Insecticide Baseline Sensitivity in Codling Moth (Lepidoptera: Tortricidae) Populations From Orchards Under Different Management Practices

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Abstract The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a key "fruit-feeding" pest of apples and is known for developing resistance to various insecticidal products applied in apple orchards. Laboratory studies were conducted to determine insecticide resistance in codling moth populations collected from various apple orchards in Pennsylvania. In particular, male moths of *C. pomonella* were collected from commercial and abandoned fruit orchards and were tested for their sensitivity levels to azinphos-methyl and methomyl via adult topical bioassays. Larval sensitivity also was examined for different insecticides (e.g., acetamiprid, novaluron, rynaxypyr) via diet-surface topical bioassays. Adult *C. pomonella* populations expressed significant differences in their sensitivity to azinphos-methyl and methomyl. Concurrent estimates of azinphos-methyl insecticide effectiveness (i.e., adult topical assays) of moths in monitoring traps showed increased tolerance in individuals captured in commercial orchards rather than in abandoned orchards. Results of larval bioassays showed differences in sensitivity to various insecticide compounds as well as differences between compounds based on the timing of mortality. After the initial assessment, however, all insecticides (except fenpropathrin) exhibited greater toxicity with increasing time.

Key Words *Cydia pomonella*, insecticide resistance, acetamiprid, apple, spinosad, novaluron

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a key "fruit-feeding" pest of apples in the mid-Atlantic region of the United States and many other apple-growing regions in the world (Geier 1964, Hogmire and Miller 2005, Joshi et al. 2011, Pfeiffer et al. 1993, Willson and Trammel 1980, Witzgall et al. 2008). The importance of *C. pomonella* began to gradually increase in the late 1990s in Pennsylvania apple orchards, after years of relatively low levels of fruit infestation (Hull et al. 2001). During the period from 1998 to 2005, *C. pomonella* was responsible for 760 rejections of fruit loads delivered to Pennsylvania fruit processors (Krawczyk 2006). In this region, the number of fruit loads rejected due to the presence of *C. pomonella* significantly increased from 62 loads in 2001 to 207 loads in 2005 (Krawczyk 2006). Numerous factors, such as lack of proper pest

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monitoring, problems with correct spray timing or coverage of insecticides, and development of insecticide resistance probably contributed to this rapid development of *C. pomonella*–related fruit losses.

In the mid-Atlantic region, pest management tactics for controlling internal fruit-feeding pests are expensive, costing approximately $494 to $864 per hectare per annum (Krawczyk and Hull 2004). Starting in the 1970s, organophosphate insecticides were the cornerstone of apple and peach insect management programs (Jones et al. 2010). Currently, different pesticide chemistries (Hull et al. 2009a, b) as well as sex-pheromone–based mating disruption chemicals (Bohnenblust et al. 2011, Joshi et al. 2008) are used for management of *C. pomonella*. Organophosphate insecticides have provided excellent control of most direct insect pests of apple, and despite their relatively broad-spectrum activity, have relatively low impact on many important natural enemies, particularly mite predators in apple orchards (Hull et al. 1997). Despite this long-term use and reliance on organophosphates, there have been relatively few reported cases of pests developing pesticide resistance. Notable exceptions include the tufted apple bud moth, *Platynota idaeusalis* (Walker) (Knight et al. 1990), the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Ahmad et al. 2002, Lawson et al. 1997), and the oriental fruit moth, *Grapholitha molesta* (Busck) (Usmani and Shearer 2001). Reduced susceptibility of *C. pomonella* male adults to azinphos-methyl, phosmet, and methomyl has been documented in Pennsylvania apple orchards (Krawczyk and Hull 2004). Various cases of resistance to organophosphates in *C. pomonella* populations were documented previously in California (Dunley and Welter 2000, Varela et al. 1993), Washington (Knight et al. 1994), and North Carolina (Bush et al. 1993). In Europe, Sauphanor et al. (1998) observed resistance to insect growth regulators in *C. pomonella* populations and reported possible cross-resistance among insect growth regulators, organophosphates, and pyrethroids.

Insecticide resistance in *C. pomonella* adults likely causes increases in abundance of this pest and consequently higher fruit infestation in apple orchards relying on conventional insecticide-based management tactics. The use of multiple pest management approaches (e.g., using pesticides with different modes of action, tactics to avoid or slow insecticide resistance development in field populations) would help with development of effective management strategies of *C. pomonella*. The main objectives of this study were (a) to determine the sensitivity of naturally occurring *C. pomonella* adult populations to azinphos-methyl and methomyl (two broad-spectrum insecticides commonly used in Pennsylvania apple orchards), (b) to determine larval baseline sensitivity of laboratory-reared *C. pomonella* populations to some newly registered pesticides, and (c) to evaluate susceptibility of *C. pomonella* to different concentrations of azinphos-methyl.

Materials and Methods

Laboratory studies were conducted during 2001–2008 to determine insecticide resistance and larval sensitivity in field populations of *C. pomonella* to different pesticides used for pest management in Pennsylvania apple orchards. Specimens of *C. pomonella* populations were collected from conventional commercial and abandoned apple orchards with an infestation history of *C. pomonella*. Abandoned orchard sites were at or adjacent to the following coordinates: N 39°29′30.49″, W 77°13′23.2968″ (Site 1), N 39°35′6.936″, W 77°8′46.0968″ (Site 2), N 39°35′28.6404″, W 77°8′38.8536″ (Site 3), N 39°35′52.0476″, W 77°8′57.6996″ (Site 4); the commercial sites were at or adjacent to the following coordinates: N 39°30′10.7964″, W 77°6′51.4008″ (Site 1), N 39°35′5.90028″, W 77°8′33.9216″ (Site 2), N 39°35′30.0984″, W 77°8′56.904″ (Site 3), and N 40°2′5.208″, W 77°8′49.272″ (Site 4) in Adams County, Pennsylvania.

Codling moth adult bioassays. During the 2001–2005 growing seasons, multiple populations of *C. pomonella* adults were collected from commercial and abandoned apple orchards and evaluated in the laboratory for their sensitivity to the frequently used broad-spectrum insecticides azinphos-methyl (Bayer CropScience, Research Triangle Park, NC) and methomyl (DuPont Crop Protection, Newark, DE). Technical grade active ingredients were used in the bioassays, and the procedures followed those of Shearer and Riedl (1994). Scenturion delta traps (Suttera, Bend, OR) baited with *C. pomonella* pheromone (1 mg of [E,E]-8,10-dodecadien-1-ol; Suttera) were deployed in orchards and checked on a daily basis. Transportation of captured adults in trap liners (adhesive surface) was as per the procedure described by Krawczyk and Hull (2004). Collected male moths, after remaining for less than 24 h in a trap and attached ventrally to the adhesive surface, were topically treated on their dorsum with 1.0 μl of solution of insecticide dissolved in acetone or acetone alone (control). The insecticides were applied using a repeating microsyringe-mounted dispenser (Hamilton Company, Reno, NV). At least five different concentrations of each insecticide were used for testing adult moth response during each bioassay. Treated moths were left on the adhesive surface at the temperature of 22 ± 1°C. Mortality was observed and recorded as per Dunley and Welter (2000) at 48 h. Moths that did not respond to a gentle touch by a camel brush were considered dead.

In 2008, adult topical bioassays were conducted in the laboratory to determine the susceptibility of the first- and second-generation *C. pomonella* adult males collected from four commercial and four abandoned orchards to various concentrations of azinphos-methyl. Large plastic delta traps (Trece Pherocon VI®, Trécé Inc., Adair, OK) baited with CM L2 lures (3.5 mg of [E,E]-8,10-dodecadien-1-ol in grey halobutyl septum; Trécé Inc.) were placed in both the commercial and abandoned orchards, and *C. pomonella* adults captured in the traps were collected on a daily basis. The trap liners were placed in all traps each evening and after approximately 12–14 h in the orchards, trap liners with captured moths were collected from all orchards and transported to the laboratory for bioassays (Krawczyk and Hull 2004). Bioassays for the first- and second-generation adults were conducted 9–16 June and 21–28 July, respectively. In both generation bioassays, 1 μl of technical grade azinphos-methyl (Bayer CropScience) dissolved in acetone was applied topically to the dorsum of each adult male. The 500-ppm stock solution was prepared by dissolving the technical grade azinphos-methyl in acetone, and consecutive concentrations were obtained by serial dilutions of the stock solution. The 500-ppm concentration was determined during preliminary studies as the lowest concentration tested causing a 100% mortality. Concentrations included in the bioassay were 500, 250, 125, 62.5, 31.2, and 15.6 ppm (Figs.1, 2) and were applied using a repeating microsyringe-mounted dispenser (Hamilton

Company). Acetone alone was used as the control, and observations of mortality were recorded after 24, 48, and 72 h (Dunley and Welter 2001). During all laboratory bioassays, temperature (~21–23°C), relative humidity (~70 %), and photoperiod (light [11 h] and dark [10 h] with approximately 3 h of dim light) were maintained in the laboratory.

Larval baseline bioassays. Neonates of *C. pomonella* from a laboratory colony were used for the larval bioassays using a lima bean (Phaseolus lunatus L.) diet (Shorey and Hale 1965). Formulated insecticides were diluted six to eight concentrations. A 0.5-ml droplet of the appropriate insecticide concentration was applied to the surface of the lima bean diet in a 28-ml plastic diet cup. The following insecticides were evaluated: acetamiprid (UPI, King of Prussia, PA), azinphos-methyl (Bayer CropScience), fenpropathrin (Valent U.S.A. LLC, Walnut Creek, CA), methoxyfenozide (Dow AgroSciences LLC, Indianapolis, IN), novaluron (Makhteshim Agan of North America, Inc., New York, NY), rynaxypyr (DuPont Crop Protection) and spinosad (Dow AgroSciences LLC). Five neonates were transferred to each cup containing the treated diet, and larval mortality was assessed 1, 4, 5, and/or 7 d later. These bioassays were conducted 21–22°C and approximately 70% relative humidity.

Statistical analysis. Mortality data from the 2001–2005 adult and larval bioassays were subjected to probit analysis using POLO PLUS (LeOra Software 2003) to provide estimates of the slopes of the probit regression, the median lethal concentration (LC_{50}), and the 90% lethal concentration (LC_{90}). Adult mortality data from the 2008 bioassays were initially subjected to probit analysis using POLO PLUS (LeOra Software 2003). However, due to higher heterogeneity and limited sample size, the POLO probit analysis could not establish the LC_{50} and LC_{90} values for the responses in this study. Therefore, the mortality data from 2008 bioassays were subjected to an analysis of covariance (ANACOVA) (Zar 1999). Mortality data (from commercial and abandoned orchards) were compared with each other at different concentrations of azinphos-methyl by determining the linear relationship of percentage of mortality and time after exposure. The slope and intercept of the linear relationships at each concentration between abandoned and commercial orchards were analyzed and compared using ANACOVA (Zar 1999). R software was used to perform this analysis, while the graphs were generated in SigmaPlot® 11 (Systat Software, Inc., Chicago, IL).

Results

Adult bioassays. The concentration-mortality responses of *C. pomonella* adults collected from various Pennsylvania apple orchards in 2001–2005 to azinphos-methyl ranged from an LC_{50} of 16.5 ppm (7.3–26.1, 95% fiducial limits) to an LC_{50} of 225.2 ppm (65.7–567.2, 95% fiducial limits) (Table 1). The greatest differences between the least sensitive (e.g., higher LC_{50}) and the most sensitive (e.g., lowest LC_{50}) adult populations to azinphos-methyl were detected in 2001 (12.8-fold difference) and 2005 (13.6-fold difference) (Table 1). The concentration-mortality responses to methomyl ranged from an LC_{50} of 17.5 ppm (5.5–28.9, 95% fiducial limits) to an LC_{50} of 96.1 ppm (63.8–130.7, 95% fiducial limits) (Table 2). Differences between the least and most sensitive populations varied from 3.4-fold in 2005 to 5.49-fold in 2001.

Table 1. Concentration-mortality responses of codling moth male adults to azinphos-methyl laboratory topical bioassays. Codling moth populations were collected from various commercial and abandoned apple orchards in Pennsylvania.

Year	Population*	*n*	Slope	LC_{50} (ppm) (FL at 0.95)	LC_{90} (ppm) (FL at 0.95)
2001	L	144	1.3 ± 0.4	16.8 (5.1–28.9)	157.8 (81.3–955.1)
	H	397	3.5 ± 0.5	215.7 (147.7–277.7)	503.8 (381.8 –842.8)
2002	L	108	2.6 ± 0.7	16.9 (7.7–25.0)	53.0 (36.1 -112.3)
	H	207	3.2 ± 0.5	108.5 (84.3-135.0)	275.9 (210.3 -431.2)
2003	L	131	2.2 ± 0.5	32.4 (15.9–49.1)	127.4 (81.1–320.2)
	H	156	2.9 ± 0.5	37.6 (27.7–49.6)	101.9 (73.2–179.3)
2005	L	83	2.1 ± 0.6	16.5 (7.3–26.1)	66.4 (40.0–233.5)
	H	137	1.1 ± 0.3	225.2 (65.7–567.2)	2,980.2 (995–96,621)

* LC_{50} denotes the 50% lethal concentration; LC_{90}, 90% lethal concentration; L, the codling moth population with the lowest LC_{50} value during a given season; H,the population with the highest LC_{50} value; FL, fiducial limits.

In the 2008 bioassays, *C. pomonella* male moths collected from the abandoned orchard system during the first generation were more susceptible to various concentrations of azinphos-methyl at 24, 48, and 72 h after exposure than the populations collected from commercial orchards (Fig. 1B–I). However, the populations from these two orchard systems were not significantly different at the highest dose (500 ppm [$F = 4.300$; df = 49; $P = 0.130$]) of azinphos-methyl (Fig. 1J). Percentage of mortality at 0 ppm was different in these two populations, which could be due to physiological and developmental differences. During the second-generation flight, *C. pomonella* from the abandoned orchard system were significantly more susceptible to all concentrations of azinphos-methyl (Fig. 2B–F).

Larval baseline bioassays. Baseline sensitivity of *C. pomonella* larvae to tested insecticides varied with the product tested (Table 3). In our observations, each tested insecticidal compound exhibited greater toxicity (lower LC_{50} and LC_{90} values) over time after the initial assessment, except for fenpropathrin (Table 3). For azinphos-methyl, despite the threefold differences (at the LC_{50} level) observed during 1- and 4-d readings, the toxic effect of this compound on neonate larvae of *C. pomonella* was very rapid. Based on the overlapping confidence limits, no differences were observed at 1- and 7-d LC_{50} values for acetamiprid, suggesting rapid kill for this compound (Table 3).

Discussion

In this study, *C. pomonella* populations collected from commercial and abandoned Pennsylvania apple orchards showed different sensitivities to various

Table 2. Concentration-mortality responses of codling moth male adults to methomyl in laboratory topical bioassays. Codling moth populations were collected from various commercial and abandoned apple orchards in Pennsylvania.

Year	Population*	*n*	Slope	LC_{50} (ppm) (FL at 0.95)	LC_{90} (ppm) (FL at 0.95)
2001	L	122	2.1 ± 0.7	17.5 (5.5–28.9)	71.7 (41.1–338.1)
	H	152	2.4 ± 0.6	96.1 (63.8–130.7)	324.9 (216.1–811.6)
2003	L	112	3.7 ± 0.9	21.2 (12.7–27.8)	46.9 (35.7–80.8)
	H	179	2.2 ± 0.4	85.1 (55.8–115.1)	333.1 (233.4–612.5)
2005	L	165	1.8 ± 0.4	20.9 (11.9–30.2)	108.5 (69.6–250.4)
	H	121	2.2 ± 0.6	72.1 (32.5–111.3)	274.9 (165.6–1174)

* LC_{50} denotes the 50% lethal concentration; LC_{90}, 90% lethal concentration; L, the codling moth population with the lowest LC_{50} value during a given season; H,the population with the highest LC_{50} value; FL, fiducial limits.

insecticides used in apple orchard pest management. Apple production and pest management programs in the United States have changed significantly during the last 30 to 40 yr. The intensive applications of insecticides sprayed on fruit orchards have caused some *C. pomonella* populations to develop resistance to a number of insecticides (Bush et al. 1993, Dunley and Welter 2000, Knight et al. 1994, Mota-Sanchez et al. 2008, Riedl et al. 1986, Varela et al. 1993). In orchards, resistant genotypes of *C. pomonella* are selected and maintained due to the continuous and heavy use of pesticides with similar modes of action (McKenzie and Batterham 1994). Due to the frequent applications of pesticides, belonging mainly to the organophosphate class (e.g., azinphos-methyl, diazinon, parathion, phosmet, etc.), *C. pomonella* has developed resistance to many of these products in different regions (Chapman and Barrett 1997; Fuentes-Contreras et al. 2007; Knight 2010; Reuveny and Cohen 2004; Reyes et al. 2007, 2009; Voudouris et al. 2011; Yang and Zhang 2015) and has emerged again as a serious pest of apples in the last few decades.

In general, *C. pomonella* populations were found less sensitive to azinphos-methyl (a predominant *C. pomonella* insecticide used in apple orchards for several decades) than methomyl insecticide in this study. In similar studies conducted in the western United States, Varela et al. (1993) observed differences in the field efficacy of insecticides when the differences between populations at the LC_{50} level were no greater than two- to threefold. The results of adult azinphos-methyl bioassays in our study showed conspicuous patterns of a higher percentage of mortality (i.e., more insecticide susceptibility) for male *C. pomonella* collected from abandoned than from commercial orchards for both first- and second-generation flights. These results indicate that *C. pomonella* populations in some Pennsylvania commercial apple orchards have developed some level of resistance to azinphos-methyl. In previous studies, Krawczyk and Hull (2005) reported resistance by *C. pomonella* to

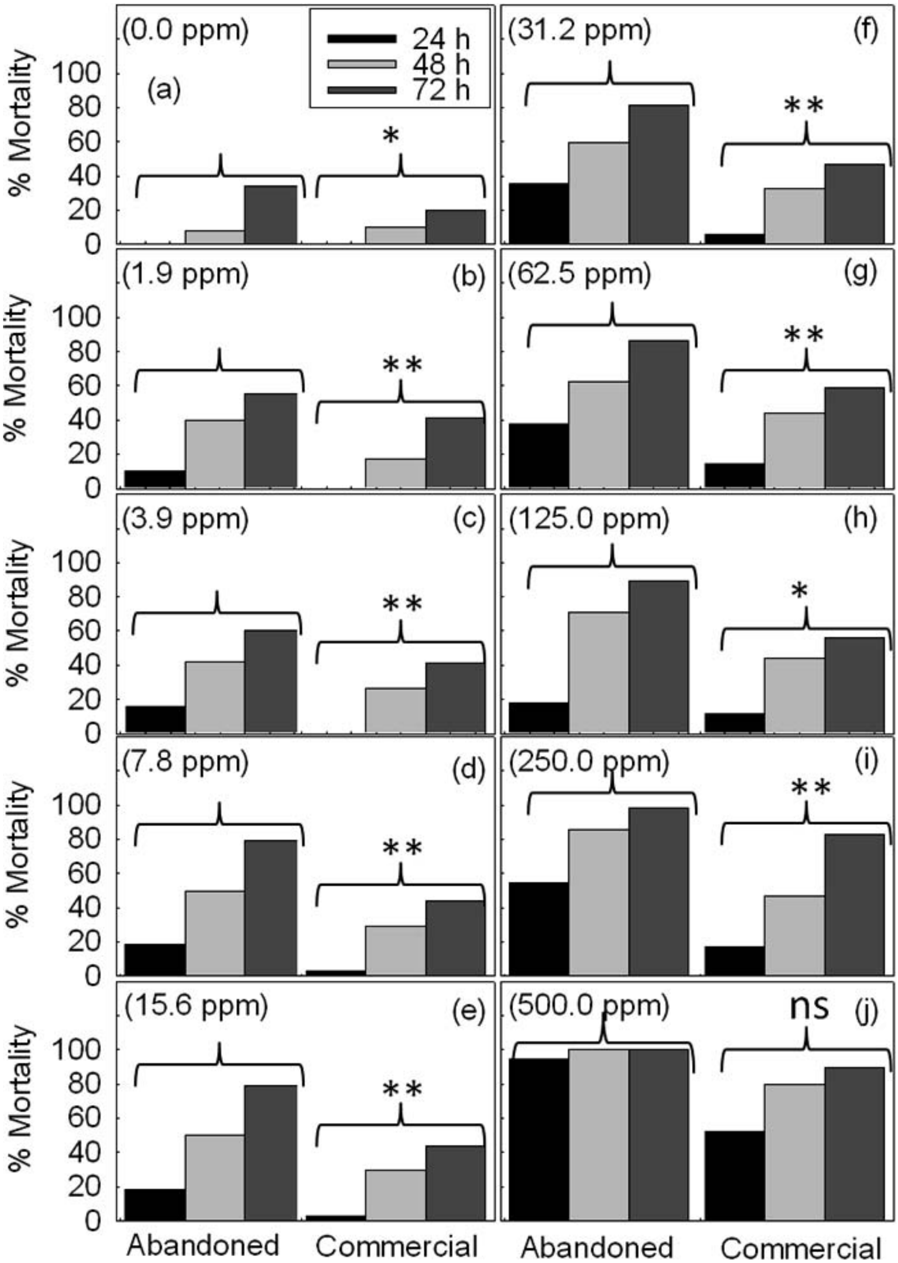

Fig. 1. Percentage of mortality of codling moth first-generation adult males to concentrations of azinphos-methyl in laboratory assays. Codling moth populations were collected from three abandoned and three commercial apple orchards in Pennsylvania during 2008. * represents $P < 0.05$, and ** represents $P < 0.01$.

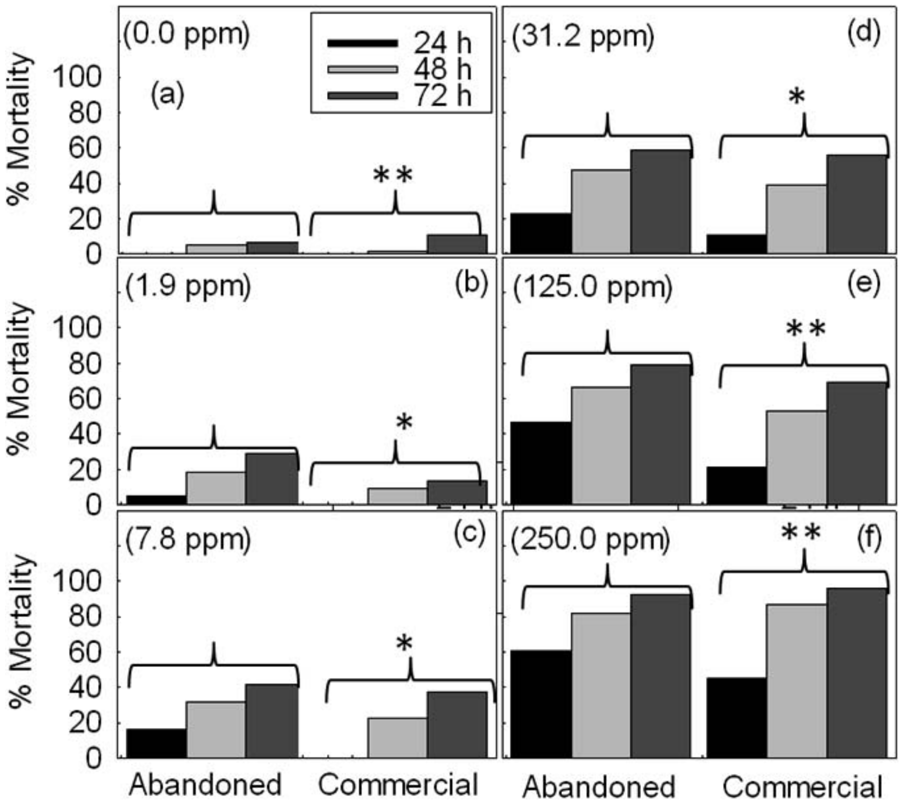

Fig. 2. Percentage of mortality of codling moth second-generation adult males to concentrations of azinphosmethyl in laboratory assays. Codling moth populations were collected from three abandoned and three commercial apple orchards in Pennsylvania in 2008. * represents $P < 0.05$, and ** represents $P < 0.01$.

various insecticides (e.g., azinphosmethyl, methomyl, phosmet) used over the past 10 to 15 yr in apple orchards in Pennsylvania. Such differences in the toxicity responses of these two populations could be a factor causing other phenological differences among *C. pomonella* populations in this region (Joshi et al. 2016).

In this study, results of larval bioassays suggest higher sensitivity of *C. pomonella* larvae to acetamiprid treatment. Similarly, azinphos-methyl treatment had a very rapid toxic effect on the neonates compared to insecticides such as methoxyfenozide and novaluron. The differences in the LC_{50} and LC_{90} levels between 1 d and later mortality readings are very important from a practical perspective of managing internal-feeding pests. In the normal orchard environment, the newly hatched *C. pomonella* larva will generally feed on the surface of fruit for less than 24 h before entering the fruit, and to prevent a larva from causing fruit injury, insecticides have to kill the larva before it enters the fruit. Even partial feeding expressed as “stings” can decrease the market value of fruits. Our bioassays

Table 3. Baseline sensitivity codling moth neonates to various insecticides as determined in laboratory diet-surface topical bioassay. Larval mortality was determined at 1, 4, 5, and/or 7 d after placement on the treated diet. Bioassays were conducted using various codling moth populations reared at least for one generation in the laboratory on thinned apple fruits.

Insecticide	Mortality at Day to Day:	*n*	Slope	LC_{50} (ppm) (FL at 0.95)	LC_{90} (ppm) (FL at 0.95)
Acetamiprid	1	299	1.5 ± 0.3	1.1 (0.5–0.6)	7.6 (5.3–13.9)
	7	264	1.9 ± 0.4	1.0 (0.4–1.6)	4.7 (3.3–8.7)
Azinphos-methyl	1	400	2.4 ± 0.3	15.4 (11.9–19.1)	52.4 (39.9–78.4)
	4	300	3.2 ± 0.5	4.9 (3.9–6.0)	12.4 (9.9–17.7)
Fenpropathrin	1	300	1.2 ± 0.3	0.1 (0.01–0.23)	1.08 (0.68–2.02)
Methoxyfenozide	1	450	0.8 ± 0.2	132.9 (61.6–652.8)	4,690 (861–213,408)
	4	450	1.9 ± 0.2	4.2 (3.2–5.2)	19.7 (14.6–29.7)
Novaluron	4	700	1.5 ± 0.4	353.0 (234.0–836.0)	2,553.0 (1,000–30,852)
	7	700	0.7 ± 0.1	0.6 (0.2–1.2)	49.6 (21.5–168.2)
Rynaxypyr	1	400	1.6 ± 0.2	0.8 (0.5–1.0)	4.9 (3.7–7.7)
	5	250	3.1 ± 0.6	0.4 (0.3–0.5)	1.1 (0.9–1.5)
Spinosad	1	240	1.1 ± 0.4	9.9 (4.0–546.2)	132.8 (20.0–1,521,655)
	4	240	2.6 ± 0.4	1.0 (0.7–1.3)	3.1 (2.2–5.6)

* LC_{50} denotes the 50% lethal concentration; LC_{90}, 90% lethal concentration; FL, fiducial limits.

suggest that only azinphos-methyl and acetamiprid were able to rapidly kill the larvae after 1 d of feeding. Other insecticides, e.g., methoxyfenozide, novaluron, and spinosad, were very active against neonate *C. pomonella* at extended mortality readings; however, their LC_{50} and LC_{90} values at 1 d (or 4 d for novaluron) were much higher than at the 4-d (or 7-d for novaluron) assessment. In addition, for the newer chemistries, such as rynaxypyr, the 1-d LC_{50} and LC_{90} values were higher than LC values at 5 d. New insecticidal products such as rynaxypyr, spinosad, and neonicotinoids have become available to the tree fruit industry and have shown promise as alternatives to organophosphates, carbamates, and pyrethroids. Some of these compounds, such as spinosad (which primarily acts by targeting binding sites of nicotinic acetylcholine receptors), provides excellent control of leafrollers and leafminers, while neonicotinoids or chloronicotinyls (imidacloprid, thiamethoxam) are primarily active against leafhoppers, aphids, and leafminers. However, other compounds from this group appear to have a broader range of activity, with potential efficacy against some lepidopteran pests (acetamiprid, thiacloprid, or clothianidin). Our findings on *C. pomonella* resistance to insecticides would be helpful in further understanding of insecticide resistance in *C. pomonella* local populations in Pennsylvania and possibly other regions where *C. pomonella* is a major pest of fruit crops.

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