Assessing Azinphos-methyl Resistance in New York State Codling Moth Populations

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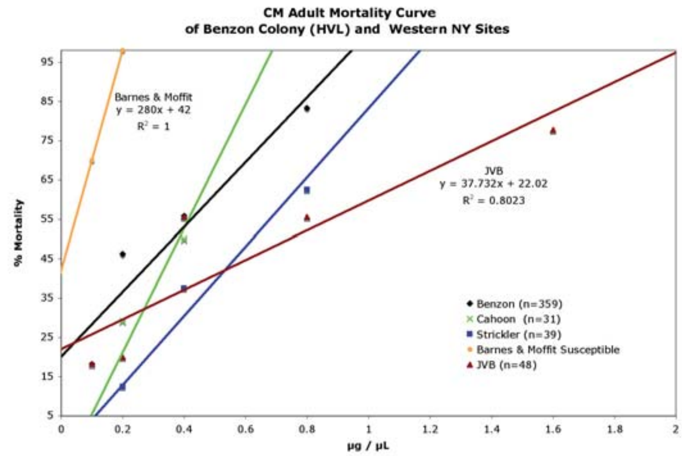

Figure 1. Effect of Guthion 50W (azinphos-methyl) on mortality of adult codling populations trapped from Western NY orchards, a laboratory strain (Benzon) and study group baseline (Barnes/Moffit).

The codling moth (CM) *Cydia pomonella* (Linnaeus), a European native, is a principal insect pest of pome fruit throughout much of the world. A member of the lepidopteran family Tortricidae, it is a bivoltine moth, having two generations in most of the U.S. including New York State. A partial third generation exists in the Pacific Northwest. Introduced to the New World during the earliest years of pome fruit production the codling moth has historically been a very difficult insect to control. It was not until the development of the synthetic insecticides, broad-spectrum materials with extended residual, contact and feeding efficacy, that economic damage imposed by this insect was, for a period, curtailed.

> **"Codling moth populations throughout the Eastern tree fruit-producing region have become increasingly tolerant to insect pest management practices. We have observed in this study greater tolerance to azinphos-methyl in adult codling moth populations trapped in commercial processing blocks when compared to Eastern NY commercial fresh market orchards."**

The larvae overwinter in cocoons on the trunk and limbs, emerging as adults during the tight cluster through bloom period of apple. Shortly after mating, the female deposits her eggs onto foliage and fruit, giving rise to larva that cause significant feeding damage to the surface, flesh, core and seed of the fruit if unmanaged. A mechanism of survival contributing to the codling moth persistent success are the well-developed feeding habits of the larva. Larva emerging from eggs laid onto the fruit often begins burrowing through the egg covering, directly into the fruit, with little to no surface activity. To evade toxins present on the surface or skin of the fruit, the newly emerged larva will chew and excrete the skin prior to entry, only then proceeding into the fruit to feed. This tactic of fruit skin disposal reduces the amount of toxin the larva consumes while feeding.

Emergence of the CM first generation larva coincides with the immigration of adult plum curculio (PC) *Conotrachelus nenuphar* (Herbst) into commercial orchards. For more than 50 years, the CM has been effectively managed through the use of the organophosphate class of insecticides against the PC. Later in the season the second generation of CM larval emergence overlaps, at least in part, with other principle fruit pests, including the obliquebanded leafroller (OBLR) *Choristoneura rosaceana* (Harris), apple maggot *Rhagoletis pomonella* (Walsh), oriental fruit moth, *Grapholita molesta*, and the lesser apple worm *Grapholita Prunivora* Walsh. Management of these insects provides fair to excellent levels of CM control depending on a number of management and biotic factors. Recent restrictions placed on the organophosphate class of insecticides, such as worker re-entry intervals, lengthened days to harvest and limits on total applications per season, have substantially reduced late season OP use. This shift in insecticide use may require the control of these insects through the use of multiple and more species-specific classes of insecticides, and incorporation of mating disruption to achieve comparable control yet increasing the cost of pome fruit production.

Insects with multiple generations, which maintain a constant presence in commercial orchards, are exposed to the selection pressure insect pest management tool impose. This constant exposure provides the mechanism for insecticide resistance development. Over the last two decades codling moth populations throughout North America have become increasingly tolerant of pest management practices, with increasing levels of insecticide resistance to the organophosphate class of chemistries used in pome fruit pest management. Insecticide resistance to azinphos-methyl (Guthion) has been well documented in codling moth populations throughout the western Unites States fruit growing regions for the past 20 years (Varela, et al., 1993; Reidhl et al., 1986, Steenwyck and Welter, 1998). Ohio, Michigan and Pennsylvania have seen increasingly high internal lepidopteran damage levels in harvested fruit. Over the past nine years western New York processing blocks of apple have seen increasing numbers of damaged fruit due to internal worm infestations. Infested fruit sampled from these orchards contained larvae from a complex of internal worm, consisting of the codling moth, the oriental fruit moth and the lesser apple worm. In 2002 infestations were found to contain predominately oriental fruit moth larvae, yet in 2005, a year in which 100 loads from 60 farms were ticketed

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from processing centers by USDA inspectors, the predominate species was and continues to be the codling moth.

Given the dramatic increase in the presence of the internal worm complex in processing fruit, we were hard-pressed to ask to the question; 'Has there been an increase in tolerance or resistance of the internal worm population in western NY' or is this simply related to a shift in specific practices used in managing processing apple blocks? Historically, insecticide resistance development in CM populations has been a reoccurring and economically devastating event, and as such, it seemed prudent to investigate state wide CM populations for levels of susceptibility to commonly used insecticides. In collaboration with the Lake Ontario Fruit Team (LOFT) and the Hudson Valley Laboratory, a study was initiated to establish the insecticidal tolerance of azinphos-methyl in regional adult codling moth populations given its seasonal use patterns. A concurrent evaluation was also made of currently used insecticides to determine the efficacy of these materials against susceptible CM adult and 1st instar larva.

To determine insect population levels of resistance to a specific chemical or insecticide class, comparison bioassay studies to both field collected and susceptible populations to the specific insecticide in question are conducted. In a classic case study, shortly after azinphos-methyl was released for use in commercial orchards, Barnes and Moffit (1963) at the University of California-Riverside conducted a resistance study on codling moth populations obtained in commercial and abandoned orchards. They determined that field populations of codling moth had a lethal dose or LD90 level of 0.158 μg/μl of azinphos-methyl, considered to be the standard susceptible level of mortality to this insecticide. Helmut Riedl conducted a follow-up study in 1985 to confirm that New York orchards still had susceptible populations of codling moth with adults taken from a site in Geneva, NY exhibiting LD90 levels of 0.350 μg/μl of azinphos-methyl. Although this concentration was two fold higher than the Barnes and Moffit study population, it was not considered to be resistant.

Results: Adult Bioassay

In our first series of tests conducted in 2008-09 on adult codling moths, we collected adults from four western NY processing orchards in Williamson and Wolcott and five Hudson Valley fresh market orchards in Marlboro, Milton, Highland, Altamont, Burnt Hills to compare levels of susceptibility to azinphos-methyl. Approximately 20-100 CM traps were set at each site to collect adults from the 1st generation flight. Moths flying between dawn and dusk were captured on pheromone trap cards and returned to the laboratory within 24 hours. Adults were then treated using 1 μl (micro liter) droplets of laboratory grade acetone, applied to our control population, and serial dilutions of 98.9% concentration of azinphos-methyl (Pestanal; Sigma-Aldrich, Atlanta, GA), dissolved in acetone to achieve rates of 0.1, 0.2, 0.4, 0.8, 1.6 part per million concentrations, equivalent to μg/μl (microgram per micro liter) doses. Applications of these concentrations were made to the dorsal thoratic plate of the moth with mortality assessed using two evaluations at 24 and 48 hours after treatment. Moths were scored as live or dead based on antenna-stimulated and or probed movement response of the adult. In order to establish differences in levels of insecticide tolerance, mortality

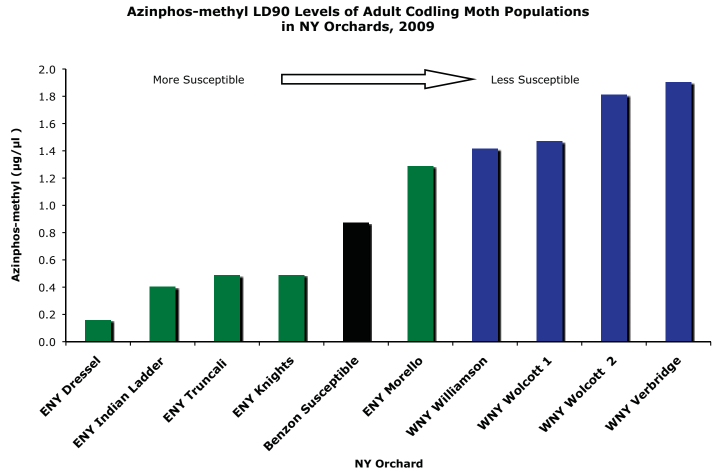


Figure 2. The concentration of Guthion 50W (azinphos-methyl) required to produce morality in 90% of the adult codling population trapped from Eastern (ENY) and Western (WNY) orchards.

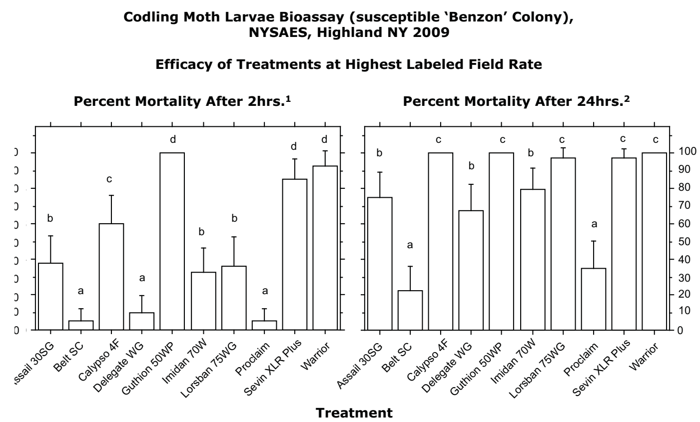


Figure 3. Percent mortality of 1st instar laboratory reared susceptible codling moth larva topically exposed to highest labeled field rate insecticides.

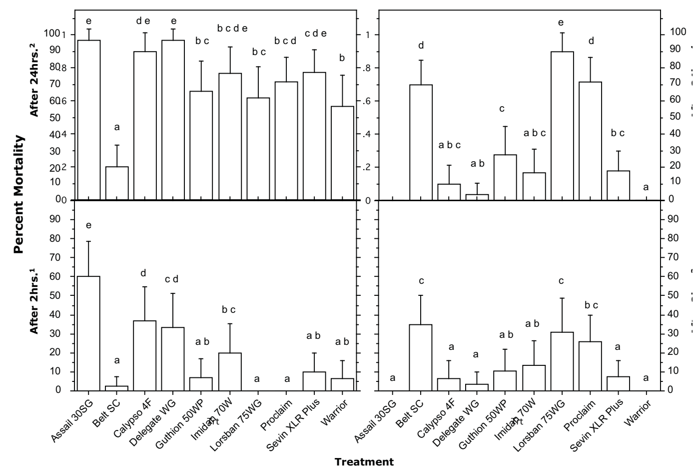


Figure 4. Percent mortality of 1st instar laboratory reared susceptible codling moth larva exposed to apple4residue using the highest labeled field rate insecticide, and the percent of apples to which CM larva had burrowed after 2 and 24 hours.

curves were used to determine degrees of CM susceptibility to azinphos-methyl (Figure 1).

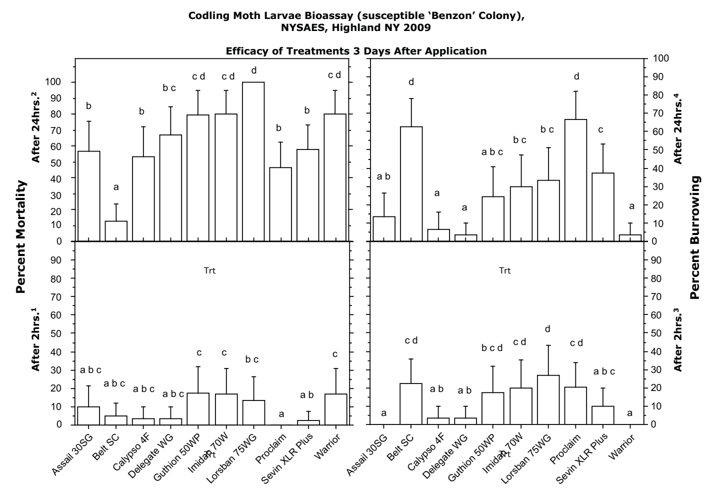
From the data generated in this portion of the study we observed lower levels of susceptibility in all study groups compared to the previous studies mentioned. Levels of reduced susceptibility were evident in western NY orchards, primarily in Verbridge and Williamson (Figure 2). Laboratory reared populations ('Benzon susceptible strain'), and populations collected from most eastern NY orchards exhibited an LD90 of ≤ 0.85 µg/µl for azinphos-methyl with the exception of the 'Morello' orchard. The western NY strains in Verbridge displayed an LD90 of 1.86 µg/µl for azinphos-methl, approximately 2.2 fold less susceptible than the Benzon strain. Given the findings that all NY strains demonstrated a higher tolerance to azinphos-methyl compared to that of the Riedl and Barnes/Moffit studies implies reduced susceptibility levels across the state, with lower levels of susceptibility in WNY processing orchards. The overall low levels of azinphos-methyl susceptibility of NYS codling moth populations coupled with reduced levels of susceptibility in these processing blocks, may have contributed, at least in part, to insecticide failure resulting in infested loads observed during the past seven years. Further evaluations in 2010 will help determine the degree to which these populations are comprised of tolerant or resistant population levels of susceptibility.

Results: CM Larval Studies of Insecticide Contact Activity

Additional studies were conducted using topical bioassays on susceptible strains of larva using conventional and new insecticide chemistries. 'Benzon' 1st instar larva were evaluated for susceptibility to 10 commercial insecticides, in which larva were placed onto moistened filter paper affixed to the lid of 25 ml plastic cups. Applications to the larvae were then made using the highest labeled field rate for each product using a micro-applicator and 1 ml syringe employing 1 µl applications. Larvae were rated for mortality and evaluated at 2 and 24-hour intervals. Larva exposed to Guthion 50WP (azinphos-methyl) exhibited 100% mortality at 2 and 24-hour evaluation intervals, statistically equivalent to Warrior (lambda-cyhalothrin), Sevin XLR (carbaryl) and Calypso (thiacloprid), which provided 92.5% & 100%, 85.0% & 97.5 % and 60.0% & 100.0% mortality levels respectively (Figure 3).

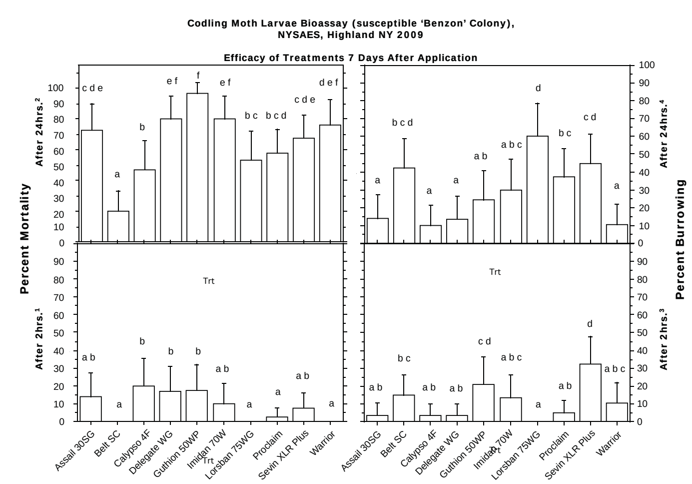
Results: CM Larval Studies of Insecticide Residual and Feeding Activity

Residue and feeding studies to 'Benzon' 1st instar larvae were conducted to evaluate 10 commercial insecticides on susceptible strains of larva. Treated 'Delicious' apple were submersed in a dilute insecticide solution using the highest labeled field rate of each insecticide for three seconds and held indoors at 70°F, offering no exposure to rain or sunlight for 1, 3, 7, 14 and 21-day periods. Ten (10) 2.5 cm apple discs were removed from two treated fruit and secured onto the lid of plastic cups, onto which were placed CM larvae. The larvae were evaluated for mortality and feeding damage 2 and 24 hour intervals (Figures 4-8). Results demonstrated that larva exposed to azinphos-methyl treated apple exhibited lower levels of mortality at both the 2 hr and 24 hour evaluation intervals compared to most other insecticides. Assail 30SG (Acetamiprid), a recently developed neonicotinoid insecticide, had significantly higher levels of fruit residual activity against 1st instar larva than other insecticides after 2 hours but not statistically different from Calypso 4F, Delegate WG, Imidan 70WP or Sevin XLR after 24 hours. The least amount of feeding after 24 hours was observed in the Assail 30SG, Calypso 4F, Delegate WG, Imidan 70WP, Sevin XLR and Warrior treatments. However, after 3 days, the organophosphates Guthion 50WP, Imidan 70WP, Lorsban 75WG and the pyrethroid Warrior demonstrated increased residual activity with the least amount of feeding observed in the Assail 30SG, Calypso 4F, Delegate WG, Guthion 50WP, and Warrior treatments. After seven days, Delegate WG, Guthion 50WP, Imidan 70WP, and Warrior demonstrated increased residual activity with the least amount



Bioassay conducted on 1st instar codling moth larva transferred to a 2.4 cm diameter red delicious apple disk dipped in a treatment solution and placed in chambers at 70ºF. (Superscript 1.[df = 3, F-value = 1.693, P-value = 0.0896]; 2.[df = 3, F-value = 10.561, P-value = 0.0001]; 3.[df = 3, F-value = 3.328, P-value = 0.0007]; 4.[df = 3, F-value = 9.874, P-value = 0.0001]).

Figure 5. Percent mortality of 1st instar laboratory reared susceptible codling moth larva exposed to apple residue using the highest labeled field rate insecticide, and the percent of apples to which CM larva had burrowed after 2 and 24 hours.



Bioassay conducted on 1st instar codling moth larva transferred to a 2.4 cm diameter red delicious apple disk dipped in a treatment solution and placed in chambers at 70ºF. (Superscript 1.[df = 3, F-value = 2.592, P-value = 0.0068]; 2.[df = 3, F-value = 7.176, P-value = 0.0001]; 3.[df = 3, F-value = 3.716, P-value = 0.0002]; 4.[df = 3, F-value = 5.134, P-value = 0.0001]).

Figure 6. Results of a 2 and 24 hour evaluation of 1st instar, laboratory reared susceptible codling moth larva showing percent mortality of larvae exposed to apple residue using the highest labeled field rate insecticide after 7 days, and the percent of apples to which CM larva had burrowed.

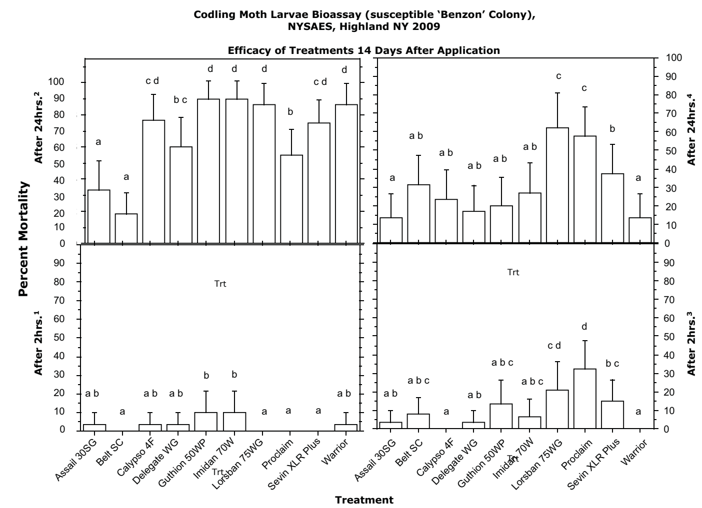
of feeding observed in the Assail 30SG, Calypso 4F, Delegate WG, Guthion 50WP, and Warrior treatments. After 21 days, the highest level of residual efficacy was observed with Assail 30SG, Lorsban 75WG and Proclaim with the least amount of feeding observed in the Delegate WG treatment, followed by Assail 30SG, Proclaim, Sevin XLR, Warrior and Calypso 4F treatments.

Discussion

In this study we've observed a 2.2 fold greater tolerance to azinphos-methyl in adult codling moth populations trapped in commercial processing blocks when compared to the majority of Eastern NY commercial fresh market orchards and a susceptible control group of adult codling moths. Yet, a number of factors may contribute to the failure of azinpho-methyl to manage the internal worm complex in other parts of the state. These also include the loss of insecticides used successively in the past to manage this insect. One in particular, Lorsban 75WG, a very effective material when used at petal fall, is no longer available for use as a foliar insecticide beyond the pre-bloom stage of apple, thus removing it from use against the 1st generation flight of codling moth. Lorsban has been found by researchers to have excellent efficacy against the adult CM, and demonstrates a negative cross-resistance to azinphos-methyl, allowing it to maintain effectiveness even when resistance to Guthion fails to control CM (Steenwyck and Welter, 1998). The movement of azinphos-methyl resistant CM individuals from western states such as Washingtom State and California, to the east by way of storage bin transport, has also been implicated as the cause of resistant population build-up, especially in and around processing centers. Another facet of discussion regarding the loss of CM control includes a possible shift in the emergence pattern of codling moth later into the season for both generations (Boivin, 1999). Pheromone trapping peaks, often called the 'B Peaks,' imply adult emergence delays, and have been observed in trap graphs of Western and Eastern orchards. A delay in adult and subsequent larval emergence would prolong the presence of newly hatching larva beyond prediction models and insecticide residual activity, allowing for increasing levels of damage.

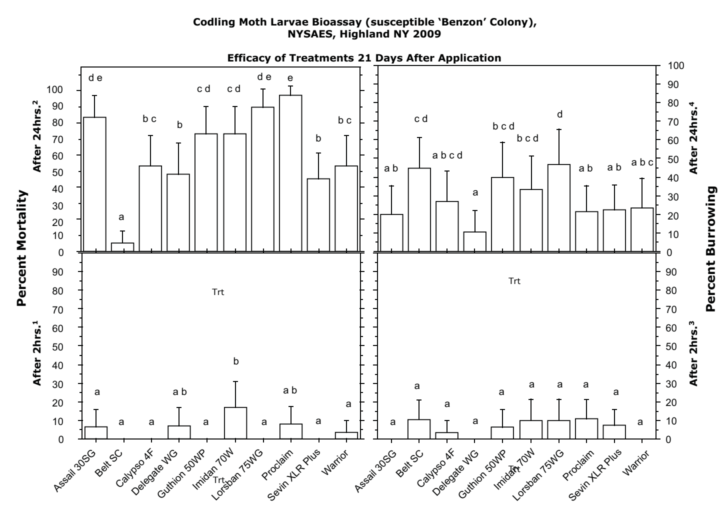
Another important factor in New York appears to be the differences in fresh market and processing production methods between regions with regards to application techniques. In general, apples grown in Western NY sites for processing are produced in larger acreages often bordering contiguous orchards of neighboring processing blocks. Large blocks have a tendency to 'promote' endemic populations. These orchards have for many years exclusively used azinphos-methyl in season long management programs. In processing blocks of apple, fruit can be acceptably sold at reduced visual standards when compared to retail fresh fruit market standards with regards to color, size and overall appearance. In some orchards this may have led to reductions in insecticide rates, alternate row application methods, stretched intervals, reductions in horticultural practices such as summer pruning, all of which hinder effective insecticide coverage and subsequent insect control. These practices promote greater insect survival, higher levels of selection pressure with regards to insecticide resistance development and ultimately less susceptible strains of insects as we have observed in this study.

Comparatively, apples grown in the Eastern part of the state are primarily produced for fresh market or pick-your-own sales, produced in smaller acreages often isolated from other orchards, surrounded by woodlands and or abandoned orchards. In most Hudson Valley orchards, the presence of these woodlots or abandoned orchards lead to higher insect pressure, increasing insecticide rate requirements. However, the pest complex migrating in from 'the edge' typically has greater levels of genetic diversity leading to greater insecticide susceptibility. Fruit quality, size and color requirements for fresh market demands methodical management including intensive crop load reductions, as sales depend on visually appealing large fruit. Intensive crop load management in fresh market fruit fosters king fruit to set



Bioassay conducted on 1st instar codling moth larva transferred to a 2.4 cm diameter red delicious apple disk dipped in a treatment solution and placed in chambers at 70ºF. (Superscript 1.[df = 3, F-value = 1.497, P-value = 0.1480]; 2.[df = 3, F-value = 11.694, P-value = 0.0001]; 3.[df = 3, F-value = 4.007, P-value = 0.0001]; 4.[df = 3, F-value = 4.570, P-value = 0.0001]).

Figure 7. Results of a 2 and 24 hour evaluation of 1st instar, laboratory reared susceptible codling moth larva showing percent mortality of larvae exposed to apple residue using the highest labeled field rate insecticide after 14 days, and the percent of apples to which CM larva had burrowed.



Bioassay conducted on 1st instar codling moth larva transferred to a 2.4 cm diameter red delicious apple disk dipped in a treatment solution and placed in chambers at 70ºF. (Superscript 1.[df = 3, F-value = 2.516, P-value = 0.0086]; 2.[df = 3, F-value = 14.843, P-value = 0.0001]; 3.[df = 3, F-value = 1.711, P-value = 0.0857]; 4.[df = 3, F-value = 2.242, P-value = 0.0194]).

Figure 8. Results of a 2 and 24 hour evaluation of 1st instar, laboratory reared susceptible codling moth larva showing percent mortality of larvae exposed to apple residue using the highest labeled field rate insecticide after 21 days, and the percent of apples to which CM larva had burrowed.

and remain, while eliciting clustered fruit to drop, significantly reduces insect harborage that would typically occur in and around the fruit cluster, offering insects less protection from insecticide applications and residue.

Conclusions

This study has demonstrated new insecticide chemistries to exhibit effective control of the early instar stages of the codling moth larva with regards to both mortality and feeding inhibition. Through the rotation of these insecticides, a different class of pest management tools can target each generation. This will effectively reduce the selection pressure exerted by any one active ingredient or insecticide class, thus reducing the resistance potential and prolonging the efficacy of these new materials for years to come.

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