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IDENTIFICATION & CHARACTERIZATION OF KEY INSECTICIDE PERFORMANCE MECHANISMS FOR THE CONTROL OF PLUM CURCULIO (*CONOTRACHELUS NENUPHAR*) IN MICHIGAN TART CHERRIES

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IDENTIFICATION & CHARACTERIZATION OF KEY INSECTICIDE PERFORMANCE MECHANISMS FOR THE CONTROL OF PLUM CURCULIO (*CONOTRACHELUS NENUPHAR*) IN MICHIGAN TART CHERRIES

By

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ABSTRACT

IDENTIFICATION & CHARACTERIZATION OF KEY INSECTICIDE PERFORMANCE MECHANISMS FOR THE CONTROL OF PLUM CURCULIO (*CONOTRACHELUS NENUPHAR*) IN MICHIGAN TART CHERRIES

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Plum curculio (*Conotrachelus nenuphar* Herbst) is a major pest of cultivated tree fruits in Eastern North America. This beetle lays eggs inside of the fruit prior to harvest, and larvae eat the flesh of the fruit prior to pupation in the soil. There is a zero-tolerance regulation in place for live larvae in processed cherries; if a single larva is detected at inspection, a grower may not submit any of that harvest for processing. This quality mandate has been met for over 50 years with the use of organophosphate insecticides (azinphos-methyl and phosmet) targeted at plum curculio adults before and during the egg-laying period. Azinphos-methyl is losing its registration in cherries in 2012, and it is vital to identify and optimize new pest management practices in order for the processed cherry industry to continue to meet the stringent market demands.

Several new classes of insecticides are registered for pest management in tree fruits. These compounds do not share the acute contact activity of the organophosphates, and it is inappropriate to insert them directly into a conventional organophosphate-based plum curculio management program. This research utilized the plant-insect-chemistry triad (PIC-Triad) to a) Identify key performance characteristics of insecticides that contribute to fruit protection b) Describe the temporal dimensions of these activities and c) explore the possibility of targeting alternative life stages. This process integrated

novel laboratory bioassays, field based bioassays and insecticide residue data to predict and evaluate the potential of insecticides for use as plum curculio control agents.

Despite being inside the fruit, egg and larval plum curculio were susceptible to insecticide poisoning. The current mainstay, azinphos-methyl was a potent ovicide and larvicide, both in the laboratory and as a "curative" agent in the field. This activity has not been reported previously, and has almost certainly contributed to past effectiveness of this material. The neonicotinoids acetamiprid, thiamethoxam and thiacloprid were also potent curative agents. The insect growth regulator pyriproxyfen was a poor curative agent, and actually increased the risk of larvae in the cherries at harvest. The pyrethroid esfenvalerate has high toxicity to all life stages, but does not penetrate cherry fruit tissue sufficiently to have curative action in the field.

In field based bioassays with adult plum curculio, neonicotinoids reduced fruit damage for up to seven days after application. This fruit protection resulted from lethal action when residues were high, but lower residue levels acted as antifeedents. Azinphos-methyl protected fruit by killing adults for 14 days after application. Indoxacarb had 14 d of active residues, but beetles were able to significantly damage fruit before becoming incapacitated by this slow-acting compound.

Future plum curculio management will rely on optimizing these and other new tools. Researchers cannot rely on laboratory studies of lethal action to predict fruit protection. Antifeedant, oviposition deterrent, and chemosterilant modalities should be evaluated in additional to lethal effects.

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Introduction

MICHIGAN ranks first in the USA for tart cherry production; it accounts for around 70% of the nation's total production and $50 million in annual receipts (Table 1.1). Almost all tart cherries are processed, either frozen or canned, and Michigan is responsible for 85% of the total USA processing capacity. The state also contributes to the national production of sweet cherries; in 2007 MI accounted for $18 million of $584 million in 2007 receipts (USDA-NASS 2005, 2008).

Table 1.1. US Tart Cherry value and production 2002-2007

	Production (Millions of Pounds)		Value (millions of dollars)	
Year	Michigan	Total	Michigan	Total
2002	15	62	7	27
2003	154	226	58	81
2004	149	213	51	71
2005	208	268	47	63
2006	180	250	35	53
2007	193	250	49	65

Source: USDA-NASS 2005, 2008

While cherries are a lucrative specialty crop, this profitability has been dependent on the prophylactic use of broad-spectrum insecticides for the control of key pest species.

The tart cherry industry has legal obligations for supplying infestation-free fruit to post-harvest processors (USDA Agricultural Marketing Service 1941a,b), and both the sweet and tart cherry industries rely on a high quality product to drive their market receipts. This standard has been in place for over a half-century, and remains the driving motivation for pest management in cherries. If any larvae are detected in processor-bound load of cherries, (i.e. 1000 lbs of cherries in a post-harvest cold water holding tank) that product is not eligible for processing. Since there are no post-harvest infestation removal technologies, a single larva can result in the loss of a grower's entire harvest. Every year, processors are forced to reject thousands of pounds of harvested cherries because of the detection of insect infestation. The major contributors to this type of loss are the plum curculio, *Conotrachelus nenuphar* (Herbst), and cherry fruit fly (*Rhagoletis* spp.). An historical progression of potent lead, arsenical, chlorinated hydrocarbon and organophosphate chemistries has been used to meet the zero-tolerance standards for pest infestation. However, the first three classes have been banned, and the 1996 Food Quality and Protection Act (FQPA) has significantly restricted the current use of organophosphates. The registration of the current mainstay, azinphos-methyl (Guthion®), is set to expire in 2012. The loss of this potent insecticide has placed the viability of the cherry industry, both inside and outside of Michigan, in jeopardy.

Both the Tart Cherry Pest Management Strategic Plan (2001) as developed by US cherry stakeholders and the Michigan Tart cherry crop profile (Jess 2003) have identified plum curculio and cherry fruit fly as top research priorities. Both are critical pests whose larvae infest the cherry fruit at harvest. There are several promising new chemistries being developed for fruit fly control, including compounds in the spinosyn (King and

Hennessey 1996, Yee and Alston 2006, Pelz-Stelinski et al. 2006) and neonicotinoid (Stelinski et al. 2001) classes. However, there are still significant gaps in our knowledge of the key performance characteristics of these and other new insecticides on plum curculio, such as life-stage activity, acute and sublethal effects and optimal timing. If the cherry industry is to survive the phase out of azinphos-methyl, the next iteration of plum curculio management requires answers to these questions.

This research addressed the challenges of cherry pest control in the face of stringent quality standards and declining access to the most potent insecticides in this post-FQPA era. The primary focus of this research was to identify and characterize the key performance mechanisms of new insecticide chemistries that are becoming available for the control of plum curculio, and the subsequent optimization of these controls for use in the field. Traditional methods such as topical laboratory assays and field plot studies do not provide all of the necessary information to accurately evaluate and understand the crop protection performance of these new chemistries. As such, I took an integrated approach that identified and evaluated a series of interactions between the plant, the insect and insecticide chemistries. Secondary goals included understanding plum curculio behavioral ecology, and validating laboratory bioassay techniques for the assessment of insecticide activity.

Cherries

Prunus cerasus, the tart or sour, cherry, is a member of the Rosacaea family. It is a small tree (less than 30') native to Europe, but was introduced in the US in the 1600's with the influx of European colonists. *Prunus avium*, the sweet cherry, was also introduced from Europe at this time. Oregon, Washington, Michigan, Pennsylvania, New

York and California are the major producers of sweet and tart cherries. Total acreage for the country is 37,000 acres for tart cherries and approximately 78,000 acres of sweet cherries. Michigan alone has 27,000 acres of tart cherry and 8,100 acres of sweet cherry production. Total cherry production values exceed $60 million annually. Sweet cherries are primary sent to fresh markets, while tart cherries are typically processed – either frozen, canned or dried (NASS 2005).

In Michigan, Tart cherry trees bloom in mid- to late-April. This is later than other stone fruits and reduces their susceptibility to frost. However, late frosts are not uncommon, and can be devastating. In 2002, Michigan harvested only 15 million pounds of tart cherries, less than 10% of the normal crop. Per pound prices that year were up, but the lack of product kept national production value at a fraction of normal totals. Harvest of the cherry fruit occurs July-August in Michigan. Fruit are shaken off of the trees mechanically, and fall into water filled chilling tanks to slow the ripening process and maintain harvest quality. For processed cherries, the fruit in the chilling tanks are inspected for insect infestation prior to being unloaded at the processor.

Production of cherries has several pest and disease challenges. In addition to the infesting pests mentioned earlier (plum curculio, *Rhagoletis* fruit flies) the American Plum Borer (*Euzohera semifuneralis*) (Lepidoptera: Pyralidae) and the Peachtree Borer (*Synanthedon exitiosa*) (Lepidoptera: Sesiidae) are also important insects to manage in cherry orchards. The borers are typically monitored with pheromone traps and controlled with insecticide sprays on the tree trunks.

Cherry leaf spot (*Blumeriella jaapii*) and Brown Rot (*Monilinia fructicola*) are the primary disease concerns. Cherry leaf spot infections can reduce tree vigor and severe

infections can lead to death during the Michigan winter. Brown Rot is a fruit quality issue more than one of overall tree health, but infections can reduce yield dramatically. Spring rains enhance infection periods for both of these diseases, and weekly fungicide cover sprays are common in Michigan.

Conotrachelus nenuphar: The Plum Curculio

Conotrachelus nenuphar (Herbst) (Coleoptera: Curculionidae) is a native to North America. It is in the Molytinae subfamily and was described by Herbst in 1797. Its current USA range is primarily east of the Rocky mountains; but there is an isolated population in Northwestern Utah (NAPIS 2004).

There are two strains of plum curculio. The northern strain has an obligate adult diapause prior to mating and oviposition. The southern strain has a facultative diapause with either a partial of full second generation (Stearns 1931, Smith 1957, Smith & Salkeld 1964). The facultative aspect of southern strain diapause is not complete, as some first generation individuals enter into diapause even under favorable conditions (Mampe & Neunzig 1967). Northern and southern strains have a mating barrier that limits the viability of progeny from mixed-strain matings (Stevenson & Smith 1961, Padula & Smith 1971). There is currently no non-destructive method for determining whether an individual is from the northern or southern strain, or whether it is in a reproductive or diapause-bound state. Reproductive status of 20d-old (or older) females can be determined by ovarial dissection (Smith & Salkeld 1964). Recently, RAPD-PCR techniques have been used to identify and correlate plum curculio genetic types with diapause tendencies (McClanan et al. 2004). Additionally, RAPD-PCR has identified

Wolbachia infections in both strains. *Wolbachia* is a maternally-transmitted, intercellular bacteria that is known to cause reduced fecundity when males have a different strain than the females (Dobson et al. 2002).

Throughout the northern part of its range (including Michigan), plum curculio is univoltine. Unmated adults overwinter in leaf litter and loose soil both in orchards and adjacent woodlots (Chapman 1938, Smith & Flessel 1968, Lafleur et al. 1987, Racette et al. 1992). Adults mate in early spring, and most females are mated well in advance of commercial crop fruit set (Smith & Salkeld 1964, Hoffmann et al. 2004). Females lay eggs inside of fruit, and eggs take roughly 3 days to hatch (Smith 1957, Mampe and Neunzig 1967). The egg laying period is fairly long; new oviposition scars are noted from May through early July if appropriate hosts are present (Reissig et al. 1998). The legless larvae eat the flesh of the fruit and take approximately 3 weeks ($400DD_{50°F}$, $215.5DD_{11.1°C}$) to complete development after eggs are laid (Smith 1957, Lan et al. 2004). When they have completed feeding, larvae exit the fruit and burrow into the soil. Pupation time is variable; soil quality, temperature and moisture are important factors. Smith (1957) observed 7000 southern strain pupae and derived a mean time between soil-entry and adult emergence to be 28 days (840 $DD_{50°F}$). After adult eclosion in August, northern strain adults may do some feeding, but are assumed to move to overwintering locations. Summer generation Southern strain beetles feed for a while after eclosion and then begin another round of oviposition (Quantance and Jenne 1912, Smith and Salkeld 1964, Gaydon 1972, Racette et al. 1992).

The host range for plum curculio is broad. It feeds and oviposits in a wide variety of wild and cultivated Rosaceous plants, including *Amelanchier, Malus, Crataegus,* and

Prunus species (Chapman 1938, Maier 1990, Brown 2005). Plum curculio feeding and oviposition also occur in blueberries (*Vaccinium spp.*) (Hallman 2003, Polavarapu et al. 2004), although this is a more limited impact relative to tree fruit. Hallman and Gould's subsequent 2004 report on 22 possible tropical host fruits identified plum curculio feeding in tropical fruits, particularly mango. However, oviposition was only documented in apple, plum, peach and loquat (*Eriobotrya japonica*) – all Rosacaea. There is evidence for regional host races within and between the strains of plum curculio. Southern strain plum curculio in Georgia's peach growing region seldom attack apples (Jenkins et al. 2006), whereas apple is considered a preferred host in northeast growing regions. Similarly, MI plum curculio readily attack both tart cherry and apples, while WV populations (also northern strain) are less likely to attack apple (Leskey and Wright 2007).

From an economic standpoint, plum curculio is an important pest in apples, peaches, plums, sweet cherries, tart cherries, and blueberries in the Eastern USA. It has been a known pest of these fruits for over 100 years, and significant efforts have been directed at its control and/or eradication in all commercial hosts. In apples, peaches, plums, blueberries and fresh-market cherries, the primary concern is one of aesthetic grading and fruit abortion. Fruit with adult feeding damage or oviposition scars is unacceptable for the fresh market, and is often diverted to secondary markets (juice, roadside) or destroyed. Additionally, infested fruit on the tree may abort in early summer. Light infestations may not impact yields significantly, but heavy infestations can cause significant fruit loss.

Because of its potential for economically important damage, plum curculio is a quarantine pest in the fruit growing regions of the western US. The presence of a small population in Box Elder Co., UT, has raised the concern of this pest's spread to the region's commercial orchards. Currently, Utah's plum curculio detections are primarily home orchards or roadside trees (D. Alston, Personal Communication).

Control Strategies for Plum Curculio

Historical context

The economic impact of plum curculio has been appreciated for over a century. An 1898 Kansas State Agricultural College bulletin notes it as a pest of primary concern in orchards, and recommends actively monitoring for oviposition injury. If adults are detected, this early bulletin recommends a spray of Paris Green (copper acetoarsenate) and Bordeaux Mixture along with mechanical jarring of trees onto sheets to collect adult beetles (Faville & Parrott 1898). Arsenicals like Paris Green were used extensively prior to DDT, and growers reverted back to lead arsenate after DDT was banned (FAO/WHO 1965).

In addition to mechanical controls and spraying, winter burning of putative hibernation sites was part of the early control suite (Quaintance & Jenne 1912, Stearns et al. 1935). This practice has fallen out of favor, and Bobb (1949) determined that winter burning was "of no value" due to the soil depth of curculio overwintering. Early spring burning, however, was seen as having utility, since beetles were mostly in the surface ground cover, duff and leaves. Burning specifically for plum curculio is not common

modern practice, although some collateral control may be seen by growers who use burning for weed suppression.

Integrated Pest Management: Monitoring

Integrated Pest Management (IPM) is a systems approach to reducing pest damage to tolerable levels. While there have been many iterations of a definition, the following, from Marcus Kogan's 1998 Annual Review of Entomology paper, offers a sense of the utility (and complexity) of IPM:

> IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment.

Insecticides are one of many tactics that are employed to reduce the economic impact of a pest. An idealized IPM program would identify and use natural enemies (predators, parasites) and cultural controls to reduce pest populations, and employ naturally resistant crop cultivars to increase the tolerance of the production system to pest damage. At the heart of an IPM program is the understanding of what the pest population level is and/or how much damage a crop is able to sustain before a grower begins to see an economic impact. When this threshold is exceeded, growers – even the most ecologically-minded ones – apply chemical controls to their crops. IPM does not start and end with chemical applications, though. Monitoring for the pest and associated damage is a foundation of

IPM practice, and it is only through monitoring and evaluation of the pest/ecosystem combination that one can optimize the benefits of the many pest control tools.

Since the economic threshold for plum curculio is so low, insecticides are still the primary control method for plum curculio. There is a strong research effort aimed at broadening the suite of IPM pest management tactics for use against this pest. In addition to identifying and optimizing insecticides, the key areas of current research include improved monitoring/trapping techniques, identification of attractant cues, and use of biological control agents.

As described previously, monitoring for oviposition damage has been a foundation for curculio control for over a century. Most growers employ professional scouts who check trees for pest presence or damage. There are also a few monitoring traps that are used in the orchard to enhance detection of adults moving into the orchard in the spring. The pyramid trap (Tedders and Wood 1994) was originally developed for pecan weevils, but has been modified for plum curculio. It is considered to be a silhouette mimic for migrating adults. A scaffold limb version of the Circle screen trap (Mulder et al. 1997) is often used in addition to the free-standing pyramid traps.

These common monitoring traps (and most use-specific traps) are often enhanced with kairomone and pheromone lures. These lures are often mixtures of benzaldehyde (Leskey et al. 2001, Piñero et al. 2001, Prokopy et al. 2003) and the male-produced aggregation pheromone, grandisoic acid (Eller & Bartelt 1996). Despite the addition of these chemistries, plum curculio monitoring traps do not operate as efficiently as many of those designed for lepidopteran orchard pests. Intense trapping and daily monitoring during the immigration period has been shown to be useful in determining the magnitude

of plum curculio movement from adjacent areas (Piñero and Prokopy 2006), but they are not sufficient to generate definitive control decisions (Leskey and Wright 2004). In-orchard sampling/monitoring for damage remains the most reliable method (Vincent et al. 1999).

Integrated Pest Management: Biological Control

Enhancing the activity of biological control agents is often an element in IPM. However, the natural enemy profile for plum curculio is still incomplete, and augmentative releases are not yet a standard practice. *Steinernema feltiae*, *Heterorhabditis bacteriophora* and *S. riobrave* are three species of nematodes that have shown effectiveness in plum curculio control. In laboratory tests, *Steinernema feltiae* and *Heterorhabditis bacteriophora* effected up to 90% larval mortality when larvae were immediately added to treated soil (Alston et al. 2005). However, larvae added to soil a week after treatment suffered only half the mortality of those placed immediately into the soil. Field studies yielded only 40% mortality for similar rates of infective juveniles. Utah-collected *S. feltiae* were as effective on larvae as commercially prepared strains, which suggests that wild populations of nematodes are present and potentially active. *Steinernema riobrave* prey on adult plum curculio, and can reduce adult emergence from pupation by 78-98% (Shapiro-Ilan et al. 2004). *Steinernema feltiae* performed well against adults in lab situations, but showed mixed results in field applications (Shapiro-Ilan et al. 2004, Alston et al. 2005, Shapiro-Ilan et al. 2008). There are formulations of steinernematid and heterorhabditid nematodes available, but they are not registered for plum curculio control.

Natural fungal communities are being evaluated for their use in plum curculio management. *Beauveria bassiana* and *Metarhizium anisopliae* have both been shown to cause plum curculio larval mortality (Alston et al. 2005, Tedders et al. 1982). However, there seems to be wide variance in activity between local isolates. A South Carolina isolate of *M. anisopliae* caused nearly 90% mortality while a California isolate only inflicted 26%. *Beauveria bassiana* has shown relatively low control utility, only 24-28% mortality in laboratory soil tests. (Tedders et al. 1982). *B. bassiana* is available as a registered product for plum curculio management.

Parasitic wasps are not well described for plum curculio. An early parasitoid record is that of *Thersilochus conotrecheli* by Cushman (1916). This was described as a readily observed insect in infested Pennsylvania orchard, and only recovered from plum curculio larvae. Since then *Nealiolus curculionis* and *Cerceris atramontensis* have also been described as using plum curculio as a host (Krombein et al. 1979). The geographic range for the non-specific *N. curculionis* (10 described hosts) is very large – Quebec to Florida and west to California. *Cerceris atramotensis* has a more limited host range and is only present from Quebec to North Carolina and west to North Dakota and Texas. *Aliolus rufus* has also been recovered from pupating plum curculio (Mampe and Neunzig 1967). None of these organisms have been utilized in an IPM program for plum curculio.

There may have been an important historic link between these pathogens / natural enemies and plum curculio, but the use of broad-spectrum pesticides and other cultural practices in fruit agriculture has probably severed the ecological tie and negated the regional scale impact of these organisms. Pesticide use in agroecosytems can reduce the numbers of natural enemies (Elzen and Elzen 1999, Norris and Kogan 2000) and

tillage can reduce habitat suitability for soil-based infectious agents (Alston, personal communication).

Integrated Pest Management: Insecticide-based control with Organophosphates

The organophosphate azinphos-methyl (Guthion®, Bayer CropScience, Research Triangle Park, NC) was registered under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in 1957. It has since become the mainstay for control of plum curculio and other major pests of fruit trees. Azinphos-methyl and its sister compound, Phosmet (Imidan®, Gowan Co. Yuma, AZ), are among the few insecticides that consistently receive 'Excellent' ratings for control of plum curculio in state extension pest management guides (Wise et al. 2008). In 2001, 89% of the nation's tart cherry acreage received at least one azinphos-methyl application (NASS 2004).

These organophosphates are highly effective at controlling plum curculio in tree fruit. They have broad-spectrum contact activity, and a long post-application residual activity – greater than 14d in ideal conditions. While azinphos-methyl has been effective for the control of plum curculio, the Food Quality and Protection act of 1996 (P.L. 104-170) has limited its use in food crops in general; many uses have been removed from the Guthion label completely. In 1990, the Guthion label allowed 8.5 lbs of formulated product per season. Cherry growers are now (2008) limited to 1.5 lbs/acre per year (Guthion 50WP, 50% AI) (Edwards 2006). Current use in orchards with a history of infestation is generally two post-bloom prophylactic sprays. The first is timed to reduce initial curculio adult populations, oviposition and associated yield loss, while the later spray is aimed at preventing infestation at the processing plant. The pre-harvest interval

for Guthion in cherries is 15 days. This pre-harvest interval is designed to assure that residues on harvested fruit meet EPA dietary tolerances, but it opens the window for infestation if coverage during this period immediately before harvest is incomplete for any reason (rain, wind, equipment). Eggs laid at 12-14 days pre-harvest in an unprotected crop would be late instars, and readily detectable by regulatory inspectors.

Total reliance on organophosphates is a scenario of concern for resistance management (Denholm and Rowland 1992). However, there is little evidence for resistance development in this pest (Vincent et al. 1999). Plum curculio may simply lack the genetic/metabolic profile for developing resistance, or the immigration and mixing of unexposed adults from unmanaged woodlots is a sufficient source of susceptible genes that negates any in-orchard selection pressure.

The Food Quality Protection Act (FQPA) of 1996

The Food Quality Protection Act of 1996 (P.L. 104-170) was a dramatic addition to the United States regulatory structure. This legislation is an amendment to FIFRA and the Federal Food, Drug and Cosmetic Act (FFDCA) and has an overall goal of making these two statutes more consistent in their treatment of pesticides, in addition to standardizing worker and consumer safety. The EPA's own summary describes this legislation's goals:

> For over two decades, there have been efforts to update and resolve inconsistencies in the two major pesticide statutes, but consensus on necessary reforms remained elusive. The 1996 law represents a major breakthrough, amending both major pesticide laws to establish a more consistent, protective regulatory scheme, grounded in sound science. It mandates a single, health-based standard for all pesticides in all foods; provides special protections for infants and children; expedites approval of safer pesticides; creates incentives for the development

> and maintenance of effective crop protection tools for American farmers; and requires periodic re-evaluation of pesticide registrations and tolerances to ensure that the scientific data supporting pesticide registrations will remain up to date in the future. (http://www.epa.gov/oppfead1/fqpa/backgrnd.htm)

As part of the FQPA, all insecticides were re-evaluated with respect to use patterns, mammalian toxicity, environmental impact, and other risk factors. This initial review was to be completed by 2006. Because of their relatively high mammalian toxicity, the organophosphate chemistries were in the first group of insecticides to be re-evaluated. The assessment of where compounds fit into FQPA tolerances has largely been through committees in collaboration with manufacturers and researchers. However, the US EPA has a tradition of public comment periods and this has been adhered to for post-FQPA decisions. Public comment has been important, because the FQPA provides for continued use of hazardous compounds if the benefits of continued use outweighed the known risks.

In 2001, after a public comment period and committee evaluation, it was determined that twenty-three uses of aziphos-methyl would be immediately cancelled (Group 1). The remaining seventeen uses were placed into two categories: Group 2 had seven uses with a 4-year phase out period, and Group 3 contained ten time-limited re-registrations. Group 2 uses include cotton, cranberries, nectarines, peaches, potatoes, caneberries and southern pine seed orchards. These uses were initially given an extension to September 30, 2006. The time-limited group (Group 3) consists of almonds, apples, blueberries, Brussels sprouts, cherries (sweet and tart), nursery stock, parsley, pears, pistachios, and walnuts. All continued uses have additional exposure mitigation instructions, including the elimination of aerial spraying, extended re-entry intervals

(REI), application limits, and buffer zones for sensitive habitats. Since this period began, Bayer Cropscience, the primary registrant of azinphos-methyl, has elected to cancel uses in cotton, caneberries, cranberries, nectarines, peaches, potatoes and southern pine seed orchards.

Azinphos-methyl use in cherry is part of the Group 3 reregistration, and there have been several extensions of its use. Because of the limited number of broad spectrum/contact active replacements, cherry stakeholders, including the Cherry Marketing Institute (CMI) and the Michigan Cherry Committee, have lobbied strongly for retaining azinphos-methyl in this high-value crop under the risk-benefit category of the review process. While lobbying extended its registration, azinphos-methyl use in cherries *will* eventually be cancelled. In 2007, the EPA announced a cancellation deadline of 2012 for all remaining uses of azinphos-methyl. The organophosphate phosmet (Imidan®) registration will remain in place, but with additional use restrictions. In addition to having weaker insecticidal action, this compound is also phytotoxic to sweet cherries. The search to identify alternatives is vitally important for the cherry industry.

Insecticide Chemistries for Plum curculio control: New classes and new opportunities

As previously described, the current grower standard for plum curculio is foliar application of organophosphate insecticides. Organophosphates are acetylcholinesterase inhibitors that work at the synaptic junction of the insect nerve where they bind to the acetylcholinesterase enzyme and impair its ability to remove the stimulatory

neurotransmitter acetylcholine from the synaptic space. Typical insect symptoms include convulsions and uncoordinated movement. Death results from overstimulation of the nervous system, ATP loss, and dehydration. Birds and mammals also use the acetylcholinesterase system in neurotransmission, and are also susceptible to the effects of organophosphate compounds (Casida 1973).

Another class of neurotoxins registered for plum curculio control is synthetic pyrethroids. These compounds bind to the nerve axon itself and inhibit the voltage gated sodium channel from closing properly after the nerve impulse has been generated (Casida et al. 1983). Keeping this ion channel open causes symptomology similar to organophosphates. Pyrethroids are typically fast-acting. There are several pyrethroids labeled for plum curculio control in cherries, and their overall efficacy is 'good' (Wise et al. 2008). However, pyrethroids have short-lived residual activity against plum curculio (5-7d) and are known to disrupt integrated mite management programs by killing or repelling key predators of pest mites (Hull et al. 1997).

Growers and researchers are looking ahead to new insecticide classes to fill the gaps left by the impending loss of organophosphates. The key classes of alternative chemistries are the Insect Growth Regulators (IGRs), Neonicotinoids, and Oxadiazines. The use of these classes are not yet established in orchard management systems, and their use for plum curculio control is not well understood, let alone optimized.

The insect growth regulators are a promising new insecticide group. In contrast to the broad contact-based toxicity of an organophosphate, lethal effects of IGRs are typically limited to immature stages. However, they can also induce an array of sublethal effects across life stages that can provide exceptional pest population reduction over time

(Hargrove & Langley 1990). The three main groups of IGRs are the Juvenile Hormone (JH) mimics, ecdysteroid agonists, and chitin synthesis inhibitors. As a class, their effectiveness appears to be dependent on careful correlation to pest biology (i.e. larval stage, reproduction, and diapause), so it is critical to have biological information that is pertinent to their use. Even though the primary pest targets of IGRs are lepidopterans, current research suggests that there are measurable effects on beetle species.

Esteem® (pyriproxyfen) is a JH mimic currently registered for use in cherries against scale insects and peach twig borer. It is also registered for use in pome fruits for codling moth and leafrollers. This chemical has been shown to cause developmental failure in immature insects (Kostyukovsky et al. 2000) and direct adult mortality across several orders (Liu & Chen 2001, Meola et al. 1996). Pyriproxifen has also been shown to interfere with diapause induction in apple blossom weevil (Zderek et al. 2000) and Colorado Potato Beetle (Koopmanschap et al. 1989). Preliminary experiments suggest that while pyriproxifen is not directly lethal to plum curculio, it does have sublethal effects on developing pupae (Hoffmann & Whalon 2003). Topical and residual doses terminate northern strain reproductive diapause at a wide range of doses (Hoffmann et al. 2007). Collectively, these data suggest that JH analogs may have a place in population-wide control of plum curculio, if properly timed with pest biology.

Ecdysteroid analogs such as tebufenozide, methoxyfenozide, and halofenozide mimic the hormonal action of 20-Hydroxyecdysone. While these non-steroidal compounds are quite different in chemical structure from molting hormone, they are able to bind to the ecdysone receptor complex and initiate the sequence of head capsule slippage, and cutuiculin/epicuticle deposition. The analogs' structure keeps them bound

to the receptor and inhibits the remainder of the molting cascade. The bursicon/sclerotization cascade does not occur, and the newly molted larva remains inside of the old skin (Retnakaran et al. 2001, 2003, Smagghe et al. 1999). Ecdysteroid agonists have ovicidal and larvicidal effects in a number of lepidopterans (Charmillot et al. 2001), and adult sterilization has been noted in leafrollers and codling moth (Sun & Barrett 1999, Sun et al. 2000). Tebufenozide and methoxyfenozide are generally considered lepidopteran-specific, but halofenozide has demonstrated profound effects on Colorado Potato Beetle development and reproduction (Farinos et al. 1999). Application of this compound to reproductive Colorado Potato Beetle adults results in resorption of oocytes in mated females.

Novaluron, lufenuron, diflubenzuron and buprofezin are chitin synthesis inhibitors (CSIs) that inhibit the complete formation of the cuticle after an insect molts (Elek 1998a). The benzoylureas novaluron, lufenuron and diflubenzuron are considered "Type 0, Lepidopteran" CSIs and buprofezin is a "Type 1, homopteran" compound by the Insecticide Resistance Action Committee (www.irac-online.org). Death is usually the result of starvation, since the muscles for the mandibles lack sufficient attachment points on the weakened head capsule. The action of CSIs is primarily on immature insects, but exposure of adults to these compounds can reduce fertility or subsequent larval development (Elek 1998a,b; Calkins et al. 1977, Wise et al. 2007a). Because of sterilization effects, lufenuron is considered a promising replacement for organophosphates in the control of the Mediterranean Fruit Fly *Ceratitis capitata* (Casaña-Giner et al. 1999). Of these compounds, only buprofezin (Applaud®) is currently registered in cherries. Novaluron (Rimon 0.83EC, Crompton, Middlebury CT)

is currently registered in apples but a stone fruit label is expected in the near future through the IR-4 process.

The neonicotinoid (or chloronicotinyl) class of chemistry has provided a number of promising products for tree fruit pest management, including thiamethoxam (Actara™), imidacloprid (Provado®), acetamiprid (Assail®) and thiacloprid (Calypso™). Like the organophosphates and pyrethroids, these chemicals are nerve poisons. Neonicotinoids bind to the nicotinic acetylcholine receptor on the post-synaptic nerve cells (Yamamoto & Casida 1999). The resulting symptoms of overstimulation are similar to those of organophosphates, pyrethroids and carbamates (Valles & Koehler 1998).

The neonicotinoids have a much lower affinity to mammalian nicotinic acetylcholine receptors (nAChR) than to insect nAChRs (Yamamoto et al. 1995, Tomizawa and Casida, 2003, 2005; Millar and Denholm 2007). As a result, they are highly selective toxins with a promising human safety profile.

Neonicotinoids are becoming well known for their movement into and through plant tissues, from both uptake from soil applications and seed treatments, and translaminar movement into leaves and subsequent tissue translocation (Buckholz and Nauen 2001, Sur and Stork 2003, Weichel and Nauen 2004, Wang et al. 2005). In apples, neonicotinoids move into the cuticle and tissue layers of fruit and leaves and have toxic effects on a wide range of pest species, including plum curculio (Wise et al. 2008). The use of these compounds appears to provide short periods of adulticidal activity, with a longer-lived deterrence of oviposition and feeding. This pattern of activity correlates well with the observed spatial-temporal characteristics of the chemical residues in field

trials. The penetration profile for neonicotinoids in cherries is not yet known, but this class may be very important if compounds show good longevity in residue profiles.

Indoxacarb (Avaunt®) is the only registered compound within the oxadiazine class and is currently available for use in pome and stone fruit production. Indoxacarb inhibits sodium movement into nerve cells. The result is paralysis and eventual death rather than overstimulation. Indoxacarb is broken down by insect carboxyl esterases to a secondary compound DCJW (N-decarbomethoxylated JW 062) which is significantly more toxic in insects than its parent compound (Wing et al. 2000, Ahmad et al. 2002, Tsurubuchi & Kono 2003, Ramasubramanian & Regupathy 2004). Indoxacarb and the DCJW metabolite also affect mammalian sodium channels, but effects are seen at micromolar concentrations, as opposed to nanomolar concentrations in insects (Zhao et al. 2003). Mammalian detoxification pathways also contribute to the selectivity of indoxacarb. Conversion to DCJW is a minor pathway, and DCJW is further metabolized and excreted (Dias 2006).

Indoxacarb may provide relief from some pyrethroid-resistant populations of tree fruit pests, since the increased esterase activity of these resistant populations can enhance the production of the DCJW metabolite after indoxacarb exposure (Ramasubramanian & Regupathy 2004). Though broadly classified as a contact insecticide, its lethal activity is greatly enhanced with ingestion for some pests, including plum curculio (Wise et al. 2002). Indoxacarb has low water solubility, and preliminary research in Michigan apples suggests it to be rain fast with a stable residue profile.

Evaluating Insecticide Performance: The PIC-Triad

The publicly mandated need for cherry fruit that is free of detectable larvae is not likely to change in the near future. Meeting this need will require continued use of pesticides as the foundation of plum curculio management, and identification of effective replacements for organophosphate insecticides. While growers are accustomed to the performance characteristics of organophosphate-based control, we must remember that the pest control mechanisms of replacement chemistries may be very different than the fast-acting contact and ingestion activity of conventional classes. In fact, one of the core challenges that researchers and growers face is to remain mindful that the labels "OP Replacement" or "OP Alternative" do not equate to simply switching "neonicotinoid" for azinphos-methyl on the spray calendar. IGRs, neonicotinoids, and oxadiazines lack the singular mode of activity on which organophosphate performance is based. In fact, many of new chemistries have multiple modes of activity that are closely linked to spatial and temporal components of the orchard ecosystem. Identifying and characterizing the critical environmental and biological parameters of these modes of activity was a primary goal of this research. The following characteristics are the main areas of interest relative to plum curculio: life stage specificity, transcuticular vs. ingestion modes of exposure, residual activity, and sublethal reproductive and behavioral effects.

Because of the linkage between these performance characteristics and the environment/plant biology, topical bench studies and small plot trials are not sufficient for evaluating these compounds. A new research philosophy has been developed at Michigan State University to more completely understand and predict field performance of new chemistries (Wise et al. 2006). This methodology is based on the idea that to

fully understand insecticide performance, the interaction of three critical elements – the insect, the plant, and the chemistry – must be considered. Without all of the PIC-Triad elements (Figure 1.1), we lack vital information regarding how a specific chemistry will perform against a pest on a particular plant.

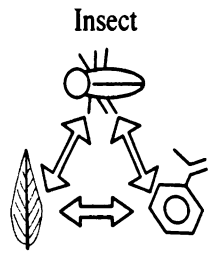

Figure 1.1. The PIC-Triad (From Wise et al. 2007)

Traditional pest control has been keenly focused on the chemical-insect interaction. This is certainly appropriate, as this interaction represents the distillation of the entire pest management process: Agent A affects Pest X. Describing this interaction in a post-OP and post FQPA environment is no longer as simple as developing LD_{50} and LC_{50} data on laboratory benchtops. Extending performance characteristics beyond contact toxicity will require a sharp and critical eye toward detection of secondary effects like sterilization and inhibition of feeding. This approach requires the expertise of behaviorists and physiologists in addition to classical probit analysis, but the benefits of identifying an effective insecticide certainly warrant the combined effort.

Behaviorists have focused on the insect-plant element of the PIC-Triad, but there are still key questions that still need to be answered relative to plum curculio life history. Insect behavior not directly linked to economic injury may become very important in optimizing new control tactics. Feeding patterns and development times become more than biological background information in the new chemical landscape. These data will equip scientists to examine the more complex and dynamic outcomes that occur when pests interact with plants and multi-mechanistic insecticides.

The plant-chemical interaction regulates the exposure profile of the insecticides to the various insect life stages. It is typically the link between the insect and the insecticide, either through contact on the plant surface as the insect crawls across it, or through ingestion of treated plant tissues. It is also the least understood of the PIC triad elements; the spatial and temporal dimensions are beyond the typical scope of pest management research. The spatial dimension focuses on the movement of the chemical onto or into the plant and the eventual locations that are reached and maintained in fruit and foliage. The temporal dimension focuses on how the proportions of active ingredient on and inside the plant (leaves and fruit) change over time, and how environmental factors like photodegradation, evaporation, hydrolysis, and biochemical metabolism influence longevity trends. Profiling a chemical's active ingredient in fruit and foliage over time relative to changes in insect behavior provides insight into the performance mechanisms that are behind observed crop protection capabilities. Profiling the residual activity and movement through plant tissues is a key way of assessing the utility of a compound in actual field conditions, even if a test plot has low pest pressure. Residue profiles provide a connection between toxicology/bioassay data and field performance to generate an assessment of a compound's effectiveness against adult insects. Interior residue profiles provide additional data related to potential ovicidal and larvacidal effects, which has not been an active area of research for plum curculio.

In summary, understanding the critical performance characteristics of new plum curculio controls is of great importance. The regulatory environment and the inherent limitations of the available compounds have added complexity to the "deliver the chemical to the insect" chain. Insect biology and behavior are more important than ever in assessing a

compound's utility. Chemical mode of action and mode of exposure are no longer limited to acute and contact-based toxicity. Plant-chemical interactions have become more than measuring rain fastness. Despite these challenges, these new insecticides hold numerous benefits: activity against OP-resistant pests, safety to orchard workers and beneficial insects, and "reduced risk" or "OP alternative" registration status with the EPA. For this reason, it is critical that we gain the knowledge of how these new compounds work with respect to life stage timing, residual activity and lethal/sublethal effects. Failure to adequately clarify their places in cherry pest management will place the cherry industry at risk.

Chapter 2

Ovicidal activity of organophosphate, oxadiazine, neonicotinoid and insect growth regulator chemistries on northern strain plum curculio (*Conotrachelus nenuphar* Herbst)

Data from this chapter was published, in part, in the Journal of Insect Science as Hoffmann et al. 2008.

Abstract

An *in vitro* method was developed for assessing ovicidal effects of the organophosphate azinphos-methyl, the neonicotinoids thiacloprid, thiamethoxam and clothianidin, the oxadiazine indoxacarb, the anthranilic diamide chlorantraniliprole and the insect growth regulators novaluron and pyriproxifen on plum curculio. The baseline survivorship of this method was 88 percent. Plum curculio eggs were most sensitive to azinphos-methyl. Thiacloprid, clothianidin and the chitin synthesis inhibitor novaluron had LC_{50} values below 100 ppm (μg/ml). Neither thiamethoxam, indoxacarb, pyriproxifen, nor chlorantraniliprole were ovicidal at 100 ppm. Octanol-water partitioning coefficients, log K_{OW}, appear to be an important indicator of ovicidal activity within the neonicotinoids. This new bioassay method eliminates the confounding of the insect-chemical and plant-chemical interactions and the results highlight the utility of a post-infestation curative approach to plum curculio management.

Introduction

The Plum curculio, *Conotrachelus nenuphar* Herbst, is an endemic pest of tree fruit in Eastern North America. The northern strain of this insect is univoltine, and has obligate adult overwintering diapause. The southern strain has a facultative diapause. Both strains are serious pests of cultivated stone and pome fruits (Quaintance and Jenne 1912, Hallman and Gould 2004).

For apples grown in the USA Great Lakes States, like Michigan and New York, the oviposition by *C. nenuphar* occurs in the 6 - 10 weeks (400 Growing Degree Days 10°C [$DD_{10°C}$]) after petal fall (Reissig et al. 1998). Eggs are laid just underneath the fruit skin after the female makes a small feeding incision. After oviposition, the female also chews a C-shaped excavation around the egg, which is thought to prevent local tissue expansion and protect the egg from being subsequently crushed (Owens et al. 1982). Eggs take 3-6 days to hatch (Smith 1957; Mampe and Neunzig 1967) and larvae are exclusively internal feeders. Whether or not the eggs hatch, the oviposition incision develops into a surface scar and can render fruit unacceptable for fresh markets. Larval presence inside of fruit is a key regulatory concern for processed commodities like tart cherries, where there are zero-tolerance standards in place for insect infestation (USDA Agricultural Marketing Service 1941a,b).

The management of this pest is overwhelmingly focused on control of adults (Smith 1964; Howitt 1993; Reissig et al. 1998). Organophosphorus insecticides (primarily azinphos-methyl) are currently the primary means of plum curculio control, but newer classes are being aggressively studied in light of the FQPA-directed phase out of the organophosphate azinphos-methyl (US EPA 2006). These new classes

(neonicotinoids, oxadiazines, Insect Growth Regulators (IGRs)) generally lack the acute adult contact toxicity of the organophosphorus compounds and require close examination to fully understand their potential uses in plum curculio management.

Post-infestation, or curative, action is one of the possible modes of activity for chemical control. The early organophosphates parathion and EPN [*O*-ethyl *O*-(*p*-nitrophenyl) phenylphosphonothioate] were identified as having some ovicidal and larvicidal activity against plum curculio (Smith et al. 1956), but this was primarily viewed as a secondary benefit of these adult-targeted materials. Currently-registered organophosphate and neonicotinoid insecticide sprays have also been shown to penetrate into apple fruit tissue at concentrations sufficient to kill the internally-feeding plum curculio larvae (Wise et al. 2007a).

Insect growth regulators also kill eggs of certain insect species. The chitin synthesis inhibitor diflubenzuron killed eggs of codling moth (Charmillot et al. 2001), and teflubenzuron and hexaflumuron were effective against eggs of the cowpea weevil (Abo-Elghar et al. 2003). The juvenile hormone analog pyriproxifen was ovicidal when applied to eggs of codling moth (Charmillot et al. 2001; Yokoyama and Miller 1991), diamondback moth (Oouchi 2005), and whiteflies (Ishaaya et al. 1994). The effectiveness of this class against plum curculio eggs has not been studied.

The current study examined the toxicity of eleven crop protection compounds to plum curculio eggs. These compounds came from several classes: organophosphates (azinphos-methyl, phosmet), neonicotinoids (thiacloprid, thiamethoxam, clothianidin, acetamiprid), pyrethroids (esfenvalerate), anthranilic diamides (chlorantraniliprole), oxadiazines (indoxacarb) and insect growth regulators (pyriproxyfen and novaluron).

The challenge of regulating chemical concentrations in the fruit required the development of an *in vitro* assay that isolated the insect-chemical interaction from other influences like varying chemical penetration and movement through plant tissues and plant metabolism of the insecticide compounds. Evaluating these fundamental insect responses can help make sense of patterns that researchers see in studies that incorporate the full insect-fruit-chemical system.

Materials and Methods

Insect Source and Maintenance

Northern strain plum curculio were collected from 5 May – 10 June 2006 in cherry and apple orchards at the Trevor Nichols Research Complex in Fennville, MI (42.5951°N, -86.1561°W) using commercially-available pyramid traps (Tedders and Wood, 1994) and a pneumatic limb shaker (Maibo Model ST-7-06, distributed by Treetools LLC, Portland, OR). Weevils were sexed according to the method of Thomson (1932) and placed into gender-separate screen cages (Model 1450 B BioQuip Products Inc., Gardena, CA) after a 2 wk mating period. Beetles were provided untreated cherry branches (*Prunus cerasus* var. Montmorency) with fruit and foliage in wetted floral foam (OASIS® Smithers-Oasis Co. Kent, OH) as food and oviposition material. When preparing to harvest a unified cohort of eggs, females were provided fresh, undamaged fruit for 24h.

Southern strain plum curculio were from a Michigan State University colony that has been maintained on green thinning apples (modified from Smith 1957). Eggs were harvested from thinning apples in 24 h cohorts.

Chemical Material

A well-plate *in vitro* method was used to determine the ovicidal toxicity of azinphos-methyl (Guthion® 50W, Bayer CropScience, Research Triangle Park, NC), phosmet (Imidan 70-W®, Gowan Co., Yuma, AZ), thiamethoxam (Actara 25WG, Syngenta, Greensboro, NC), thiacloprid (Calypso™ 4F, Bayer CropScience), clothianidin (Clutch™ 50 WDG, Arysta LifeScience North America USA, Cary, NC), acetamiprid (Assail® 30SG, Cerexagri, Inc., King of Prussia, PA), esfenvalerate (Asana® XL, DuPont, Wilmington, DE), indoxacarb (Avaunt® 30 WG, DuPont, Wilmington, DE), chlorantraniliprole (Altacor™, DuPont), pyriproxifen (Esteem® 35WP, Valent Agricultural Products, Walnut Creek, CA) and novaluron (Rimon® 0.83 EC, Chemtura Corporation, Middlebury, CT). Formulated materials were prepared in distilled water with 0.125% (by volume) Latron B-1956® (Rhome and Haas, Philadelphia, PA) as a surfactant; control treatments were water and surfactant only. Initial survey concentrations were prepared at 100, 10, and 1.0 and 0.1 ppm (μg / ml) AI. Initial survey activity (if any) informed the concentrations used for secondary screening for LC_{50} calculations.

Egg Bioassay

The egg toxicity assays were set-up in 96 well cell plates (Corning Inc., Corning, NY). Every other perimeter cell of the plate had 300 μL of distilled water in order to minimize desiccation of the interior experimental cells. This plate setup allowed for six treatments (plus a control) of 10 cells per treatment. A 4mm x 8mm square of Whatman® #1 filter paper (Springfield Mill, Maidstone, Kent, England) was inserted in

each of the interior wells, along with 30 µL of chemical solution (or water control). This initial amount was sufficient to keep eggs hydrated through the incubation period without the need for additions. There was always a set of 8-10 control eggs per replicate to correct for method mortality and variations in incubation conditions.

Eggs were harvested from the fruit using a needle-like probe, forceps, and a dissecting microscope (Model 47 50 61 Carl Zeiss Inc. Oberkochen, Germany), and then placed on the filter paper 2-4 mm above the solution level, one egg per well (Figure 2.1). If eggs ruptured in transfer, a new paper was placed in the well. Although eggs were not directly in contact with the liquid, wicking action of the filter paper was sufficient to keep the eggs hydrated throughout the incubation period (ca. 5 d for untreated eggs). Plates were kept at 22 ± 4°C and 16:8 L:D. and observed twice daily for larval hatch. Hatched larvae were recorded and removed (along with the filter paper that the egg was placed on) daily for 10 days.

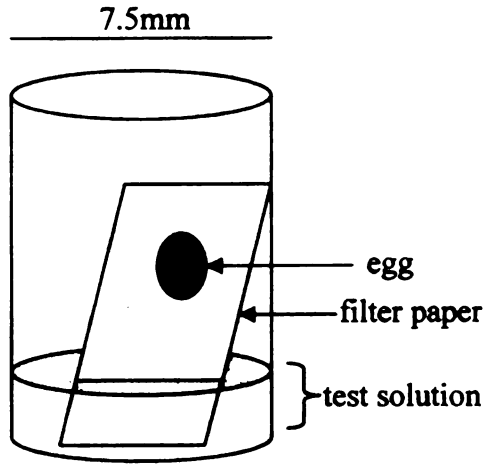

Figure 2.1. Single well of the egg bioassay.

Data Analysis

Egg hatch data were adjusted for untreated mortality (Abbott 1925); replicates in which the controls suffered >30% mortality were excluded from the analysis. LC_{50} values were calculated using PROC PROBIT in SAS (SAS Institute 2006). Confidence limits and slopes of regression lines were also derived from this procedure.

Results

The hatching percentage of the control cells (including replicates that were excluded from LC_{50} analysis) was 86.1 ± 3.0 S.E. ($N = 354$). Egg desiccation was not observed in the controls and normal hatch began 5 d after females were first provided cherries for oviposition. Plum curculio eggs have a relatively soft chorion, and egg rupture during harvesting and transfer was not uncommon. Sharpened forceps were appropriate for peeling back the fruit skin, but blunted metal probes (14 mm length, tapering to 0.25 mm tip) worked the best for the ultimate extraction and transfer to the filter paper.

Azinphos-methyl, esfenvalerate and novaluron were the most toxic to plum curculio eggs of the screened compounds, although eggs were much less sensitive to novaluron (Table 2.1). Phosmet was less active than its sister compound azinphos-methyl. Activities of the neonicotinoids thiacloprid and clothianidin were similar, but neither thiamethoxam nor acetamiprid was active against plum curculio eggs. Neither the oxadiazine indoxacarb, the anthranilic diamide chlorantraniliprole, nor the IGR pyriproxifen reduced egg hatch at the concentrations used.

Discussion

The 96-well plate *in vitro* method was an effective way to incubate eggs. An efficient *in vitro* ovicidal assay is an important tool for evaluating new insecticides for the control of plum curculio. This method had very little control mortality, and is a robust screening technology for ovicides. The well-plate method would be appropriate for any system where eggs are laid inside of plant tissue and can be extracted without

Table 2.1. Toxicity profiles for ten compounds applied to plum curculio eggs. Mortality was determined after 10 d of incubation

Compound	Chemical Class	Log K_{ow}	Field application rate (µg/ml) [b]	n	LC_{50} (in µg/ml) (95% CL)	Slope ± SE	Chi-Square of slope parameter (df = 1)	P- value for slope > 0
Azinphos-methyl	Organophosphate	2.96	1200	186	0.44 (0.27, 0.65)	2.84 ± 0.74	14.67	0.0001
Phosmet [d]	Organophosphate	2.78	2100	183	2.06 (0.37, 4.20)	2.11 ± 0.61	11.9	0.0006
Thiacloprid	Neonicotinoid	1.26	299	274	57.55 (15.27,4802)	0.60 ± 0.15	15.56	<0.0001
Thiamethoxam	Neonicotinoid	-0.13	103	90	11,537 [b]	0.21 ± 0.22	0.95	0.33
Clothianidin	Neonicotinoid	0.7	224	180	32.70 (6.07, 18577)	0.28 ± 0.10	8.13	0.0044
Acetamiprid [d]	Neonicotinoid	0.8	180	90	6259 [b]	0.34 ± 0.30	1.34	0.25
Esfenvalerate [d]	Pyrethroid	6.22	36	218	0.49 (0.26, 0.80)	1.14 ± 0.33	46.81	<0.0001
Chlorantraniliprole [d]	Anthranilic diamide	2.86	131	58	NA [c]	NA [c]	--	--
Indoxacarb	Oxadiazine	4.65	157	32	NA [c]	NA [c]	--	--
Pyriproxifen	Insect growth regulator	5.37	131	104	NA [c]	NA [c]	--	--
Novaluron	Insect growth regulator	4.3	310	170	0.44 (0.13, 1.08)	0.44 ± 0.14	28.69	<0.001

a. Using maximum fruit tree (apple, cherry) labeled AI/acre at 100 gallons (378 L) spray volume;

Not all compounds are currently labeled for application against plum curculio

b. LC_{50} is extrapolated as it is above the upper concentration limit that was tested (100 µg/ ml), confidence limits cannot be estimated

c. Treatments showed no variation nor difference from controls

d. Southern Strain, laboratory-reared beetles used these compounds, all other assays used field-collected northern-strain beetles

damaging the developing embryos. With this technique, field-based efficacy data, residue analyses and baseline toxicity data can be linked to more completely evaluate the potential for targeting eggs with insecticides.

Application and residue data from field applications are required to put these *in vitro* data into context. Labeled application rates for these compounds are shown in Table 2.1. After a field-rate foliar spray, azinphos-methyl was recovered from the outer 2 mm of apple flesh at 1.76 ppm, and this dosage significantly reduced larval emergence from fruit treated after egg hatch (Wise et al. 2007a). Wise et al. (2006) found that the LD_{50} for topical azinphos-methyl exposure to plum curculio adults was 0.16 μg/ beetle. The LC_{95} for azinphos-methyl in the current ovicidal study was 1.68 ppm μg/ ml. Collectively, these life-stage specific studies suggest that azinphos-methyl performance is likely achieved through a combination of adult, egg, and larval activity. Wise et al. (2007a) recovered 0.01 ppm thiacloprid and 0.05 ppm novaluron from the outer 2 mm of apple flesh after treatment with labeled rates of these compounds. These recoveries are markedly less than the LC_{50} concentrations demonstrated for eggs. Thiacloprid did show a curative effect in larval-targeted field-based applications, but no effect was observed with novaluron applications to infested apples (Wise et al. 2007a). Susceptibility to these compounds may depend on the exposed life stage.

It should be noted that insecticide residues inside of fruit that would act as ovicides are transient, and occur early in the season relative to harvest. The reported residues in penetration studies are a result of labeled application protocols, and harvested materials meet the legal thresholds for insecticide residue concentrations.

Despite sharing the same target site and mode of action, the variation in ovicidal action among the tested neonicotinoids is striking. Ovicidal activity of this class against plum curculio correlates well with the octanol-water partitioning coefficient (log K_{OW}) of these compounds. Since the lipid layers of the insect chorion provide a general barrier to hydrophilic (low- log K_{OW}) materials (Smith and Salkeld 1966), compounds like thiamethoxam (log K_{OW} = -0.13) are unlikely to reach target sites within the embryo. Thiacloprid and clothianidin both have positive partitioning coefficients and are therefore better able to move through the chorion. It should be noted that thiamethoxam is a precursor to clothianidin, and is converted to clothianidin in both plants and insects (Nauen et al. 2003). Foliar application of thiamethoxam may provide both a surface residue profile of the parent compound, as well as ovicidal activity of the conversion product after it has penetrated into the plant tissue. Formulated clothianidin is not currently labeled for use in cherry orchards.

The variable ovicidal activity profile across neonicotinoids has been noted in other studies as well. Acetamiprid (log K_{OW} = 0.8) was highly effective against bollworm eggs, while thiamethoxam and imidacloprid (log K_{OW} = 0.57) both showed less activity (Kilpatrick et al. 2005). In multicolored Asian lady beetles, acetamiprid and imidacloprid were both highly toxic to eggs while thiamethoxam had no significant effect (Youn et al. 2003). Plum curculio eggs appear to be unaffected by acetamiprid, despite a "favorable" partitioning coefficient. This may be due to enhanced detoxification of acetamiprid's *cyano* functional group. The cyano-substituted neonicotinoids are less toxic to honeybees relative to the nitro-substituted neonicotinoids (Iwasa et al. 2004).

Partitioning coefficients are not absolute predictors of activity, though. The oxadiazine indoxacarb, and anthranilic diamide chlorantraniliprole are highly lipophilic, but completely inactive against plum curculio eggs. Indoxacarb compound is primarily an ingestion-active material (Wing et al. 2000), so it is not surprising that it does not work against the embryonic stage. Chlorantraniliprole targets the ryanodine receptors and induces the release of intracellular calcium stores (Bloomquist 1996, Cordova et al. 2006). This compound is primarily a lepidopteran-active compound, and ingestion appears to be the primary mode of exposure. Phosmet should also show a strong activity given its lipophillic character ($\log K_{OW} = 2.78$). This compound has an alkaline hydrolysis half-life of 7 h in water solution with a pH of 7.4 (Freed et al. 1992). The trials reported here used water with a pH of 3.8. This likely stabilized the test solutions, but the synaptic target sites are not developed until later in the incubation period (Chapman 1998); this aging period may have lowered the actual exposure concentration to the nervous system target sites relative to what was initially put in the wells.

Comprehensive control of plum curculio in the absence of azinphos-methyl will likely require a suite of tactics and life-stage targets. Although adult control during the growing season will likely remain the mainstay, investigation of alternative avenues are needed to completely understand the impact of field treatments on curculio populations. Curative activity represents one such approach, but it is not appropriate for all of the crops that are susceptible to plum curculio damage. Fresh market commodities must meet high consumer quality demands and oviposition scarring is not acceptable for many consumers. However, processed markets (juices, canned and frozen fruits) do not have

these aesthetic concerns. A curative approach would allow these crops to meet the principal mandate of infestation-free fruit.

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Chapter 3

Using an artificial diet for pesticide assays on plum curculio larvae

Abstract:

A 96-well plate bioassay using pinto bean flour-agar diet was developed to evaluate chemicals for their potential as plum curculio larvicides. Control survivorship for this method was 84% for northern strain plum curculio and 82% for southern strain beetles. Diet concentrations of 1.0 ppm (μg/ ml) azinphos-methyl, phosmet, thiamethoxam, thiacloprid, acetamiprid, esfenvalerate, and novaluron caused significant mortality to plum curculio larvae after 10 d exposure. Concentrations of 0.1 ppm of all of the neonicotinoids were toxic to larvae, but not for the organophosphates. Indoxacarb did significantly kill larvae at 1.0 ppm, but the length of survivors was significantly reduced relative to controls. Pyriproxifen had no observed effects at 1.0 ppm. Data from this type of laboratory assay can be correlated with existing field residue trials to screen compounds for curative potential against internally-feeding pests.

Introduction

Plum curculio, *Conotrachelus nenuphar* (Herbst), is a major pest of pome and stone fruit in Eastern North America. Adults move into orchards in the spring, mate, and lay eggs in developing fruit (Racette et al. 1992). Eggs are laid just under the surface of the fruit skin, and the legless larvae are exclusively internal feeders. Eggs take approximately 5 d to hatch and larvae feed for 10-14 d before leaving the fruit and burrowing into the soil to pupate (Smith 1957, Mampe and Neunzig 1967, Hoffmann et al. 2008).

Larval feeding can induce fruit abortion in apples and peaches and can cause direct crop losses (Levine and Hall 1975). Surface scarring from oviposition can cause indirect losses due to reduced grading of fruit destined for the fresh market. This scarring does not directly impact the processed fruit product, but there is a zero-tolerance for infested processed tart cherries (USDA Agricultural Marketing Service 1941a,b); a single larva at inspection is sufficient to require rejection of an entire harvest load.

Plum curculio management is historically centered on killing adults during the spring and early summer oviposition period. The organophosphate azinphos-methyl (Guthion® 50W, Bayer CropScience, Research Triangle Park, NC) is the primary compound for control, and has excellent contact and residual activity. However, the organophosphate class is heavily scrutinized, and azinphos-methyl is scheduled to be phased out due to Food Quality & Protection Act (1996) regulatory changes. There are compounds in other insecticide classes (neonicotinoids, pyrethroids, oxadiazines) registered for control of plum curculio, but there is not enough knowledge of their key performance characteristics to confidently replace existing organophosphate-based

management programs. Maximizing the effectiveness of these new compounds may guide us to new strategies and tactics in plum curculio integrated pest management, especially for those materials with sublethal effects or compounds like indoxacarb that need to be ingested for optimal performance (Schnepf et al. 1998, Wing et al. 2000, Bravo et al. 2007, Wise et al. 2006, Desneux et al. 2007). Optimization is also economically important given ranges of application rates and the costs of chemicals, equipment, and time.

Previous research suggests that egg and larval stages inside the fruit are potential targets for plum curculio pest management tactics. Post-oviposition curative (eradicant) activity of chlorinated hydrocarbons and early organophosphates was identified in peaches and plums as the orchard industry transitioned away from lead and arsenical pesticides for plum curculio control (Driggers and Darley 1949, Driggers 1950, Smith et al. 1956). The more contemporary organophosphate azinphos-methyl and the neonicotinoids thiamethoxam and thiacloprid also penetrate apple skin and reduce plum curculio larval emergence (Wise et al. 2007a).

Field-based studies provide important efficacy data and residue-mortality corollaries. However, they lack the ability to adequately control pesticide concentrations inside the fruit for toxicology studies. Controlled laboratory studies can assist in identifying compounds that have curative activity prior to investing space and time for field trials. Artificial diets have been useful in assessing the toxicity of many compounds for fruit-feeding insects. Methods for insecticide incorporation vary; surface treatments can be done with liquid aliquots or spray towers (Sauphanor et al. 1998, Ahmad and Hollingworth 2004, Stará and Kocourek 2007), or the treatments can be fully

incorporated into the medium during preparation (Knight et al. 2001, Ioratti et al. 2006, Reuveny and Cohen 2004). A wheat germ and pinto bean-based artificial diet has been described for plum curculio larvae (Yonce et al. 1971, 1973). This diet is similar to the diet used for codling moth rearing (*Cydia pomonella* L.) (Ahmad and Hollingworth 2004). The main differences are that the Yonce diet includes wheat germ and formaldehyde, and lacks a vitamin mixture and Fabco (Bio-serv, Frenchtown, NJ).

A diet adequate for both species would be useful for researchers that are working on both species in the laboratory. This paper describes an artificial diet-based method for screening insecticide compounds for larvicidal activity against plum curculio, and reports responses to compounds currently used in tree fruit protection.

Methods

Insects. Two strains of plum curculio were used for these assays. Reproductive generation Northern strain plum curculio adults were collected by limb jarring at the Michigan State University Trevor Nichols Research Complex in Fennville, MI (42.5951°N, -86.1561°W) and from trap collections in Manistee county, MI. Fennville adults were collected 2 May – 8 June 2007 with a pneumatic limb shaker (Maibo Model ST-7-06, distributed by Treetools LLC, Portland, OR). Manistee county individuals were collected using pyramid traps (Tedders and Wood 1994) in production organic apple and cherry orchards. Southern strain plum curculio were used from a continuous Michigan State University colony that has been maintained on green thinning apples (modified from Smith 1957) with no insecticide selection pressure. Adults aged 1-4 wk post-eclosion were used as source material for southern strain eggs.

For each strain, males and females were kept together for 2 wk to encourage mating. Females were then placed into ventilated plastic containers and provided with green thinning apples for feeding and oviposition; sex determination was done according to Thomson (1932). When preparing to harvest a unified cohort of eggs, females were provided fresh apples for 24 h. Eggs were collected from fruit within 12 h of this oviposition period and incubated on moistened filter paper (Hoffmann et al. 2008).

Chemical Material. The formulated materials used were: azinphos-methyl (Guthion® 50W, Bayer CropScience, Research Triangle Park, NC), phosmet (Imidan 70-W®, Gowan Co., Yuma, AZ), acetamiprid (Assail® 30SG, Cerexagri, King of Prussia, PA), thiamethoxam (Actara® 25WG, Syngenta, Greensboro, NC), thiacloprid (Calypso™ 4F, Bayer CropScience), indoxacarb (Avaunt® 30 WG, DuPont, Wilmington, DE), esfenvalerate (Asana® XL, DuPont, Wilmington, DE), novaluron (Rimon® 0.83 EC, Chemtura Corporation, Middlebury, CT), and pyriproxifen (Esteem® 35WP, Valent Agricultural Products, Walnut Creek, CA). Stock solutions of approximately 100 ppm (μg / ml) AI in distilled water (pH 3.5-4.5) were added to the diet to arrive at the final concentration for the complete diet mixture. Because of limited insect material, northern strain larvae were only tested at 1.0 ppm. Southern strain larvae were tested at 1.0 ppm; tests of additional, lower concentrations were guided by results of northern and southern strain assays.

Larval Bioassay. Assays were performed in sterile 96-well plates (Life Sciences Products, Inc., Frederick, CO) with perimeter wells filled with 300 µl water to reduce desiccation. For each larva, a core of artificial diet (0.3 g) (modified from Ahmad and Hollingworth 2004) was placed in a well of the 96-well plate. On a 100 g basis, the solid components of this diet consisted of 77.1 g pinto bean flour, 11.6 g brewer's yeast, 7.2 g agar, 1.4 g vitamins (Vanderzant mix), 1.2 g ascorbic acid, 0.7 g methyl paraben, 0.4 g sorbic acid, and 0.4 g Fabco mold inhibitor. These materials (except pinto beans) were purchased from Bio-Serv (Frenchtown, NJ). Dry materials were mixed into 181 ml water; agar was boiled in 130.3 ml water until thickened. Solid-water and agar-water mixes were combined and mixed vigorously for 30 s. The total mixture was poured to fill the bottom of a 100 mm diam x 15 mm depth petri dish and allowed to set. Once the mixture set and cooled, a 6 mm ID (#3) stainless steel cork borer was used to cut cores out of the diet and place them into wells in a sterile 96-well plate. Two holes were pressed with a probe as starter tunnels for larvae. For insecticide-treated diet, stock insecticide solutions were prepared separately and added to the diet solid-water mixture prior to the agar addition. Spiked-diet concentrations were calculated as the active ingredient proportion of the total mass for all ingredients.

Within 12 h of hatching, neonates were transferred with a blunted probe to the surface of the diet – one per well of the 96-well plate. If a larva had not initiated tunneling after 30 min, it was replaced with a fresh individual. Each replicate had 8-20 larvae, each treatment dosage had at least three replicates. A control replicate was run in parallel with each treatment replicate. After 10 d, larvae were recovered from the diet using probes and a dissecting microscope. Larval survivorship was recorded and length

was measured to the nearest mm. Larvae were considered dead if they did not move in response to being probed with forceps. Larvae that escaped or could not be found in the diet were not considered in the analysis.

Survivorship of individual treatments to their parallel controls was compared using PROC MIXED in SAS (SAS Institute, 2006). Percent survivorship was arcsin square-root transformed prior to analysis. For treatments with larval survivorship, length of recovered live larvae was compared to the length of larvae in the parallel control using PROC GLM.

Results

Control survivorship for northern and southern strains were 84.1 and 81.9 percent, respectively. Mean larval length for northern and southern strains after 10 d was 7.4 and 7.3 mm, respectively. The current standard control compounds phosmet and azinphos-methyl were significantly toxic at 1.0 ppm (Table 3.1). However, survivorship for larvae exposed to these organophosphate compounds at 0.1 ppm was not different than the controls. The length of surviving larvae for phosmet (0.1 or 1.0 ppm) or azinphos-methyl (1.0 ppm) was also not different from the controls (Table 3.1).

There were no survivors at the 1.0 ppm exposure level for the neonicotinoids thiacloprid, acetamiprid and thiamethoxam or the pyrethroid esfenvalerate. Thiamethoxam and esfenvalerate also caused 100% mortality at 0.1 ppm. There were survivors of thiacloprid and acetamiprid treatments at 0.1 ppm, but this survivorship was still significantly less than that of the controls ($P < 0.01$ for each treatment) (Table 3.1). Thiacloprid and acetamiprid survivors were significantly smaller than the controls ($P <$

Table 3.1. Northern and southern strain Plum curculio larval survivorship and larval lengths after 10 d exposure to insecticide-incorporated diet.

			Percent Survivorship (by replicates)				Survivor Length (mm) (by individual)			
Plum Curculio strain	Compound	ppm	n [a.]	Treatment Mean (± SE)	Control Mean (± SE)	ANOVA P – value (df_{num}, df_{den})	n [b.]	Treatment Mean (± SE)	Control Mean (± SE)	ANOVA P – value (df_{num}, df_{den}) [c.]
Northern	Control (overall)		14		84.1 (3.7)		127		7.39 (0.14)	
	Azinphos-methyl	1.0	3	0 (0)	85.7 (9.8)	0.0194 (1, 2)				
	Phosmet	1.0	3	12.7 (0.9)	87.5 (12.5)	0.0279 (1, 2.06)	3	5.67 (0.33)	5.93 (0.44)	0.7971 (1, 16)
	Thiamethoxam	1.0	3	0 (0)	74.0 (4.0)	0.0272 (1, 1)				
	Thiacloprid	1.0	3	0 (0)	96.3 (3.7)	0.0060 (1, 2)				
	Esfenvalerate	1.0	3	0 (0)	71.3 (5.8)	0.0529 (1, 1)				
	Indoxacarb	1.0	3	43.5 (10.5)	85.0 (10)	0.0596 (1, 1)	18	2.28 (0.23)	8.23 (0.22)	<0.0001 (1, 37.1)
Southern	Control (overall)		34		81.9 (2.7)		212		7.30 (0.09)	
	Azinphos-methyl	0.1	2	79.0 (1.0)	80.5 (9.5)	0.8241 (1,1)	15	6.60 (0.36)	7.43 (0.23)	0.0676 (1, 27)
	Phosmet	0.1	3	75.3 (2.6)	84.0 (11.0)	0.4163 (1, 2)	19	7.11 (0.34)	7.15 (0.22)	0.9054 (1,37)
		1.0	3	4.7 (4.7)	70.3 (9.4)	0.0355 (1, 2)	1	7.0 (0)	7.4 (0.75)	0.8379 (1, 4)
	Thiamethoxam	0.1	3	0 (0)	82.0 (9.1)	0.0050 (1, 2)				
	Thiacloprid	0.1	3	9.0 (4.5)	73.0 (17.0)	0.0003 (1, 2)	2	2 (0)	7.8 (0.2)	< 0.0001 (1, 9)
	Acetamiprid	0.1	6	32.2 (8.8)	85.2 (7.4)	0.0071 (1, 6)	13	4.1 (0.56)	7.0 (0.36)	< 0.0001 (1, 34.4)
		1.0	3	0 (0)	82.0 (9.1)	0.0219 (1, 2)				
	Esfenvalerate	0.1	4	0 (0)	94.3 (5.7)	0.0011 (1, 2)				
	Indoxacarb	0.1	3	38.3 (9.6)	83.3 (6.2)	0.0166 (1, 2)	9	5.22 (0.52)	7.14 (0.38)	0.0089 (1, 29)
	Pyriproxifen	1.0	3	96.0 (4.0)	73.3 (9.0)	0.0083 (1, 2)	21	7.47 (0.30)	7.61 (0.24)	0.8191 (1, 35.2)
	Novaluron	0.25	3	14.3 (14.3)	85.7 (14.3)	0.0441 (1, 2)	3	3.0 (0.58)	7.75 (0.25)	< 0.0001 (1, 9)
		1.0	4	0 (0)	94.3 (5.7)	0.0011 (1, 2)				

[a.] Number of replicates for each treatment – dose combination. Some treatments were done on the same day and shared untreated control replicates.

[b.] Number of live individual larvae that were assessed for length

[c.] ANOVA for treatment-control comparison. Degrees of freedom (numerator, denominator) have been adjusted using Satterthwait's approximation for experiments that had incomplete pairing of treatment-control replicates.

0.0001 for thiacloprid 0.1 ppm; $P < 0.0001$ for acetamiprid 0.1 ppm). Larvae surviving 0.1 ppm thiacloprid were 2 mm in length and those surviving 0.1 ppm acetamiprid were 4.1 mm long; survivors from the respective controls averaged 7.8 and 7.0 mm in length (Table 3.1). Some of the survivors of the neonicotinoid treatments showed obvious signs of poisoning – uncoordinated movement when placed on a flat surface and persistent mandibular tremors.

Indoxacarb did not cause significant mortality at 1.0 ppm ($P = 0.0596$), but the significant difference between treated and untreated larval length ($P < 0.0001$) prompted an additional test at 0.1 ppm (Table 3.1). There was a significant effect on survivorship at this lower rate ($P = 0.0166$), as well as a significant reduction in final larval size relative to the controls ($P = 0.0089$). Live recovered larvae exposed to 1.0 ppm averaged only 2.3 mm in length , while those in the 0.1 ppm treatments were 5.2 mm; respective controls averaged 8.23 and 7.14 mm in length. The lack of a survivorship effect for the 1.0 ppm treatment is probably due to low power; more replication might provide better variance estimates and improve the power to separate means.

The insect growth regulators had varied effects. Larval survivorship after exposure to 1.0 ppm pyriproxifen (a juvenile hormone mimic) was actually significantly greater than that of the controls, although the length of survivors did not differ. There were no survivors of exposure to novaluron (a chitin synthesis inhibitor) at 1.0 ppm and survivorship was significantly reduced to 14.3% ($P = 0.0441$) at 0.25 ppm (Table 3.1). The average length of novaluron survivors was only 3 mm, compared to 7.8 mm for the controls. One of the recovered dead larvae had incompletely molted prior to dying and had two head capsules.

Discussion

There have been successes in rearing of plum curculio (oviposition to larval emergence) on artificial diet (Yonce et al. 1971, 1973), but thinning apples remain the standard for rearing this insect in colony. Preliminary evidence suggests that larvae are able to pupate successfully after being reared on the diet reported in this study, but the goal of this study was to identify an appropriate substrate for short-term comparative toxicity assays. There is evidence that using artificial diet for complete rearing of plum curculio induces colony-level changes in metabolism; there was a dramatic increase in survivorship to pupation after five generations of rearing on artificial diet (Yonce et al. 1973). These changes in ability to use food resources might also translate into life histories and bioassay responses that do not represent those of a beetle with a genetic history of being raised on actual fruit.

The diet reported here has efficiency benefits, since it is already being used for the maintenance and resistance profiling of codling moth colonies. The materials for this assay are also readily available and relatively inexpensive, but there are a few comments that need to be made regarding the 96-well plates. While they were generally easy to work with, the plates suffered a few drawbacks: drying out along the outer cells (even with water in the border cells) and escaping larvae. A possible improvement would be to use bioassay trays with adhesive lids that separated each cell (such as Bio-serv BAC128). These would eliminate the possibility of escaping larvae and probably minimize moisture loss, or at least make it more uniform across all of the cells. Moisture loss could be further reduced by placing these trays in a humidified growth chamber or over a water bath.

Targeting immature life stages may become an important part of future plum curculio management. Plum curculio eggs are susceptible to a number of insecticides, including the neonicotinoid thiacloprid, the organophosphates azinphos-methyl and phosmet, and the pyrethroid esfenvalerate (Hoffmann et al. 2008). Using compounds as part of a curative approach to plum curculio management requires the pesticide compounds to penetrate the fruit cuticle and move into the flesh of the fruit. Insecticide residues have been reported in early-season apple fruits for a number of registered insecticides. One day after being sprayed with a labeled rate of formulated compound, both azinphos-methyl and thiamethoxam were recovered from the outer 2 mm of apple flesh at concentrations (1.76 and 0.1 ppm, respectively) that were 100% lethal to plum curculio larvae in the artificial diet study reported here (Wise et al. 2007a). Thiacloprid was highly lethal to larvae in artificial diet at 0.1 ppm and in curative activity field studies this compound significantly reduced plum curculio larval emergence from infested fruit at a maximum apple flesh concentration of 0.01 ppm. The maximum indoxacarb residue in the Wise et al. (2007a) study of apples was 0.19 ppm, and there was no difference in larval emergence between indoxacarb-treated and untreated apples. This corresponds well with the lack of lethality in this study's 0.1 ppm indoxacarb.

Northern strain larvae had a higher survivorship rate at 1.0 ppm than southern strain larvae at the same concentration.

Novaluron's effectiveness against larval plum curculio in the laboratory is similar to that observed in other larval bioassays. There were no survivors of third-instar red flour beetle *Tribolium castaneum* (Herbst) at 1.0 ppm applications (Kostyokovsky and Trostanestsky 2006). It has an LC_{90} of 0.54 ppm in foliar applications against the

Egyptian armyworm *Spodoptera littoralis* (Boisduval) (Ishaaya et al. 2003), and 56% of a laboratory colony of codling moth (*Cydia pomonella* L.) died after feeding on diet mixed with 1.0 ppm novaluron (Reuveny and Cohen 2004). The current formulation of novaluron (Rimon 0.83EC) has not proven effective in plum curculio field-based curative trials in apples. While application rates exceed 300 ppm, Wise et al. (2007a) recovered a maximum of only 0.07 ppm from the interior flesh of apples; less than the lowest dose tested in the laboratory. It is likely that novaluron's limited curative activity is due to insufficient penetration into the flesh of the fruit.

Pyriproxifen was not acutely lethal to plum curculio in this assay, but there may be sublethal developmental effects that would only be apparent if larvae were given complete rearing conditions. In the following chapter, I describe how plum curculio larvae emerging from pyriproxifen-treated cherries were significantly heavier than those emerging from untreated fruit.

Surviving, but developmentally delayed, larvae inside of the fruit may be an important consideration for control choices. Larvae surviving indoxacarb treatment in this study were significantly undersized after 10 d. Larvae in field exposures may have a long development time after indoxacarb exposure and use of this compound could result in unexpected infestation at harvest time. Alternatively, the undersized larvae may die as a result of the chronic exposure and be unobservable at inspection. These implications are important and additional experiments need to be done to verify the actual outcomes for this unique compound.

This study underscores the comprehensive activity of azinphos-methyl against plum curculio. While the adulticidal effects are well known, it is likely that this

compound has also been killing eggs and larvae inside of tree fruit. Post-organophosphate integrated pest management will need to take this multi-stage action into consideration. Achieving azinphos-methyl's level of control will probably require a treatment program that shares azinphos-methyl's breadth of life stage targets. This study suggests that thiacloprid, thiamethoxam, acetamiprid, novaluron, and esfenvalerate would be good candidate compounds for a larval-targeted curative strategy if they were properly timed, and actually penetrate sufficiently into fruit tissue.

This approach does not prevent cosmetic injury to the fruits, and is best suited for use in commodities where there is a minimal economic cost to surface damage to the fruit. Processed fruits, like peaches and tart cherries, are primarily concerned with flesh quality, and surface damage is secondary. The curative strategy is not strictly limited to processed fruits. Plum curculio causes fruit abscission in apples during the time of normal thinning (Levine and Hall 1977), and these dropped fruit contribute to maintaining local plum curculio populations. Even though curative sprays (pre- or post June drop) do not prevent cosmetic fruit injury, they will support the control of future plum curculio populations.

This larval assay technique, along with ovicidal assays (Hoffmann et al. 2008) provide useful tools for dormant-season screening and prioritization of new insecticides. In addition, these procedures may also provide tools for resistance monitoring. Plum curculio are being increasingly exposed to neonicotinoids as part of fruit pest management programs, either incidentally or as the actual target organism. Persistent exposure to any single mode of action represents a high-risk scenario for resistance development (Denholm and Rowland 1992). Plum curculio did not develop

organophosphate resistance over the 50 years of organophosphate pressure, but they may possess the latent genetic potential for neonicotinoid resistance.

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Chapter 4

Curative activity of insecticides against plum curculio in tart cherry

Abstract

Tart cherry branches were infested with plum curculio eggs and treated with insecticides to target large larvae, neonates, and eggs. The organophosphates azinphos-methyl and phosmet and the neonicotinoid thiamethoxam reduced larval emergence rates by over 90% for all targets. Few surviving larvae were found inside fruit after over 30 days. Acetamiprid and thiacloprid also had curative activity, and yielded greater than 75% reductions in emergence and few surviving larvae after 30 days. Pyriproxyfen reduced larval emergence, but 66% of fruit treated to target late-instars still had live larvae after 30 days. Novaluron, chlorantraniliprole and esfenvalerate had no curative activity. Indoxacarb had some curative activity, but all targeted life stages had larval emergence. Internal and external residues were analyzed for these compounds and help define the penetration and curative potential of these materials. The unlabeled curative activity of azinphos-methyl has played an important but unexplored role in meeting federal standards for infestation-free tart cherries at processing. As this compound is phased out, new integrated pest management programs for this pest will need to address the loss of azinphos-methyl's curative activity.

Introduction

Plum curculio (*Conotrachelus nenuphar* Herbst) is a major pest of commercial tree fruit in eastern North America. Northern strain adults emerge from overwintering prior to fruit set, mate, and begin ovipositing in apple, cherry and peach fruit as soon as the fruit begin expanding (Racette et al. 1992, Lafleur and Hill 1987, Hoffmann et al. 2004) Larvae develop in the flesh of the fruit for 2-3 wk and drop into the soil to pupate. Adults emerge in August, feed and enter an obligate diapause in adjacent woodlots and covered areas (Smith and Flessel 1968, Lafleur et al. 1987, Racette et al. 1992). Southern strain beetles have overlapping generations in the field, and have a facultative winter diapause (Stearns 1931, Chapman 1938).

Plum curculio take approximately three weeks at typical field temperatures to complete development after eggs are laid (Smith 1957, Lan et al. 2004). The specific developmental thresholds and degree day requirements for southern strain plum curculio combined egg and larval development are $215.5DD_{11.1°C}$ (Lan et al. 2004). The thresholds for egg hatch have not been developed, but it is estimated to be three days at 80°F for southern strain beetles (Smith 1957).

Processed tart cherries are under strict regulatory guidelines for infestation-free fruit at harvest (USDA Agricultural Marketing Service 1941a, b). Tart cherries are a valuable U.S. specialty crop, with $50-80 million in national production value (NASS 2006, 2008). Over 75% of this production value comes from eastern states with economically important plum curculio populations. In most settings, this weevil has been managed by adult-targeted foliar organophosphate sprays during the oviposition period. Foliar sprays of the organophosphates azinphos-methyl (Guthion® 50W, Bayer

CropScience, Research Triangle Park, NC), and phosmet (Imidan® 70-W, Gowan Company, Yuma, AZ) have been used since the 1950s (Forsythe and Rings 1965, Smith and Fiori 1959). The compounds provide excellent fruit protection in tart cherries and other tree fruit, with low levels of resultant surface damage and oviposition scarring.

Many agricultural uses of organophosphates such as chlorpyrophos and methyl parathion, have been phased out as a result of the Food Quality and Protection Act of 1996 (FQPA). The current regulatory framework has set the final phase out date for the remaining azinphos-methyl uses at 2012 (US EPA 2006). Phosmet has met current tart cherry regulatory requirements with extended reentry intervals (REI) and pre-harvest intervals (PHI). Phosmet still fits a tart cherry curculio management program with these use updates, but the next FQPA review cycle may impose additional limits that preclude its utility as an effective product. Phosmet cannot be used in sweet cherries because of phytotoxicity issues.

In the absence of organophosphates, the near-term chemical controls for plum curculio control are likely to come from the neonicotinoid, oxadiazine, pyrethroid and insect growth regulator classes. Despite the EPA designation of many of these compounds as "OP replacement" and good field performance data, these compounds are different in terms of mode of action and pest spectrum than the organophosphates (Wise and Gut 2004, Wise et al. 2006).

Reduced oviposition injury is a proxy for curculio control, but it is not the only way to manage plum curculio populations or actual economic impact in tree fruit. The neonicotinoids thiacloprid and thiamethoxam have demonstrated post-oviposition curative activity against larval plum curculio in apples (Wise et al. 2007a). This type of

curative (or "eradicant") activity was also noted in the 1950s, as the chlorinated hydrocarbons and early organophosphates were being intensely evaluated as replacements for arsenic-based insecticides. Sprays of dieldrin, parathion or EPN killed cherry fruit fly maggots inside of host fruit (Sherman 1951, Cox 1952, Frick and Simkover 1953) and parathion, EPN, dieldrin, and benzene hexachloride (BHC, or hexachlorohexane- HCH) similarly reduced larval plum curculio emergence after application to infested prunes (Cox 1949, 1951; Smith et al. 1956). Similar results were seen in peaches; BHC and parathion were effective curative agents for plum curculio (Driggers and Darley 1949, Bobb 1950, Driggers 1950), codling moth (*Cydia pomonella* L.) and oriental fruit moth (*Grapholita molesta* Busck) (Driggers 1950).

As tart cherry pest management transitions away from the most potent adulticidal compounds, it is once again important to look at the entire suite of life stage targets that are afforded by current and upcoming pest management tools. Many of these compounds have been evaluated in the laboratory for their direct effects on plum curculio eggs (Hoffmann et al. 2008) and larvae. While laboratory experiments may demonstrate activity, these findings do not equate to field performance. The complete plant-insect-chemical linkage (Wise et al. 2007a) is what governs field efficacy, and this cannot be fully simulated in the laboratory.

This chapter reports on the field efficacy and residue profiles of several crop protection compounds used as curative agents against plum curculio. The general classes include the organophosphates, neonicotinoids, oxadiazines, pyrethroids, insect growth regulators and anthranilic diamides. Targeted life stages include eggs, hatchling larvae and late instars still in the cherry fruit on the tree.

Materials and Methods

Study location & Plant material. Caged field trials in 2006 were done at the Trevor Nichols Research Complex (TNRC) in Fennville, MI. 2007 trials were done at the Clarksville Horticultural Experiment Station (CHES) in Clarksville, MI and the Northwest Horticultural Research Station (NWHRS) in Traverse City, MI. At each of these research stations, limb sleeve trials were conducted on Montmorency tart cherry trees (*Prunus cerasus* var. Montmorency) that had been bearing for at least 5 years.

Insect Material. There were three study periods for this research, 2005, 2006 and 2007. For all periods, northern strain plum curculio were collected cherry and apple orchards at the Trevor Nichols Research Complex in Fennville, MI using commercially-available pyramid traps (Tedders and Wood, 1994) or by limb jarring onto tarps. Collections were made in April and May during warm evenings (> 10°C) with calm winds. Beetles were held together for one week after field collection to ensure that females were mated. Weevils were sexed according to the method of Thomson (1932) and placed into gender-separate screen cages (Model 1450 B BioQuip Products Inc., Gardena, CA) lined with paper towels. Cages were kept outside in an effort to keep the beetles synchronized with the orchard phenology. Beetles were provided untreated cherry branches (*Prunus cerasus* var. Montmorency) with fruit and foliage in wetted floral foam (OASIS® Smithers-Oasis Co. Kent, OH) for food. Plant material in cages was replaced every two to three days, and water was added daily. Females were given only water and foliage for 24 h prior to being placed on trees in sleeve cages.

Chemical material. Formulated compounds from several insecticide classes were prepared in 500 ml batches and placed in spray bottles immediately prior to spraying in the field (Table 4.1). Concentrations applied were labeled field rates applied at 100 gallons per acre (935 L/ Ha). Latron B 1956® (a spreader-sticker, Loveland Industries, Inc., Greeley CO) was added to all sprays at 0.125 ml per L spray volume.

General methods. To generate uniform cohorts of eggs on fruit, female plum curculio were placed in sleeve cages on untreated branches that had at least 30 cherry fruit on them. Polyester netting (0.8 mm mosquito netting, American Home & Habitat Inc., Squires, MO) was used to make the cylindrical limb sleeve cages (53.3 cm diam, 91.4 cm length). The cages had a drawstring on one end to close tightly around the proximal end of the tree branch; the distal end was folded several times and closed with large binder clips (ACCO brands Lincolnshire, IL). Females were allowed 48 – 96 h to oviposit on the fruit, after which time the female plum curculio (and any other insects) were removed from each sleeve cage. Sleeve cages remained sealed on the branches after oviposition to prevent additional damage. Each sleeve cage was considered an experimental replicate.

Treatments were applied using a 500 ml industrial spray bottle, and fruit and foliage were sprayed to drip (usually around 150 ml/ branch). Fruit was allowed to remain on the trees until larvae in sentinel untreated cherries neared maturity. Damaged fruit was brought back to the laboratory and placed individually into 1 oz (29.6 ml) rearing cups with lids (Bio-Serve, Frenchtown, NJ). Lids were perforated four times with a probe to provide ventilation. Fruit from the same branch were placed together in 30-cup trays (Bio-Serve, Frenchtown, NJ). Rearing cups were observed twice daily for

Table 4.1. Formulated compounds and concentrations used for curative sprays. All preparations were based on 935 L/Ha spray volume (100 gallons/ acre).

Formulated name	Chemical Class	Active ingredient	Company	Formulated Rate / acre	g AI / Ha	Active Ingredient Concentration (µg / ml)	Life stage target
Guthion® 50WP	Organophosphate	Azinphos-Methyl	Bayer CropScience Triangle Park, NC	2 lbs	1120	1200	Large larvae, neonate, egg
Imidan® 70W	Organophosphate	Phosmet	Gowan® Company Yuma, AZ	2.5 lbs	1967	2100	Neonate
Actara® 25WG	Neonicotinoid	Thiamethoxam	Syngenta Crop Protection, Inc. Greensboro, NC	4.5 oz	79	84	Large larvae, neonate, egg
Calypso™ 4F	Neonicotinoid	Thiacloprid	Bayer CropScience Triango Park, NC	4.5 fl oz	158	168	Large larvae, neonate, egg
Assail® 30SG	Neonicotinoid	Acetamiprid	Cerexagri-Nisso LCC. King of Prussia, PA	5 oz	105	112	Large larvae, neonate, egg
Avaunt®	Oxadiazine	Indoxacarb	I.E. du Pont De Nemours, and Co. Wilmington, DE	6 oz	126	135	Large larvae, neonate, egg
Asana®XL	Pyrethroid	Esfenvalerate	I.E. du Pont De Nemours, and Co. Wilmington, DE	12.8 fl oz	74	79	Neonate
Rimon® 0.83EC	Benzoylphenyl urea	Novaluron	Makhteshim Agan of North America, Inc. NY	20 fl oz	145	155	Large larvae, neonate, egg
Esteem® 35WP	Juvenile Hormone Mimic	Pyriproxyfen	Valent U.S.A. Corporation Walnut Creek, CA	5 oz	122	131	Large larvae, neonate, egg
Altacor™ WG	Anthranilic diamide	Chlorantraniliprole	I.E. du Pont De Nemours, and Co. Wilmington, DE	5 oz	122	131	Neonate

larval emergence and emerged larvae were weighed on a Mettler AE 50 analytical balance (Mettler-Toledo, Inc., Columbus OH). After 2-3 wk, cherries were beginning to desiccate, and remaining fruit were dissected for presence of alive and dead larvae. In 2006, live larvae were weighed on an analytical balance and in 2007 they were measured (length) with a ruler.

Statistical Analysis. Proportions of larvae emerged and remaining in the cherry were arcsine-square root transformed and analyzed using PROC MIXED in SAS (SAS Institute 2006). Tukey's HSD adjustment was used for multiple comparisons, and Dunnett's test was used for least-squared means comparisons to the untreated control. Mean masses or lengths per replicate (sleeve) were analyzed using PROC MIXED and mean separations were adjusted using Tukey's HSD.

Residue collection and analysis. Undamaged fruit were collected from sprayed branches 24 h after chemical application and frozen for residue analysis. Fruit from 2006 were dissected in a -20°C cold room to separate the skin, outer 1 mm layer of flesh and next 1 mm flesh. Frozen fruit were cut in half and a 2 mm cork borer was used to cut cores from the inside of the cherry out through the skin, and sections were cut with a razor blade. Approximately 0.5 g material was dissected for each section. After sectioning, samples were held in 10 ml dichloromethane at -20°C until laboratory workup.

The fruit residues for 2007 were analyzed as surface vs. interior fractions using a sonication and homogenization technique (Wise et al. 2007a). After fruit were collected and frozen, they were weighed (approximately 10 g per sample) and sonicated for 30 s in

60 ml acetonitrile. This material was collected and anther 25 ml volume of acetonitrile was used to rinse the vial. The remaining fruit was placed into 60 ml dichloromethane; both the acetonitrile and dichloromethane fractions were stored at -20°C until laboratory workup.

Dichloromethane and fruit samples were homogenized (Model Pro200, Procienctific Inc., Monroe, CT), rinsed with dichloromethane (3 x 20 ml) and run through a sodium sulfate column to remove water. The column was rinsed with two volumes of 20 ml dichloromethane. The collected dichloromethane was reduced to 2 ml volume placed in a 2.5 ml gas chromatography vial. Acetonitrile fractions were also passed through sodium sulfate and reduced to 2 ml by rotary evaporation.

Thiamethoxam and thiacloprid residues were determined using a Waters 2690 Separator Module HPLC, with a Waters 2487 dual-wavelength absorbance detector. The column was a C18 reversed-phase column with 4.6 mm bore and 5 mm particle size. Flow rates were set at 1 ml / min. For thiamethoxam, the mobile phase started at 90:10 water:acetic acid (0.1%) in acetonitrile and reduced to 70:30 between 12 and 13 min at 35°C. The detector for thiamethoxam was set at 255 nm. For thiacloprid, the mobile phase started as a 30:70 ratio of 0.4 ml HCl (35%) in water : acetonitrile and ramped to 25:75 at 4 min and 10:90 between 4 and 9 min. The mobile phase was brought back to 30:70 at 13 min. The detector was set at 242 nm for thiacloprid.

Gas chromatography was used for azinphos-methyl, phosmet, acetamiprid, indoxacarb, novaluron, pyriproxyfen, chlorantraniliprole and esfenvalerate. The equipment used was an Agilent 6890 gas chromatograph with a 5973N Mass Spectra Detector. The column was a Zebron ZB-5ms 30 m, 0.25 mm I.D. with 0.25µm film

thickness. The oven temperature program was: 5 min at 115°C, ramp of 9°C / min to 280°C, ramp of 30°C/ min to 310°C. The inlet was kept in pulsed splitless mode at 200°C, with 78324 PA and a pulse pressure of 103421 Pa. The purge flow (helium) was 50 ml/ min. The mass detector was set to scan at a minimum of 28 Da up to the maximum molecular mass of the molecule of interest.

Areas under the chromatographic curve for the compounds of interest were integrated. Standard curves and initial sample masses were used to determine ppm recoveries (μg analyte / g sample).

Assay setup

2006. Treatments in were timed to target late-instar, neonate and egg stages of plum curculio. All three timings were assessed with three replicates each of six treatments and a control. The treatments were azinphos-methyl, thiamethoxam, thiacloprid, indoxacarb, pyriproxyfen and novaluron.

Large larval target. To assess the susceptibility of large larvae to curative treatments, we allowed a cohort of larvae to grow on the tree prior to any foliar insecticide applications. Six females were placed in each of 23 sleeve cages on 1 June 2006. Weather over the next few days was cool, so females were allowed 96 h to oviposit before being removed from the branches (93% recovery). Larvae were allowed to develop in fruit without treatment until June 19 (137.2 $DD_{10°C}$, 279 $DD_{50°F}$ after oviposition began), when each of the seven treatments (Table 4.1) was randomly applied to three branches. The remaining untreated branches were checked daily to determine the developmental stage

of untreated eggs and larvae. At the time of spraying, larvae in sentinel sleeved cherries were 5-6 mm in length and easily visible (mature larvae are approximately 9 mm). Cherries were removed from trees on 22 June 2006 and stung fruit were placed individually in rearing cups. At this time, larvae in sentinel cherries were 5-8 mm in length and none had emerged in the sleeve cages. After 14 d, cherries were beginning to dry down significantly, and remaining fruit were dissected for presence of larvae.

Neonate target. Neonate susceptibility to curative applications was tested by applying foliar insecticides immediately after egg hatch. On 13 June 2006, seven females were placed in each of 23 limb screens. On 15 June, females were removed (94% recovery). Sentinel control cherries showed >75% larval hatch on 19 June (56.7 $DD_{10°C}$, 134 $DD_{50°F}$ after oviposition began), and cherries were sprayed that day. Stung cherries were brought back into the lab on 30 June and placed in rearing cups. After 20 d, cherries were dissected for the presence of larvae.

Egg target. On 23 June 2006, four females were placed into each of 24 screens and allowed to oviposit for 3 d. Sprays targeting eggs were applied 3 h after females were removed (90% recovery) from the branches on 26 June (7.8 $DD_{10°C}$, 46 $DD_{50°F}$ after oviposition began). Fruit were harvested from the trees on 10 July. Larval emergence was monitored until July 26, when all cherries were dissected for unemerged larvae.

2007. Studies in 2007 were targeted at neonates and designed to evaluate additional insecticide products. Due to a late spring freeze, there was limited availability of fruit statewide. CHES and NWHRS were less impacted than the TNRC facility.

CHES neonate target. On 30 May 2007, six females were placed on each of 16 branches for 24 h of oviposition. Almost all (95%) of the females were recovered and eggs were allowed to hatch before being sprayed on 6 June 2007 (53.3 $DD_{10°C}$, 128 $DD_{50°F}$ after oviposition began). There were five replicates of acetamiprid, four replicates of esfenvalerate, and seven untreated branches. Fruit were removed from the tree on 15 June and observed daily for larval emergence. Cherries were dissected for remaining larvae on 24 July 2007.

NWHRS neonate target. On 7 June 2007, six females were placed on each of 18 branches for 24 h of oviposition. Only three females were not recovered. Eggs were allowed to hatch, and branches were sprayed on 12 June 2007 (54.4 $DD_{10°C}$, 130 $DD_{50°F}$ after oviposition began). There were four replicates of chlorantraniliprole, three replicates of acetamiprid, three of esfenvalerate, four replicates of phosmet and four untreated branches. Fruit were removed from the tree on 20 June and observed daily for larval emergence. Cherries were dissected for remaining larvae on 26 July 2007.

Results

2006.

Large larval target. The number of damaged cherries per limb cage ranged from 42 to 93, but there was no significant difference in the number of stings (81.1 ± 3.48 SEM) or stings per cherry (1.37 ± 0.04 SEM) between the treatment branches. The emergence rate for larvae was 302 larvae from 1258 damaged cherries. The first larva emerged two days after cherries were harvested from the trees.

The untreated control had 80.7 ± 8.6 SEM percent larval emergence on a per-cherry basis. There was a significant difference in the proportion of emerged larvae across treatments ($F = 38.69$; d.f. = 6, 14; $P < 0.0001$). Cherries treated with novaluron showed no difference in total larval emergence rate relative to the controls, but all other treatments had significant reductions from the untreated control (Figure 4.1). Average emergence rates were under 20% for indoxacarb, and under 5% for the remaining treatments. Only two larvae emerged from 195 azinphos-methyl-treated cherries.

The masses of emerged larvae varied significantly ($F = 4.72$; d.f. = 6, 9; $P = 0.0191$); larvae emerging from the pyriproxifen treatment were the most massive (0.023 ± 0.004 g); larvae from untreated cherries averaged 0.0168 ± 0.0003 g (Figure 4.2). The heaviest pyriproxyfen-exposed emerged larva was 0.0370 g. Larvae emerging from the thiamethoxam and azinphos-methyl treatments had the lowest masses at 0.010 and 0.006 g, respectively.

When cherries were dissected after 14 d, 66% (± 2.0 SEM) of the pyriproxifen-treated cherries still had live larvae inside of them. This internal infestation rate was significantly greater than that of the controls (7.5 ± 3.8 larvae per cherry) ($F = 13.08$; d.f.

= 6, 14; $P < 0.0001$). None of the live-larvae infestation rates for the other treatments were different from the untreated control, although they were lower than that of pyriproxyfen (Figure 4.3). Masses of live larvae that were still inside of the cherries after 14 d varied significantly by treatment (F = 4.96; d.f. = 6, 9; $P = 0.0212$), with those recovered from the pyriproxyfen treatments being the heaviest at 0.019 ± 0.001 g (Figure 4.4).

Dead larvae were found inside of the cherries. Thiamethoxam-treated cherries yielded 100 dead larvae from 221 fruit. These larvae were dry, 5 to 7 mm long and typically flattened against the pit of the fruit. Less than ten larvae were found in other treatments, no dead larvae were found in the pyriproxyfen treatments.

Neonate target. The number of damaged cherries per limb cage ranged from 29 to 79, but there was no significant difference in the number of stings (61.9 ± 5.2 SEM) or stings per cherry (1.42 ± 0.06 SEM) between the treatment branches. The overall emergence rate for larvae was 262 larvae from 900 damaged cherries. A single larva had emerged in a limb screen on the day of harvest.

The untreated control had 72.5 ± 8.1 SEM percent larval emergence rate. There was a significant difference in the proportion of emerged larvae across treatments (F = 23.15; d.f. = 6, 14; $P < 0.0001$). Cherries treated with novaluron or pyriproxyfen showed no difference in larval emergence rate, but all other treatments had significant reductions from the untreated control (Figure 4.5). There were no larvae from 119 azinphos-methyl-treated cherries, and emergence from thiamethoxam and thiacloprid treatments was around 2%. Larvae emerging from the pyriproxifen treatment were significantly heavier

than the other treatments (0.034 ± 0.001 g) ($F = 55.80$; d.f. = 5, 9; $P < 0.0001$) (Figure 4.6). The heaviest pyriproxyfen-exposed emerged larva was 0.0567 g.

When cherries were dissected, an average of 39.6% of the pyriproxifen-treated cherries yielded live larvae. This internal infestation rate was significantly greater than that of the controls ($F = 11.95$; d.f. = 6, 14; $P < 0.0001$) (Figure 4.7). Novaluron treated cherries also showed a significantly increased live-larvae infestation rate when compared only to the untreated replicates (Dunnett's test). No larvae were recovered from cherries treated with neonicotinoids, or azinphos-methyl. Masses of live larvae that were still inside of the cherries were not significantly different ($F = 6.33$; d.f. = 3,4; $P = 0.0534$). Live larvae were only found in one of three replicates for indoxacarb and the untreated control, which limits the power of this comparison (Figure 4.8). The heaviest pyriproxifen-exposed larva was 0.0459 g.

Dead larvae were recovered in all treatments. Indoxacarb had the highest recovery rate having the highest average rate (27%). Dead larvae recovered from indoxacarb were 1 – 4 mm in length. No dead larvae were recovered from azinphos-methyl treatments.

Egg target. The number of damaged cherries per limb cage ranged from 19 to 72. Fruit had begun to soften, and plum curculio were no longer making stereotypical oviposition scars; oviposition marks were impossible to distinguish from feeding marks. The overall emergence rate for larvae was 185 larvae from 839 damaged cherries. Seven larvae had emerged in limb screens on the day of harvest.

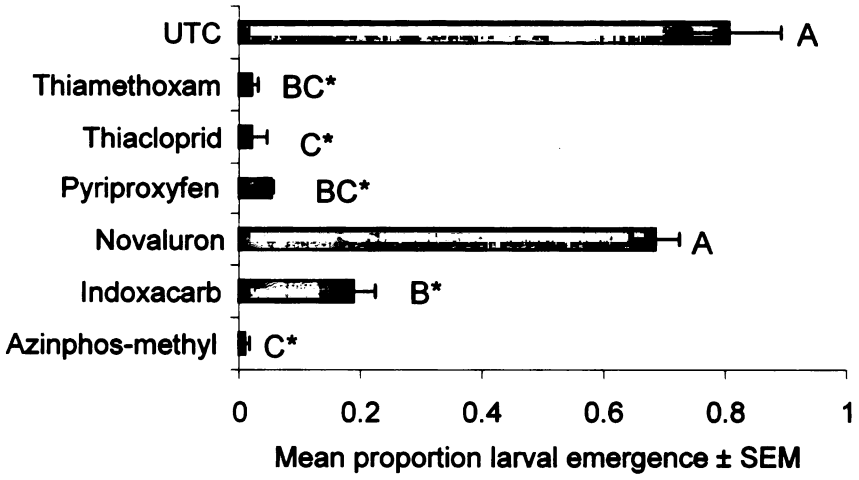

Figure 4.1. Mean proportion of larvae (per cherry) emerged from fruit treated to target late-instar plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

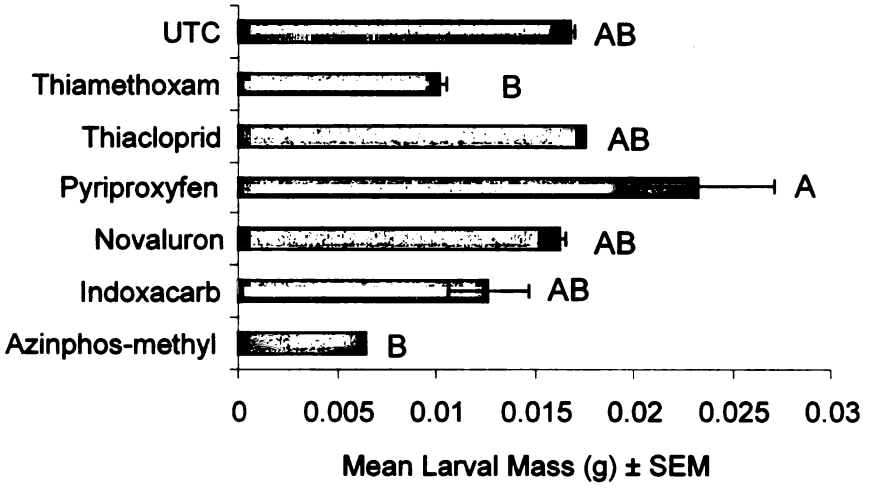

Figure 4.2. Mean mass of larvae emerged from fruit treated to target late-instar plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD).

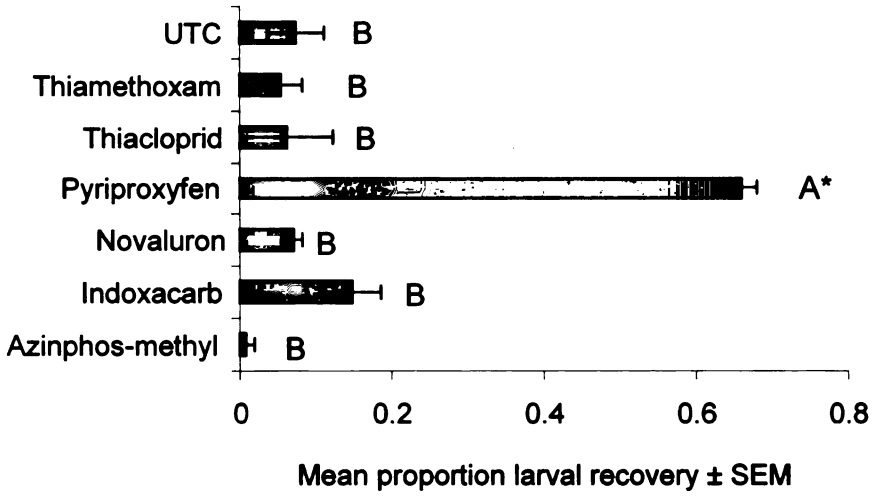

Figure 4.3. Mean proportion of live larvae (per cherry) recovered from dissected fruit (36 d post-oviposition) treated to target late-instar plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

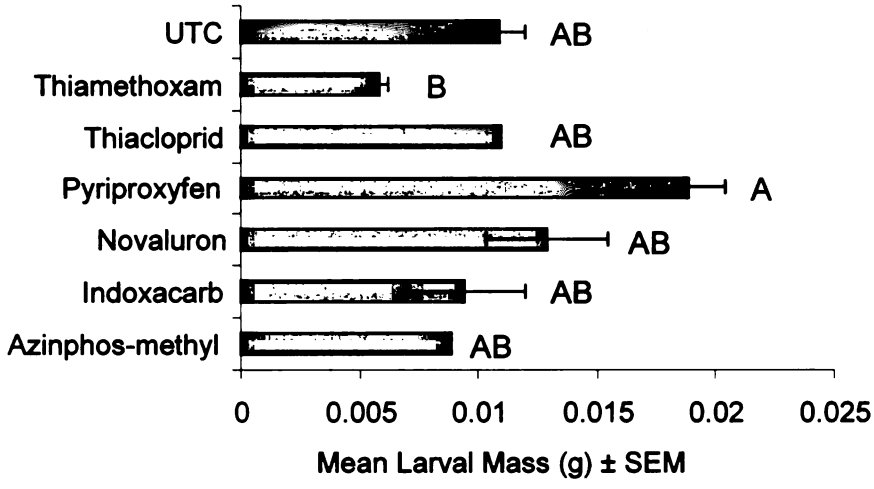

Figure 4.4. Mean mass of live larvae dissected from cherries (36 d post oviposition) treated to target late-instar plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD).

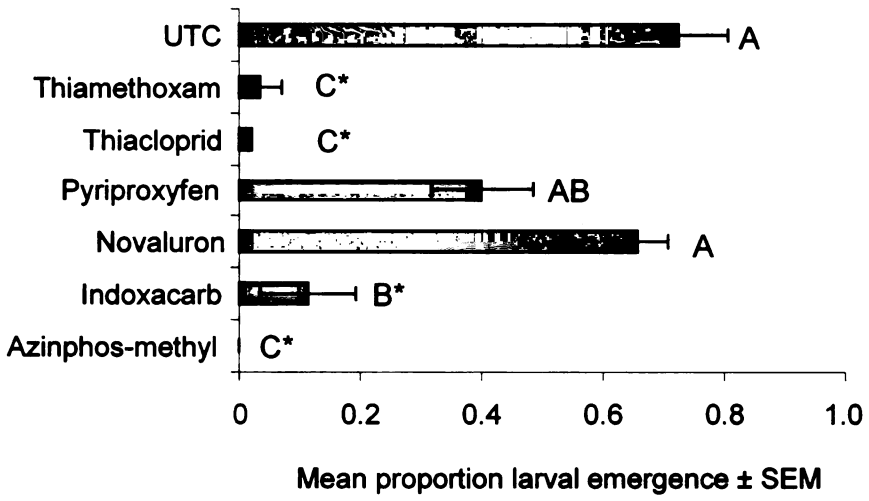

Figure 4.5. Mean proportion of larvae (per cherry) emerged from fruit treated to target neonate plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

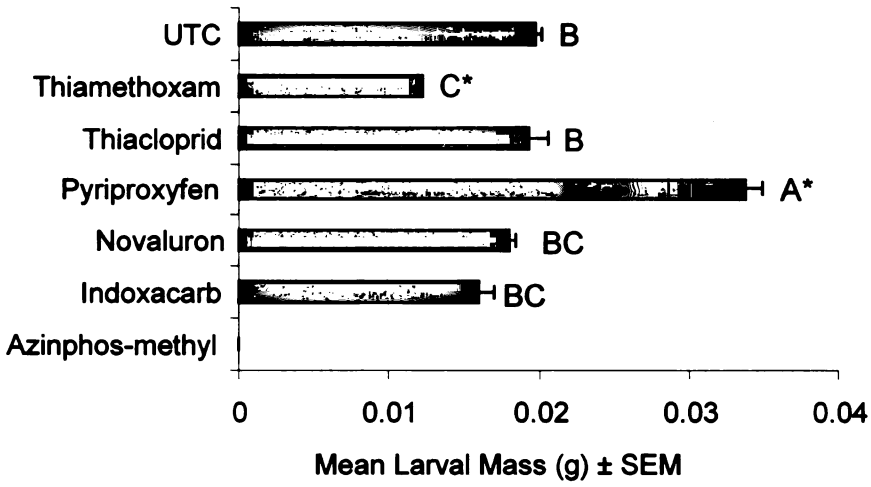

Figure 4.6. Mean mass of larvae emerged from cherries treated to target neonate plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison. No larvae emerged from azinphos-methyl treated cherries

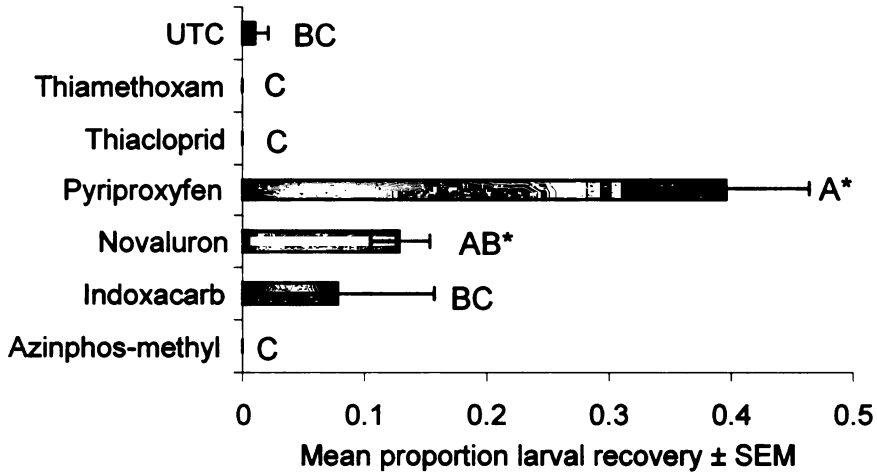

Figure 4.7. Mean proportion of live larvae (per cherry) recovered from dissected fruit (37 d post oviposition) treated to target neonate curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

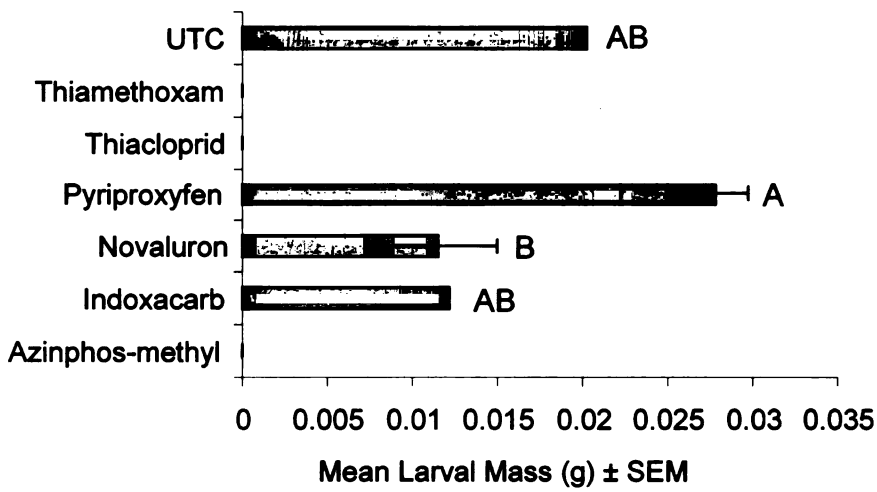

Figure 4.8. Mean mass of live larvae dissected from cherries (37 d post oviposition) treated to target neonate plum curculio. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD).

The untreated control cherries had 46.2 percent average larval emergence rate. The emergence rates for the insect growth regulators pyriproxyfen and novaluron were no different from the untreated control, but thiamethoxam, thiacloprid, and azinphos-methyl showed significant rate reductions ($F = 13.09$; d.f. = 6, 14; $P < 0.0001$) (Figure 4.9). No larvae emerged from any of the thiamethoxam or azinphos-methyl treatment replicates (106 and 97 total cherries, respectively). Larvae emerging from the pyriproxifen treatment were significantly heavier than the other treatments ($F = 66.25$; d.f. = 4, 10; $P < 0.0001$) (Figure 4.10). The heaviest pyriproxyfen-exposed emerged larva was 0.0627 g, three times the average mass of larvae from untreated cherries. Average larval mass from thiacloprid, novaluron, and indoxacarb treatments was no different from that of untreated cherries.

When cherries were dissected, an average of 16.2% of the pyriproxifen-treated cherries had larvae inside of them, whereas 2% of the untreated cherries had larvae inside. This internal infestation rate was significantly greater than that of the controls ($F = 8.26$; d.f. = 6, 14; $P = 0.0006$) (Figure 4.11). Novaluron treated cherries also showed a significantly increased live-larvae infestation rate when compared only to the untreated replicates (Dunnett's test). No larvae were recovered from cherries treated with thiamethoxam, or azinphos-methyl. Larvae that were still inside pyriproxyfen-treated cherries were significantly heavier than those from other treatments ($F = 57.44$; d.f. = 4,6; $P < 0.0001$) (Figure 4.12). The heaviest pyriproxifen-exposed larva still inside the cherry was 0.0522 g. Living indoxacarb-exposed larvae were notably intoxicated, with rapid mandibular movement and inability to move in a coordinated manner. Over half of the

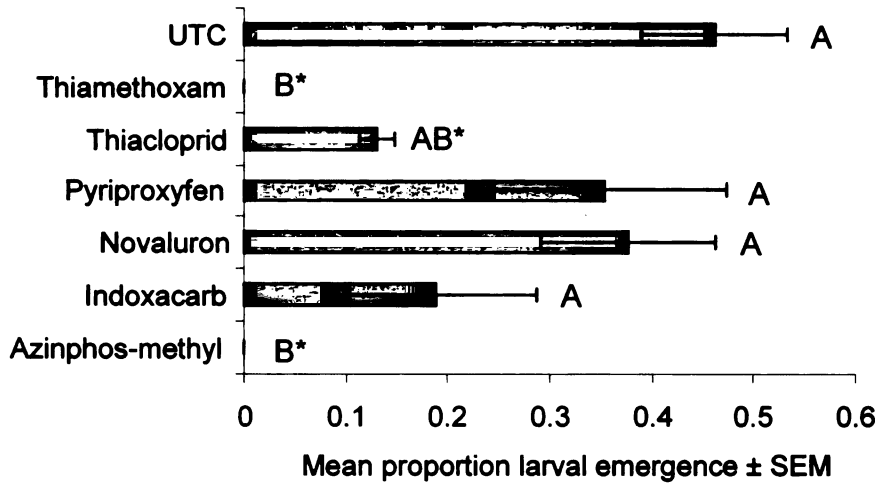

Figure 4.9. Mean proportion of larvae (per cherry) emerged from fruit treated to target plum curculio eggs. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

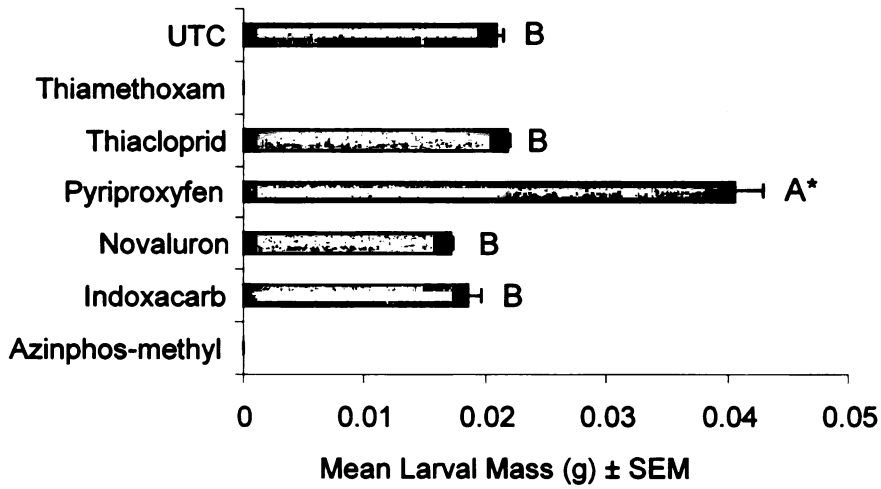

Figure 4.10. Mean mass of larvae emerged from cherries treated to target plum curculio eggs. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

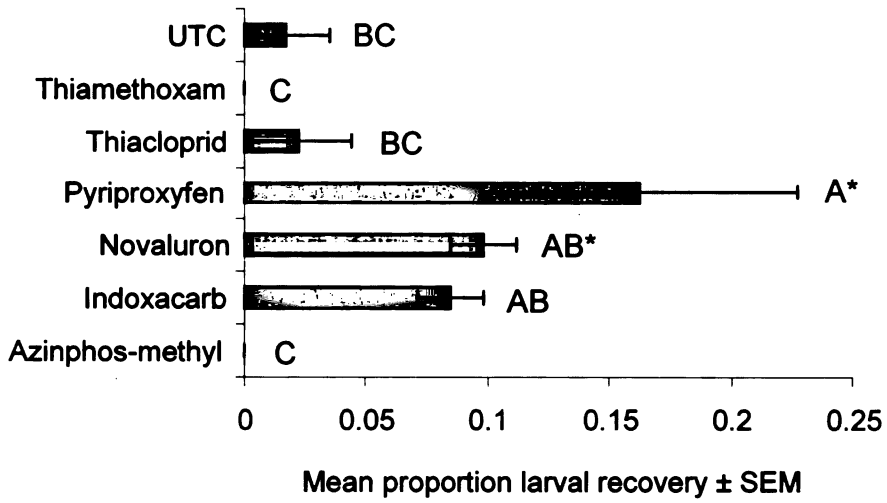

Figure 4.11. Mean proportion of live larvae (per cherry) recovered from dissected fruit (33 d post oviposition) treated to target curculio eggs. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

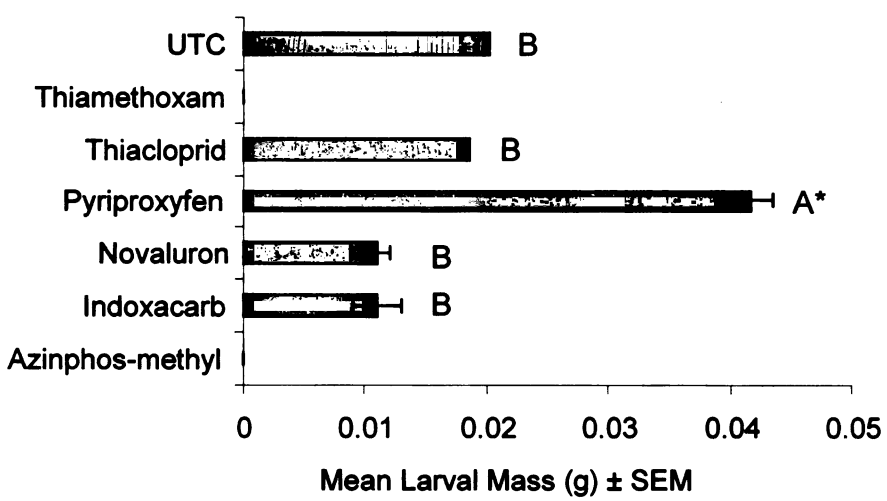

Figure 4.12. Mean mass of live larvae dissected from cherries (33 d post oviposition) treated to target plum curculio eggs. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

living larvae in the novaluron treatments (12 total) had anatomical deformities such as mandibles that were fused to the head rather than articulated.

The recovery rate of dead larvae from dissected fruit varied from zero to 15%. We did not find dead larvae inside pyriproxyfen or untreated cherries. The dead larvae found in the thiamethoxam and azinphos-methyl treatments were typically neonates found in the oviposition mark, with little or no evidence of tunneling.

2007.

CHES neonate target. The number of damaged cherries per limb cage ranged from nine to 24, but there was no significant difference in the number of stings per cherry (1.30 ± 0.08 SEM) between the treatment branches. The overall emergence rate for larvae was 97 larvae from 247 damaged cherries. The first larva emerged the day after fruit were brought back into the laboratory.

The untreated control had 56.6 ± 6.4 SEM percent larval emergence rate. The acetamiprid treatments had significantly lower emergence rates than esfenvalerate or the untreated controls ($F = 17.73$; d.f. = 2, 11; $P = 0.0004$) (Figure 4.13). There was no significant difference between the average masses of emerged larvae across the treatments (0.0191 ± 0.001 g; $P = 0.4312$).

Only esfenvalerate-treated and untreated cherries had living larvae at the time of dissection; the average infestation rate was four and three percent, respectively. There was no significant difference between the treatments ($P = 0.2265$). There were only four living larvae recovered, and there was no significant difference in the lengths of the recovered larvae (5 mm, $P = 0.333$). Dead larvae were recovered in all treatments.

Larvae from untreated cherries were 4-6 mm in length. For acetamiprid and esfenvalerate treatments, recovered larvae were all hatchlings in the oviposition scar.

NWHRS neonate target. The number of damaged cherries per limb cage ranged from six to 50, but there was no significant difference in the number of stings per cherry (1.37 ± 0.04 SEM) between the treatment branches. The overall emergence rate for larvae was 170 larvae from 433 damaged cherries. The first larva emerged three days after fruit were brought back into the laboratory.

The untreated control had 73.9 ± 8.8 SEM percent larval emergence rate. No larvae emerged from acetamiprid-treated cherries (57 total fruit) and only nine larvae emerged from 104 phosmet-treated fruit. This was significantly less than the 19.3% emergence from the untreated controls ($F = 20.83$; d.f. = 4, 13; $P < 0.0001$) (Figure 4.14). Cherries treated with esfenvalerate and chlorantraniliprole showed no significant reduction in emergence rate from the untreated control. There was no significant difference between the masses of emerged larvae (0.0172 ± 0.002 g; $P = 0.0567$).

Less than 10% of cherries from any treatment had larvae inside of them when they were dissected (overall mean: 2.5%), and there was no significant difference between the treatments ($P = 0.1611$). Three of the four chlorantraniliprole replicates had live larvae remaining inside the fruit, but only a single larva was recovered from phosmet, esfenvalerate or untreated control replicates; no live larvae were found in acetamiprid treatments. There was no significant difference in the lengths of the recovered larvae (4.04 mm, $P = 0.071$). Dead larvae were recovered in all treatments except the untreated controls. For acetamiprid and phosmet, larvae died as hatchlings in

the oviposition scar. Dead larvae in chlorantraniliprole and esfenvalerate treatments ranged from neonates to 5 mm larvae.

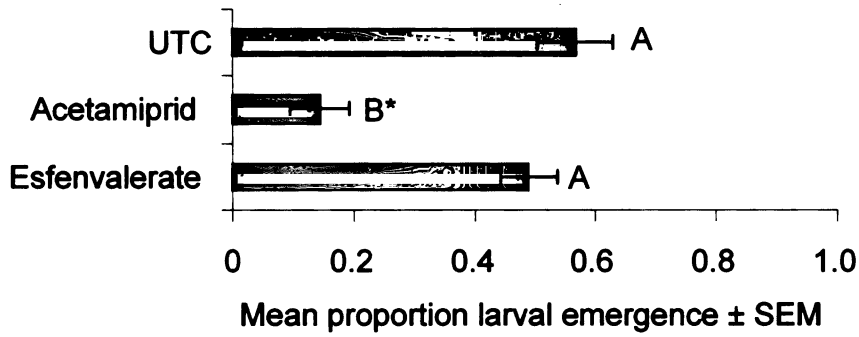

Figure 4.13. Mean proportion of larvae (per cherry) emerging from fruit treated to target neonate plum curculio at CHES in 2007. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

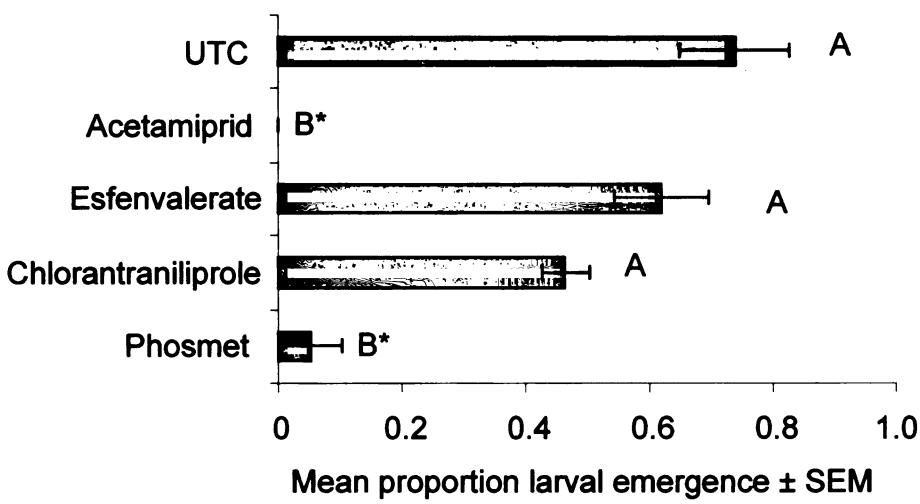

Figure 4.14. Mean proportion of larvae (per cherry) emerging from fruit treated to target neonate plum curculio at NWHRS in 2007. Treatments with different letters are significantly different at $\alpha = 0.05$ (Tukey's HSD). Treatments with a * are significantly different from the control ($\alpha = 0.05$) using a Dunnett's comparison.

Residue Recovery

Chemical residue recoveries of the 2006 tissue sections were highest for the skin sections and generally decline with the more interior residues (Table 4.2). Azinphos-methyl had the highest residue recovery rate, as 24.2 ppm was recovered from the skin section. All compounds were recovered in the inner 1 mm of fruit tissue.

Thiamethoxam and thiacloprid residues were approximately evenly split between interior and skin locations. Pyriproxyfen was overwhelmingly a surface residue, with rates of less than 0.1 ppm recovered from the internal sections. Internal sections of azinphos-methyl, novaluron and indoxacarb had about 10% of the total residues recovery

2007 trials evaluated residues using the sonication and homogenization method. The neonicotinoid acetamiprid had high levels of penetration, and significantly more was bound, rather than dislodgeable, residue (Table 4.3) . Esfenvalerate was recovered at very low levels from any fractions. About half of the chlorantraniliprole recovered residues are inside the fruit, whereas acetamiprid and phosmet are overwhelmingly on the surface.

Table 4.2. Mean residue recovery (± SEM) from cherry fruit tissue 24 h post application for six insecticide compounds using the dissection method. Data are from 2006 Trial at Trevor Nichols Research Complex.

Compound Applied	Tissue	n	ppm recovered [a.]
Thiamethoxam	Inner 1 mm	3	1.21 (0.34)
	Outer 1 mm	3	2.09 (0.71)
	Skin	3	3.52 (1.40)
Thiacloprid	Inner 1 mm	3	0.13 (0.03)
	Outer 1 mm	3	0.16 (0.03)
	Skin	3	0.42 (0.05)
Pyriproxyfen	Inner 1 mm	3	0.09 (0.01)
	Outer 1 mm	3	0.04 (0.01)
	Skin	3	4.06 (0.36)
Novaluron	Inner 1 mm	3	0.13 (0.27)
	Outer 1 mm	3	0.42 (0.17)
	Skin	3	4.45 (1.37)
Indoxacarb	Inner 1 mm	3	0.14 (0.11)
	Outer 1 mm	3	0.25 (0.12)
	Skin	3	4.41 (2.28)
Azinphos-methyl	Inner 1 mm	3	1.17 (0.18)
	Outer 1 mm	3	2.93 (0.20)
	Skin	3	24.23 (2.13)

[a.] μg analyte / g fruit sample

Table 4.3. Mean residue recovery (± SEM) from cherry fruit tissue 24 h post application for six insecticide compounds. Data are from 2007 trials at CHES and NWHRS.

Compound	Residue location	n samples	mean ppm [a.]
Acetamiprid	Internal	4	0.66 (0.09)
	External	4	0.02 (0.01)
Esfenvalerate	Internal	7	0.01 (0.004)
	External	7	0.01 (0.002)
Chlorantraniliprole	Internal	1	0.45
	External	1	0.7
Phosmet	Internal	1	1.85
	External	1	18.77

[a.] μg analyte / g fruit sample

Discussion

This set of experiments serves as a field validation of previously reported laboratory experiments that show plum curculio eggs and larvae are susceptible to many of the insecticides that are used to control adults of this species. Field residue recoveries for these compounds make strong cases for which materials have the potential to be curative agents. In order for compounds to be effective curative compounds, insecticides must get to the target stage at a minimum effective concentration. When residue data from these field experiments are paired up with the effective concentrations for controlled laboratory studies, we are able to gain insights into each compound's potential and realized activity.

Azinphos-methyl and phosmet caused near complete elimination of live internal infestation at all application timings. The recovery rate of internal residues exceeded the observed level of effect in laboratory studies (Hoffmann et al. 2008, Chapter 2, Chapter 3). Egg LC_{50} values for azinphos-methyl and phosmet were 0.44 and 2.06 ppm, respectively (Chapter 2), and larval effective concentrations for neonates were between 0.1 and 1.0 ppm.

The neonicotinoids acetamiprid, thiamethoxam, and thiacloprid were all exceptionally active curative agents, and these compounds are recovered from fruit tissue at rates > 0.1 ppm. This concentration was found to cause significant mortality in spiked-diet trials (Chapter 3 of this document). Like the organophosphates, the neonicotinoids were effective at reducing larval emergence at egg, neonate, and large larva-targeted application timings. Plum curculio have a 4-8 wk oviposition period (Reisseg et al. 1998) and eggs, hatchlings and growing larvae are simultaneously present in an orchard, rather

than distinct cohorts. The wide window of neonicotinoid curative efficacy suggests that these compounds will be quite forgiving as growers incorporate them into their pest management practices.

Thiamethoxam is converted to clothianidin in insects and plant tissue, and direct neural studies with thiamethoxam show significantly reduced binding affinities to insect nAChRs relative to other neonicotinoids (Nauen et al. 2003). However, the observed activity of this compound was similar to that of the other tested neonicotinoids. It is likely that the activity seen with thiamethoxam application is due to the action of clothianidin on eggs and larvae. Clothianidin (Clutch® 50 WDG; Valent U.S.A. Corp., Walnut Creek, CA) is not currently labeled in cherries, but it is labeled in apples at a rate of 1.5 oz active ingredient per acre (105 g / Ha). The application rate of thiamethoxam in apples for control of plum curculio is equivalent to 79 g/ Ha. This superficial analysis suggests that apple growers may be best served by applying a full rate of clothianidin and getting almost 25% more a.i. on the trees. However, thiamethoxam is one of the only neonicotinoids to have a negative log*P* (*Log* K_{OW}) value (-0.13). This represents the octanol-water partitioning coefficient, and negative values represent greater partitioning into the aqueous phase. The hydrophilic character of thiamethoxam may actually contribute to its penetration through the fruit cuticle into the fruit flesh where it is later metabolized to the more lipophilic clothianidin (Log*P* = 0.7). The residue analysis technique used for this study are unable to differentiate between clothianidin and thiamethoxam residues.

Novaluron does not appear to have curative activity in the field at current application rates. Fruit tissue recoveries for this compound (> 0.13 ppm) compare

closely to the effective concentrations tested seen in laboratory studies (Chapter 3), but there was no observed reduction in mortality. The lowest concentration tested for larvae was 0.25 ppm and the LC_{50} for eggs was determined to be 0.44 ppm (Hoffmann et al. 2008). The overall emergence pattern was not different from untreated fruit; emergence patterns from the novaluron replicates were similar to those observed in the untreated controls. After over 30 d post oviposition, there *was* an increased infestation rate (approximately 10%) relative to untreated fruit for cherries that were treated to target the egg and neonate stages. These live larvae were small (4 – 6 mm) but still visible. This trend was not seen in novaluron treatments that targeted large larvae. These data suggest that novaluron retards larval development if they are exposed as early instars. Applications of this compound within 40 d of harvest may increase the chance of larval infestation at harvest. Early season applications (petal fall, shuck off) of this chitin synthesis inhibitor should still be considered; the season long potential of sterilizing egg-laying females (Wise et al. 2007a) outweighs the slight chance of an infested cherry remaining on the tree for 40 d. It would be appropriate to use a known curative agent after a novaluron application.

Esfenvalerate is another compound that showed curative potential in the laboratory, but failed in the field. The reasons for this failure are much easier to determine than for novaluron; there was extremely low recovery of this compound from fruit interior tissue. Two of the samples had no detectable residue. This lack of recovery could be due to inefficient penetration through the cherry cuticle, or it could be a function of metabolic breakdown of this compound within the fruit tissue after penetration (Mikami et al. 1985). Ester hydrolysis is the major metabolic pathway for esfenvalerate,

and both plants and animals possess enzymes that mediate this process. Separating these possibilities would likely involve looking for esfenvalerate metabolites in fruit tissue after surface treatment.

No matter what the cause, larvae are clearly not receiving sufficient exposure of this potent compound. If this exposure is limited by esfenvalerate penetration through the fruit cuticle, optimized spray adjuvants may dramatically improve its efficacy. If the lack of efficacy is due to plant metabolism, it is unlikely that this compound will ever be a curative agent.

When one simply examines the emergence rate from pyriproxyfen treated cherries, it appears that there is a reduction relative to untreated fruit. However, this juvenile hormone analog has an unexpected effect of keeping a significant proportion of larvae feeding inside the fruit, even 37 d after oviposition. These larvae are larger than any normal mature larva (up to four times the average normal mass) and would be easily visible to an inspector looking for insect infestation at a processing plant. The increase in size may be due to supernumerary molts, or a disruption of the behavioral signaling for the larvae to exit the fruit and begin pupation behaviors. Larvae that emerged from fruit on their own were also significantly larger than those from untreated replicates.

Pyriproxyfen is currently registered for use against San Jose scale in cherries. I would strongly recommend against the pre-harvest use of this compound in cherry orchards. Plum curculio larvae appear to be sensitive to this compound across the development period, and the likelihood of infestation at harvest is substantial at any treatment timing. If application of pyriproxyfen is necessary, I recommend a concurrent

spray of a highly effective curative agent (one of the neonicotinoid insecticides, or an organophosphate).

Indoxacarb was a relatively weak curative agent in these field trials. This compound is not a potent larvicide at 1.0 or 0.1 ppm, with only 50-60% reductions of larval survivorship when reared in artificial diet (Chapter 3). It had no measurable effect on plum curculio eggs when they were incubated in 100 ppm solution (Hoffmann et al. 2008). The insecticidal action of indoxacarb is mostly from the decarbomethoxylated metabolite DCJW. This metabolism does take time and is most efficient after oral, rather than topical, exposure to indoxacarb (Wing et al. 2000). Live, but significantly lighter, larvae were recovered inside of fruit at all treatment timings. Indoxacarb is a known feeding inhibitor (Wing et al. 2000, Tillman et al. 2001) and general paralytic. These actions probably work together to retard larval growth in plum curculio.

Chlorantraniliprole, a new ryanodine receptor activator, was not effective as a curative agent. There was no significant reduction in larval emergence and nine live larvae were still found in the fruit over 8 wks after eggs were laid (only one was found in untreated fruit). These larvae inside the fruit could be a results of sublethal intoxication and slowing of feeding and resultant growth. In susceptible insects, this anthranilic diamide causes the release of stored calcium into intracellular spaces, causing uncontrolled muscle contraction and a rigid paralysis (Bloomquist 1996, Cordova et al. 2006). This compound is not shown to be an effective ovicidal agent at 100 ppm (Chapter 2) and analytical recovery of this compound was 0.45 ppm.

Live larvae in dissected fruit were light in color and their movement facilitated discovery. However, dead larvae were not easy to find, even under a microscope; large

larvae were often desiccated and flattened against the pit, and dead neonates were too small to notice without dedicated assessment. An inspector at a processing plant would be unlikely to detect larvae in this condition. There were several different people dissecting these cherries, and their ability to locate dead larvae varied. As such, the number of dead larvae inside the fruit is an unreliable measure for analysis, and has been treated as an item of commentary. Similarly, the number of oviposition scars is known to be inaccurate, as evidenced by some individual fruit having more larvae emerge than there were oviposition scars.

Fruit quality in replicates treated with neonicotinoids or organophosphates was generally quite good if the treatments were timed to target eggs or neonates. Oviposition scars were obvious when the fruit were removed from the trees, but there was no frass or tunneling that typically highlight infested fruit. These fruit also remained intact after removal from the tree much longer than untreated fruit. Damaged fruit from all experiments targeting large larvae were clearly infested, and would likely have fallen off of the tree prior to harvest even if the larvae inside were dead.

The sonication and dissection methods do treat skin residues differently. The sonication method dislodges surface residues, but analyte that is physically inside the skin tissue is not extracted. The dissection method did not use any surface extraction, and the dislodgable and embedded skin residues are viewed together. As a result, the sonication method may actually inflate the "internal" residue values for compounds that have a high affinity for the cuticle and skin tissue. It should also be noted that method recoveries are typically around 50%; 0.1 ppm detection probably represents an actual concentration of 0.2 to 0.5 ppm.

Curative (or "eradicant") potential of many insecticides was documented in the 1950's, during the transition from lead and arsenical compounds to the chlorinated hydrocarbons and organophosphates. The chlorinated hydrocarbons, carbamates and organophosphates proved to be exceptional fruit protectants, with outstanding activity against plum curculio adults (Forsythe and Rings 1965, Hagley and Chiba 1980, Smith and Fiori 1959). This activity was such that there was little perceived need to understand or develop a curative strategy to enhance the efficacy of these materials. Even though the activity was ignored, the data presented here, in conjunction with published reports (Wise et al. 2007a) demonstrate that curative activity resulting from foliar applications of azinphos-methyl has provided population control well beyond the officially-targeted adult stage.

With the 2012 phase out of azinphos-methyl, growers, researchers, and other pest management stakeholders are in a transition period similar to the one in the 1950s. Unlike last century's transition, there may not be compounds with the rapid adulticidal action and resultant fruit protection of the organophosphates. Despite this clear change in the types of tools available, many stakeholders are still blindly holding on to the single-minded paradigm of adult control. We cannot afford to ignore potential pest management tactics just because they have not been "officially" used before. Regardless of the stated targets on the pesticide labels, curative activity has been part of plum curculio management for over fifty years, and any viable post-azinphos-methyl pest management system needs to incorporate this mode of activity.

Resistance management

An important discussion point for this strategy is that of resistance management. Of the tested materials, the only non-organophosphate curative agents come from the neonicotinoid class. These compounds share the same physiological target site and mode of action. If plum curculio were to develop resistance to one compound, it is likely that the related compounds would have reduced efficacy (Nauen and Denholm 2005, Prabhaker et al. 2005, Mota-Sanchez et al. 2006, Millar and Denholm 2007). Plum curculio have been annually exposed to broadcast application of organophosphates for over 50 years without any reports of resistance. This does not preclude the genetic capacity for neonicotinoid resistance, and consistent exposure to one mode of action is a high-risk scenario for development of resistance (Denholm and Rowland 1992).

Northern strain plum curculio resistance management is helped by the annual influx of susceptible genes from untreated woodlots and unmanaged orchards. Organophosphate-based management practices effectively sterilize the orchard during the growing season, with few survivors moving back into wooded overwintering sites potentially spreading resistance genes the following spring.

As a group, the neonicotinoids are used in all tree fruits and provide good to excellent activity against a wide variety of fruit pests (Wise et al. 2008). This class has the capacity to fill the plum curculio curative role of the organophosphates during the phase out period and beyond the 2012 use expiration of azinphos-methyl. Supportive integration with compounds like pyrethroids can help "clean up" adult populations and minimize resistance concerns. If current understandings of the sterilization capacity of novaluron are borne out (Wise et al. 2007a), early-season application of this chitin

synthesis inhibitor could serve as an effective roadblock for the spread of resistance genes in the orchard.

Acknowledgments

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Chapter 5

Plum curculio mortality and associated fruit injury after exposure to field-weathered insecticides on tart cherry branches

Abstract

Plum curculio, *Conotrachelus nenuphar* (Herbst) adults were exposed to field-aged residues of thiamethoxam, acetamiprid, thiacloprid, indoxacarb or azinphos-methyl on tart cherry (*Prunus cerasus* L. var Montmorency). At 1, 3, 7, and 14 d post-application, bioassays were used to assess beetle mortality and plant tissue injury after 96 h of exposure. Azinphos-methyl had lethal activity and significant fruit protection at 14 d post application. Fruit protection in azinphos-methyl treatments likely comes from acute contact activity. All of the neonicotinoids had lethal activity at 3 d post treatment, with acetamiprid activity extending to 7 d. Antifeedant and oviposition deterrent effects were seen with thiamethoxam and thiacloprid; damage incidence was significantly reduced in the absence of significant beetle mortality. Thiamethoxam and acetamiprid penetrated into leaf and fruit tissue and were detected in the interior tissues at 14 d post application, but interior thiacloprid residues were not detected after day 1. Indoxacarb provided some fruit protection out to 7 d post application, and intoxicated beetles at 14 post application, but the slow action of this compound allowed significant damage to occur before beetles are incapacitated. Indoxacarb was only detected as a surface residue after the first day post-application. Fruit protection in azinphos-methyl-based plum curculio management was due primarily to acute contact activity. Neonicotinoids are the likely replacement

class for the organophosphates class, but fruit protection is due to a combination of lethal and sublethal activities.

Introduction

Plum curculio, *Conotrachelus nenuphar* (Herbst) is a key pest of eastern North American tree fruits. In northern regions, plum curculio are univoltine and unmated adults overwinter in leaf litter and loose soil both in orchards and adjacent woodlots (Chapman 1938, Smith & Flessel 1968, Lafleur et al.. 1987). Adults emerge from the soil in spring after soil temperatures consistently remain above 50°F (Bobb 1949) and mate in early spring, with most females mated well in advance of commercial crop fruit set (Smith & Salkeld 1964, Racette et al. 1992, Hoffmann et al. 2004). Females lay eggs inside of fruit, and eggs take 3-6 days to hatch (Smith 1957, Mampe and Neunzig 1967). The egg laying period for the northern strain is fairly long, with new oviposition scars noted from May through early July if appropriate hosts are present (Reissig et al. 1998). The legless larvae eat the flesh of the fruit and take approximately 3 weeks to complete development (Smith 1957, Lan et al. 2004). When they have completed feeding, larvae exit the fruit and burrow into the soil. Pupation time varies, with soil quality, temperature and moisture being important factors (Chen and Scherm 2007). After adult eclosion in August, northern strain adults may feed, but are assumed to move to overwintering locations (Lafleur et al. 1987). Summer generation Southern strain beetles feed for a while after eclosion and then begin another round of oviposition (Gaydon 1972).

The host range for plum curculio is broad, with feeding and oviposition identified in wild and cultivated Rosaceous plants, including *Amelanchier, Malus, Crataegus,* and

Prunus species, (Quaintance & Jenne 1912, Chapman 1938, Maier 1990). The assumed ancestral native hosts are Canada Plum (*Prunus nigra*), Wild Plum (*P. americana*), and *P. mexicana* (Chapman 1938). Plum curculio feeding and oviposition are also common in blueberries (*Vaccinium spp.*), which are native plant to eastern North America (Tomlinson 1951, Polavarapu et al. 2004). Hallman and Gould's 2004 report on 22 possible subtropical and tropical host fruits identified plum curculio feeding in tropical fruits, particularly mango. However, oviposition was only documented in apple, plum, peach and loquat (*Eriobotrya japonica*) – all Rosacaea.

Plum curculio feeding and oviposition on fruit are major causes of economic losses in commercial tree fruit crops east of the Rocky Mountains. Plum curculio adults have been observed to feed on leaves, flowers and fruits structures as soon as they arrive into orchards (Chouinard et al. 1993). On fruits, this feeding damage presents as punctures with gouged-out subsurface flesh (Fulton 1928). Oviposition injury by plum curculio is readily identifiable by the c-shaped incision that the females chews into the fruit skin. This behavior is thought to inhibit local tissue growth and reduce the risk of larvae getting crushed as the fruit develop (Chapman 1938). The egg is actually laid in a small secondary incision within the crescent (Fulton 1928). In apples, cherries, and plums this mark can become a large, corky scar as the fruit expands. In peaches, however, this scar is often obscured by the fruit's pubescence. Left unmanaged, plum curculio damage can exceed 90 percent, even if untreated areas are adjacent to chemically-managed orchards (Oatman et al. 1966).

For fresh markets, the oviposition scar (regardless of any internal damage by larvae) is sufficient to reduce the value of the harvested fruit. The oviposition scar is not

a major issue in processed fruit, but there is a marketing mandate for infestation-free processed tart cherries at harvest (USDA Agricultural Marketing Service 1941a,b). There are no disinfestation procedures currently available for tart cherries, so growers are under significant pressure to keep their entire orchards free of plum curculio infestation at harvest.

Current control tactics for plum curculio are centered on insecticide-based population control during the oviposition period. Fruit are monitored for evidence of beetle activity (feeding damage, oviposition scars) and controls are initiated at the first sign of beetle presence, after the pollination period is completed. Other tactics, such as trapping or biological control are not sufficient to meet the quality demands. In apples and cherries, the organophosphate azinphos-methyl (Guthion®, Bayer Cropscience) is the current mainstay. Azinphos-methyl has given growers excellent plum curculio control since the late 1950s (Bobb 1957, Smith and Fiori 1959, Snapp 1960, Forsythe and Rings 1965); its rapid knockdown of plum curculio and long residual activity (>10 d) were identified early in its use in plum curculio management (Smith and Fiori 1959). However, this compound is being phased out as part of the 1996 Food Quality and Protection Act framework; the registration is currently set to expire in 2012 (US EPA 2006). A related compound, phosmet (Imidan®, Gowan Co.) is also known to offer good protection against plum curculio (Forsythe and Rings 1965, Hagley and Chiba 1980). Phosmet registration is expected to continue beyond that of azinphos-methyl, but rate restrictions and increased pre-harvest intervals may reduce its utility.

There are non-organophosphate compounds that show promise for control of plum curculio damage during the oviposition period. A number of compounds in the

neonicotinoid class show promise as fruit protection compounds. Applications of the neonicotinoids thiamethoxam and thiacloprid have shown >80% reduction in apple fruit damage (Wise et al. 2006). The neonicotinoids acetamiprid and imidacloprid are currently labeled for plum curculio "suppression" rather than "control." The oxadiazine indoxacarb (Avaunt®, DuPont, Wilmington, DE) has also demonstrated fruit protection in field trials (Wise et al. 2006). A wide array of pyrethroids are registered for plum curculio control. These compounds typically have a short pre-harvest interval, which makes them valuable tools for late season control; large, visible larvae may still be in the fruit if eggs are laid within two to three weeks of harvest. However, there are concerns about pyrethroid use and mite management programs (Hull et al. 1997).

Despite this array of tools, there are significant gaps in our understanding of how these various compounds achieve fruit protection. The organophosphates are well known for their acute contact activity, but it cannot be assumed that all classes and compounds perform similarly. Traditional toxicity bioassays on adult insects suggest the potential for killing the pest, but these tests do not assess the realized crop protection potential. Sublethal behavioral effects, antifeedant or repellent activities may be quite effective crop protection mechanisms which can be overlooked in laboratory toxicity screenings. Antifeedant activity has been reported in neonicotinoids (Nauen et al. 1998, Drinkwater 2003, Wise et al. 2007b, Tansey et al. 2008) and many botanical extracts are behaviorally active against insect pests (Isman 2006). On the other end of the spectrum, field efficacy trials focus on observing plant tissue endpoints, and do not address the question of how any observed crop protection is actually achieved.

Intensifying economic competition demands that application recommendations for new compounds be optimized. In order to have the most complete assessment of crop protection potential, plant-insect and plant-chemical interactions should be observed in parallel with the traditional insect-chemical interaction. This PIC-triad (Wise et al. 2007a) is a particularly useful framework for the current generation of insecticides. It integrates the key processes and guides our description of a compound's pest management potential as residues degrade or move into new plant tissue. This paper utilizes the PIC-triad framework in describing the performance characteristics of six compounds for plum curculio control.

Materials and Methods

Insect Source and Maintenance. Northern strain plum curculio were collected from 5 May – 10 June 2005 in cherry and apple orchards at the Trevor Nichols Research Complex (TNRC) in Fennville, MI (42.5951°N, -86.1561°W) using beating trays or commercially-available pyramid traps (Tedders and Wood, 1994). Beetles were held together for one week after field collection to ensure that females were mated. Weevils were sexed according to the method of Thomson (1932) and placed into gender-separate screen cages (Model 1450 B BioQuip Products Inc., Gardena, CA) lined with paper towels. Cages were kept outside in a shaded area near the cherry orchard so that beetle and plant physiology stayed synchronized. We provided beetles with untreated cherry branches (*Prunus cerasus* var. Montmorency) with fruit and foliage in wetted floral foam (OASIS® Smithers-Oasis Co. Kent, OH). Plant material in cages was replaced every two to three days, and water was added daily.

Orchard. Experimental trees were in a tart cherry (*Prunus cerasus* L. var Montmorency) orchard at the TNRC that was planted in 1994. The site is on a slight slope north-south, with trees at the southern edge at a higher elevation than those at the northern edge. Trees in this orchard received maintenance sprays of tebuconazole (Elite® 45; Bayer Cropscience, Research Triangle Park, NC) for cherry leaf spot (*Blumeriella jaapii* Rehm) and powdery mildew, chlorthalonil (Bravo Weather stik®; Syngenta, Greensboro, NC) for cherry leaf spot and powdery mildew, and fertilizer (Mora-leaf® Plus 20-20-20; Wilbur-Ellis Co., Yakima, WA). No insecticides were applied to experimental plots other than those described below.

Insecticides. There were five formulated materials used in these field trials: azinphos-methyl (Guthion® 50W, 1120 g AI/ Ha, Bayer CropScience, Research Triangle Park, NC), acetamiprid (Assail® 70WP, 167 g AI/ Ha, Cerexagri, King of Prussia, PA), thiamethoxam (Actara® 25WG, 79 g AI/ Ha, Syngenta, Greensboro, NC), thiacloprid (Calypso™ 4F, 158 g AI/ Ha, Bayer CropScience), and indoxacarb (Avaunt®, 126 g AI/ Ha, DuPont, Wilmington, DE). Insecticides were tank mixed at 935 L/Ha (100 gallons/acre) concentrated spray and sprayed with an FMC 1029 sprayer.

Field Application. Chemicals were applied on 31 May 2005 in a randomized, complete block design, with four blocks and one treatment replicate per block. The blocking criterion was based on topography; trees at the top of the hill seemed to be slightly more advanced that trees at the bottom. Cherry fruit were at 8-10 mm in diameter at the time of application. There were four trees per treatment, and each treatment was separated

from neighboring trees within rows by at least two trees, with a row between treatments. Control trees were in an adjacent orchard section, separated by at least 4 trees or two rows from the area receiving insecticide treatments.

Bioassays. Terminal branches were harvested and brought into the laboratory to evaluate lethal and sublethal effects of field applications. One branch per tree was removed at 4 h, 3 d, 7 d and 14 d post application. Each shoot was pruned to ten fruit and ten leaves and placed in water-soaked floral foam (Smithers-Oasis Co., Kent, OH) inside a clear plastic 946 ml container (Fabri-Kal, Kalamazoo, MI). Foam was covered with 3 mm of melted paraffin wax to maintain tissue turgor and keep beetles from burrowing into the foam. The center 8 cm diam of the plastic lids was cut out and replaced with nylon mesh to minimize condensation inside the bioassay chamber and also minimize the potential for fumigation effects.

Five male and five female plum curculio were placed into each bioassay container for 96 h. Beetles were observed at 12, 24, and 96 h after introduction for symptoms of poisoning, and were scored as unimpaired, dead, or intoxicated. After 96 h, beetles were removed from the container and the plant tissue was evaluated for damage. Number of oviposition marks and feeding punctures on the fruit were counted, as was the number of feeding events on leaf tissue.

Surface and Interior Residue Analysis. At each post-treatment interval, treated fruit and leaf samples were taken from the field with bioassay collections and immediately frozen. Samples (approximately 10 g) were weighed and placed into 120 ml circular vials

(Qorpak, Bridgeville, PA) and surface residues extracted with two 60 ml washes of acetonitrile. For the first wash, leaves or fruit were sonicated in the solvent for 30 s. After surface extraction, vials were filled with 60 ml dichloromethane. All samples were held at -28°C until final workup for residue analysis.

At the MSU Pesticide Analytical Laboratory, leaf and fruit samples (in dichloromethane) were homogenized for 30 s (Model Pro200, ProScientific Inc., Monroe, CT), rinsed with dichloromethane (3 x 60 ml), filtered through a sodium sulfate column and collected in a round-bottom flask. The column was rinsed with an additional 40 ml dichloromethane (2 x 20 ml). The round bottom flask was reduced to dryness by rotary-evaporation and 2 ml solvent added. The sample was put through a 0.45 mm filter and collected in a 2.5 ml gas chromatography vial.

Thiamethoxam and thiacloprid residues were determined using a Waters 2690 Separator Module HPLC, with a Waters 2487 dual-wavelength absorbance detector. The column was a C18 reversed-phase column with 4.6 mm bore and 5 mm particle size. Flow rates were set at 1 ml / min. For thiamethoxam, the mobile phase started at 90:10 water:acetic acid (0.1%) in acetonitrile and reduced to 70:30 between 12 and 13 min at 35°C. The detector for thiamethoxam was set at 255 nm. For thiacloprid, the mobile phase started as a 30:70 ratio of 0.4 ml HCl (35%) in water : acetonitrile and ramped to 25:75 at 4 min and 10:90 between 4 and 9 min. The mobile phase was brought back to 30:70 at 13 min. The detector was set at 242 nm for thiacloprid.

Gas chromatography was used for acetamiprid, azinphos-methyl, indoxacarb. The equipment used was an Agilent 6890 gas chromatograph with a 5973N Mass Spectra Detector. The column was a Zebron ZB-5ms 30 m, 0.25 mm I.D. with 0.25μm film

thickness. The oven temperature program was: 5 min at 115°C, ramp of 9°C / min to 280°C, ramp of 30°C/ min to 310°C. The inlet was kept in pulsed splitless mode at 200°C, with 78324 PA and a pulse pressure of 103421 Pa. The purge flow (helium) was 50 ml/ min. The mass detector was set to scan at a minimum of 28 Da up to the maximum molecular mass of the molecule of interest.

Statistical Analysis. The proportion of live beetles across the 96 h observational period was analyzed by a repeated-measure ANOVA using PROC MIXED in SAS (SAS 2006). For the repeated measures, the response variable was the arcsin-transformed percentage of alive beetles; dead and intoxicated insects were treated together. The class variables that were treated as "repeated measures" were the observation time (1 h, 12 h, 96 h) and the replicate tree from which the limb samples were taken. Contrasts were evaluated for significant observation time/treatment/day ($P < 0.05$) interactions. Post-hoc evaluation of the 96 h indoxacarb "intoxicated" proportions was done using PROC MIXED in SAS with treatment tree as the repeated measure.

Plant damage measures (fruit feeding, oviposition, leaf feeding) were transformed and analyzed separately using PROC MIXED in SAS. The replicate tree was treated as the repeated measure. Significant treatment/day interactions were explored using LSMEAN contrasts.

Results

Survivorship, Mortality and Intoxication

Beetle response depended on the treatment, age of residue, and exposure to the residue. After partitioning the repeated measures, there was a significant effect of treatment, day, and observation hour main effects as well as treatment x day, treatment x hour, and treatment x day x hour interactions (Table 5.1) on the number of unimpaired beetles.

Table 5.1. Statistical fixed effects and nested interactions for repeated measures analysis of unimpaired, intoxicated and dead plum curculio after beetles were exposed to tart cherry branches 1, 3, 7, and 14 days after insecticides were applied to source trees. There were six insecticide treatments and beetles were observed after 12, 24 and 96 h of exposure .

Variable	Effect	Numerator DF	Denominator DF	F-value	Pr > F
Unimpaired Beetles	Treatment	5	71	71.56	<0.0001
	Treatment * Day	18	71	13.36	<0.0001
	Treatment * Hour	12	142	40.21	<0.0001
	Treatment * Day * Hour	36	142	2.5	<0.0001
Intoxicated beetles	Treatment	5	213	41.44	<0.0001
	Treatment * Day	18	213	3.72	<0.0001
	Treatment * Hour	12	213	22.45	<0.0001
	Treatment * Day * Hour	36	213	2.36	<0.0001
Dead beetles	Treatment	5	71	82.09	<0.0001
	Treatment * Day	18	71	10.40	<0.0001
	Treatment * Hour	12	142	30.50	<0.0001
	Treatment * Day * Hour	36	142	2.42	`0.0001

Interactions can be challenging to describe, but contrast analysis of the treatment/day/hour interaction allows us to make relevant survivorship comparisons between the treatments and the parallel untreated controls. There was no significant difference in the number of unimpaired beetles between any of the untreated treatment-time combinations. Most notable is that beetles showed no reduction in survivorship after 1 h exposure to residues of any age (Figure 5.1). Even azinphos-methyl did not show a significant reduction in survivorship until the 24-hour observation period.

The neonicotinoids thiamethoxam, thiacloprid and acetamiprid all showed significant 96-hour reductions in unimpaired beetles for the 1 d and 3 d aged residues (Figure 5.1). Acetamiprid was the only neonicotinoid to have a significant reduction in 96-h unimpaired beetles after exposure to 7 d-old residues, and none of the neonicotinoids reduced the number of healthy insects at 14 d post application. Within each specific field-aged bioassay, the time-mortality patterns were different across the neonicotinoids. For the 1-day and 3-day bioassay, Thiamethoxam and thiacloprid reduced survivorship after 24 h exposure, but acetamiprid effects did not become apparent until the insects had been exposed to plant tissue for 96 h. While acetamiprid takes longer to act, it appears to retain efficacy in the field for a longer period of time. Intoxication was not a significant behavior after exposure to the neonicotinoids in general (Figure 5.2). The number of intoxicated beetles in the neonicotinoid treatments was different from the control treatments for only the thiacloprid treatment at the 96-hour observation on 3 –d residues.

Azinphos-methyl reduced beetle survivorship after 24 h for all post-application residue ages (Figure 5.1). There was no significant measure of intoxication at any time interval (Figure 5.2); any beetle not alive was not moving and assumed to be dead.

The survivorship pattern of effect for indoxacarb was most similar to that of azinphos-methyl. The number of unaffected beetles was significantly different from the untreated replicates at the 96-hour observation period for all of the residue ages tested (Figure 5.1). The only other observation period to show an effect was the 24-h observation of 3-d old residues. This pattern was not due to mortality, however; the number of dead beetles in indoxacarb treatments was not significantly different from that

of the controls at any observation period across residue ages (Figure 5.3). The measure of unimpaired beetles is due completely to the intoxication of the plum curculio adults (Figure 5.2). Effected adults were uniformly on their backs, slowly moving their legs. However, their flight musculature was not similarly compromised; when intoxicated beetles were disturbed (by shaking the bioassay container), they would skim inverted across the floor of the container. Even after 120 h (data not shown) of exposure to 1-d old residues, beetles impaired in the indoxacarb treatments had still not succumbed.

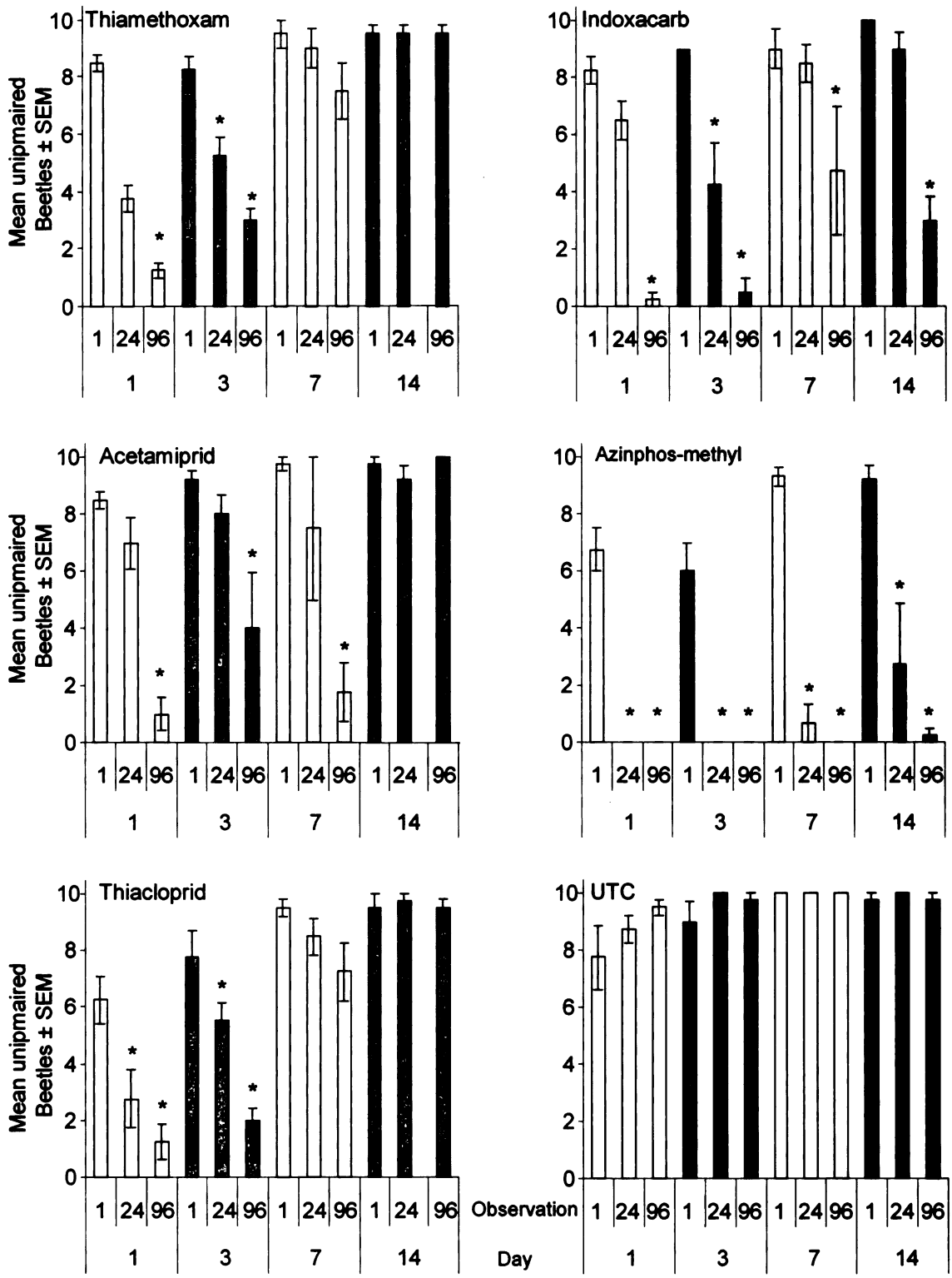

Figure 5.1. Mean number of unimpaired plum curculio observed at three intervals (1, 24, 96 h) within four post application intervals (1, 3, 7, 14 d). Bars with * above them designate significant difference ($\alpha < 0.05$) from untreated control at the same post-treatment day/observation combination.

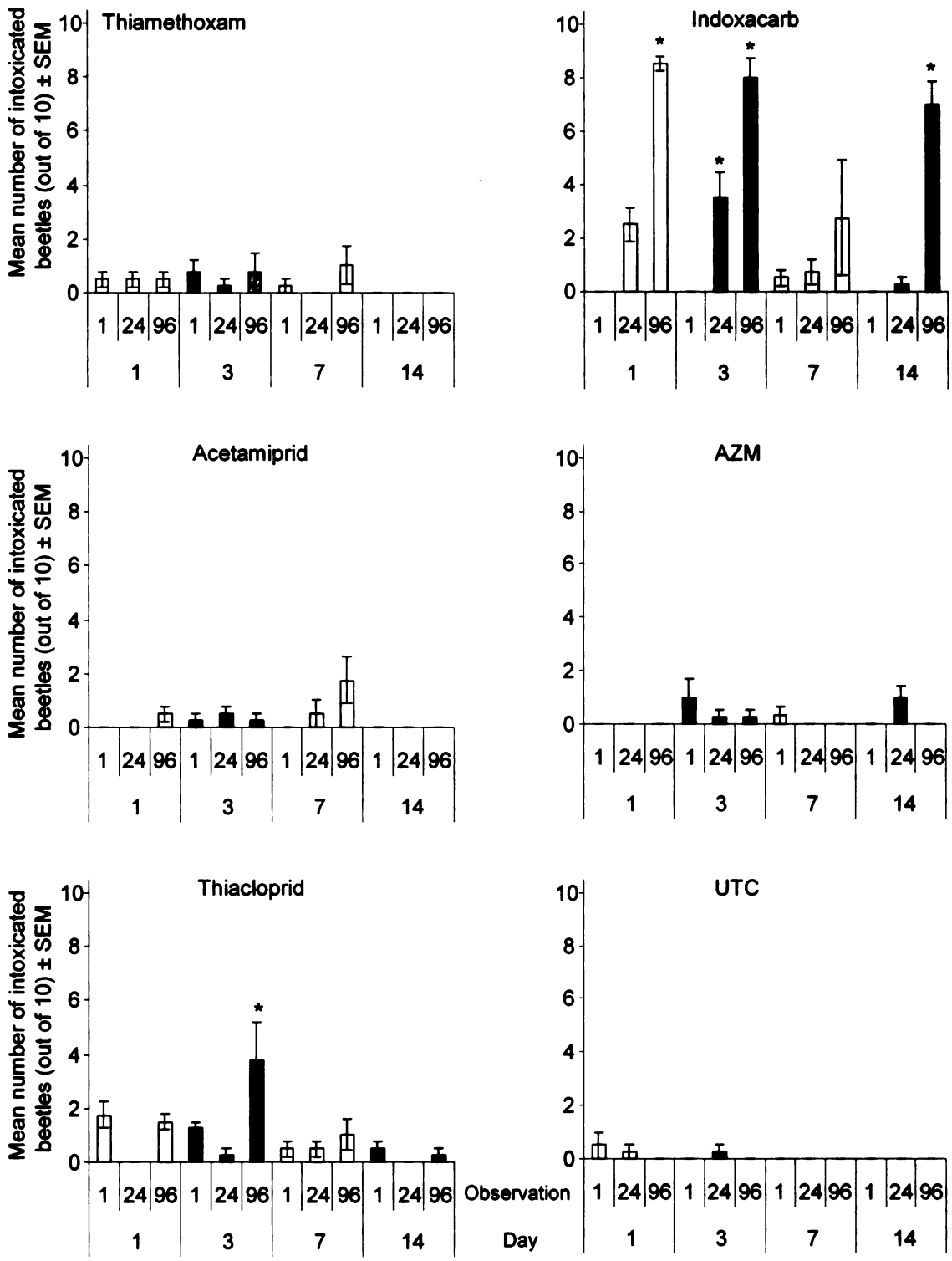

Figure 5.2. Mean number of intoxicated plum curculio observed at three intervals (1, 24, 96 h) within four post application intervals (1, 3, 7, 14 d). Bars with * above them designate significant difference ($\alpha < 0.05$) from untreated control at the same post-treatment day/observation combination.

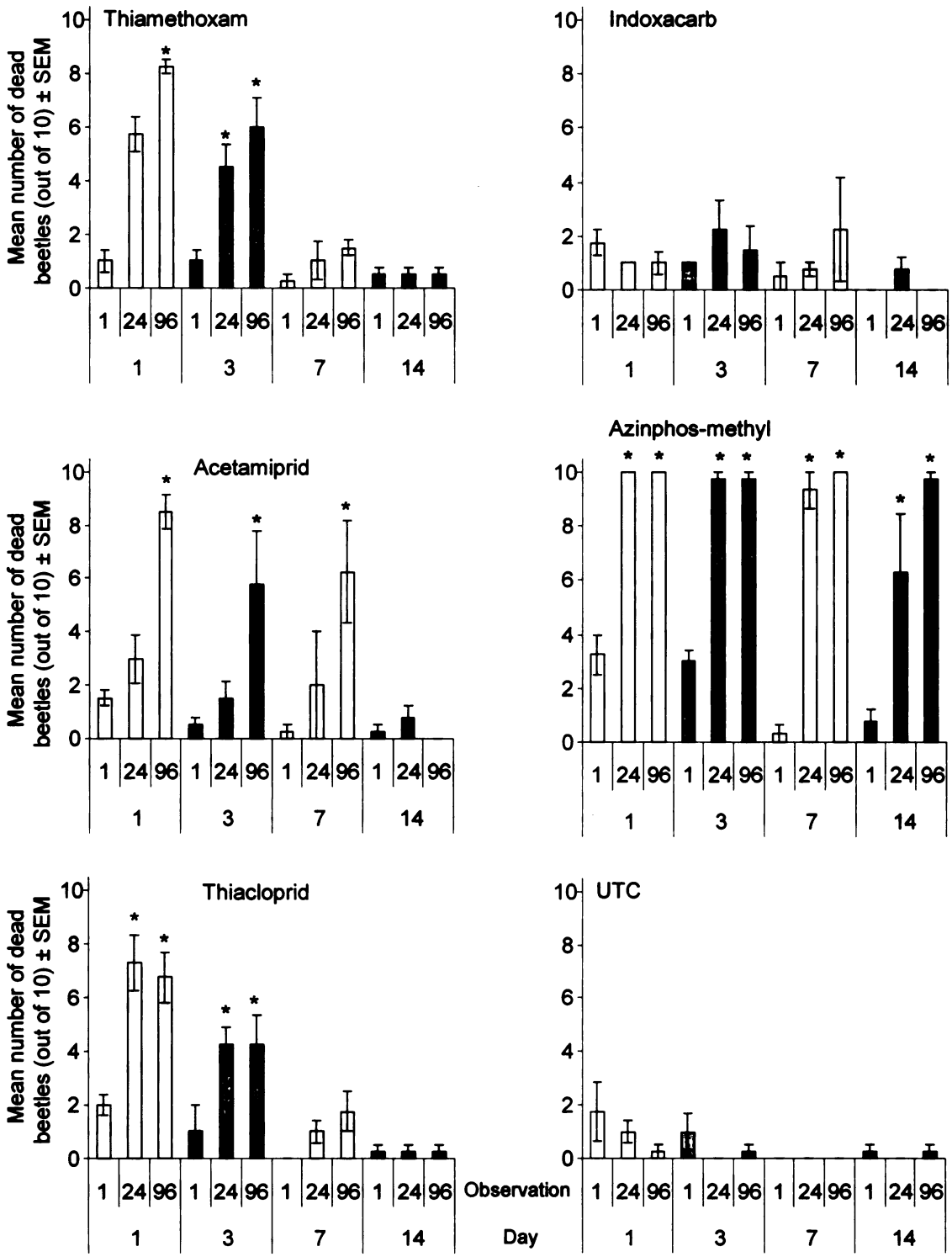

Figure 5.3. Mean number of dead plum curculio observed at three intervals (1, 24, 96 h) within four post application intervals (1, 3, 7, 14 d). Bars with * above them designate significant difference ($\alpha < 0.05$) from untreated control at the same post-treatment day/observation combination.

Plant Tissue Damage

Beetles fed on the field-collected leaves and fruit that were brought into the laboratory. Plant tissue damage after 96 h of beetle exposure could be broken up into leaf damage, fruit feeding damage and fruit oviposition damage. Leaf damage was minimal in terms of surface area. Damaged area was always less than 5% and was either a few feeding holes or some margin feeding. Single leaf feeding events could not be separated, so the most accurate measure was the proportion of leaves that were damaged; these data were arcsin-square root transformed for analysis. Leaf feeding in the untreated controls declined over the 14 days of the experiment (Figure 5.4). There was a significant effect of treatment ($F = 10.03$; d.f. = 5, 25.6; $P < 0.0001$) and post-treatment day ($F = 10.57$; d.f. = 3, 31.6; $P < 0.0001$) but all of the treatments behaved similarly over time (no significant treatment x day interaction). Leaf feeding overall declined over the course of the experiment; the least-squared means comparisons show that the proportion of damaged leaves (across all treatments) on post-treatment days one and three were significantly higher than that of days seven and fourteen. The mean proportion of leaf damage was highest for the untreated control and acetamiprid, azinphos-methyl, thiacloprid and thiamethoxam all had significantly reduced proportions of damaged leaves relative to the control (figure 5.4).

Unlike leaves, the proportion of untreated fruit with damage (of any type) did not vary significantly across the post-treatment intervals (Figure 5.5). There was an effect of which treatment was applied to the fruit ($F = 10.49$; d.f. = 5, 56.8; $P < 0.0001$) and of which post-treatment day the trials were done ($F = 21.40$; d.f. = 3, 36.8; $P < 0.0001$) but there was not a significant interaction between these two main effects. All of the

chemical treatments had a significantly lower cell mean for proportion oviposition than the untreated controls, but were not significantly different from one another. On average, days one and three post treatment had a significantly lower proportion of fruit damage than days seven and fourteen post application.

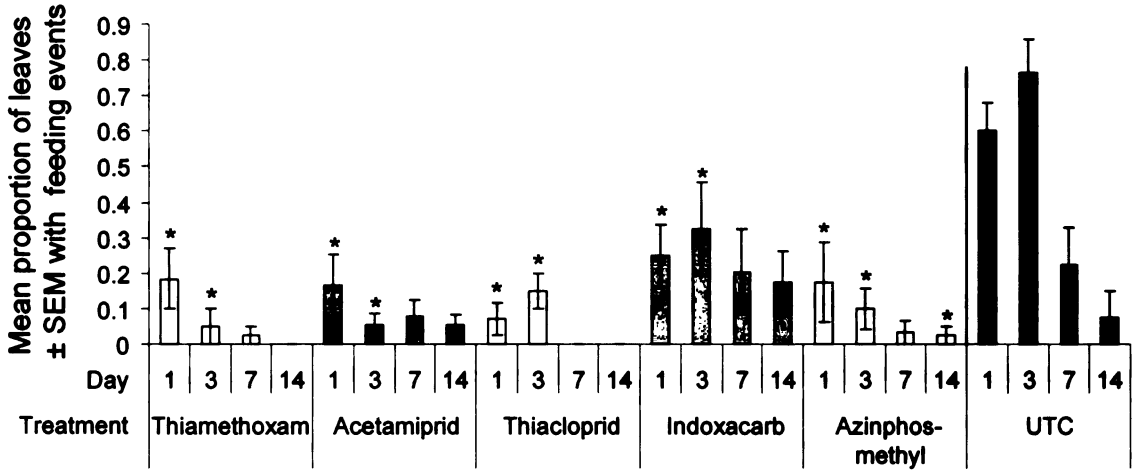

Figure 5.4. Mean proportion of insecticide-treated tart cherry leaves with feeding damage after plum curculio were allowed to feed on insecticide-treated branches for 96 h. Beetles were exposed to branches 1, 3, 7, and 14 d after insecticides were applied. Bars with * above them designate significant difference ($\alpha < 0.05$) from untreated control at the same post-treatment day.

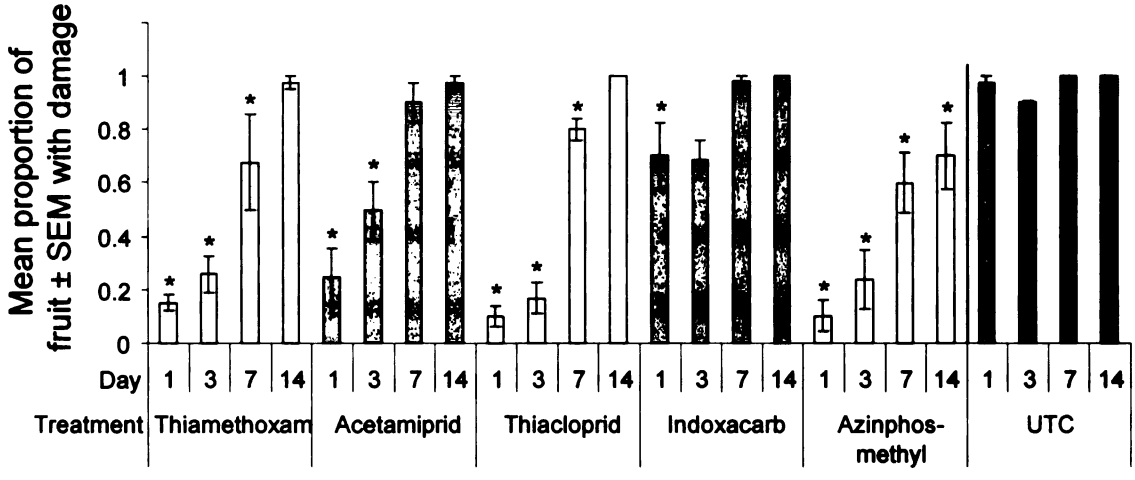

Figure 5.5. Mean proportion of tart cherry fruit with feeding or oviposition damage after plum curculio were allowed to feed on insecticide-treated branches for 96 h. Beetles were exposed to branches 1, 3, 7, and 14 d after insecticides were applied. Bars with * above them designate significant difference ($\alpha < 0.05$) from untreated control at the same post-treatment day.

Fruit feeding and oviposition are both ingestion processes and combining these events for analysis gives an overall view of impact on fruit protection and possible anti-feedant effects. Overall feeding was significantly impacted by treatment ($F = 42.70$; d.f. = 5, 23.8; $P < 0.0001$) (Figure 5.6A) day ($F = 61.84$; d.f. = 3, 28.8; $P < 0.0001$) and the treatment x day interaction ($F = 3.26$; d.f. = 15, 36.3; $P = 0.0018$). Thiamethoxam and azinphos-methyl both reduced the number of fruit damage events all the way out to day fourteen. Acetamiprid, thiacloprid and indoxacarb significantly reduced the number of events (relative to the untreated replicates) out to day seven.

Individual fruit feeding and oviposition events were easier to determine on fruit than for leaves. Counts of oviposition scars were square-root transformed for analysis. There were significantly more oviposition scars in the controls ($F = 9.77$; d.f. = 5, 16.8; $P = 0.0002$) (figure 5.6B) and there was a significant effect of post-treatment interval ($F = 24.20$; d.f. = 3, 24.20; $P < 0.0001$). There was no significant day x treatment interaction. The mean number of oviposition scars for the seven and fourteen day post-treatment timing were significantly higher than the number of scars in the two earliest periods. The mean oviposition counts for indoxacarb treatments were not different from the controls, but thiamethoxam, thiacloprid, acetamiprid, and azinphos-methyl all had lower values for overall oviposition when compared to the control. Fruit in indoxacarb treatments had lower oviposition counts than the controls only at the first day after pesticide application (Figure 5.6B). Oviposition scar counts in thiamethoxam and thiacloprid treatments were significantly lower than the relevant controls at both one and three days after insecticide application. Acetamiprid and azinphos-methyl treatments yielded significantly fewer oviposition events through seven days post-application. There was a significant

reduction in the number of oviposition scars in the untreated fruit on the third day after treatment, relative to the other untreated post-application intervals. This reduced incidence of oviposition is possibly due to unsuitable laboratory conditions or a synchronicity in egg maturation in the colony.

The incidence of fruit feeding was significantly different across treatments and controls ($F = 44.84$; d.f. = 5, 31.9; $P < 0.0001$) (Figure 5.6C). There was a significant effect of post-treatment day on the number of observed feeding sites controls ($F = 58.43$; d.f. = 3, 29.2; $P < 0.0001$) as well as significant interaction between these two main effects controls ($F = 2.98$; d.f. = 15, 34 ; $P = 0.0041$). Unlike the measurements for oviposition, the untreated fruit showed no difference in non-ovipositional feeding events across the study periods. Azinphos-methyl and thiamethoxam reduced the number of feeding events in all four study periods. Acetamiprid and thiacloprid reduced fruit feeding up to day seven post-application and the effects of indoxacarb were only observed in residues aged one and three days (Figure 5.8).

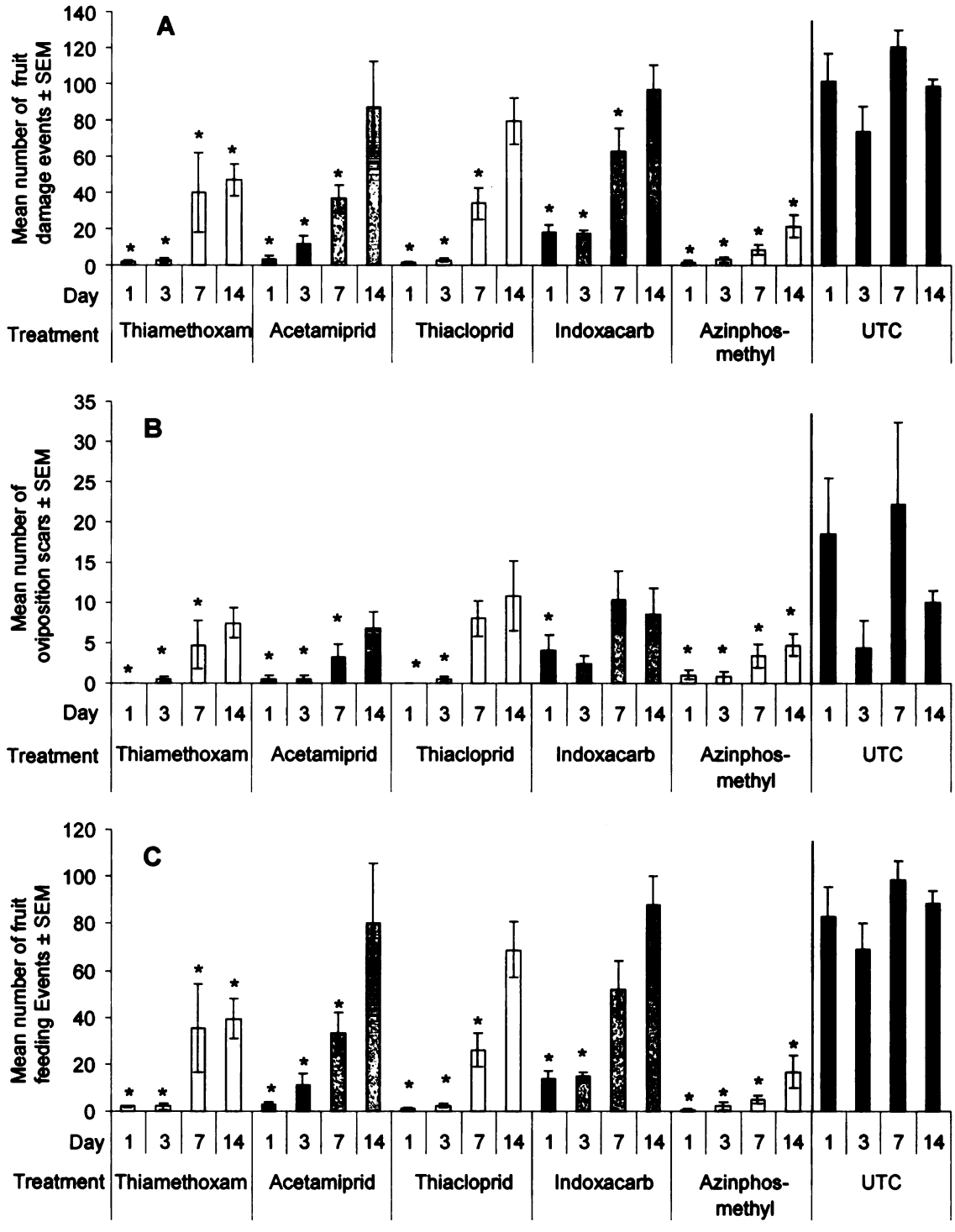

Figure 5.6 (A-C). Mean number of A) total fruit damage events (feeding plus oviposition); B) Oviposition scars; C) Feeding events from plum curculio after beetles were exposed to insecticide-treated branches for 96 h. Beetles were exposed to branches 1, 3, 7, and 14 d after insecticides were applied. Bars with * above them designate significant difference ($\alpha < 0.05$) from untreated control at the same post-treatment day.

Residue profiling

Insecticide residues of all compounds were recovered from fruit and leaf tissue (Table 5.2). For the neonicotinoids thiamethoxam and acetamiprid, there is a well defined pulse of material into the interior of fruit in leaves that is not observed in thiacloprid. Thiacloprid had the highest residue levels of any compounds on the leaf surfaces. Indoxacarb had leaf surface residues around 1 ppm throughout the study period, but no detectable interior residues in either fruit or leaves after the first day post-application. Fruit surface residues of indoxacarb decreased from 80 to 30 ppb across the first three study observations and was not detected at 14 d. Azinphos-methyl was detected at a maximum average surface residue of 45.4 ppm, and maximum internal residues of 1.4 and 1.1 ppm in leaves and fruit, respectively. Even 14 d after application, there was still an average of 0.44 ppm azinphos-methyl recovered from the fruit interior.

Table 5.2. Insecticide residue recoveries (ppm ± SEM) from cherry leaf and fruit tissue at four periods after application with an airblast sprayer

		Leaf		Fruit	
Compound	Day	Surface	Interior	Surface	Interior
Thiamethoxam	1	47.62 (13.52)	26.63 (10.75)	15.58 (9.00)	1.01 (1.01)
	3	42.85 (16.60)	8.87 (6.05)	10.22 (7.53)	nd
	7	9.20 (4.95)	nd	9.75 (6.42)	52.69 (45.16)
	14	nd	3.56 (3.56)	0.13 (0.13)	2.12 (2.12)
Acetamiprid	1	1.64 (0.70)	2.01 (0.61)	1.22 (0.40)	3.00 (0.20)
	3	2.21 (2.21)	0.48 (0.14)	2.34 (0.81)	0.19 (0.19)
	7	0.49 (0.34)	8.10 (3.01)	0.52 (0.12)	1.18 (0.46)
	14	0.52 (0.27)	2.39 (1.09)	0.09 (0.03)	0.31 (0.18)
Thiacloprid	1	234.70 (42.40)	(no samples)	9.82 (1.16)	5.92 (3.61)
	3	74.26 (7.82)	nd	10.00 (NA)	nd
	7	31.97 (4.06)	0.42 (0.42)	nd	nd
	14	9.87 (3.48)	nd	nd	nd
Indoxacarb	1	2.02 (0.47)	0.01 (0.00)	0.08 (0.01)	nd
	3	1.37 (0.19)	nd	0.06 (0.01)	nd
	7	0.34 (0.22)	nd	0.03 (0.02)	nd
	14	1.14 (1.14)	nd	nd	nd
Azinphos-methyl	1	45.36 (10.46)	0.07 (0.03)	2.57 (0.64)	0.06 (0.01)
	3	6.87 (6.87)	1.40 (0.23)	5.74 (5.27)	1.10 (0.06)
	7	12.22 (2.85)	0.05 (0.01)	0.08 (0.04)	0.06 (0.06)
	14	1.39 (0.71)	0.08 (0.03)	nd	0.44 (0.04)

Discussion

This longitudinal study of aging residues allows us to identify and separate lethal and sublethal modes of fruit protection. Fruit protection in azinphos-methyl treatments is best explained by acute lethal activity due to contact with surface residues; there are significant reductions in survivorship, but very little feeding. Azinphos-methyl provided significant levels of fruit protection all the way through the 14 d study period. Even at the last sample day, beetle mortality in azinphos-methyl treatments was nearly 100%. The only measure of fruit protection that was not significantly reduced at day fourteen was the number of oviposition scars. In optimal weather conditions, the period of activity for azinphos-methyl probably extends at least another few days beyond the 14 d study period reported here.

The neonicotinoids and indoxacarb do not share this same direct relationship between mortality and fruit protection. Closer examination of the patterns of mortality and plant tissue damage in the neonicotinoid treatments yields insights into the lethal and sublethal mechanisms of fruit protection afforded by the class. For thiamethoxam and thiacloprid, there no reduction in the number of unimpaired beetles relative to the untreated controls at day seven, but the incidence of fruit damage (feeding, oviposition scars, proportion fruit damage) was reduced. Thiamethoxam's effect of reducing fruit damage events extended out to 14 d post-application. This fits with previous observations that neonicotinoids can have a sublethal, antifeedant effect (Nauen et al. 1998, Drinkwater 2003, Wise et al. 2006, 2007b; Tansey et al. 2008) and closely parallels observations made of plum curculio response to field-aged residues in apples. Seven days after application to apple trees, there was no significant mortality in thiacloprid or

thiamethoxam treatments, but significant reductions in fruit feeding and oviposition marks, and this pattern extended out to 14 d for thiamethoxam and fruit feeding (Wise et al. 2006). The data shown here cannot specifically define the span of this sublethal activity, other than to say that it fits somewhere between 3 d and 14 d post application for thiamethoxam and thiacloprid. There are still surface residues present on fruit or leaf tissue at 14 d for both of these compounds, and interior residues greater than 1 ppm for thiamethoxam. It is likely that leaf surface residues are the major contributor; leaves contribute most of the surface area in the bioassay (and in an orchard). There were no fruit interior residues after the first day post application for thiacloprid, which is identical to the pattern found in apples (Wise et al. 2006). If ingestion is a major mode of exposure for thiacloprid, it should be used early in the season

Unlike the other two neonicotinoids, beetles exposed to 7 day residues of acetamiprid did couple significantly lower numbers of healthy beetles relative to the untreated controls with significantly reduced counts of oviposition and feeding. The proportion of fruit with damage was not reduced, though; beetles may have detected the insecticide after a few feeding events and moved to a different fruit until they finally succumbed. Neither increased mortality nor fruit protection was evident at 14-d post application in acetamiprid treatments. It is possible that there was a period between seven and fourteen days post application where there were antifeedant levels of acetamiprid residue present on the fruit. It should be noted that plum curculio exposed to acetamiprid take longer to demonstrate signs of poisoning than the other two neonicotinoids tested. Acetamiprid residues remained fairly consistent over the time period, and were always above 0.1 ppm.

Neonicotinoids are currently the likely replacement compounds for azinphos-methyl in plum curculio management programs. While this class lacks the long lived lethal activity of the organophosphates, the neonicotinoids to have a suite of sublethal antifeedant, oviposition deterrent, and curative activities (Chapter 4) that should allow for successful management of this pest. Data from apple maggot field and laboratory trials also suggest neonicotinoid oviposition deterrent activity. Thiacloprid reduced apple maggot infestation rates in field trials at residual rates that were not toxic to adults in laboratory (Reissig 2003). It is important to note that there is likely to be more plum curculio damage in programs that rely on neonicotinoids than in the traditional organophosphate programs, especially as residues age into the "antifeedant" levels. However, oviposition damage should be treated specially in light of the known curative activity of these compounds. Existing oviposition scars may not represent a live-infestation risk at harvest. Scouting methods may have to be modified to adjust for this reality.

Indoxacarb provided some fruit protection on the first and third day after fruit were sprayed. There were fewer feeding events at the third day after application, but the proportion of indoxacarb-treated fruit damaged was not different than the untreated controls. Even though the final proportion of impaired and dead beetles exceeded 75% at the third and fourteenth post application day, beetles were still able to feed extensively prior to succumbing. This long delay in effect was also noted in field controlled dose studies (Wise et al. 2006); direct application of 10 μg indoxacarb (one-half the LD_{50} dose) to plum curculio yielded a lethal half-time of 114 h.

Indoxacarb cannot be applied before or during bloom due to its toxicity to honeybees. Given this constraint, indoxacarb activity against adult plum curculio would be optimized between petal fall and shuck off. Plum curculio move into the orchards in advance of bloom and do feed on tree tissues (leaves, floral parts) at this time (Racette et al. 1992, Chouinard et al. 1993, Hoffmann et al. 2006, Appendix 3). Developing fruit are somewhat protected by the cherry shuck, and applications well in advance of shuck off would provide the necessary exposure route for this ingestion-activated compound while minimizing the risk of fruit damage during the exposure period. Intoxicating levels of leaf surface residues persisted to 14 d post application in this study, but care should be taken to monitor damage on fruit after shuck off. Without the treated shuck as a barrier, the individual fruit probably have limited chemical protection. Application of indoxacarb after shuck off probably represents an excessive risk for fruit damage. Indoxacarb has limited curative potential (chs 2, 3 & 4) and applications after fruit are exposed would require a follow-up spray to kill eggs and larvae.

It is not clear whether exposure to field residues of indoxacarb results in actual mortality of effected plum curculio. In this field-based study, intoxicated plum curculio did eventually die, but it may have been from dehydration in the laboratory assay chamber rather than direct toxicity. In the orchard, impaired beetles would likely fall to the ground, where morning dew and rain events might provide enough moisture to support beetles through the process of detoxification. This cycle of exposure-detoxification might continue until indoxacarb residues dropped below an effective level.

Penetration of an insecticide into fruit tissue and bioavailability to an insect walking or feeding on plant tissue depend on the interaction between the compound and

the tissue. Compounds vary in their ability to move through the waxy layer and into the cuticle and subsurface tissues. This mobility is based on the intrinsic chemical properties of both the plant tissue surface and the pesticide that govern the steps of sorption onto/into the cuticular waxes, diffusion through the cuticle and desorption into the inner tissue (Kirkwood 2001, Buchholz 2006). A single compound may have varying properties across plant types (Chowdhury et al. 2001), and the plant cuticular properties may change dramatically during leaf development (Jetter and Schaffer 2001, Belding et al. 2000). The research shown here is a snapshot of one period during the development of cherry fruit and foliage. Fruit and leaf surface and interior chemistries do change over the entire course of development (Ishida et al. 1997, Knoche et al. 2004) and it is likely that they will have different relative permeabilities as the season progresses. As a result, insect response to pesticide applications may not be consistent across plant phenology.

It needs to be noted that the insecticide applications described here meet current pre-harvest label requirements. The residues that are recovered from fruit tissue are transient, and treated fruit are expected to meet pesticide residue tolerances by the time fruit are harvested.

Fresh market fruit crops are principally valued according to their size, color and degree to which they are free of surface damage. Surface damage is often an indicator of internal insect infestation and can also serve as an entry point for pathogens. Fruits such as apples and peaches can spend weeks or even months in storage, and it is important to minimize all agents that affect the integrity of the fruit. Consumers demand whole fruit with an intact appearance, and because of this growers and researchers have focused their insecticide evaluations on addressing this specific endpoint. Field trials of insecticides in

Michigan apples are evaluated throughout the season for damage by a whole suite of herbivorous insects: codling moth, oriental fruit moth, plum curculio, leafrollers, green fruitworm and tarnished plant bug.

Evaluating insecticides in processed tart cherries requires a somewhat different approach. Because of the zero-tolerance regulatory framework, the primary at-market concern for this crop is actual arthropod infestation. Feeding and other surface damage during the growing season are certainly concerns because of the yield reduction and tree health, but surface damage is not the major driver of tart cherry market value. As such, an important measured endpoint should be the infestation rate under different treatment regimes. The results of this study mark the infestation *potential* after treatment with several different insecticides, but do not address the final infestation rate. Even for those materials and timings that did have higher rates of oviposition, we should consider that female plum curculio do not lay an egg in every oviposition scar, and eggs are susceptible to curative action of insecticides that penetrate through the fruit skin.

Acknowledgements

I thank Amanda Carper for maintaining our northern strain plum curculio source colony and Jason Seward for managing the pesticide applications. I also thank Tom Guest for his assistance in preparing the residue samples and Dr. Christine Vandervoort and the MSU Pesticide Analytical Laboratory for supporting the analytical chemistry.

Chapter 6: Bringing it all together.

Tart cherry management has relied on the acute lethal action of organophosphate compounds for over half a century. While this activity alone can significantly reduce the damage potential of any pest population, there are a range of possible crop protection modalities that do not require fast acting insecticides. Antifeedant activity, oviposition deterrence, and sterilization are three key non-lethal means of fruit protection. These modalities are a challenge to identify. Measuring sublethal activities requires more time than dosing insects and counting dead individuals after 48 h. They also require researchers to have a more detailed understanding of the possible outcomes and specific means to measure these outcomes.

Laboratory bioassays establish the baseline expectations for acute toxicity but they are not complete predictors of actual field performance. In order to truly assess and optimize a chemical control tactic, one must consider all of the elements of the PIC triad (Wise et al. 2006, 2007a). This collection of studies on plum curculio (*Conotrachelus nenuphar* Herbst) has used this PIC-triad model to explore the potential modes of activity responsible for fruit protection with the current set of insecticides in tart cherry. These studies range from novel bioassay methods for plum curculio egg and larva toxicity, field-based bioassays, and insecticide residue recoveries from fruit and leaf tissue in the field. This composite view provides deeper insights into the crop protection potential than any one set of experiments.

Commentary on Insecticide Classes

These studies have assessed nine compounds for management of plum curculio. Not all of these compounds have received the full laboratory-field-residue assessment, but even preliminary results can help us predict some patterns of utility for plum curculio control in tart cherries and other susceptible fruits.

Organophosphates

Azinphos-methyl and phosmet are known for their strong lethal action as plum curculio contact adulticides. Both of these compounds were toxic to egg and larval life stages in the laboratory and reduced larval emergence from infested cherry fruit in field curative studies. These findings on azinphos-methyl are very important. This compound is viewed as the standard for curculio management, and the success of this compound has been due to a combination of potent adulticidal activity as well as a dramatic, and yet undescribed, curative activity. The curative activity establishes another baseline of activity for future tactics as this class of insecticides is withdrawn from agricultural use.

Neonicotinoids

The neonicotinoids are likely to be the next set of "workhorse" tools for plum curculio control. Their laboratory adulticidal activity does not match that of azinphos-methyl, but the observed prophylactic fruit protection stems from initial lethal activity and sublethal antifeedant activity as residue levels decline. This antifeedant activity translates directly into oviposition protection, since females feed on fruit as part of the oviposition process.

There is quite a bit of variation across this class when measuring the intrinsic toxicity to eggs, which makes general claims on curative potential a challenge if one only looks at ovicidal activity. Acetamiprid, thiacloprid, and clothianidin are all lethal to eggs at concentrations below 100 ppm, whereas thiamethoxam is ineffective. When one incorporates larval bioassay data, the neonicotinoids perform more uniformly; larvae are susceptible to all of these neonicotinoids at 1 ppm levels. At the field level, these compounds perform quite well, with greater than 70% reduction in larval emergence when targeting eggs, neonates or late instars.

The neonicotinoid class is an important proving ground for ideas relating to plant-chemical and insect-chemical interactions. While the toxicological mode of action is uniform across the class, the chemical properties (size, polarity, water solubility, etc.) vary greatly across the different compounds. These properties define mobility across barriers in plant (cuticle, cell walls) and insect (chorion, exoskeleton, gut wall) tissues, and as such, we cannot assume uniform effectiveness, even though these compounds share a molecular target within the insect. Given the variation in chemical properties across this class, I think the neonicotinoids represent a model series for evaluating penetration dynamics of insecticides. These studies are made more interesting by metabolic processes like the conversion of the hydrophilic thiamethoxam to the lipophilic clothianidin. This ambivalent property may be an important part of thiamethoxam's efficacy.

The broad use of neonicotinoids in orchard systems for pest management demands an increased attention to resistance management in the entire orchard pest complex. Incidental insecticide exposure to pest insects that were not the intended target

of the insecticide application represents a known resistance-development scenario. The suite of pests in the orchard should be a consideration for IPM developers when they are developing rate and timing recommendations.

Pyrethroids- Esfenvalerate

Only one pyrethroid, esfenvalerate, was tested against plum curculio. This compound was highly lethal to eggs and larvae in the laboratory yet failed in the field. It appears that the active ingredient in the current formulation (Asana® XL) does not move sufficiently into the fruit flesh; extremely low residues (or zero) levels of this compound were detected from fruit interior samples. The results of the esfenvalerate curative studies illustrate the value of the plant-insect-chemical framework, rather than sole reliance on laboratory or field efficacy data that focuses only on adult activity. This integrated framework tells us that we have a highly active compound that is simply not getting to the eggs and larvae.

Low internal residues for esfenvalerate are also seen in apple fruit after June foliar applications (Wise, Hoffmann and Vandervoort, unpublished). This compound is used for the control of other fruit pests but it is unlikely that it is serving a curative purpose in apples.

Because of the extremely favorable activity of esfenvalerate against plum curculio eggs and larvae in the laboratory (Chapters 2 & 3) and good activity against plum curculio adults (Wise et al. 2008), I view esfenvalerate as a "compound of special interest." In its current Asana® XL formulation, it is probably not serving as a curative

compound, but reformulation or specific tank mixes with surfactants or spreader/stickers might dramatically improve its curative activity.

Oxadiazines- Indoxacarb

Indoxacarb is the only member of the oxadiazine class that was tested against plum curculio. This compound has a much slower course of toxicity than most other compounds, with post-exposure killing times on the order of days, instead of hours. This property is likely due to the post-ingestion bioactivation step required for this compound to become toxic. As such, this compound should *not* be used in conjunction with materials (like neonicotinoids) with antifeedant effects.

The ingestion requirement for this compound also poses problems for timing it appropriately. If applications are timed after fruit are available, there could be unacceptable levels of damage before beetles actually succumb. A late bloom/petal fall application of this compound is the right phonological timing for the beetle since plum curculio are actively feeding on cherry flower tissues. However, indoxacarb is highly toxic to honeybees, and a bloom application is not appropriate. A petal fall timing would fit the plum curculio's phenology, but I have concerns about the behavioral fit. Spraying while the shuck is still on does expose beetles to the insecticide, but when the shuck falls off, the fruit is left completely exposed with no insecticide coverage. Rain and weathering events would further minimize the effectiveness of this strategy.

Juvenile Hormone Mimics- Pyriproxyfen

The juvenile hormone mimic pyriproxyfen does not cause acute mortality in plum curculio adults, eggs or larvae, but it does have potent sublethal effects that suggest that its use in pre-harvest tart cherries should be strictly avoided. Infested cherries treated with pyriproxyfen had dramatically reduced larval emergence. Superficially, this is a demonstration of efficacy. However, dissection of these infested cherries a week after larvae emerged from untreated cherries yielded high numbers of large, live plum curculio larvae in the fruit. The physiological cascades that drive pre-pupal behavior seem to be disrupted to the point where pyriproxyfen-treated larvae continued to feed inside the fruit despite being an appropriate size for pupation. This could be disastrous in the field, especially if growers relied on adult-targeted sprays during the traditional 2-week "window" prior to harvest. If pyriproxyfen had been used earlier in the season, larvae from 3-4 weeks prior to harvest could still be in the cherries at harvest time.

Pyriproxyfen is a useful tool for controlling scale insects in tree fruit, but pre-harvest use of this compound should be avoided in tart cherry orchards with a plum curculio population. If pyriproxyfen sprays are vital for the control of other pests, I would strongly recommend that growers incorporate compounds with plum curculio curative activity to guard against the enhanced infestation risk after pyriproxyfen treatment.

Pyriproxyfen may have utility as a post-harvest population-level control strategy for plum curculio. Summer-emerged northern strain females initiate productive mating and egg laying behaviors after exposure to contact and residual levels of pyriproxyfen (Hoffmann et al. 2007). This switch to reproductive behavior may reduce their cold

hardiness; consequent winter kill may reduce the plum curculio population pressure the following season.

This post harvest use of pyriproxyfen may induce injury in adjacent crops that still have fruit on the tree – particularly apples. Plum curculio females might move to these trees and lay eggs in the maturing apples. It is unknown whether curculio find these larger fruits acceptable, and the risk needs to be explored further. There are insecticides being used for control of other apple pests (Codling moth, leafrollers, Oriental fruit moth) during August/September, and these might provide sufficient control of a second generation of plum curculio.

Benzoylureas - Novaluron

Novaluron was a potent ovicide and larvicide in laboratory studies. The field studies for this compound suggest limited fruit curative activity however. Like esfenvalerate, this reduced efficacy appears to be a product of limited penetration into fruit tissue. Novaluron did not show curative activity when applied to thinning apples, and less than 0.07 ppm novaluron was recovered from the flesh of treated fruit (Wise et al. 2007a).

While novaluron may have limited utility in a post-oviposition curative application, there are promising data on its use as a pre-oviposition chemosterilant for plum curculio. There was a 93% reduction in larval emergence from rearing apples after female plum curculio were exposed to fruit treated with 240 ppm novaluron (Wise et al. 2007a). An early season spray of this material may not reduce oviposition scarring (Ki

Kim, personal communication) but it could reduce the population of summer-emerged adults.

Anthranilic Diamide- Chlorantranilprole

Development trials of chlorantrianilprole have not demonstrated high levels of plum curculio protection in apple field trials (Wise, personal communication). But we cannot ignore the potential for alternative modes of crop protection. Unfortunately, chlorantranilprole does not have laboratory-based ovicidal activity, and field curative assays targeted at neonates did not significantly reduce larval emergence.

The Curative Approach

This series of studies started out as a descriptive study of the residue on and within fruit and how adult plum curculio behavior and mortality changes with these changing residues. Limiting strategies to adult control ignores the life stage of actual concern in cherries. Simply put, plum curculio adult control in tart cherries is *useful but not vital*; reducing live larval infestation is the real necessity.

This philosophical point is a challenge for researchers and growers who work primarily in curculio-susceptible fresh-market fruits like apples, peaches, plums, sweet cherries and blueberries. The curative approach is seen as irrelevant for fresh markets and their demand for blemish-free fruit; damaged fruit are no longer economically valuable and not worth protecting. While growers who supply fresh market fruit are reasonable in their focus on prophylactic tactics to meet consumer quality demands, I suggest that integrating a curative tactic is far from irrelevant. As part of a population-

reduction strategy, this tactic could serve to greatly minimize F1 recruitment, and it is likely that growers are relying on curative activity without even realizing it.

In apples, the period of major plum curculio egg laying is during the month of June. Apples damaged by plum curculio are more likely to drop off the tree during the "June drop" phenological event, than undamaged fruit (Levine and Hall 1977, 1978). These damaged apples, both on and off of the trees, are a definite contribution to late-season and subsequent-year plum curculio populations. From an economic standpoint, curative control of plum curculio at these periods makes good economic sense.

In most orchards, growers have probably been reliant on curative activity for decades without even realizing it. Azinphos-methyl and neonicotinoids are recommended for June cover sprays to control codling moth, potato leafhopper, leafrollers, rose chafer, and green apple aphid often contain neonicotinoids or azinphos-methyl (Wise et al. 2008). The neonicotinoids thiacloprid and thiamethoxam have known curative activity in apples (Wise et al. 2007a), and acetamiprid has shown significant reduction in larval emergence from infested apples (Wise & Hoffmann unpublished).

In processed tart cherries, integrating a curative approach with a solid adulticidal program is likely to reduce the number of insecticide applications against plum curculio. If orchards have plum curculio activity, current programs typically spray at petal fall and have up to three additional cover sprays in the 10 weeks prior to harvest to reduce adult activity and possible damage. An integrated program would place the compounds that most effectively reduce viable oviposition damage early in the season and save curative compounds for later in the season if monitoring indicates that viable oviposition is still continuing. An early season application of azinphos-methyl or phosmet (while available)

would likely give 10-14 days of lethal action against adults, with the penetrating residue killing eggs or larvae from this early period. This early timing would also be appropriate for chemosterilant compounds. Monitoring for additional damage is important in the two to three weeks after these applications. Determining the effectiveness of chemosterilant strategies and residual curative action will require scouts to assess the viability of eggs inside of oviposition scars. But this information may guide growers to delay the next set of sprays (adult/curative target neonicotinoids) until viable oviposition is detected again. Data suggest that the timing of curative sprays of neonicotinoids is forgiving; eggs and small larvae are equally susceptible to this class. The collective lethal and antifeedant activity of neonicotinoids extends about seven days after application. After this point, monitoring would begin again for *new* oviposition scars, probably every three to five days to maximize a spray's chance to kill larvae before significant fruit damage occurs.

Such an approach will be information intensive and may not save growers money. Integrated pest management makes no claims on being the most financially efficient approach, but it is one that seeks to provide the greatest benefits to the grower, the environment and society.

One of the guiding principles of integrated pest management is that of targeting the most susceptible life stage. Plum curculio larvae have been ignored as a target for decades, even in the face of true curative activity. With the loss of azinphos-methyl, eggs and larvae inside the fruit may actually represent the stages of greatest susceptibility to the next generation of tools. This research has rediscovered this mode of activity and I feel that the maturation of this strategy will bring positive results for growers at the post harvest processing plant.

Generalizing the process

Insecticide performance is inherently linked to a specific crop. The previous summaries relate to the plant-insect-chemical interactions of plum curculio in cherry. We cannot assume insecticide efficacy in other crops based on these cherry results. Apples, peaches, sweet cherries and tart cherries have their own characteristic cuticular wax profiles and permeabilities. Surface chemistry profiles also change as fruit develops (Knoche et al. 2001, Knoche et al. 2004, Peschel et al. 2007) and can differ between varieties of the same fruit (Belding et al. 2000).

This variability makes it a challenge to make accurate predictions whether *compound A* is going to have plum curculio curative activity in *crop W*. This gap in data represents an opportunity for plant physiologists to gather and model data on the penetration of compounds in fruits with various surface characteristics. A multivariate approach could identify and quantify the key physical properties that govern a pesticide's penetration potential.

Absent this type of model of insecticide mobility, we must be satisfied with single-crop, single variety trials. The process outlined in this thesis is reasonably efficient in addressing the key performance characteristics of a set of compounds:

1) Baseline toxicity studies: These studies should be done on all possible life stages. These studies may already be published for some compounds. There is a wide breadth of possible sublethal responses to insecticide exposure (antifeedant, oviposition deterrent, sterilant); researchers need to explore insect responses even if basic toxicity studies are not promising. For compounds that are notably toxic, these

baseline data can be combined with residue data to determine whether current application timings and methods achieve the toxic concentrations.

2) Residue profile analysis: The spatial and temporal aspects of insecticides are what make the physiological effects biologically relevant.

3) Field-based bioassays: These studies put all three elements of the plant-insect-chemical triad together. Treated plant material is exposed to the insect of interest in the laboratory. This approach allows for observation of sublethal effects and other behavioral outcomes that would be missed by endpoint-based damage assays. Assessing field residues as they age and analyzing the spatial dynamics of residues provides the most comprehensive use of field material.

Throughout this outlined process, researchers must remain focused on the desired outcomes for the system of interest. For plum curculio in tart cherries, the outcome is fruit that have no detectable live larvae in them at harvest. Other systems may look to reduce incidence of feeding or levels of infestation or disease transmission. Efficient research should always be asking how a proposed study moves practice toward this outcome.

This process is guided by the PIC-Triad concept, which is a systems-based model for understanding the suite of an insecticide's performance mechanisms prior to deployment. There have been criticisms that this approach does not support the concept

of Integrated Pest Management, because of the focus on insecticides. I argue that insecticides are only a single component to this model, and research that comprehensively addresses these plant-insect-chemical interactions provides highly enriched performance data that feed directly into IPM decision making. We cannot make sound management decisions if we do not understand the full potential of the tools at our disposal

The PIC-Triad approach has yielded valuable insights on novel chemical uses, tactics and optimizations for plum curculio control. Post-infestation curative activity and sublethal feeding inhibition are two key modes of fruit protection that have been identified using this integrated approach. These mechanisms would have gone unobserved with traditional lethality bioassays. The next step for researchers is to take these observations, optimize their use in the field, and integrate them with other pest management tactics. The use of these new tactics will be information based, and monitoring for pest damage and efficacy should

This single-pest approach may only represent base level of IPM (Kogan 1998), but the PIC-Triad can be extended to look at the effects of control tactics across pest categories (insect, weed, disease). To this end, a the interactions would be generalized to Crop, Pest, and Tactic elements that are interacting not only with each other but also with elements such as water quality, nontarget organisms and other environmental health metrics.

On Azinphos-methyl

The long history of azinphos-methyl use is coming to an end in tree fruits. But we are only now coming to appreciate the extent of this compound's activity. For some reason, the "eradicant" activity that was noted in the chlorinated hydrocarbons and parathion (Cox 1949, Driggers and Darley 1949, Smith et al. 1956) was never really explored for azinphos-methyl. I feel that researchers and growers were blinded by the obvious acute lethality and long-lasting residual action, and assumed that the high levels of control were due solely to these properties. Another possibility is that post-DDT concerns about insecticides in fruit effectively halted continued research and silenced publication of these important findings. While the research presented here brings this curative activity back into light, it also widens the gap in control that will need to be filled when azinphos-methyl is finally gone. Hopefully, the research community will be able to think creatively when assessing future crop protection tools – lethal benchtop activity is unlikely to be the important mode of fruit protection. I also hope that the agricultural and consumer community is open to the concept of using the inevitable transient residues in the fruit to enhance the final quality and return on growers' investment.

On "Zero Tolerance"

I have some final comments to make about the zero-tolerance mandate in processed tart cherries. I feel that this standard provides no public health benefit. Cherries that are actually damaged by plum curculio or other organisms get sorted prior to processing. The remaining material is boiled and sanitized to the point where I doubt

that a plum curculio larva would even be recognizable by a consumer. Plum curculio larvae fall into the classification of "filth" by the Federal Food, Drug and Cosmetic Act. However, I am unable to find any study that claims harm by ingestion of plum curculio larvae. I have personally eaten several grams of plum curculio larvae (both cooked and raw) with no ill effects. Given that insects are a major source of protein around the world, I think it is a relevant question as to whether plum curculio larvae represent a true "filth" contaminant in our food supply.

Harmful contamination in our contemporary food, drug, and even toy supply has been distressingly common in the last five years. *E. coli* contamination in spinach, beef and even peanut butter have regularly sickened people and mandated recalls. Contaminated heparin syringes and lead-tainted toys from China have sickened and killed dozens of people in the US (Lipton and Barboza 2007, Bogdanich 2008). All food items should certainly be inspected for reasonable health concerns but I do not feel that the occasional plum curculio larva represents that type of concern. There is no spoilage issue since the larva is dead, and the entire mix has been heat and pressure sterilized.

Another way to frame the topic is that of relative risk: is an unseen plum curculio larva in a cherry good a greater risk than the insecticides that are being sprayed to completely eliminate the potential of that unseen larva? It is difficult to move between assessing the individual's risk and the public, or even environmental, risk. But that challenge does not mean that policy makers can avoid the big picture. Thousands of acres are sprayed with tons of insecticides each year to control fruit-feeding pests. Some pests, like plum curculio, can be managed well – but not perfectly – with reduced rates or numbers of applications. Accepting a percentage of infestation, rather than a simple

zero-tolerance might allow growers to significantly reduce sprays while still providing a high-quality product to the marketplace.

Tart cherry harvest for processing is currently governed by a marketing order that aims at a specific per-pound pricing. States are given specific production quotas and overproduction is diverted to secondary markets (fresh, dried) or simply not harvested. Relaxed infestation standards would probably increase the amount of fruit available to processing. I am not an economist, and can neither predict nor evaluate the impact of increased availability of tart cherries to the processed marketplace. Would the marketing order reduce the price paid per pound of processed cherries? Is consumer demand sufficient for increased production? Would growers actually come out behind if there were more cherries in the marketplace? Would the USA be able to build a stronger export market? Would foreign concerns of contamination weaken the export market? Does the production limitation generated by the zero-tolerance standard actually stabilize the USA cherry growing system? These policy and economic questions require real data to pursue. I hope that policy makers incorporate potential environmental and health risks of insecticides as part of these data.

New directions for plum curculio control

The integrated pest management of plum curculio will likely remain reliant on foliar sprays for some time. Trapping and chemical attractant research has been very active in the last decade (Eller & Bartelt 1996, Leskey & Prokopy 2000, Leskey et al. 2001, Piñero and Prokopy 2003, Prokopy et al. 2003, Prokopy et al. 2004, Leskey et al. 2005), but researchers are still looking for a potent pheromone or kairomone that can

raise trapping efficiency to the standards set by many lepidopteran mating-disruption systems.

Biological control is also an active research area, primarily in the area of entomophathogenic nematodes and fungi. Some species of *Steinernema* nematode are showing promise as agents against the soil-dwelling life stages of plum curculio (Olthof and Hagley 1993; Shapiro-Ilan et al. 2002, 2004, 2008; Alston et al. 2005) and there are *Metarhizium* and *Beauveria* fungi isolates that also have some activity against plum curculio (Alston et al. 2005, Tedders et al. 1982). Parasitic wasps of plum curculio have been identified (Mampe and Neunzig 1967, Krombein et al. 1979), but their impact will probably never be sufficient to control plum curculio on its own.

One class of insecticides that has not been evaluated against plum curculio is the ingestion-active *Bacillus thuringiensis* toxins. While the insecticidal proteins produced by these bacteria are primarily active against lepidopteran and dipteran pests, There have been advances in identifying coleopteran-active *B.t.* strains and protein-modifications (Herrnstadt et al. 1986, Reed et al. 2001, Weathersbee et al. 2003, Walters et al. 2008). Plum curculio adults could be an important target for *B.t.* applications, and its relative safety to bees would allow this ingredient to be used very early in the season when beetles are in the orchard and feeding on floral tissues, but fruit is not yet exposed. This would give a much longer effective period before fruit are exposed than a post bloom application of indoxacarb, another ingestion-active insecticide that is *not* safe to pollinators. The pre-bloom period is currently devoid of any plum curculio management practices and a *B.t.* product (either a variant or a modified Cry-protein) at this timing could have dramatic crop protection benefits. The development of a curculio-active *B.t.*

product would be a major breakthrough in support of organic tree fruit production. Plum curculio is one of the most challenging pests for growers of eastern organic apples and cherries. There is already a receptive market for this pesticide in organic production; *Bacillus thuringiensis* is currently used for control of several lepidopteran pests of apple.

Closing comments

At the fundamental level, I hope that my research findings can be incorporated into integrated pest management programs and reduce the costs of providing high quality food to consumers. The most obvious cost is the investment in agricultural inputs. Optimized insecticide sprays reduce these costs for the grower (and eventually the consumer) by putting the right material in the right place and the right time. But agricultural production has non-monetary costs as well: the impacts of landscape changes and agricultural chemicals on the ecosystem are difficult to measure, but are no less important. I believe that incorporating a curative approach into tart cherry pest management will reduce insecticide applications with no loss in harvest fruit quality. This is a significant reduction in cost to the environment, and holds to the integrated pest management philosophy of maximizing the benefits to producers, society and the environment.

Quod erat dictum

EJH

May 2008

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2008-08

Title of thesis or dissertation (or other research projects):

Identification & Characterization of key insecticide performance mechanisms for the control of plum curculio (*Conotrachelus nenuphar*) in Michigan tart cherries

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Eric James Hoffmann

Date 6/18/08

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Table a1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Plum Curculio, *Conotrachelus nenuphar* (Herbst) Northern Strain	Trevor Nichols Research Complex Fennville, MI Series 30 May 2007 - 15 June 2007 coll. Eric J Hoffmann					5	5		MSU Entomology
Plum Curculio, *Conotrachelus nenuphar* (Herbst) Southern Strain	Trevor Nichols Research Complex Fennville, MI 2 February 2008 From Laboratory colony					5	5		MSU Entomology

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Eric James Hoffmann

Date 10/12/2008

Voucher No. 2008-08

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator

17 June 2008
Date

Table a2.1. Insecticides used for this dissertation research

Formulated name	Active ingredient	Chemical Class	% active ingredient	CAS Number	Company
Guthion ® 50WP	Azinphos-Methyl	Organophosphate	50%	86-50-0	Bayer CropScience
Imidan ® 70W	Phosmet	Organophosphate	70%	732-11-6	Gowan ® Company
Actara ® 25WG	Thiamethoxam	Neonicotinoid	25%	153719-23-4	Syngenta Crop Protection, Inc.
Assail ® 30SG	Acetamiprid	Neonicotinoid	30%	135410-20-7	Cerexagri-Nisso LCC.
Calypso ™ 4F	Thiacloprid	Neonicotinoid	40.4%	111988-49-9	Bayer CropScience
Clutch ™ 50WDG	Clothianidin	Neonicotinoid	50%	210880-92-5	Valent U.S.A. Corporation
Avaunt ®	Indoxacarb	Oxadiazine	30%	173584-44-6	I.E. du Pont De Nemours, and Co.
Asana ® XL	Esfenvalerate	Pyrethroid	8.4%	66230-04-4	I.E. du Pont De Nemours, and Co.
Altacor ™	Chlorantraniliprole [a]	Anthranilic diamide	35%	500008-45-7	I.E. du Pont De Nemours, and Co.
Rimon ® 0.83EC	Novaluron	Benzoyl urea	9.3%	116714-46-6	Makhteshim Agan of North America, Inc.
Esteem ® 35WP	Pyriproxyfen	Juvenile Hormone Mimic	35%	95737-68-1	Valent U.S.A. Corporation

a. Also known as Rynaxypyr™

Table a2.2. Pesticide manufacturer contact information

Company	Address and Telephone number
Bayer CropScience	P.O. Box 12014, 2 T.W. Alexander Dr. Research Triangle Park, NC 27709 1-866-99-BAYER
Cerexagri-Nisso LCC.	630 Freedom Business Center, Suite 402 King of Prussia, PA 19406 1-800-438-6071
Gowan ® Company	370 S. Main Street, Yuma Arizona 85364 928-783-8844
I.E. du Pont De Nemours, and Co.	Wilmington, Delaware 19898 1-888-638-7668
Makhteshim Agan of North America, Inc.	551 Fifth Avenue, Suite 1100 New York, NY 10176 800-825-0312
Syngenta Crop Protection, Inc.	Greensboro North Carolina, 27409 1-800-334-9481
Valent U.S.A. Corporation	P.O. Box 8025 Walnut Creek CA 94596 1-800-6-Valent

Appendix 3

Feeding phenology of northern strain plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae), in Montmorency cherries

Eric J Hoffmann, Mark E Whalon & John C Wise

2006 Meeting of the Entomological Society of America. December 2006. Indianapolis, IN.

Abstract

Field-collected male and female northern strain plum curculio (*Conotrachelus nenuphar* Herbst) were studied in laboratory bioassay chambers to better understand the relationship between weevil feeding/oviposition and Montmorency tart cherry developmental phenology.

Background: Plum curculio

- Endemic pest of eastern North American tree fruit.
- The northern strain present in Michigan has an obligate adult winter diapause
- Internal feeding by larvae causes substantial economic loss.
- Impact of adult feeding in Michigan tart cherry production is still understudied
- Knowledge of feeding phenology is important when considering ingestion-active insecticides.

Research Questions

- What types of early season damage do plum curculio do to cherry tissue?
- Are there differences between male and female feeding rates on various tissues?
- Can we detect overwintering-generation feeding?

Methods

- Reproductive generation male and female northern strain plum curculio were collected by limb jarring in untreated orchards at the MSU Trevor Nichols Research Complex in Fennville, MI.
- Insects were housed in outdoor cages with cherry branch clippings for feeding and oviposition.
- Overwintering generation adults were reared from infested plum fruit collected earlier in the summer.
- Ten fruit and ten leaves of tart cherry (*Prunus cerasus v.* Montmorency) were placed into 950ml containers with wet floral foam.
- One beetle / bioassay container (replicate) for 3 days, 10-20 males and females / assay.
- Seven assays throughout season (Table 1).

Table a3.1. Dates and tree phenology for feeding bioassays

Date	Phenology	Generation
May 6, 2006	Late bloom	Reproductive
May 18, 2006	Petal fall-shuck split	Reproductive
May 26, 2006	9 mm fruit	Reproductive
July 15, 2006	Harvest 1	Reproductive
July 21, 2006	Harvest 2	Overwintering
Aug 11, 2006	Post harvest	Overwintering
Sept 4, 2006	Post harvest	Overwintering

Statistical Methods. Slicing and contrasts (Proc GLM SAS) were used to analyze within-sex and across-phenology effects and interactions for significant overall ANOVA analyses. Mean-varience dependence of count data was corrected by log-transformation prior to analysis. Untrasformed means are shown.

RESULTS

Ovule feeding through the shuck occurred during bloom, and males did this more often than females ($F1,9= 2.31$, $P = 0.17$). Only one female fed on the ovule, while 3 males engaged in this activity.

Males: 2.3 events per replicate ± 1.2 SE

Females: 0.4 events per replicate ± .04 SE

Sepal feeding only occurred during bloom. There was no significant difference between male and female feeding rates ($F1,9= 1.46$, $P = 0.26$).

Males: 8.8 events ± 3.1 SE

Females: 13.6 events ± 3.3 SE

Stamen feeding (anther or filament) was not observed.

Petal feeding was not observed.

Leaf stem feeding was not observed.

Late season (Aug, Sept) overwintering generation damage was not observed on buds, leaves, stems or bark

For feeding graphs, Different CAPITAL LETTERS represent significant differences ($P < 0.05$) across phenologies for females, different *italicized lower case letters* represent differences across phenologies for males. * Represents a significant difference between males and females for a given phenology.

Fruit Stem feeding (Figure a3.1) occurred at all of the plant stages and was not significantly different between males and females across the periods evaluated.

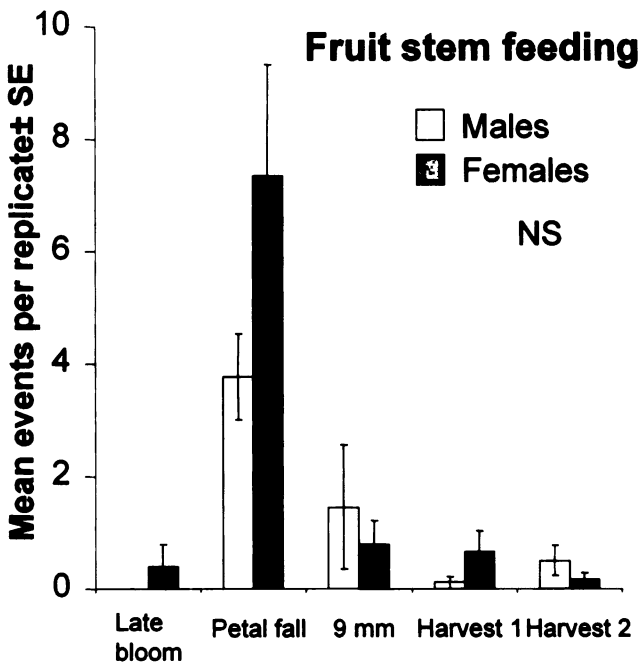

Figure a3.1. Fruit stem feeding events after plum curculio adults were exposed to branches for 72 h

Shuck feeding (Figure a3.2 A) varied by plant stage but not beetle sex within a plant stage. It was more common at late bloom than the later periods.

Fruit feeding (Figure a3.2 B) varied by fruit stage and beetle sex. There was more non-ovipositional feeding on fruits at shuck split than at other periods.

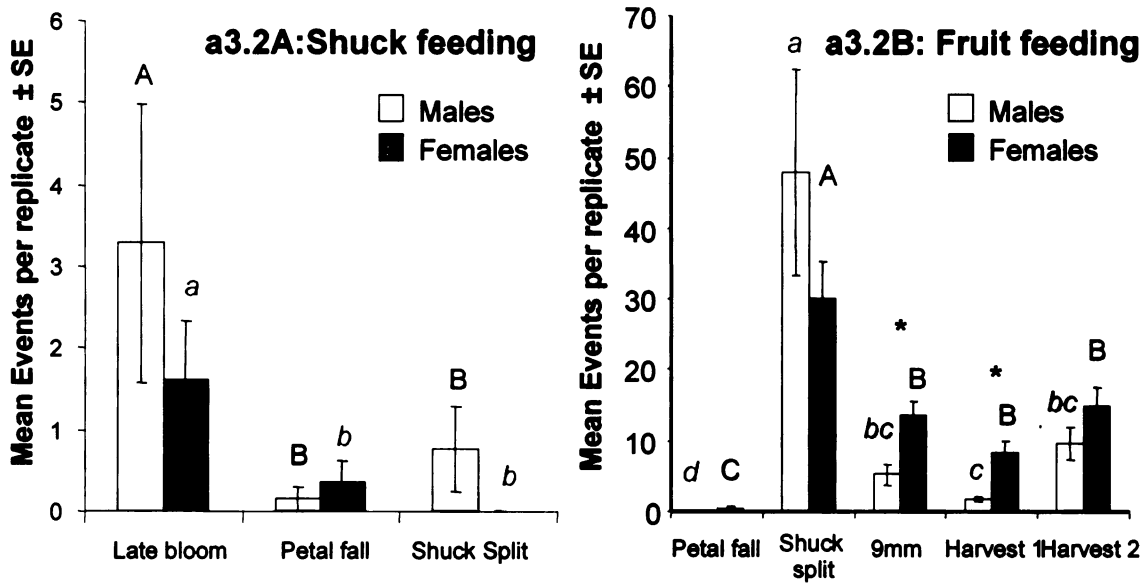

Figure a3.2 (A & B) Feeding profiles for plum curculio on tart cherry shucks (A) and tart cherry fruit (B) across plant phenology. Different CAPITAL LETTERS represent significant differences ($P < 0.05$) across phenologies for females, different *italicized lower case letters* represent differences across phenologies for males. * Represents a significant difference between males and females for a given phenology.

Female egg laying and oviposition scars (Figures a3.3, a3.4)were highest after the fruit were exposed from the shuck. It should be noted that there was egg laying on fruit that were still in the shuck.

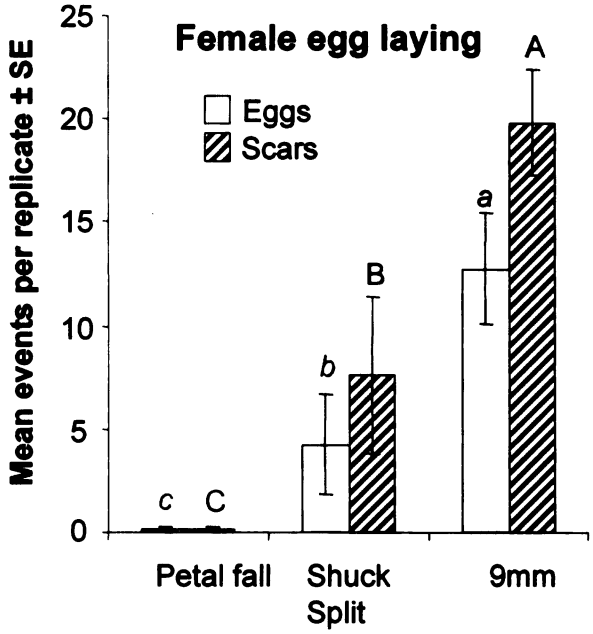

Figure a3.3. Number of egg laying events after plum curculio females were exposed to tart cherry fruit for 72h.

Leaf blade damage was low, but consistent across the different assay periods.

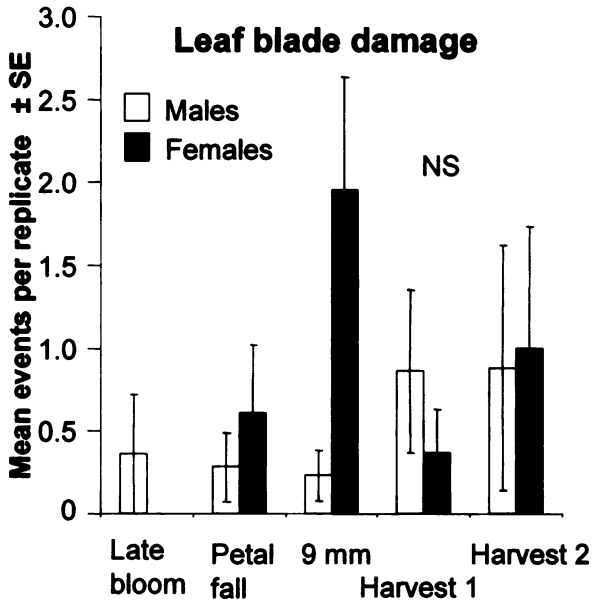

Figure a3.4. Leaf blade damage events after plum curculio were exposed to tart cherry fruit for 72h.

Discussion

Plum curculio early season feeding patterns do not suggest any nutrient limitations; feeding on high-protein sources such as anthers was not observed. Ovule feeding through the shuck was unexpected, and may have been a source of protein for the few beetles that did this. Feeding seems to be related to water content of tissues; shuck and sepal lobe tissues were primarily eaten during bloom, when turgid and green.

Leaf feeding was much less prevalent than expected, with less than 2 incidents per 10 leaves. Leaves may be unpalatable for plum curculio adults. Previous observations suggested petal feeding can be extensive but this was not observed. Assays were started after peak bloom and petals fell off of flowers during the assay, making damage hard to assess. Frost damage was present on many flowers, which may have reduced the palatability of petals.

Pre-bloom damage can be significant. The assays reported here were not initiated until bloom, and may have missed overwintering generation feeding if it occurs during the pre-bloom and early blooming period. Alternatively, overwintering adults may already have had sufficient energy stores and not needed to feed. Late season plum curculio damage in cherries was difficult to assess here. Fruit remaining on the trees were heavily damaged by cherry fruit fly infestation.

In general, feeding persists throughout the growing season, especially on fruit. There does not appear to be a pre-harvest period of non-feeding that precludes use of ingestion-active insecticides.

Conclusions

- Economically significant plum curculio feeding damage begins well in advance of fruit sizing. Females and males chew through the shuck (fused sepals) and feed on the ovule.
- Fruit feeding is the most common type of feeding at the phenologies observed.
- Oviposition can occur as soon as the fruit is externally accessible.
- Males and females show few detectable differences in feeding habits.
- Some harvest-period damage by overwintering beetles was observed on leaves and fruit, but no damage in August or September.

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