	H dilute	4	81	*
Acetamiprid 70WP	167	0.25%	2002	TF 24	H dilute	4	79	*
Acetamiprid 70WP	167	1.0%	2002	TF 24	H dilute	4	77	ns
Acetamiprid 70WP	84		2002	TF 24	H dilute	4	69	*
Acetamiprid 70WP	167		2002	CV 18	H dilute	4	94	ns
Acetamiprid 70WP	167	0.25%	2002	CV 18	A 234	4	79	ns
Acetamiprid 70WP	167	0.25%	2002	CV 18	A 935	4	72	ns
Acetamiprid 70WP	167	0.25%	2002	CV 19	H dilute	4	79	---
Acetamiprid 70WP	235	0.25%	2002	TF 26	H dilute	4	88	*
Acetamiprid 70WP	167	0.25%	2003	TF 24	H dilute	4	80	*
Acetamiprid 70WP	167	0.25%	2003	TF 24	H dilute	4	70	*
Acetamiprid 70WP	167	0.25%	2003	TF 26	H dilute	4	75	*
Acetamiprid 70WP	167	0.25%	2003	CV 18	H dilute	4	79	---
Acetamiprid 70WP	167		2003	TF 24	H dilute	4	72	*
Acetamiprid 70WP	167	0.25%	2003	TF 24	H dilute	4	82	*
Acetamiprid 70WP	167	1.0%	2003	CV 19	H dilute	4	79	ns
Acetamiprid 70WP	167	0.25%	2003	CV 18	A 935	4	80	---
Acetamiprid 70WP	167	0.25%	2003	TF 16	A 935	4	84	---
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	4	92	*
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	4	87	*
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	6	95	*
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	4	91	*
Thiacloprid 480SC	211		2000	TF 24	H dilute	6	71	*
Thiacloprid 480SC	211		2001	CV 18	H dilute	8	97	ns
Thiacloprid 480SC	281		2001	CV 18	H dilute	8	97	ns
Thiacloprid 480SC	211		2002	CV 19	H dilute	5	79	---
Thiacloprid 480SC	211		2002	CV 19	H dilute	5	77	---
Thiacloprid 480SC	136		2003	TF 24	H dilute	4	57	*
Thiacloprid 480SC	136	0.25%	2003	TF 24	H dilute	4	56	*
Thiacloprid 480SC	203		2003	TF 24	H dilute	4	55	*
Thiacloprid 480SC	203	0.25%	2003	TF 24	H dilute	4	60	*
Thiacloprid 480SC	269		2003	TF 24	H dilute	4	72	*
Thiacloprid 480SC	269	0.25%	2003	TF 24	H dilute	4	72	*
Thiacloprid 480SC	168	0.25%	2003	TF 24	H dilute	4	43	*
Thiacloprid 480SC	168	0.25%	2003	TF 24	H dilute	4	53	*
Thiacloprid 480SC	203	0.25%	2003	TF 24	H dilute	4	50	*
Thiacloprid 480SC	269	0.25%	2003	TF 24	H dilute	4	58	*
Thiacloprid 480SC	203	0.25%	2003	TF 26	H dilute	4	76	*
Thiacloprid 480SC	203	0.25%	2003	TF 24	H dilute	4	72	*
Thiacloprid 480SC	203	0.25%	2003	CV 18	A 935	4	84	---
Thiacloprid 480SC	203	0.25%	2003	CV 18	A 935	4	89	---
Thiacloprid 4F	210	0.25%	2004	TF 24	H dilute	4	95	*
Clothianidin 50WDG	70/105 <sup>e</sup>		2003	TF 24	H dilute	6	6	*
Clothianidin 50WDG	105/214 <sup>e</sup>		2003	TF 24	H dilute	4	5	*
Clothianidin 50WDG	105	0.25%	2004	TF 24	H dilute	6	46	*
Clothianidin 50WDG	105	0.25%	2004	TF 24	H dilute	4	37	*
Clothianidin 50WDG	210	0.25%	2004	TF 24	H dilute	6	49	*
Clothianidin 50WDG	210	0.25%	2004	TF 24	H dilute	4	28	*

\* Mean fruit injury level was significantly higher (= less crop protection) than the OP standard treatment mean; ns, difference not significant; '---' indicates that a standard treatment was not included in the test.

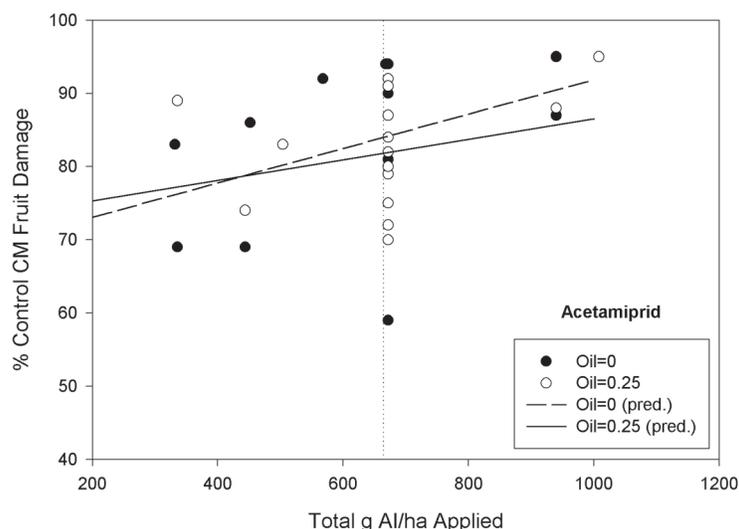
<sup>a</sup>Locations: TF, Tree Fruit Research and Extension Center (home farm); CV, WSU Columbia View Farm.

<sup>b</sup>Method: H, Handgun, A, Airblast

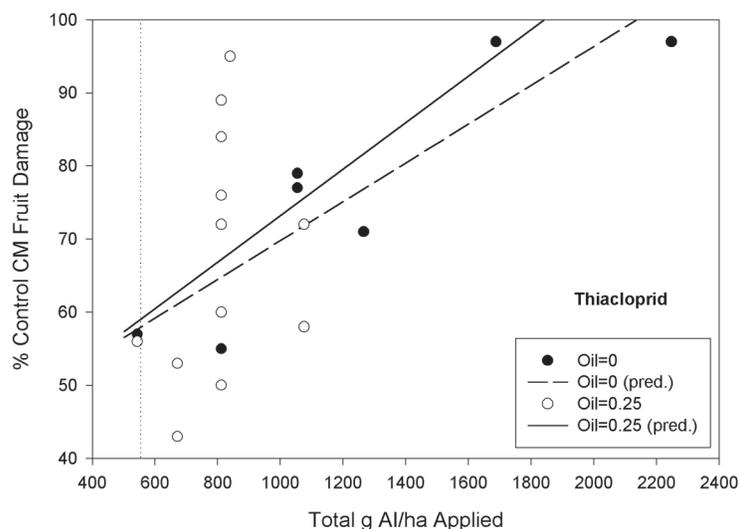
<sup>c</sup>Percentage of fruit damage relative to the untreated check; values <100 indicate poorer control.

<sup>d</sup>The OP standard in this trial was phosmet.

<sup>e</sup>Rates for the 1st and 2nd generation of codling moth, respectively.



**Figure 1.** Effect of rate on the efficacy of acetamiprid, 1999-2004. Without oil,  $y = 68.38 + 0.023x$ . With 0.25% oil,  $y = 72.56 + 0.014x$ . The dotted vertical line indicates the maximum allowable label rate per season.



**Figure 2.** Effect of rate on the efficacy of thiacloprid, 2000-2004. Without oil,  $y = 43.27 + 0.027x$ . With 0.25% oil,  $y = 41.47 + 0.032x$ . The dotted vertical line indicates the maximum allowable label rate per season.

area compared to those deposited by the field application method. While thiacloprid, and possibly the other two neonicotinyl insecticides, might provide some mortality of *C. pomonella* eggs laid on residues, the levels of mortality are not sufficient to warrant shifting timing away from the primary target, the neonate larvae.

The laboratory and field-aged residue bioassays described above provide a foundation for understanding how new insecticides proposed for the apple pest control system function against lepidopteran pests. Using these techniques, candidate insecticides can be screened rapidly and inexpensively against different pests

and different life stages of those pests. When compared with insecticides known to be effective against a pest or particular pest stage, the relative toxicities of candidate insecticides can be characterized through dose-response bioassays. Further, because these tests are conducted with known susceptible populations that are maintained under controlled conditions, reference data are also available for use in future comparisons with field-collected populations when resistance issues arise. The field-aged residue bioassays also provide insights into the longevity of candidate insecticides' residual activities and can help establish re-treatment intervals for initial field trials.

In this study with neonicotinyl insecticides, bioassay data from both laboratory and field experiments helped eliminate the need and expense of conducting field trials against leafrollers and *L. subjuncta*. Further, bioassays revealed the toxicity of neonicotinyl insecticides to *C. pomonella* eggs when applied topically, indicating that they are more than just larvicides. This may help explain why the neonicotinyl insecticides are more effective management tools than might be expected from their larvicidal activity alone. Separating the effects of an insecticide on insect life stages using bioassays also provides clues as to how to best target more susceptible stages and time applications in field trials. This information is difficult to obtain from field trials without greatly increasing sample time and intensity, because pest life stages typically overlap during the course of a treatment regime.

#### *C. pomonella* field trials, efficacy

Acetamiprid provided a moderate to high degree of fruit protection in all field trials over five years (Table 5), with an average of 82.2% (range 59-95%) reduction in percentage *C. pomonella* fruit injury when compared to the untreated check. The relative percentage reduction in fruit damage was positively related ( $F = 4.03$ ,  $P = 0.05$ ,  $R^2 = 0.13$ ) to the total g AI/ha applied (Fig. 1). Although some of the treatments included a 235 g AI/ha rate, the current label limits the rate to 167 g AI/ha. The addition of oil to acetamiprid did not affect the percentage relative control ( $F = 0.15$ ,  $P = 0.71$ ).

Fruit protection from thiacloprid was more variable than acetamiprid in all field trials over four years, with an average of 71.0% (range 43-97%) reduction in percentage fruit injury compared to the untreated check. The greater variation compared to acetamiprid was in part due to the lower rates of thiacloprid that were used in some trials. The highest levels of control achieved (97%) were in treatments where eight applications were made (1,800 to 2,394 g AI/ha/year); this exceeds the amount allowed by the current label (maximum of 280 g AI/ha per application; 560 g/ha/year). The level of control with the higher rates (>260 g AI/ha) was generally better than at lower rates, but still varied from 58-72% control for four applications. The effect of total g AI/ha applied significantly affected the percentage control of *C. pomonella* ( $F = 8.53$ ,  $P = 0.01$ ,  $R^2 = 0.39$ ), however, the addition of oil did not ( $F = 1.71$ ,  $P = 0.21$ ) (Fig. 2).

Although fewer trials were performed with clothianidin, results suggest that it is less effective than the other two neonicotinyl insecticides. Relative reduction in percentage fruit injury averaged 28% (range 5-49%) (Table 5). This lower level of control in field trials would not be predicted if only the results from the larval

bioassay were considered, where clothianidin was similar to acetamiprid and thiacloprid. However, bioassay results for toxicity to adults, eggs, and length of residual control of larvae were all lower for clothianidin, and the combination of reduced effects from all routes of exposure was likely sufficient to adversely affect field performance.

In all field trials where the mean percentage of injured fruit in a neonicotinyl insecticide treatment was significantly different from the organophosphate standard (azinphosmethyl or phosmet), control with the neonicotinyl insecticides was always inferior (Table 5). The level of control as measured by percentage fruit injury with azinphosmethyl averaged 94.2% over all trials (data not shown). In the absence of organophosphate insecticide resistance, azinphosmethyl is still the most effective insecticide tested against *C. pomonella*. The field trial results are consistent with the high toxicity indices in larval bioassays, as well as the long residual control in field-aged residue bioassays with azinphosmethyl.

## Conclusions

Of the three neonicotinyl insecticides examined, acetamiprid had the most consistently high level of activity against *C. pomonella* in small-plot field trials, while thiacloprid tested at its higher rates provided levels of control that were very similar. With the high *C. pomonella* pressure present in the test orchards (average of 60.1% fruit injury in the untreated controls over five years), the activities of these products were slightly inferior to azinphosmethyl. However, in commercial orchards, where *C. pomonella* pressure is not as severe as in these trials, it is likely that acetamiprid and thiacloprid would provide commercially acceptable levels of *C. pomonella* control. These products do not provide control of *C. rosaceana*, *P. pyrusana* or *L. subjuncta*. The neonicotinyl insecticides have been shown to provide control of many indirect pests of apple, notably aphids, leafhoppers and some hemipteran pests (Beers *et al.* 2002a; Beers *et al.* 2002b; Beers *et al.* 2002c). Therefore, applications timed for *C. pomonella* control would likely have the added benefit of suppressing some of these pests.

None of the neonicotinyl insecticides are likely to be used as stand-alone, four-spray programs for season-long control of *C. pomonella* in the western US. Although the acetamiprid label allows such a program, current economics do not favor it. However, withdrawal of organophosphate insecticide use from tree fruit pest management would dramatically alter the economic scenario.

The range of apple pests controlled by neonicotinyl insecticides makes resistance management an ongoing challenge. In addition to *C. pomonella* control there are multiple opportunities throughout the season for use of one or more of the neonicotinyl insecticides. Using multiple applications of the same chemistry group would likely hasten the onset of resistance and lead to possible loss of the entire class for apple pest control. Acetamiprid and thiacloprid are effective alternatives to organophosphate insecticides for *C. pomonella* control, which is likely to be their primary use in western apple orchards. To preserve their effectiveness for as long as possible, all neonicotinyl insecticides should be considered as a single product type and use should be restricted to not more than two applications per year, and only during one *C. pomonella* generation per year.

A selective apple pest management program would use all available control tactics to obtain the highest level of crop protection at the lowest economic cost, with considerations for farm worker safety and environmental stewardship. There currently exists an array of pest control tactics (mating disruption, organophosphate insecticides, neonicotinyl insecticides, insect growth regulators, and *C. pomonella* granulosus virus) that can form the foundation of a selective, effective and stable apple pest management program for Washington growers. Many of these control tactics are effective against leafrollers as well as *C. pomonella* (e.g., spinosad, pyriproxyfen, methoxyfenozide, and difluorobenzamide). By integrating neonicotinyl insecticides into a multi-tactic apple pest management program it should be possible to implement a sound resistance management strategy for all products while achieving excellent control of key pests with limited detrimental effect on biological control of secondary pests.

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