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Role of neonicotinyl insecticides in Washington apple integrated pest management. Part II. Nontarget effects on integrated mite control

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Abstract

The effect of neonicotinyl insecticides on integrated mite control in Washington apple was examined from 2000-2004. In a series of 20 field trials (54 treatments) designed primarily to look at efficacy against the codling moth, *Cydia pomonella*, nearly half of the treatments using four or more applications of acetamiprid had peak mite densities exceeding the economic threshold of 5 mites per leaf. Overall, acetamiprid treatments had 4.6-fold higher mite densities than the standard organophosphate insecticide treatment. Of the treatments with high mite populations, *Panonychus ulmi*, the European red mite, and *Tetranychus urticae*, the twospotted spider mite, were the dominant species in roughly equal numbers of cases. Only 11.1% of the thiacloprid treatments exceeded 5 mites per leaf; these experimental treatments included eight applications, whereas the current label restricts the number of applications at the rate for *C. pomonella* to two applications. One out of six clothianidin treatments caused a significantly higher mite density than the standard treatment; however, this material appeared to suppress predatory mites. Neonicotinyl insecticides did not eliminate predatory mites, but they inhibited their ability to respond normally to increasing prey populations. In field trials designed specifically to examine mite population densities where neonicotinyl insecticides were used, significantly higher levels of tetranychid mites occurred in one or more acetamiprid treatments (one, two or four applications) in five out of six trials. In the sixth trial (in a commercial orchard), only two acetamiprid applications were made, and mite populations were low in all treatments. While elevated mite densities were more likely to occur with four applications, in one case it occurred following a single application. The predominant tetranychid mite species (either *P. ulmi* or *T. urticae*) varied from trial to trial; however, there was no apparent bias regarding stimulation of the two species. Horticultural mineral oil was used with acetamiprid in some trials in an attempt to mitigate mite outbreaks. However, the addition of oil did not counteract the tendency of acetamiprid to increase tetranychid mite populations, and in one trial, had a negative effect on predatory mite densities. Seasonal tetranychid mite density was positively related to the total grams AI (or number of applications) of acetamiprid, thus reducing the number of applications per season should lower the probability of mite outbreaks.

Keywords: acetamiprid, *Aculus schlechtendali,* clothianidin, *Cydia pomonella, Galandromus occidentalis,* imidacloprid, *Panonychus ulmi, Tetranychus urticae,* thiacloprid, thiamethoxam, *Zetzellia mali*

Abbreviation:

AI	active ingredient
CMD	cumulative mite days

Introduction

Organophosphate insecticides came into widespread use in orchards shortly after their introduction after World War II. Since that time, numerous orchard pests have developed resistance to this group of compounds. In addition, some of the natural enemies associated with orchard pests have also become resistant to organophosphate insecticides, allowing them to be effective in the orchard ecosystem. Predatory mites are a salient example of acquired tolerance, which formed the basis of Washington's integrated mite control program (Hoyt 1969; Hoyt and Burts 1974). This program was implemented in the 1960s, and is still functional today. Although organophosphate insecticides are used primarily for lepidopteran pests today, the organophosphate-based program used on Washington apples has been relatively stable for decades, both in terms of key pest control and integrated mite control.

Several factors provided the impetus to restructure the apple pest management program. The first was the registration and successful implementation of mating disruption for the key pest of apple, codling moth *Cydia pomonella* (L.) (Brunner *et al.* 2002). Use of this tactic began in 1991, and is now employed on about 50% of Washington's apple acreage (J. F. Brunner, unpublished).

While the technique was successful, it became apparent that supplemental insecticidal controls were needed for moderate to high populations. Organophosphate use was reduced, but not eliminated under mating disruption.

The second factor was development of organophosphate resistance in codling moth populations in the western United States (Knight *et al.* 1994; Varela *et al.* 1993), with correlated cross-resistance to other compounds (Dunley and Welter 2000). Although mating disruption was viewed as an important component of a resistance management strategy (Dunley and Welter 2000), effective supplements were still necessary. Resistance, along with increasing worker safety and environmental concerns about organophosphate insecticides, accelerated the need for alternative control tools.

Two groups of insecticides emerged as likely candidates for organophosphate insecticide replacements for *C. pomonella* control. The insect growth regulators were toxic to both codling moth and other tortricid pests, but were recommended for use only in low to moderate populations (Smith *et al.* 2004). More recently, the neonicotinyl insecticides have been extensively tested for *C. pomonella* control (Brunner *et al.* 2005) and some have been found to be effective. The increasing restrictions on organophosphate insecticide use have prompted increasing use of alternative pesticides.

This paper presents part of an ongoing effort to investigate the effect of organophosphate replacement chemistries on integrated mite management, specifically the neonicotinyl insecticides. Data on mite densities were taken in conjunction with efficacy trials targeting *C. pomonella* (Brunner *et al.* 2005). When effects on mite populations were noted in the early field trials additional specific trials were conducted to evaluate the effect of neonicotinyl insecticides on mites. The objectives of these experiments included direct comparison of various neonicotinyl insecticides, evaluation of reduced numbers of applications and the addition of horticultural mineral oil as an adjuvant.

Materials and Methods

Cydia pomonella field experiments

C. pomonella treatments were applied with either a handgun sprayer (Parker Mfg, Wenatchee, WA) or an airblast sprayer (Rears Pak-Blast, Rears Mfg., Eugene, OR). Handgun plots consisted of one to five trees in a single row, and were sprayed to runoff to obtain thorough coverage. Airblast plots were either multiple trees in a single row, or multiple trees per row in three rows. Six of the 20 *C. pomonella* trials were applied using airblast at a rate of 935 liters/ha (see tables). Both handgun and airblast plots had buffer trees and rows in the experimental layout to ensure treatments did not contaminate neighboring plots.

Spray applications were directed at the first and second generation of *C. pomonella*. The first application for each generation was based on a degree-day model (Beers *et al.* 1993). Subsequent applications for the generation were made at an interval of days based on the length of residual control being evaluated. The total number of applications per season ranged from four to eight. The neonicotinyl insecticides evaluated were acetamiprid (Assail, Cerexagri, www.cerexagri.com); thiacloprid (Calypso, Bayer CropScience, www.bayercropscience.com); imidacloprid (Provado, Bayer CropScience); thiamethoxam (Actara, Syngenta Crop Protection, www.syngenta.com); and clothianidin (Clutch, Arvesta, www.arvesta.com). In most of the field trial experiments, the standard was azinphosmethyl (Guthion, Bayer CropScience), but in one case it was phosmet (Imidan, Gowan Co., www.gowanco.com).

Mite populations were assessed by picking 20 to 50 leaves per plot. Two or three mite samples were taken at approximately monthly intervals beginning in June or July, and the mite sample with the highest population was reported.

Mite-specific field experiments

The treatment regime of the mite-specific experiments was similar to the *C. pomonella* field experiments, except that all were applied airblast, and in five of the six trials, two applications per generation (four per season) were made. In the sixth trial (in a commercial orchard), two applications were made against the first generation, but only spot treatments were made against the second generation (see tables). The rate of oil was standardized at 1% vol:vol, and the rate of acetamiprid was the full label rate (167 g AI/ha) in all but one treatment. An additional difference between the *C. pomonella* field experiments and the mite-specific experiments was that a neonicotinyl insecticide was substituted for one, two, or all four of the cover sprays, with phosmet used for the remaining cover sprays (in the *C. pomonella* field experiments, the same material was used for all cover sprays). In addition to acetamiprid the thiacloprid, two other neonicotinyl insecticides were tested in one trial: imidacloprid (Provado, Bayer CropScience); and thiamethoxam (Actara, Syngenta Crop Protection). Esfenvalerate (Asana, DuPont, www1.dupont.com), a pyrethroid known to be toxic to predatory mites, was evaluated in two trials.

Mite densities were assessed by randomly selecting 20 to 60 leaves per plot, with the exception of one of the commercial orchard trials, where 100- to 200-leaf samples were taken from the 2-ha plots, avoiding the plot borders. The major differences between the two groups of experiments was that in the mite-specific trials, phosmet was the standard organophosphate insecticide used (vs. azinphosmethyl), and mite samples were taken at 1- to 2-wk intervals (vs. 2 to 3 times per season).

Mite counts

Mites were removed from leaves with a leaf-brushing machine (Leedom Enterprises, Mi-Wuk Village, CA), and mites that fell on a revolving glass plate were counted with a binocular microscope. The mite species evaluated by this method included the phytophagous tetranychids European red mite, *Panonychus ulmi* (Koch); twospotted spider mite, *Tetranychus urticae* Koch; McDaniel spider mite, *Tetranychus mcdanieli* McGregor; the predatory mites *Galandromus occidentalis* (Nesbitt) and *Zetzellia mali* Ewing; and the eriophyid apple rust mite, *Aculus schlechtendali* (Nalepa). In the mite-specific trials, the variable cumulative mite-days (CMD) was used to summarize the seasonal densities for these trials, using the method described by Beers and Brunner (1999), i.e.,

$$\mathrm{CMD}=\sum 0.5(P_a+P_b)D_{a\text{-}b}$$

where P_a is the population density (mean mites per leaf at time *a*),

P_b is the population density at time *b*, and $D_{a\text{-}b}$ is the number of days between evaluations.

Experimental design and analysis

Experiments were randomized complete block designs, with three to five replications. Mite densities in the *C. pomonella* trials were analyzed using analysis of variance and Fisher's Least Significant Difference mean separation. Data from the mite-specific experiments (CMD) were analyzed using analysis of variance (SAS Institute 1982) and the Waller-Duncan *k*-ratio *t*-test mean separation. Data were tested for homogeneity of variance using Levene's (1960) test. Data with unequal variances were transformed ($\log(y+0.5)$) prior to analysis. The effect of addition of oil to acetamiprid (three trials) on tetranychid and predatory mite seasonal densities was tested with contrast statements.

Meta-analysis of mite-specific experiments

Analyses were performed on the metadata from the mite-specific field trials. Only those trials with a sufficient number of counts to accurately calculate the cumulative mite days and an organophosphate standard (i.e., four trials, 20 treatments) were included. Sufficient data were available for acetamiprid only. The response variable CMD in the neonicotinyl treatment (CMD_{nn}) was corrected for the CMD in the standard treatment (CMD_{std}) using the following formula:

$$CMD_{corr} = (CMD_{nn} - CMD_{std})/CMD_{std}$$

Corrected CMDs were tested for homogeneity of variance using Levene's (1960) test. Data were analyzed using a homogeneity-of-slopes model in PROC GLM (SAS Institute 1982) and *F*-tests used to determine significance. The independent variable was total grams of AI per hectare applied over the season (as a continuous variable) and the use of oil as an adjuvant (as a class variable).

Results and Discussion

C. pomonella field experiments

There was a trend toward higher mite populations in treatments consisting of acetamiprid than in the organophosphate standard. Of the 30 treatments where mite data were taken, however, only six had statistically higher mite populations than the standard (azinphosmethyl or phosmet) and seven were higher than the untreated control (Table 1). Conversely, in four cases, mite populations were statistically lower in the acetamiprid treatment than the standard, although neither the standard nor the control exceeded 5 mites per leaf in these cases. Some of the higher mite populations occurred in trials where composite mite data were taken, thus no statistical comparison is possible; however mite densities in these acetamiprid treatments averaged 6- to 15-fold higher than the standard and control, respectively. The acetamiprid treatments exceeded Washington's economic injury threshold of 5 mites per leaf (Beers *et al.* 1993) in 14 of 30 cases (47%), whereas the standard treatment exceeded this threshold in 2 of 27 cases (7.4%). Overall, the acetamiprid treatments had 4.6- to 5.4-fold higher mite densities than the standard and the control, respectively. There was no relationship between the predominant mite species and the overall density; high populations were just as likely to be *P. ulmi* as *T. urticae*.

The average predatory mite densities in the standard and control were similar to those in the acetamiprid treatments, although in 7 of 20 cases (35.0%), the population was significantly lower in the acetamiprid treatment than the standard, and in 14 of 23 cases (60.8%) it was lower than the control treatment. In only one case was the predatory mite density in the acetamiprid treatment significantly higher than the control. In general, acetamiprid appeared to suppress predatory mites, but this effect was not consistent. In several of the early trials, where composite samples were taken, predatory mite densities were relatively high in the acetamiprid treatments. However, in a normal predator-prey relationship, predatory mite densities should have been consistently higher where tetranychid mite densities were higher (that is, significantly higher than the control).

Only 2 out of 18 thiacloprid treatments showed a tendency to have increased mite densities, and these were the treatments applied eight times during the season, a total of 1,688 or 2,248 g AI/ha (Table 1). These were composite samples, so statistical separation was not possible. While we have fewer data on thiacloprid than on acetamiprid, it appears to have less of a tendency to cause mite outbreaks, especially given the current label restrictions (maximum of 560 g AI/ha per season). It is more difficult to draw conclusions concerning the six treatments of clothianidin. Only one treatment had significantly higher tetranychid mite densities than the standard and the control. However, this material does appear to suppress predatory mites. Four of the six treatments had predatory mite densities that were lower than the standard. In one case, the clothianidin treatment had predatory mite densities that were higher than the control, but this treatment also had a very high level of phytophagous (prey) mites.

These data also confirm the findings of Hoyt (1969) and Beers and Brunner (1999) that organophosphate insecticides no longer perturb integrated mite control on Washington apples. In only 3 of 22 trials (13.6%) was the tetranychid mite density significantly higher in the organophosphate standard than in the control, and the density in these three standard treatments never exceeded 2.7 mites per leaf. In the two cases where the predatory mite density in the standard was significantly different from the control, the density was higher in the standard. Survival of *G. occidentalis* in organophosphate treatments in the current study is generally higher than in Hoyt's (1969) study, reflecting the continuing selection pressure with organophosphate insecticides.

While not conclusive, the trend in tetranychid mite populations observed in the *C. pomonella* field trials was sufficient to warrant a closer investigation of possible mite perturbation following the use of neonicotinyl insecticides.

Mite trial #1, experimental orchard

Mite densities were moderate overall in this experiment. The highest density of tetranychid mites occurred in the two acetamiprid treatments (5 mites per leaf). The peak density in the control reached 2 mites per leaf but was usually <0.5 mites per leaf. *P. ulmi* was the predominant tetranychid species. The high rate of acetamiprid had significantly higher seasonal densities of tetranychid mites than the untreated control (Table 2). The low rate

Table 1. Effect of neonicotinyl insecticdes applied at a codling moth timing on tetranychid and predatory mites in field trials, 2000-2004

Insecticide	Rate (g AI/ha)	Horticultural mineral oil (vol:vol)	Year	block no.[a]	Applicatio method volume[b]	No. applications season	% ERM in NN[c]	Total tetranychid mites						Total predatory mites					
								Mites/leaf			Comparison of means			Mites/leaf			Comparison of means		
								NN	Std	Ck	NN-Std	NN-Ck	Std-Ck	NN	Std	Ck	NN-Std	NN-Ck	Std-Ck
Acetamiprid 70WP	118		2000	CV 18	A 935	4	6	5.76	0.96	1.04	---	---	---	6.24	4.52	4.44	---	---	---
Acetamiprid 70WP	118		2000	CV 18	A 935	4	47	4.56	1.36	1.40	---	---	---	2.92	3.44	1.68	---	---	---
Acetamiprid 70WP	78		2000	TF 24	H dilute	4	77	5.84	1.08	0.28	---	---	---	1.28	0.84	1.08	---	---	---
Acetamiprid 70WP	118		2000	TF 24	H dilute	4	33	9.44	1.08	0.28	---	---	---	3.76	0.84	1.08	---	---	---
Acetamiprid 70WP	78	0.25%	2000	TF 24	H dilute	4	59	3.52	1.08	0.28	---	---	---	3.60	0.84	1.08	---	---	---
Acetamiprid 70WP	118	0.25%	2000	TF 24	H dilute	4	61	6.00	1.08	0.28	---	---	---	2.00	0.84	1.08	---	---	---
Acetamiprid 70WP	167		2001	TF 24	A 935	4	17	82.75	2.83	4.41	*	*	ns	0.16	3.10	2.56	*	*	ns
Acetamiprid 70WP	167		2001	TF 24	H dilute	4	80	34.10	3.90	7.50	---	---	---	0.20	2.60	7.70	---	---	---
Acetamiprid 70WP	84	0.25%	2002	TF 24	H dilute	6	77	3.05	3.25	4.75	ns	ns	ns	0.05	0.10	0.70	ns	*	*
Acetamiprid 70WP	84	0.25%	2002	TF 24	H dilute	4	55	0.55	3.25	4.75	*	*	ns	0.05	0.10	0.70	ns	*	*
Acetamiprid 70WP	84	1.0%	2002	TF 24	H dilute	4	74	4.95	3.25	4.75	ns	ns	ns	0.35	0.10	0.70	ns	ns	*
Acetamiprid 70WP	167		2002	TF 24	H dilute	4	83	0.60	3.25	4.75	*	*	ns	0.15	0.10	0.70	ns	*	*
Acetamiprid 70WP	167	0.25%	2002	TF 24	H dilute	4	83	1.80	3.25	4.75	ns	*	ns	0.00	0.10	0.70	ns	*	*
Acetamiprid 70WP	167	1.0%	2002	TF 24	H dilute	4	98	22.25	3.25	4.75	*	*	ns	0.30	0.10	0.70	ns	*	*
Acetamiprid 70WP	84		2002	TF 24	H dilute	4	40	0.55	3.25	4.75	*	*	ns	0.05	0.10	0.70	ns	*	*
Acetamiprid 70WP	167	0.25%	2002	CV 18	H dilute	4	2	12.70	1.05	2.10	ns	ns	ns	0.50	0.70	2.05	ns	*	ns
Acetamiprid 70WP	235	0.25%	2002	TF 26	H dilute	4	49	7.20	5.40	5.30	ns	ns	ns	0.25	0.15	0.75	ns	ns	ns
Acetamiprid 70WP	167	0.25%	2003	TF 24	H dilute	4	0	1.15	0.55	0.25	ns	ns	ns	0.15	0.60	0.90	ns	*	ns
Acetamiprid 70WP	167	0.25%	2003	TF 24	H dilute	4	15	2.85	0.85	0.30	ns	ns	ns	1.12	1.55	0.25	ns	ns	ns
Acetamiprid 70WP	167	0.25%	2003	TF 26	H dilute	4	67	0.30	2.65	0.65	*	ns	*	0.20	2.15	0.20	*	ns	*
Acetamiprid 70WP	167	0.25%	2003	CV 18	H dilute	4	81	1.85	---	2.70	---	ns	---	1.35	---	2.25	---	ns	---
Acetamiprid 70WP	167		2003	TF 24	H dilute	4	90	0.55	0.20	0.40	ns	ns	ns	2.95	1.60	1.35	ns	ns	ns
Acetamiprid 70WP	167	0.25%	2003	TF 24	H dilute	4	100	0.05	0.20	0.40	ns	ns	ns	0.15	1.60	1.35	*	*	ns
Acetamiprid 70WP	167	1.0%	2003	CV 19	H dilute	4	0	0.00	0.70	0.25	ns	ns	*	0.05	1.05	2.00	*	*	ns
Acetamiprid 70WP	167	0.25%	2003	CV 18	A 935	4	69	16.67	---	2.56	---	*	---	1.00	---	0.39	---	*	---
Acetamiprid 70WP	167	0.25%	2003	TF 16	A 935	4	1	3.00	---	1.13	---	ns	---	0.08	---	0.25	---	ns	---
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	4	0	7.00	0.80	0.80	*[d]	*[d]	ns	0.10	0.80	0.50	*	*	ns
Acetamiprid 70WP	118	0.25%	2004	TF 24	H dilute	4	0	42.90	3.40	1.00	*	*	ns	0.00	0.60	0.40	*	*	ns
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	6	0	23.40	3.40	1.00	*	*	ns	0.00	0.60	0.40	*	*	ns
Acetamiprid 70WP	167	0.25%	2004	TF 24	H dilute	4	0	84.90	20.40	4.30	*	*	ns	1.30	1.70	1.90	ns	ns	ns
Thiacloprid 480SC	211		2000	TF 24	H dilute	4	90	0.76	0.20	0.28	---	---	---	0.20	0.12	0.16	---	---	---
Thiacloprid 480SC	211		2001	TF 24	H dilute	8	72	19.80	3.90	7.50	---	---	---	0.60	2.60	7.70	---	---	---
Thiacloprid 480SC	281		2001	TF 24	H dilute	8	74	24.10	3.90	7.50	---	---	---	1.90	2.60	7.70	---	---	---
Thiacloprid 480SC	136		2003	TF 24	H dilute	4	69	1.60	0.55	0.25	ns	ns	ns	1.00	0.60	0.90	ns	ns	ns
Thiacloprid 480SC	136	0.25%	2003	TF 24	H dilute	4	0	0.75	0.55	0.25	ns	ns	ns	0.15	0.60	0.90	ns	ns	ns
Thiacloprid 480SC	203		2003	TF 24	H dilute	4	42	0.60	0.55	0.25	ns	ns	ns	1.35	0.60	0.90	ns	ns	ns
Thiacloprid 480SC	203	0.25%	2003	TF 24	H dilute	4	0	0.65	0.55	0.25	ns	ns	ns	0.15	0.60	0.90	ns	*	ns
Thiacloprid 480SC	269		2003	TF 24	H dilute	4	62	1.85	0.55	0.25	ns	ns	ns	0.90	0.60	0.90	ns	ns	ns
Thiacloprid 480SC	269	0.25%	2003	TF 24	H dilute	4	4	2.55	0.55	0.25	ns	ns	ns	0.35	0.60	0.90	ns	ns	ns
Thiacloprid 480SC	168	0.25%	2003	TF 24	H dilute	4	0	0.30	0.85	0.30	ns	ns	ns	0.20	1.55	0.25	ns	ns	ns
Thiacloprid 480SC	168	0.25%	2003	TF 24	H dilute	4	0	1.55	0.85	0.30	ns	ns	ns	0.55	1.55	0.25	ns	ns	ns
Thiacloprid 480SC	203	0.25%	2003	TF 24	H dilute	4	17	0.30	0.85	0.30	ns	ns	ns	0.35	1.55	0.25	ns	ns	ns
Thiacloprid 480SC	269	0.25%	2003	TF 24	H dilute	4	0	0.50	0.85	0.30	ns	ns	ns	0.60	1.55	0.25	ns	ns	ns
Thiacloprid 480SC	200	0.25%	2003	TF 26	H dilute	4	92	2.60	2.65	0.65	ns	ns	*	1.50	2.15	0.20	ns	ns	*
Thiacloprid 480SC	203	0.25%	2003	TF 24	H dilute	4	0	0.00	0.20	0.40	ns	ns	ns	0.10	1.60	1.35	*	*	ns
Thiacloprid 480SC	203	0.25%	2003	CV 18	A 935	4	94	3.44	---	2.56	---	ns	---	0.39	---	0.39	---	ns	---
Thiacloprid 480SC	269	0.25%	2003	CV 18	A 935	4	94	2.89	---	2.56	---	ns	---	0.44	---	0.39	---	ns	---
Thiacloprid 480SC	210	0.25%	2004	TF 24	H dilute	4	0	4.10	3.40	1.00	ns	ns	ns	0.10	0.60	0.40	*	ns	ns
50WDG	70/105[e]	0.25%	2003	TF 24	H dilute	6	0	0.85	0.85	0.30	ns	ns	ns	0.05	1.55	0.25	*	ns	ns
50WDG	105/214[e]	0.25%	2003	TF 24	H dilute	4	17	3.00	0.85	0.30	ns	ns	ns	0.45	1.55	0.25	ns	ns	ns
50WDG	105	0.25%	2004	TF 24	H dilute	6	0	28.50	0.80	0.80	*	*	ns	1.00	0.80	0.50	ns	*	ns
50WDG	105	0.25%	2004	TF 24	H dilute	6	0	3.70	0.80	0.80	ns	ns	ns	0.30	0.80	0.50	*	ns	ns
50WDG	210	0.25%	2004	TF 24	H dilute	6	0	3.60	0.80	0.80	ns	ns	ns	0.20	0.80	0.50	*	ns	ns
50WDG	210	0.25%	2004	TF 24	H dilute	4	0	0.50	0.80	0.80	ns	ns	ns	0.10	0.80	0.50	*	*	ns

*Treatment means were significantly different: NN (neonicotinyl) vs. std (standard), NN vs. ck (check), or std vs. ck.

— Indicates data not available to make statistical comparison; 2000-2001 composite samples without replication; 2002-2003 standard treatment not included in test.

ns, not significantly different

[a] Locations: TF, Tree Fruit Research and Extension Center (home farm); CV, WSU Columbia View Farm.

[b] Method: H, Handgun, A, Airblast (liters/ha).

[c] Species composition; figure in column is percentage European red mite, remainder was twospotted spider mite.

[d] Mean comparison statistically different at $P = 0.10$ but not at $P=0.05$.

[e] Rates for the 1st and 2nd generation of codling moth, respectively.

of acetamiprid and esfenvalerate treatments had slightly elevated tetranychid mite densities, but they were not significantly different from the control. All three treatments suppressed predatory mite densities in relation to the control. There were high levels of *Z. mali* in relation to the control in the high rate of acetamiprid; it appears that this species is better able to respond to increasing prey density

Table 2. Effect of acetamiprid and esfenvalerate on phytophagous and predatory mite densities, September 2000

			Cumulative mite days						
Treatment	g AI/ha	CM cover sprays[x]	Twospotted spider mite[y]	European red mite	Total tetranchid mites[y]	*G. occidentalis*[y]	*Z. mali*[y]	Total predatory mites	Apple rust mite[y]
Acetamiprid 70WP	167 g	C1,C2,C3,C4	33 a	181 a	274 a	12 c	24 a	36 b	25,592 a
Acetamiprid 70WP	85 g	C1,C2,C3,C4	4 ab	164 ab	206 ab	21 b	14 ab	35 b	22,793 ab
Esfenvalerate 0.66EC	92 g	C1,C2,C3,C4	9 ab	69 ab	95 ab	23 b	10 ab	33 b	20,780 ab
Untreated check	-----	-----	3 b	30 b	35 b	68 a	2 b	70 a	17,225 b

Means within columns not followed by the same letter are significantly different (Waller-Duncan *k*-ratio *t*-test).
Experimental design: RCB, 4 replicates, three-tree, single row; sampled 20 leaves/plot (center tree); airblast 1,871 liters/ha.
Tetranychid mite species composition (seasonal average): 71% ERM, 22% TSM, 7% MCD.

[x] Cover sprays: C1, 23 June; C2, 6 July; C3, 24 July; C4, 10 August 2000.

[y] Data transformed log(y+0.5) due to unequal variances.

Table 3. Effect of neonicotinyl insecticides on phytophagous and predatory mites, Aug 2001

			Cumulative mite days						
Compound	g AI/ha	CM cover sprays[x]	Twospotted spider mite[y]	European red mite[y]	Total tetranychid mites[y]	*G. occidentalis*[y]	*Z. mali*	Total predatory mites[y]	Apple rust mite
Acetamiprid 70WP	167 g	C1,C2,C3,C4	93 a	136 a	234 a	15 c	14 cd	29 b	33,650 a
Thiacloprid 480SC	140 g	C1,C2,C3,C4	23 bc	38 bc	62 bc	60 ab	12 cd	72 a	20,916 bc
Thiamethoxam 25WDG	96 g	C1,C2,C3,C4	9 cd	18 bc	30 bc	55 ab	43 ab	99 a	18,008 c
Imidacloprid 1.6F	112 g	C1,C2,C3,C4	6 de	17 cd	25 cd	33 bc	68 a	101 a	23,532 abc
Acetamiprid 70WP	168 g	C1,C2	20 b	32 b	53 b	16 c	14 cd	30 b	28,981 ab
Esfenvalerate 0.66EC	84 g	C1,C2,C3,C4	9 cd	8 bcd	17 cd	17 c	2 d	19 b	26,376 abc
Phosmet 70WP	4,182 g	[all]	1 e	2 d	4 d	57 ab	30 bc	86 a	20,200 bc
Check	---	---	3 de	4 d	8 d	85 a	21 bcd	105 a	22,035 bc

Means within columns not followed by the same letter are significantly different (Waller-Duncan *k*-ratio *t*-test).
Experimental design: RCB, 4 replicates, 5 tree (single row); sampled 20 leaves/plot; airblast 1,871 liters/ha.
Tetranychid mite species composition (seasonal average): 59% ERM, 38% TSM, 3% MCD.

[x] Cover spray in this column refers to acetamiprid; all other cover sprays in that treatment were phosmet; all treatments received four cover sprays: 1st cover, 24 May; 2nd cover 28 June; 3rd cover 19 July, 4th cover 9 Aug, 2001.

[y] Data transformed log(y+0.5) due to unequal variances.

in acetamiprid-treated plots. Apple rust mite densities were higher in the high rate acetamiprid treatment, perhaps due to lower *G. occidentalis* densities.

Mite trial #2, experimental orchard

Mite densities were low in the control throughout the season, never exceeding 0.3 mites per leaf, which is typical of minimally sprayed apple orchards in Washington. The peak density was 5 mites per leaf, which occurred late in the season in the four-application treatment of acetamiprid. The standard organophosphate treatment, phosmet, had low mite populations that were never different from the control. The seasonal cumulative tetranychid mite days (Table 3) reflected the elevated levels of mites in the acetamiprid four-application treatment, which were ca. 3.8-fold higher than the next highest treatment (thiacloprid). The acetamiprid two-application treatment had substantially lower cumulative tetranychid mite days than the acetamiprid four-application treatment, however, it was still higher than the control. Cumulative tetranychid mite populations in the imidacloprid, esfenvalerate and phosmet treatments were not different than the control.

Esfenvalerate and the two acetamiprid treatments had among the lowest seasonal densities of predatory mites (Table 3). However, there was a corresponding increase in tetranchid mite densities only in the acetamiprid treatments. While the thiacloprid treatment had a slightly elevated tetranychid mite population, the predatory mite population was not significantly different from the control. The predatory mite populations in the phosmet, thiamethoxam and imidacloprid treatments were not different than the control. Apple rust mite densities were high, but with no consistent trend in differences among treatments. However, a significantly higher rust mite density occurred in the acetamiprid four-application treatment, perhaps due to lower predatory mite densities.

Mite trial #3, experimental orchard

Mite populations were relatively high in this trial, peaking near 70 mites per leaf in one of the treatments (acetamiprid+oil, four applications). All treatments containing acetamiprid in one or more *C. pomonella* sprays (with the exception of acetamiprid +oil, two applications) increased the mite populations relative to the control and standard (Table 4). The addition of oil to acetamiprid did not reduce the seasonal mite densities (F=2.60, P=0.13), and tended to suppress predatory mites (F=11.38, P=0.005). The

Table 4. Effect of acetamiprid (with or without oil) on phytophagous and predatory mite densities, September 2002

			Cumulative mite days						
Treatment	g AI/ha	CM cover sprays[y]	Twospotted spider mite	European red mite	Total tetranychid mites	*G. occidentalis*	*Z. mali*	Total predatory mites	Apple rust mite
Acetamiprid 70WP	167 g	C2	852 ab	123 ab	975 ab	35 b	118 a	152 a	5,278 a
Acetamiprid 70WP[x]	167 g	C2	871 ab	208 a	1,079 ab	37 ab	31 ab	68 ab	2,494 a
Acetamiprid 70WP	167 g	C1,C2	1,012 ab	43 ab	1,055 ab	37 ab	94 ab	130 ab	4,944 a
Acetamiprid 70WP[x]	167 g	C1,C2	541 bcd	144 ab	685 bc	61 a	7 b	69 ab	2,897 a
Acetamiprid 70WP	167 g	C1,C2,C3,C4	1,395 a	79 ab	1,476 a	36 ab	64 ab	100 ab	3,931 a
Acetamiprid 70WP[x]	167 g	C1,C2,C3,C4	817 abc	172 ab	988 ab	27 b	21 ab	48 b	3,288 a
Phosmet 70WP	3,923 g	[all]	105 d	44 ab	150 c	21 b	42 ab	63 b	7,038 a
Untreated check	---	---	182 cd	25 b	209 c	32 b	32 ab	65 ab	4,629 a

Means within columns not followed by the same letter are significantly different (Waller-Duncan *k*-ratio *t*-test).
Experimental design: RCB, 3 replicates, 3 rows × 4 trees; sampled 40 leaves/plot; airblast 935 liters/ha.
Tetranychid mite species composition (seasonal average): 13% ERM, 87% TSM, 0% MCD.

[x] Tank mixed with Orchex 796 Horticultural Spray Oil at 1% vol:vol.

[y] Cover spray in this column refers to acetamiprid; all other cover sprays in that treatment were phosmet; all treatments received 4 cover sprays: C1 (250 DD), 1 June; C2 (+21 days), 21 June; C3 (1250 DD), 20 July; C4 (+21 days), 8 Aug, 2002.

Table 5. Effect of acetamiprid (with or without oil) on phytophagous and predatory mite densities, September 2003

			Cumulative mite days						
Treatment	g AI/ha	CM cover sprays[y]	Twospotted spider mite[z]	European red mite	Total tetranchid mites	*G. occidentalis*	*Z. mali*	Total predatory mites	Apple rust mite
Acetamiprid 70WP	167 g	C2	0 d	43 b	43 bc	30 bc	15 abc	45 ab	7,593 a
Acetamiprid 70WP[x]	167 g	C2	0 cd	72 ab	72 bc	27 cd	15 abc	42 ab	7,975 a
Acetamiprid 70WP	167 g	C1,C2	1 c	69 ab	70 bc	20 de	11 bc	31 b	7,192 a
Acetamiprid 70WP[x]	167 g	C1,C2	0 d	50 b	50 bc	18 de	30 ab	48 ab	7,871 a
Acetamiprid 70WP	167 g	C1,C2,C3,C4	11 b	148 ab	160 ab	12 e	32 a	44 ab	7,524 a
Acetamiprid 70WP[x]	167 g	C1,C2,C3,C4	41 a	213 a	254 a	19 de	15 abc	34 b	7,679 a
Phosmet 70WP	3,923 g	[all]	0 d	6 b	6 c	38 b	12 bc	51 ab	6,741 a
Untreated check	-----	-----	0 d	26 b	26 bc	59 a	1 c	60 a	4,754 b

Means within columns not followed by the same letter are significantly different (Waller-Duncan *k*-ratio *t*-test).
Experimental design: RCB, 5 replicates (3 rows × 6 trees); sampled 40 leaves/plot; airblast 1,871 liters/ha.
Tetranychid mite species composition (seasonal average): 92% ERM, 8% TSM, 0% MCD.

[x] Tank mixed with Orchex 796 Horticultural Spray Oil at 1% vol:vol.

[y] Cover spray in this column refers to acetamiprid; all other cover sprays in that treatment were phosmet; all treatments received 4 cover sprays: C1 (250 DD), 21, 22 May; C2 (+21 days), 15 June; C3 (1250 DD), 17 July; C4 (+21 days), 7 Aug 2003.

[z] Data transformed $\log(y+0.5)$ due to unequal variances.

predatory mite densities in the acetamiprid treatments were not significantly different from the control, but were low relative to the prey population. Apple rust mite densities did not differ among treatments. *Z. mali* densities were relatively high in several acetamiprid treatments, although not significantly different from the control.

Mite trial #4, experimental orchard

Tetranychid mite populations did not begin to increase until early August, and peaked a few weeks later. The densities in the control and the phosmet treatments never exceeded 1 mite per leaf during the season, and are typical of non-perturbed orchards. The highest seasonal tetranychid mite levels occurred in the acetamiprid four-application treatments, either with or without oil (Table 5). All other acetamiprid treatments had slightly or moderately elevated mite densities in relation to the phosmet standard, although they were not significantly different than the untreated control. Predatory mite densities rose with the pest mite population in August, with little discernable difference among treatments. The untreated control had the highest levels, but the mean for the four-application acetamiprid treatment was not significantly lower. Only the two-application acetamiprid treatment and four-application acetamiprid + oil treatments had slightly depressed predatory mite densities. The densities of *Z. mali* were high relative to the control in two acetamiprid treatments. The addition of oil to the acetamiprid treatments did not affect either cumulative tetranychid densities ($F = 1.69$, $P = 0.20$) or cumulative predatory mite densities ($F = 0.02$, $P = 0.89$). Apple rust mite densities were significantly lower in the control than in the other treatments, possibly due to the higher levels of *G. occidentalis*.

Table 6. Effect of acetamiprid (with or without oil) on phytophagous and predatory mite densities, commercial orchard, September 2002

			Cumulative mite days						
Treatment	g AI/ha	CM cover sprays[y]	Twospotted spider mite	European red mite	Total tetranychid mites	*G. occidentalis*	*Z. mali*	Total predatory mites	Apple rust mite
Acetamiprid 70WP	167 g	C1,C2	7 a	1 a	8 a	35 a	49 a	84 a	5 a
Acetamiprid 70WP[x]	167 g	C1,C2	3 a	1 a	4 a	71 a	34 a	106 a	5 a
Check (MD only)[y]	-----	-----	0 a	1 a	1 a	52 a	43 a	95 a	2 a

Means within columns not followed by the same letter are significantly different (Waller-Duncan *k*-ratio *t*-test).
Experimental design: RCB, 3 replicates (2 ha each); sampled 100-200 leaves/plot; airblast 1,871 liters/ha.
Tetranychid mite species composition (seasonal average): 36% ERM, 64% TSM, 0% MCD.
[x] Tank mixed with Orchex 796 Horticultural Spray Oil at 1% vol:vol.
[y] Low codling pressure in this block necessitated only spot treatment with azinphosmethyl during the second generation. CM cover spray dates were: 1st cover (1C) 31 May; 2nd cover (2C) 21 June; 3rd cover 26 July; 4th cover 12-19 August, 2002.

Table 7. Effect of acetamiprid (with or without oil) on phytophagous and predatory mite densities, commercial orchard, September 2003

			Cumulative mite days						
Treatment	g AI/ha	CM cover sprays[y]	Twospotted spider mite	European red mite	Total tetranychid mites	*G. occidentalis*	*Z. mali*	Total predatory mites	Apple rust mite
Acetamiprid 70WP	167 g	C2	8 ab	0 a	8 ab	14 a	0.0 a	14 a	1,301 ab
Acetamiprid 70WP[x]	167 g	C2	6 ab	3 a	9 ab	18 a	0.1 a	18 a	1,706 ab
Acetamiprid 70WP	167 g	C1,C2	8 ab	8 a	16 ab	20 a	0.1 a	20 a	1,414 ab
Acetamiprid 70WP[x]	167 g	C1,C2	4 ab	1 a	4 ab	14 a	0.1 a	14 a	1,275 b
Acetamiprid 70WP	167 g	C1,C2,C3,C4	8 ab	2 a	10 ab	7 a	0.0 a	7 a	1,820 a
Acetamiprid 70WP[x]	167 g	C1,C2,C3,C4	15 a	5 a	20 a	20 a	0.1 a	20 a	1,605 ab
Imidan 70WP	3,923 g	[all]	2 b	6 a	9 ab	16 a	0.1 a	16 a	1,389 ab
Untreated check	-----	-----	4 ab	1 a	5 b	12 a	0.1 a	12 a	1,569 ab

Means within columns not followed by the same letter are significantly different (Waller-Duncan *k*-ratio *t*-test).
Experimental design: RCB, 5 replicates (3 rows × 9 trees); sampled 60 leaves/plot; airblast 935 liters/ha.
Tetranychid mite species composition (seasonal average): 26% ERM, 71% TSM, 3% MCD.
[x] Tank mixed with Orchex 796 Horticultural Spray Oil at 1% vol:vol.
[y] Cover spray in this column refers to acetamiprid; all other cover sprays in that treatment were phosmet; all treatments received 4 cover sprays: C1 (250 DD), 20 May; C2 (+21 days), 23 June; C3 (1250 DD), 18 July; C4 (+21 days), 9 Aug 2003.

Mite trial #5, commercial orchard

Tetranychid and predatory mite populations were very low throughout the trial (Table 6), which is reflected in the low seasonal mite day accumulations. There was no indication of mite population increase due to the acetamiprid two-application treatment during the first generation. This was the first large-block commercial trial with acetamiprid and, coincidentally, the first where there has been no indication of elevated mite densities. Previous trials have been conducted in smaller blocks on experimental orchards, which were somewhat destabilized in terms of integrated mite control.

Mite trial #6, commercial orchard

Tetranychid mite populations never exceed 1 mite per leaf during the course of the season in any of the treatments (Table 7). Although some minor statistical differences occurred among treatment means (the untreated control had the lowest cumulative mite populations, and the acetamiprid four-application+oil treatment the highest), these levels would have no economic significance for the producer. Predatory mite densities did not differ among treatments, and there were no consistent trends in apple rust mite densities. The addition of oil to acetamiprid did not affect cumulative tetranychid mite densities ($F = 0.05$, $P = 0.82$) or cumulative predatory mite densities ($F = 0.70$, $P = 0.41$).

Meta-analysis of mite trials

In both analyses (tetranychid and predatory mites), the interaction term (oil x total g AI) was non-significant (data not shown). The tetranychid mite seasonal density (CMD) in the acetamiprid treatments increased with increasing rates of acetamiprid (total g AI/ha applied) ($F = 4.30$, $P = 0.05$, $R^2 = 0.24$) (Fig. 1). It should be noted that these data all used spray timings targeting *C. pomonella*, and the effect of other spray timings, targets, and rates has not been evaluated. In addition, since all acetamiprid applications were made at the same (full label) rate, the total g AI/ha is functionally equivalent to the number of applications. However, these data do suggest that increasing the number of applications (or total amount) of acetamiprid will increase the risk of a tetranychid mite outbreak. The effect of oil as an adjuvant was not significant (F=0.44, P=0.52). Neither the total g AI applied ($F = 1.41$, $P = 0.25$, $R^2 = 0.11$) nor the addition oil ($F = 0.18$, $P = 0.68$) had a

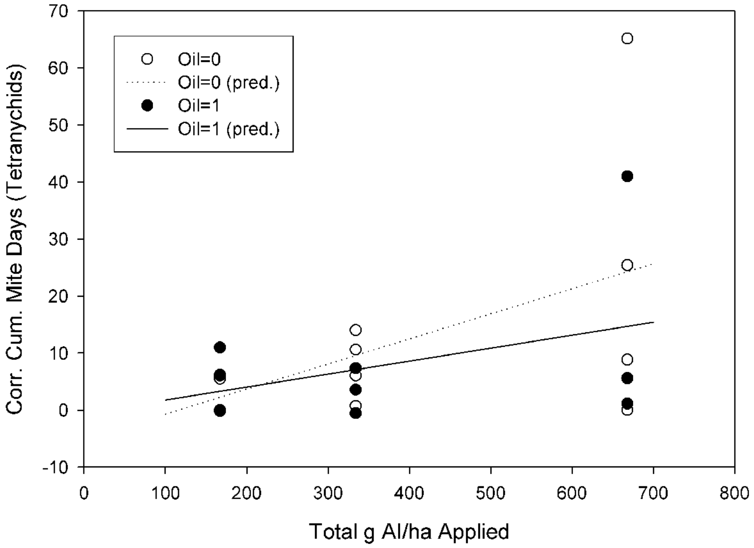

Figure 1. Effect of rate of acetamiprid (with oil, $y = -0.505 + 0.023x$; or without oil, $y = -5.095 + 0.044x$) on cumulative tetranychid mite days.

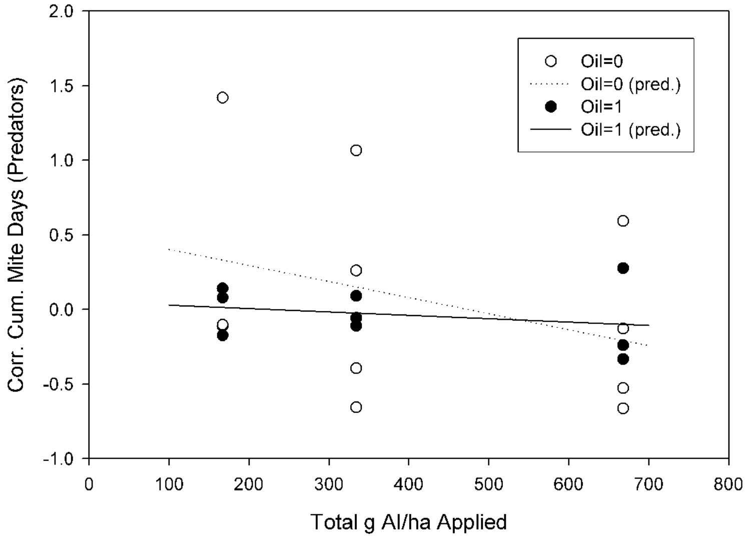

Figure 2. Effect of rate of acetamiprid (with oil, $y = 0.052 - 0.00023x$; or without oil, $y = 0.509 - 0.00108x$) on cumulative predatory mite days.

significant effect on the seasonal densities of predatory mites (Fig. 2).

Conclusions

Both the *C. pomonella* and the mite-specific field trials provide evidence of increasing probability of tetranychid mite outbreaks following the use of neonicotinyl insecticides. Most of the trials reported here were conducted in experimental orchards, some of which had destabilized predator-prey relationships. Under these circumstances, a moderate increase in mite densities occurred in about half of the cases, with severe outbreaks much less common. The absence of tetranychid mite outbreaks in two commercial orchards following limited numbers of applications is encouraging; however, the effect of using multiple neonicotinyl insecticides in the same year, or for multiple years, has not been explored on a larger scale.

There was no evidence from either the *C. pomonella* trials or the mite-specific trials that the stimulation effect acts differentially on *P. ulmi* vs. *T. urticae*. Cases of high tetranychid densities consisted of about equal incidence of both species. In the mite-specific trials, *P. ulmi* was the dominant species in three tests, and *T. urticae* the dominant species in the other three tests. The populations were comprised of a mixture of both species, but for a given treatment, high densities of the dominant species usually had correspondingly high densities of the other species.

The trends for proportions of predatory mite species were inconsistent. *G. occidentalis* is normally the dominant predatory mite species in Washington apple orchards, with *Z. mali* present in smaller numbers (Beers *et al.* 1993). However, in several acetamiprid treatments, *Z. mali* outnumbered *G. occidentalis* (e.g., Tables 2, 3, and 4), indicating these two species may be affected differentially. However, the *Z. mali* density was also abnormally high in some of the standard and control treatments, making it difficult to draw conclusions about predatory mite species.

Of the neonicotinyl insecticides tested, acetamiprid appears to have the greatest tendency to cause increased tetranychid mite populations, although the reason for this is unclear. Biological control of mites in Washington orchards has three primary components: tetranychid prey mites, an alternate prey species, apple rust mite, and one or more species of predatory mites (of which *G. occidentalis* predominates) (Beers *et al.* 1993; Hoyt 1969). Use of materials acutely toxic to *G. occidentalis* is cited most frequently as the cause of mite outbreaks, and Washington's integrated mite management program has been built on limited use of such materials (e.g., carbamates and pyrethroids) (Beers *et al.* 1993). Unlike these materials, acetamiprid is not acutely toxic to *G. occidentalis* although it does appear to be somewhat repellent in preliminary tests (E. H. Beers, unpublished) and, in fact, acetamiprid did not eliminate *G. occidentalis* in any of our trials. Although the mechanism cannot be elucidated by these field trials, acetamiprid appeared to interfere with *G. occidentalis'* normal functional or numerical response to increasing prey populations. There was no evidence that acetamiprid reduced densities of the alternate prey species, apple rust mite, which is also associated with perturbation of biological mite control. If anything, apple rust mite densities were higher in acetamiprid treatments, possibly because *G. occidentalis* densities were reduced.

Mechanisms other than release from biological control have been explored to explain mite outbreaks, specifically those that operate directly on the tetranychid species. One such mechanism is irritation, or stimulation of locomotor activity and thus dispersal throughout the tree (Davis 1952; Jones 1990). Another mechanism is pesticide-induced stimulation of reproduction, or hormoligosis (Luckey 1968). This mechanism is frequently discussed in association with mite outbreaks, with members of several groups of insecticides (organochlorines, carbamates, and organophosphates) being implicated (Bartlett 1968; Dittrich *et al.*

1973; Huffaker *et al.* 1969; Maggi and Leigh 1983). Hormoligosis in mites has been explored more recently with neonicotinyl insecticides (Ako *et al.* 2004), with no apparent effect in laboratory tests. Conversely, hormoligosis has been demonstrated for both pest (James and Price 2002) and predatory (James 1997) mite species, thus the potential net effect remains unclear.

Acetamiprid shows the most consistently high level of activity against *C. pomonella* in small-plot field trials (Brunner *et al.* 2005), and thus is the most likely of the neonicotinyl insecticides to replace organophosphate treatments. Since its registration in 2002, use of acetamiprid has increased to 25% of Washington's apple acreage (NASS 2004). However, azinphosmethyl and phosmet are still used on 78 and 12% of the acreage, respectively. Replacement of the organophosphate insecticides with neonicotinyl insecticides in the tree fruit market will greatly expand their use. In addition to their efficacy on codling moth, the neonicotinyl insecticides have activity on aphids, leafhoppers, and the mullein plant bug, *Campylomma verbasci* (Beers *et al.* 2002a; Beers *et al.* 2002b; Beers *et al.* 2002c), which will promote further uses. In this organophosphate-replacement scenario, the tendency of this class of insecticides to perturb integrated mite control will likely have widespread effects.

Although currently only the acetamiprid label allows four applications per year, the total number of possible applications of the neonicotinyl insecticides will expand with each new registration. Regardless of the target, multiple applications of neonicotinyl insecticides will likely promote resistance in one or more orchard pest species. For this reason, integrated control and resistance management strategies should be implemented for the entire pest complex. Limiting neonicotinyl use to one to two applications per season, and within one generation of *C. pomonella*, will have the dual benefit of delaying the onset of resistance and reducing the probability of mite outbreaks.

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