DEVELOPMENT OF BAIT-SPRAY CONTROL TACTIC FOR PEST MANAGEMENT OF CODLING MOTHS USING THE PEAR ESTER LARVAL ATTRACTANT KAIROMONE; 2005 FIELD TRIALS

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ABSTRACT

Six years ago, we reported here the discovery of a new, host plant volatile-derived, kairomonal attractant for the codling moth, ethyl (2*E*, 4*Z*)-2,4-decadienoate, termed the “pear ester” (PE). This kairomone is a single compound that is highly potent in attracting both adult females and males and newly hatched neonate CM larvae. This lure has a high degree of species specificity, being non-attractive to non-target beneficial insects. Our goal is to discover and develop new, effective, environmentally safe tactics to control and manage CM pest populations by improving insecticide effectiveness while fostering resistance management. This year we have made progress in developing and field evaluating the efficacy of the PE as a micro-encapsulated sprayable adjuvant, used to enhance the performance of insecticides through an “attract and kill,” (or kairomone attractant with insecticide) bait-spray tactic targeting neonate CM. Food Quality Protection Act 1996 will soon restrict/ban use of organophosphate (OP) insecticides. Alternative insecticides must be made more effective and affordable. Having found that neonate CM larvae are highly attracted to PE, we hypothesized that bait-sprays of kairomone + insecticide might attract - kill larvae more effectively. This year, a micro-encapsulated sprayable formulation, PE-MEC, was tank-mixed as an adjuvant with reduced rates of insecticides and applied by a handgun-sprayer. The field trial consisted of spraying eight single-tree replicates per treatment in a 20 acre walnut orchard. Treatments were the insecticides-alone verses insecticide + PEK-MEC adjuvant. Four insecticides were tested, including two OPs - Lorsban and Imidan, an insect growth regulator – Intrepid, and a microbial virus – Cyd-X. Results were significant, with PE-MEC adjuvant + insecticide treatments reducing CM harvest damage by 77%, 88%, 53% and 49% (respectively) below the damage incurred with insecticides alone. Navel orangeworm damage was also significantly reduced for the OP insecticide + PE-MEC treatments. We believe that the PE compound can be used to manage CM in attract and kill bait-spray applications, by allowing for the death and removal of neonate larvae. Furthermore, the ability to selectively attract, intercept, and remove/kill CM larvae, immediately after hatching when they are in their crawling search for walnuts, will promote an increased temporal and spatial exposure to insecticides. This should provide a critical and environmentally rational means of enhanced control with low volumes of applied insecticide in bait-sprays. This new attract and kill tactic should also help foster insecticide resistance management. Furthermore, the development of kairomone-based control systems will help to promote food safety and help eliminate the invasion and colonization by *Aspergillus* species and the production of aflatoxin in walnuts.

INTRODUCTION

The codling moth (CM) is the key pest of walnuts, damaging the husk, shell, seed coat and kernal of walnuts, thereby allowing for the invasion and colonization by *Aspergillus* species and the production of aflatoxin. Alternative control measures that are both effective and affordable are needed to replace the current reliance in walnut pest management on organophosphate (OP) insecticides. The use of OP insecticides is facing both regulatory restriction and banning by the EPA (Food Quality Protection Act of 1996), and the increasing/alarming incidence and levels of insect resistance. Our goal is to discover and develop new, effective, and environmentally safe, reduced-pesticide tactics to control and manage CM pest populations. Semiochemicals (behaviorally active chemicals) in particular "kairomones" or host-plant volatiles, provide a potential for improving or creating alternative codling moth (CM) control tactics. Our approach is to develop (1) various low-rate higher efficacy mating disruption tactics and (2) various low-volume insecticide, or non-insecticidal, control tactics based on the principle of "attracticide" or "Attract and Kill" (AK), a combination, or mixing, of attractant and killing agent - device. AK bait-spray tactics for other pest species, *e.g.* walnut husk fly, corn rootworm species, Colorado potato beetle, tephritid fruit flies, etc., are so successful due primarily to the fact that not just males but both females and larvae of the target pest are attracted to the AK bait.

Eight years ago our research team discovered a pear-derived odor, the "pear ester" (PE) [(2*E*, 4*Z*)-2,4-decadienoate] that is a highly selective and potent CM attractant kairomone (Light et al. 1999, 2001, & Light et al. Walnut Research Reports 1999-2003, Light and Knight 2005). This CM kairomone has biological and chemical attributes that make it very appealing to function as the attractant for bait-spray targets. These attributes of the CM kairomone are: (1) it attracts both male and female CM, including both virgin and mated females (Light et al. 2001), (2) it's highly attractive to newly hatched neonate CM larvae (Knight and Light 2001) and can stimulate larval "wandering" disrupting nut/fruit finding (Pasqualini et al. 2005), (3) CM larvae are highly sensitive and responsive to minute amounts (Knight and Light 2001), (4) it both stimulates oviposition by mated females (Knight and Light, 2004) and can confuse/disrupt the placement of eggs (Pasqualini et al. 2004), (5) it's species-specific, *i.e.*, no beneficial insects are attracted (Knight and Light 2004), and (6) micro-encapsulated (MEC) formulations exhibit excellent emission rates, longevity, and stability, remaining attractive for a month in orchards.

This project investigated the development of novel control methods using the PE kairomone as a micro-encapsulated (PE-MEC) bait-spray adjuvant to directly attract and kill newly-hatched neonate larvae prior to their finding and penetrating nut hosts. Field evaluation of the attractiveness, release rates, longevity, and stability of various MEC formulations of the kairomone (synthesized by Trécé, Inc.) were conducted through several seasons (1999 – 2004) in replicated walnut orchard plots. This sprayable MEC formulation and its application rates (grams/acre) have been evaluated for sprayable uses as attractants in bait-spray applications (Light et al. Walnut Research Reports 2003, Chicon et al., unpublished) and as an adjuvant - synergists for sprayable pheromones (Light et al. Walnut Research Reports 2002 - 2005). ARS and Trécé, Inc. are partners in researching and developing monitoring and control products based on this kairomone. This partnership is founded through a CRADA (Cooperative Research and Development Agreement) and a jointly held, co-authored patent (July 2001) for use of this kairomone. From 2003 to present, an exemption for Experimental Use Permit from the EPA and a Research Authorization permit from the CA-DFA were attained for both bait-spray and mating disruption application experiments using the pear ester kairomone without crop destruct.

Newly hatched nut-seeking neonate larvae are the traditional target of conventional insecticide tactics. CM neonates are known to be more attracted to, prefer, and feed/penetrate more readily into pome-fruits, both apples and pears, over walnuts. CM larvae are ‘slow’ and relatively ‘hesitant’ to ‘accept’ and feed/penetrate into walnut husks (Mills Walnut Research Reports 2000). Our intent is to exploit this CM larval preference by presenting pear ester in AK bait-spray formulation droplets to larvae in a walnut orchard environment to increase larval temporal and spatial exposure to and thereby lethality of insecticides. Furthermore, the ability to selectively attract, intercept, and remove/kill CM larvae, immediately after hatching when they are in their crawling search for walnuts, will provide a critical and environmentally-rational means of direct control with low volumes of insecticide. We believe that the PE compound can be used in a MEC formulation to augment insecticides in an AK strategy against neonate larvae. This experimental PE-MEC formulation has been shown to enclose/encapsulate, retain, stabilize, and then emit/evaporate the PE attractant over a prolonged period of greater than a month. The goal of these field trial AK bait-spray applications of PE-MEC spray was to use it as a tank-mixed adjuvant to augment various commercial insecticides, especially new alternatives to OPs, and to determine if the bait-spray tactic shows any improvement in the insecticides’ efficacy, target species specificity, and resistance management attributes.

OBJECTIVES

1. Conduct bait-spray application and efficacy trials on replicated individual trees in a commercial walnut orchard using low rate applications of PE-MEC adjuvant paired with various insecticides, and evaluate their Attrack and Kill control efficacy and damage suppression of moderate or greater populations of CM. (This objective will be the focus of this report).

2. Conduct and evaluate in replicated field tests the ability of a combination of pheromone and PE kairomone in a hand-applied polymeric dispenser formulation to augment and improve the Mating Disruption control efficacy when compared with hand-applied commercial pheromone products (*e.g.*, Isomate CTT ties) in apple orchards with moderate populations of CM. (Research was successful and will be reported through WOPDMC).

3. Develop, evaluate, and optimize the use of attract-and-kill insecticide-coated bait-stations or mesh panels (panels, spheres, or trap-like structures) using PE + Pheromone Combo-Lures as the lure-attractant agent. (Research was successful, will be reported elsewhere and is ongoing in both CA and WA in cooperation with Dr. Alan Knight).

PROCEDURES

Test objective was to conduct experiments that would reveal the potential synergism and/or improvement in control efficacy of insecticide sprays when using the PE-MEC spray adjuvant targeted against neonate newly-hatched codling moth (CM) and navel orangeworm (NOW) larvae. Prior 2003 test results using the PE-MEC insecticide spray adjuvant with standard full label-rates of two insecticides were unclear with no statistically significant improvement in efficacy demonstrated (*Walnut Research Report 2003*). Therefore for the 2005 season, the spray tests were designed to challenge the insecticides' effectiveness by using low label-rates or reduced rates of up to ½ the standard label-rate of insecticide. The use of these reduced rates of insecticides was to allow the contribution of the tank-mixed PE-MEC spray adjuvant to be potentially revealed and distinguished in a statistical manner. The general design was comparing reduced or ½ label-rates of insecticides-alone *vs*. reduced or ½ rate insecticides tank-mixed with PE-MEC adjuvant (Trécé, Inc.). Three rates (approx. 0.2, 0.6, & 1.7 grams/acre) of the PE-MEC adjuvant were variously tested in combination with the four insecticides. Three classes of registered or permitted insecticides were tested, including two organophosphates (*Lorsban*, Dow CropScience and *Imidan*, Gowan, Inc.), an insect growth regulator (*Intrepid*, Dow CropScience), and a biological – microbial using a formulation of the CM granulosis virus (*Cyd-X Virus*, Certis, Inc.).

Insecticide treatments tested numbered thirteen in total and were:
Lorsban (choropyrifos) ½-rate alone (2.3 pints/acre);
Lorsban 4E ½-rate + *PE-MEC* 0.2 gram/acre;
Lorsban 4E ½-rate + *P**E** -MEC* 0.6 gram/acre;
Lorsban 4E ½-rate + *PE-MEC* 1.7 gram/acre

Imidan 70-W (phosmet) ½-rate alone (2.8 lbs/acre);
Imidan 70-W ½-rate + *PE-MEC* 0.6 gram/acre;
Imidan 70-W ½-rate + *PE-MEC* 1.7 gram/acre
(Buffered to 5.5 – 6.0 pH)

Intrepid (methoxyfenozide) low label-rate alone (11.3 fl. oz/acre);
Intrepid 2F low label rate + *PE-MEC* 0.6 gram/acre

Cyd-X Virus (CM granulosis virus) mid label-rate (3.4 fl oz/acre);
Cyd-X Virus mid label-rate + *PE-MEC* 0.6 gram/acre

PE-MEC alone, 0.2 gram/acre;
PE-MEC alone, 0.6 gram/acre;
PE-MEC alone, 1.7 gram/acre.

Blank-MEC alone (comparable to 0.6 gram/acre).

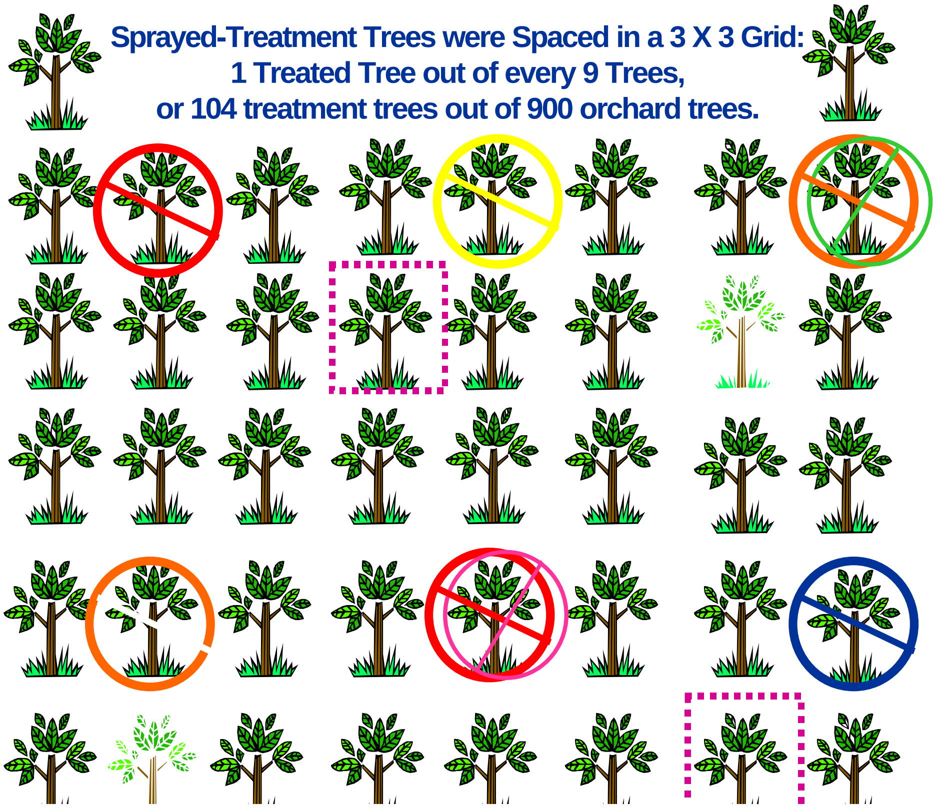
Application rates for the PE-MEC adjuvant tested were approx. 0.2, 0.6, and 1.7 grams of PE a.i./acre. All insecticide rates are shown in Table 1 for: the reduced rates used, their equivalent rates per acre and the specified Label rate range and the standard residual longevity for these label-rates. The Imidan treatments were buffered to 5.5 – 6.0 pH using Trifol buffer.

Table 1. Reduced Spray Rates Used, Equivalent Rates per Acre, and Label Rates:

Test Insecticide or Adjuvant	**Rate Used per 8 Trees (28 gal tank mix)**	**Equivalent Rate/Acre Used in Test**	**Label specified Rate/Acre**	**Label specified Residual Activity**
Lorsban-4E (Choropyrifos) Dow AgroScience	6.4 fl oz/ 8 trees	36 fl oz/ Acre or 2.3 pints/ Acre (56% of Label)	4 pints/ Acre	10 – 14 Days
Intrepid 2F (Methoxyfenozide) Dow AgroScience	2.0 fl oz/ 8 trees	11.3 fl oz/ Acre or 0.7 pints/ Acre (70% of lowest Label)	16 – 24 fl oz/ Acre	10 – 21 Days
Imidan 70-W (Phosmet) Gowan Inc.	8.0 oz/ 8 trees or 0.5 lb/ 8 trees	45 oz/ Acre or 2.8 lbs/ Acre (65% of lowest Label)	4.3 to 8.5 lbs/ Acre	21 Days
Cyd-X Virus Certis Corp.	0.6 fl oz/ 8 trees	3.4 fl oz/ Acre (mid-Label rate)	1 – 6 fl oz/ Acre	7 Days
PE-MEC (pear ester –MEC) Trece, Inc.	Low: 0.8 ml /8trees Mid: 2.4 ml/8 trees High: 6.0 ml/8 trees	Low: 4.5 ml / Acre Mid: 13.5 ml/ Acre High: 33.8 ml/ Acre	Low: 0.23 gm/ Acre Mid: 0.68 gm/ Acre High: 1.69 gm/ Acre	21 – 30+ Days
Blank-MEC (unfilled MEC) Trece, Inc.	Mid: 2.4 ml/ 8 trees	Mid: 13.5 ml/ Acre	Mid: 0.0 gm/ Acre	(capsules remain?)

Test design: Study was conducted in a mature, 20 acre commercial' Chandler' variety walnut orchard, located 2.5 miles north of Esparto, CA. Test orchard was nearly square in layout, comprised of 31 rows with 30 trees/row or planting of 47 trees/acre. The 13 treatments were replicated eight times (total of 104 sprayed trees) in a randomized complete block design, distributed throughout the 20 acre (930 total trees) orchard (Figure 1, see end of report). Treatment trees were spaced on a 3rd tree X 3rd tree separation, or having two 'buffer' non-sprayed trees separating in all directions the treatment trees (Figure 2).

Figure 2. Illustration of the 3 X 3 tree grid spacing and distribution of treatment trees (different circles) throughout the orchard. Dashed-line delineated trees represent the random selection and placement of 'Control" trees from among the orchard's un-sprayed trees.



Application methods: An ATV-mounted gasoline engine – diaphragm pump sprayer (Honda - ??) operating at 110 psi was used to apply by handgun (Spray Systems, Inc., D5 nozzle) foliar sprays at 3.5 gal/tree (28 gal / 8-tree treatment) (1 gal/minute) or an equivalent spray volume of 165 gal/acre.

Application timing and required number of applications were predicted and scheduled based on both male monitoring (pheromone- and Combo- baited traps) and female monitoring (kairomone DA-baited traps). DA-trap captures were sexed to accurately determine female flight initiation and emergence. Setting biofixes and determining timing decisions were based on a combination of capture activity of DA-traps (Pherocon DA, Trécé, Inc.), pheromone-traps (Pherocon L2), and Combo-Lure traps (Pherocon CM-DA, a lure composed of both DA and pheromone) in the test orchard and in neighboring orchards. Two groupings of the three lure traps (hung three trees apart in a group) were located in the southeast and northwest quarters of the orchard. Traps were set out for the period of March 20 to mid-October, hung at *ca*. 18 feet high, checked weekly (at least three times per week during the 1st flight) and lures replaced every two months. The goals of the CM population monitoring were: (1) determining initiation of female flight and then setting spray application timing 155 DD after, and (2) assessment a continuation of a flight period and population pressure to proper time supplemental insecticide sprays. The spray applications were timed for onset or peak in egg hatch that follows by approximately 155 DD the biofix of sustained female CM emergence - flight, as determined, or predicted, by monitoring female capture activity using DA traps prior to and through the 1st, 2nd and 3rd flights. Egg hatch and application timing was determined by using degree-days (DD) following the male (pheromone trap) and female (DA trap) biofixes utilizing the CM computer model (single sine horizontal cutoff with a lower threshold of 50° F and an upper threshold of 88° F) available at the UCD IPM website. Maximum and minimum air temperatures were obtained from the Esparto CIMIS weather station. A flight's biofix was the date on which CM initiated a sustained flight activity and sunset temperature was 62° F or greater. Cool weather, rain delays, and the late emergence of female CM caused the first spray applications to be conducted at the peak egg hatch after the peak of the 1B flight. Spray applications were conducted six times for all insecticides, while the virus insecticide was applied an additional two times. Treatment applications were sprayed on June 7 and 10 for the post 1B flight, July 6–8, July 19–22, and August 2–5 for the 2nd flight, and August 23–26 and September 7–9 for the 3rd flight (Figure 4). See Table 2 for the specific dates for the particular insecticide spray application.

Table 2. Spray application dates and Day-Degree accumulation from female codling moth 1B flight biofix, Esparto, CA, 2005.

TREATMENT	JUNE		JULY									AUGUST						SEPTEMBER		
	7	10	6	7	8	14	19	20	21	22	27	2	4	5	23	25	26	7	8	9
Lorsban		X	X				X					X				X			X	
Lorsban + PE 0.2		X	X				X					X				X			X	
Lorsban + PE 0.6		X	X				X					X				X			X	
Lorsban + PE 1.7		X	X				X					X				X			X	
Imidan	X			X				X					X				X			X
Imidan + PE 0.6	X			X				X					X				X			X
Imidan + PE 1.7	X			X				X					X				X			X
Intrepid	X		X						X				X		X					X
Intrepid + PE 0.6	X		X						X				X		X					X
Virus		X				X			X		X			X	X			X		
Virus + PE 0.6		X				X			X		X			X	X			X		
PE-MEC					X					X				X	X			X		
Blank-MEC										X				X	X			X		
degree days accumulated since female biofix (5/4/05)	684	745	1362	1389	1417	1593	1750	1778	1808	1834	1988	2164	2220	2251	2740	2789	2817	3143	3164	3184

Pre-harvest canopy count damage assessments September 15-16 and 19-20, 2005.
Pre-harvest nut knock-down on September 21, 2005. Grower harvest on October 10-12, 2005.

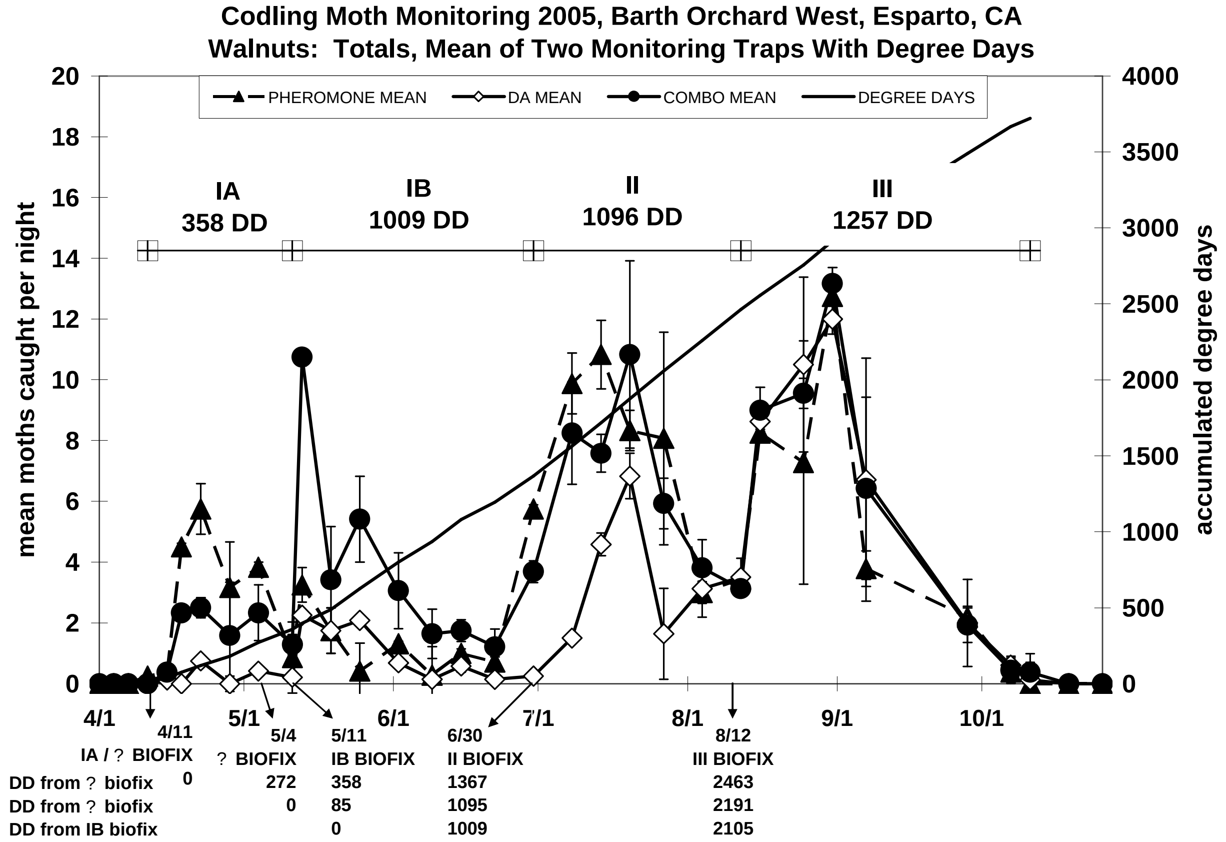
Control Efficacy – Damage Assessment. In addition to the spray treatment trees, 20 trees were randomly picked as 'Control trees' from the 826 un-sprayed 'buffer trees' in the orchard grid design (Figure 1). To evaluate and compare efficacy of the treatments verse the un-sprayed controls, CM-caused and navel orangeworm (NOW)-caused nut damage was assessed through drop nut surveys through the season and just prior to harvest through both canopy count visual surveys and nut knock-down sampling. During the 1st and 2nd flights, "drop nut" surveys determined the numbers of drop nuts per tree and instars of CM present to predict next generation emergence. Just prior to harvest (Sept. 15–16 and 19–20), 3rd flight "Canopy Counts" were conducted to estimate the percentage of infested nuts per tree. Visual examinations of 100 nuts at a height of 6 to 18 feet were conducted on the west-side of each treatment tree and control tree. All suspect infested nuts were removed and placed in labeled bags. Husk, shell and kernels were examined for CM and NOW damage, age or flight period of infestation and instar presence. The day after the canopy count assessments (Sept. 21) a random nut knock-down assessment was conducted. This 'nut knock-down' sampling was conducted two weeks prior to the shake-down harvest (October 10–12) in order to assure that knocked-down nuts had intact/attached husks, so that assessments of husk-confined damage and larval presence could be accomplished. Usually this level of detailed damage assessment is not attainable once the husks split and separate from the nut just prior to the shake-down harvest, that also separates husks from their associated shelled nut. The 'knock-down' sampling was conducted by hired laborers using 12 foot poles to randomly knocked-down 100+ nuts at a height of 6 – 20 feet from the east-side of each treatment tree and control tree. All knocked-down nuts were placed in labeled bags, the followed by husk, shell and kernel examinations for CM and NOW damage and instar presence. Both canopy count and knock-down collected nuts had their husks, shells and kernels examined to classify any damage present and scored it as to: (1) source of damage/defect whether worm, mold or shrevel, or combinations, (2) location of damage in husk, shell, and/or kernel, (3) presence and number of larvae and species whether CM or NOW (specific marking, webbing and often multiple worms of many age – classes), (4) if NOW then evidence or not of prior CM infestation, and (5) age - class or particular flight period of damage (fresh – new 3rd flight with moist frass, prior – mid-season 2nd flight with dried frass and new mold, or old – 3rd flight with dried, shriveled and highly/powdery mold).

RESULTS AND DISCUSSION

Codling Moth Population Monitoring, Prediction of Subsequent Flights and Spray Application Timing.

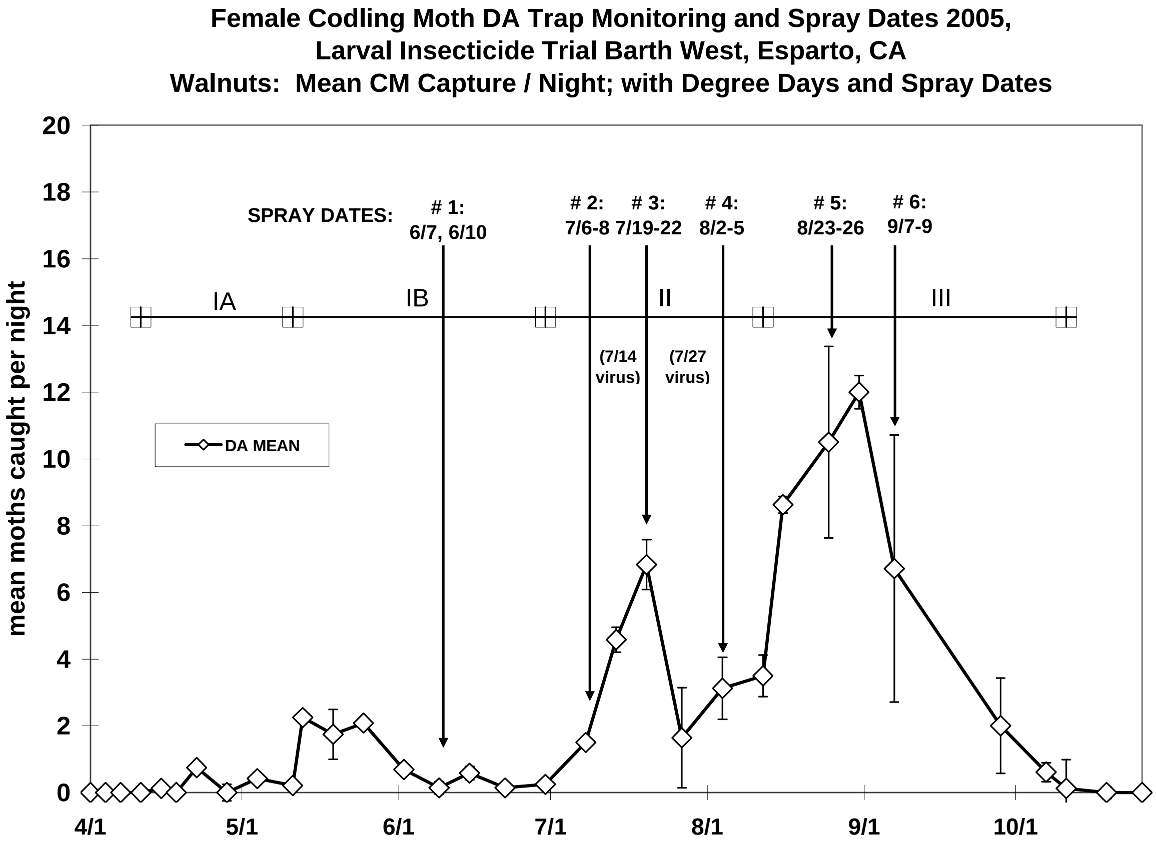
The 2005 spring was characterized, as similarly for the last four years, by cool and wet weather, with sunset temperatures not exceeding 62° F until mid-April. These cool wet conditions are believed to retard the emergence of female CM, which requires a sustained period of sunset temperatures exceeding 62° F. However, sunset temperatures in Yolo Co. during April did exceed the male 58° F temperature threshold for flight activity, contributing to the occurrence of a distinct period of 'protandry', *i.e.*, male emergence preceding females'. In this Esparto, CA orchard, male biofix was observed on April 11, based on the strong male 1A flight initiation detected by both pheromone-baited traps and Combo-Lure-baited traps (Figure 3).

Figure 3. Comparison and resolution of Codling Moth emergence, biofix determination, and flight pattern as delineated through the monitoring of male activity using pheromone-baited and Combo-Lure-baited traps and the monitoring of female activity using PE kairomone DA-baited traps in relation to Degree-Day accumulation.



The initial female biofix was not registered until on May 4, followed by a concerted sustained flight on May 11, the biofix date for the 1B flight. Again as reported in prior reports (Walnut Research Reports, 2000 – 2003), female emergence was delayed during the predominately male-attributable 1A flight. Only the PE-based 'DA'-Lure-baited traps can and did detect the 1A and 1B biofixes and true emergences of the over-wintered female moths. Thus, the initiation of the experimental bait-spray AK field trials was accordingly started after the peak of female 1B flight emergence and the secession of the prolonged spring rains (Figure 4). All three lure types, pheromone, Combo and DA, detected the 1B and 3rd flights in synchrony, though the detection of male emergence for the 2nd flight preceded that of females by *ca*. week. Over the summer experimental period, the eight replicate trees per treatment received six spray applications (eight applications for the virus treatments). The spray applications were timed for the periods of hatch and emergence of neonate codling moth larvae that occurred after the peak of the 1B flight and then continued throughout the relatively intense and prolonged later flights, in which the intensity of the 2nd flight dropped but did not pause as the 3rd flight took-off (Figures 3 and 4). Because of (1) the prolonged high trap capture levels and strong population intensity/pressure and (2) the shortened and restricted residual concentration of the lower rate insecticide applications, it was necessary to a perform repeated spray applications during the 2nd and through the 3rd flights, due to sustained high trap capture rates of CM females.

Figure 4. The dates of experimental spray applications (arrows) in relationship to female Codling Moth emergence, biofix – initiation of flights, and flight peak, duration and intensity as delineated using PE kairomone - DA-baited traps.



Bait-Spray Field Trials: Insecticides Augmented with the Kairomone PE-MEC Adjuvant, 2005.

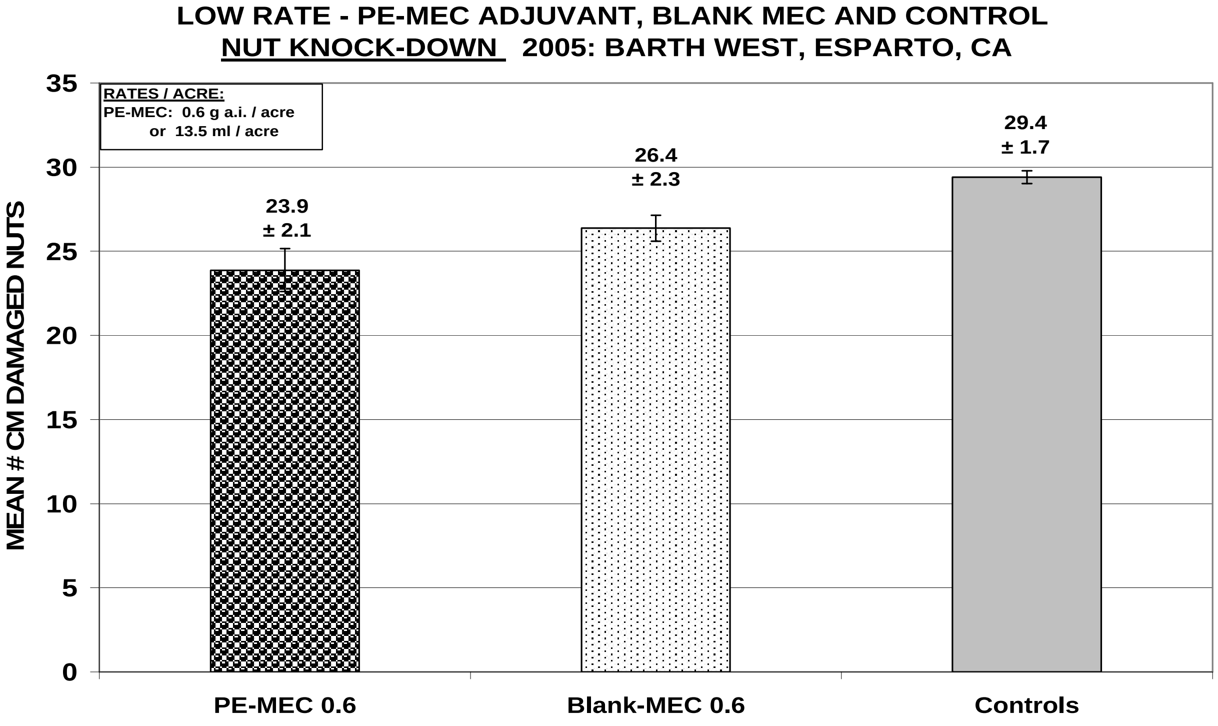
"Bait-spray" studies on both walnuts and apples were conducted this year by seven research teams worldwide using the kairomone PE-MEC adjuvant formulation tank-mixed with conventional insecticides applied by either spray-gun or commercial fan-sprayers. Not to be reported here are the six experimental studies that were conducted on apples and pears, including USA studies in Medford, OR, Yakima and Wenatchee, WA, and ongoing foreign studies in Italy and Argentina.

Results of a second year of experiments conducted in walnut orchards will be reported here. Prior test results in 'Payne' variety walnuts were reported in 2003 (cooperative research conducted with Dr. Bob Van Steenwyk and UCCE Farm Advisor Bill Coates, see *Walnut Research Report 2003*) showed that using the PE-MEC spray adjuvant caused no or only slight, but not statistically significant, improvement in efficacy when tested with two insecticides, Imidan and Intrepid, at their standard full label-rates. This year's spray tests were designed to challenge the insecticides' effectiveness by using low label-rates or reduced-rates of down to ½ the standard label-rate of insecticide. Use of reduced-rates of insecticides was intended to allow the contribution of the tank-mixed PE-MEC spray adjuvant to be potentially revealed and distinguished in a statistical manner. The general design was comparing reduced or ½ label-rates of insecticides-alone *vs*. reduced or ½ rate insecticides tank-mixed with PE-MEC adjuvant. The insecticides tested were the organophosphates - Lorsban and Imidan, the insect growth regulator Intrepid, and a CM granulosis virus - Cyd-X Virus. The PE-MEC was tested at up to three rates (approx. 0.2, 0.6, & 1.7 grams PE a.i./acre) with Lorsban and Imidan, but for the other treatments a standard rate of 0.6 grams PE a.i./acre was used.

The walnut test orchard was a mature ‘Chandler’ orchard (*ca*. 20 acres overall) that has a traditionally moderate CM population managed for over five years under a BIOS-based strategy attaining usually Class 1 harvest nuts yields (< 5% damage). For this year’s experiment, the entire orchard received no grower-applied insecticide sprays. Approximately 88% of the orchard’s trees were not experimentally treated by us with insecticides, and thereby provided two-tree “buffer zones” separating the treatment tree locations. From these 826 unsprayed trees, 20 randomly-selected trees from throughout the orchard were designated as “control trees” (numbered black dots in Figure 1) for damage assessment prior to harvest to allow for a comparison of their incurred damage with that of the spray treatment trees. Prior to the June 7 initiation of the test spray program, ‘drop-nut’ damage assessments were preformed in May through early June and established that the CM population appeared to be consistent/uniform throughout the orchard. However, the population level/activity was at that time low and just starting to increase after the first sustained CM female emergence at May 11, the biofix of the 1B flight. A combination of harvest damage - infestation assessments, both **‘canopy-count’** and pole **‘knocked-down’** nuts, were conducted in mid – late September (15–21), three weeks prior to harvest shake-down of nuts by the grower. These earlier-than-harvest damage assessments were required to assure that husk damage, both through-the-husk and solely confined-to-the-husk, would be available/accessible for evaluation along with the shell and kernel incurred damage.

Damage assessment of ‘controls’ and MEC – alone ‘standards.’ 2005 was a high pressure CM year for this orchard, with ‘control’ trees averaging 29.4% ± 1.7% CM damage in ‘knock-down’ sampling and 18.9% ± 1.5% CM damage in ‘canopy-count’ surveys (Tables 3 and 4). NOW attributable damage (presence of larvae with thoracic ‘u’-shaped marking and/or webbing production characteristics) averaged 6.8% ± 1.1% in knock-down sampling and 5.8% ± 0.8% in ‘canopy-count’ surveys (Tables 5 and 6). Of total ‘worm’ damage found, CM damage represented 76.9% for ‘knock-down’ sampling (69.2% for ‘canopy-count’ surveys), while NOW damage represented 23.1% for ‘knock-down’ sampling (30.8% for ‘canopy-count’ surveys). Virtually all NOW damage and infestation presence was attributable to their following, both in time and entrance ‘hole’, a prior shell penetration and kernel infestation by CM larvae. Thereby all CM damage reported represents both CM alone damage and that of dual CM – NOW damage (Tables 3 and 4).

Figure 5. Mean percent (± SEM) of Codling Moth caused walnut damage incurred on ‘control’ – non-sprayed trees (20 replicate trees) verses damage observed for the two treatments without insecticide, PE-MEC-alone spray treatment and “Blank-MEC” spray treatment (8 replicate trees each) as determined through nut knock-down sample collections (100+ nuts/replicate tree) followed by husk, shell and kernel evaluations.

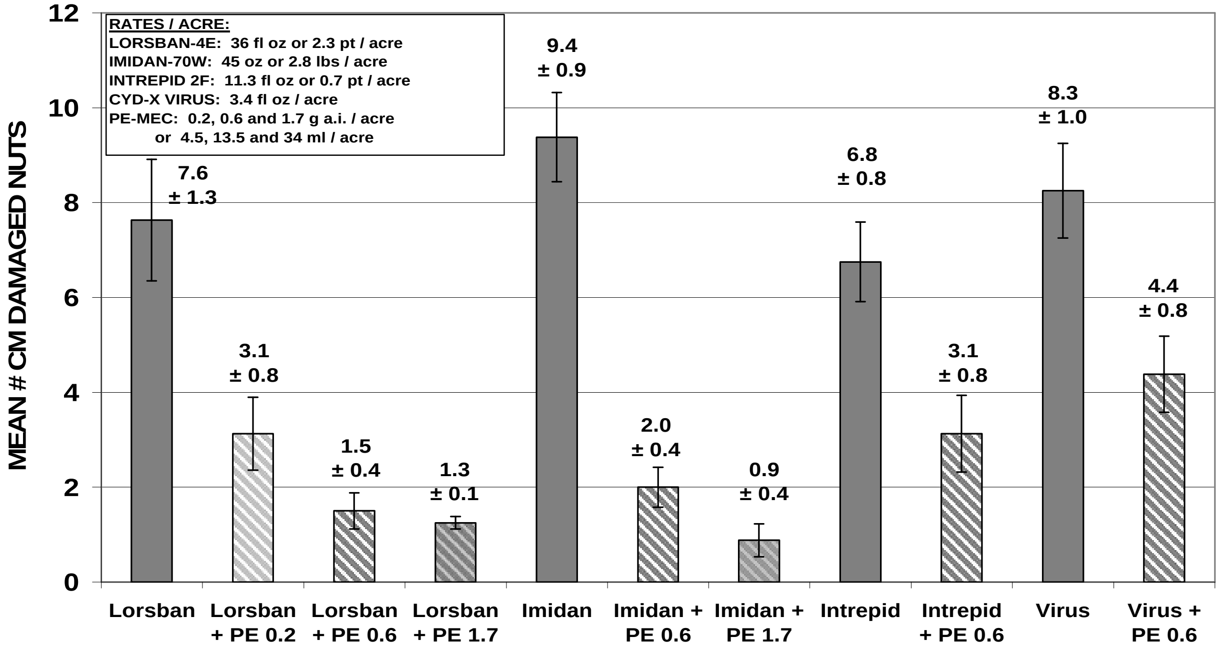


The CM damage rates for the tested standards, PE-MEC and ‘Blank-MEC’ were the same, averaging 23.9% ± 2.1% and 26.4% ± 2.3% respectively for the ‘knock-down’ sampling and 26.4% ± 3.0% and 28.4% ± 1.6% respectively for the ‘canopy-count’ surveys (Tables 3 and 4). These rates of incurred CM damage on the PE-MEC and ‘Blank-MEC’ treated trees are not statistically different from the rate of damage on ‘control’ trees for the ‘knock-down’ sampling (Table 3 and Figure 5), but are just statistically different from the rate of damage on ‘control’ trees for the ‘canopy-count’ surveys (Table 4). The nature of the visual inspections in the ‘canopy-count’ surveys make these surveys more subjective, while the random poling down of nuts by hired laborers makes the ‘knock-down’ sampling more objective in nature. However, most of the insecticide spray treatments (see below) have damage results that are comparable for both ‘knock-down’ sampling and ‘canopy-count’ surveys (Tables 3 and 4). No differences were found in NOW infestations between the PE-MEC and ‘Blank-MEC’ treated trees and ‘control’ trees for both the ‘knock-down’ sampling and ‘canopy-count’ surveys. Thus, it’s concluded that neither the PE-MEC nor the MEC formulation itself had any direct effect on either CM or NOW infestations or ability to attack walnuts. The spraying at the neonate hatch periods with PE-MEC adjuvant did not appear to “stimulate” egg deposition and/or directly effect larval survival in comparison to the nearby un-sprayed ‘control’ trees. However, we could not assess nor determine any effects of the PE-MEC adjuvant on female attraction to treated trees or perhaps stimulation of egg laying frequency on those kairomone treated trees.

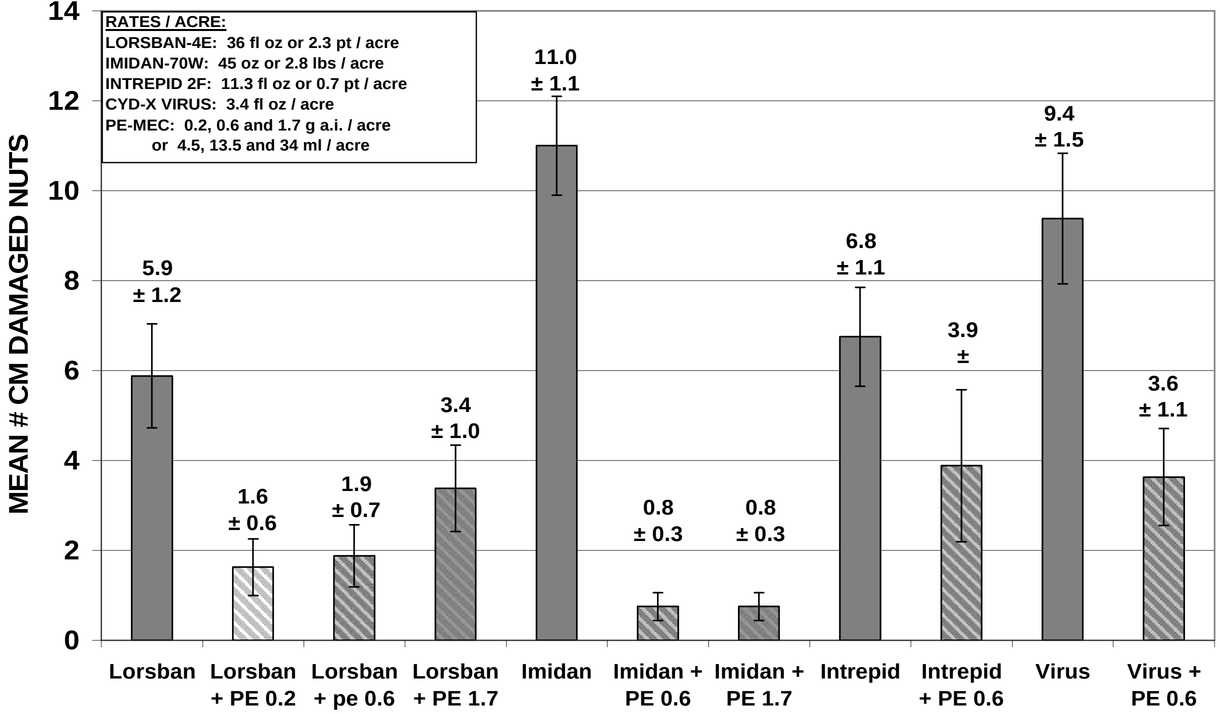
Damage assessment for the experimental spray treatments. Both 'knock-down' sampling and 'canopy-count' surveys show the expected, that these registered and/or experimental (CM virus) insecticides when applied alone at these reduced or low rates do significantly decrease both CM (Tables 3 and 4) and NOW (Tables 5 and 6) damage in comparison to non-sprayed control trees. The tank-mixing of the PE-MEC adjuvant with these four different insecticides at reduced or low rates caused highly significant reductions in CM damage below the damage found with the paired insecticide-alone applications in the 'knock-down' sampling assessments (Tables 3 and Figure 6). Generally, the same significant reduction effects for the use of the PE-MEC adjuvant were observed in the 'canopy-count' survey data (Table 4 and Figure 7). The exceptions being two treatments, the highest rate of the PE-MEC with Lorsban and with Intrepid, where damage rates recorded in the 'canopy-count' survey were reduced in both cases by 43% below insecticide-alone, but these reductions were not statistically significant ($P > 0.05$) (Table 4 and Figure 7).

The trials with the OP insecticides involved testing various tank-mixed rates of the PE-MEC, with Lorsban paired with 0.2, 0.6 and 1.7 grams PE/acre and Imidan paired with 0.6 and 1.7 grams PE/acre. For the 'knock-down' sampling assessments a significant improvement and decrease in CM damage percentage was observed for the OP treatments combined with the higher rate(s) of the PE-MEC over those with the lower experimental rate (Table 3). CM damage percentage for treatments of Lorsban combined with either 0.6 or 1.7 grams PE/acre were significantly lower ($P < 0.05$) than Lorsban + 0.2 grams/acre, while Imidan + 1.7 grams PE/acre was found to significantly ($P < 0.05$) lower CM damage over Imidan + 0.6 grams PE/acre (Table 3). However, no significant differences between the PE-MEC rates were observed for CM damage in the 'canopy-count' survey assessments or for NOW damage in both damage assessments.

Figures 6 and 7. Mean percent (± SEM) of Codling Moth caused walnut damage incurred on low and reduced-rate insecticide – alone treatment trees *verses* trees treated with insecticides + PE-MEC adjuvant (8 replicate trees each) as determined by nut knock-down (Figure 6, top) and canopy count (Figure 7, bottom) coolections followed by husk, shell and kernel evaluations.



LOW RATE - INSECTICIDES WITH PE-MEC ADJUVANT
NUT KNOCK-DOWN 2005: BARTH WEST, ESPARTO, CA

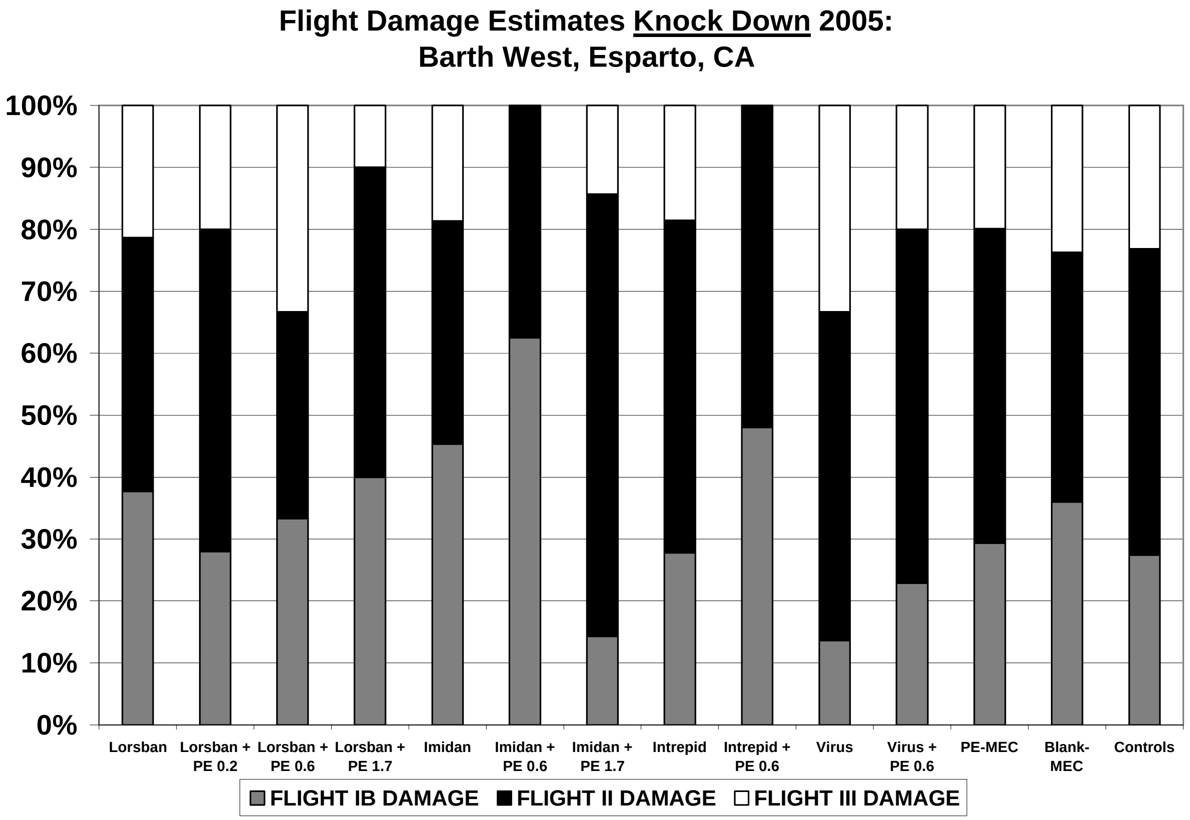


LOW RATE - INSECTICIDES WITH PE-MEC ADJUVANT
CANOPY COUNT 2005: BARTH WEST, ESPARTO, CA

All four insecticides applied alone significantly reduced NOW damage below the levels found for the 'control' trees, as determined by both damage assessment procedures (Tables 5 and 6). The tank-mixing of the PE-MEC adjuvant with either of the OP insecticides, Lorsban or Imidan, at *ca*. ½ label rate caused <u>highly significant reductions</u> in NOW damage below the damage found with these insecticides alone in both the 'knock-down' sampling and 'canopy-count' survey assessments (Tables 5 and 6). However, the control efficacy against NOW of either the IGR – Intrepid or the virus - Cyd-X were not significantly improved by the inclusion of the PE-MEC with the insecticide spray (Tables 5 and 6). The PE has not been found to attract NOW adults in our eight years of PE trap attractant studies. Though studies have not been conducted, there is no biological basis to suspect that PE would attract or affect in any manner the behavior and host selection of NOW larvae. Thus, the observed improvement in NOW control by the addition of the PE-MEC adjuvant to the two OP insecticides is presumed to be an accompanying attribute of the direct control improvement effects on CM larvae (adults also?) and/or their behaviors. One might speculate that the OP insecticides would kill CM more quickly and with less feeding/ingestion damage and penetration holes to the walnut husk (and shell) than either the IGR or the virus, which are reported to require more temporal and spatial exposure or direct consumption ingestion by CM larvae to acquire a lethal dose of material or particles. Thereby, IGRs and viruses might allow more damage and boring holes into husks and shells, thereby allowing more successful entry of the following NOW larvae. Thus, the efficacy of the OP contact poisons was improved for both CM and NOW by the use and apparent effects of the PE-MEC adjuvant.

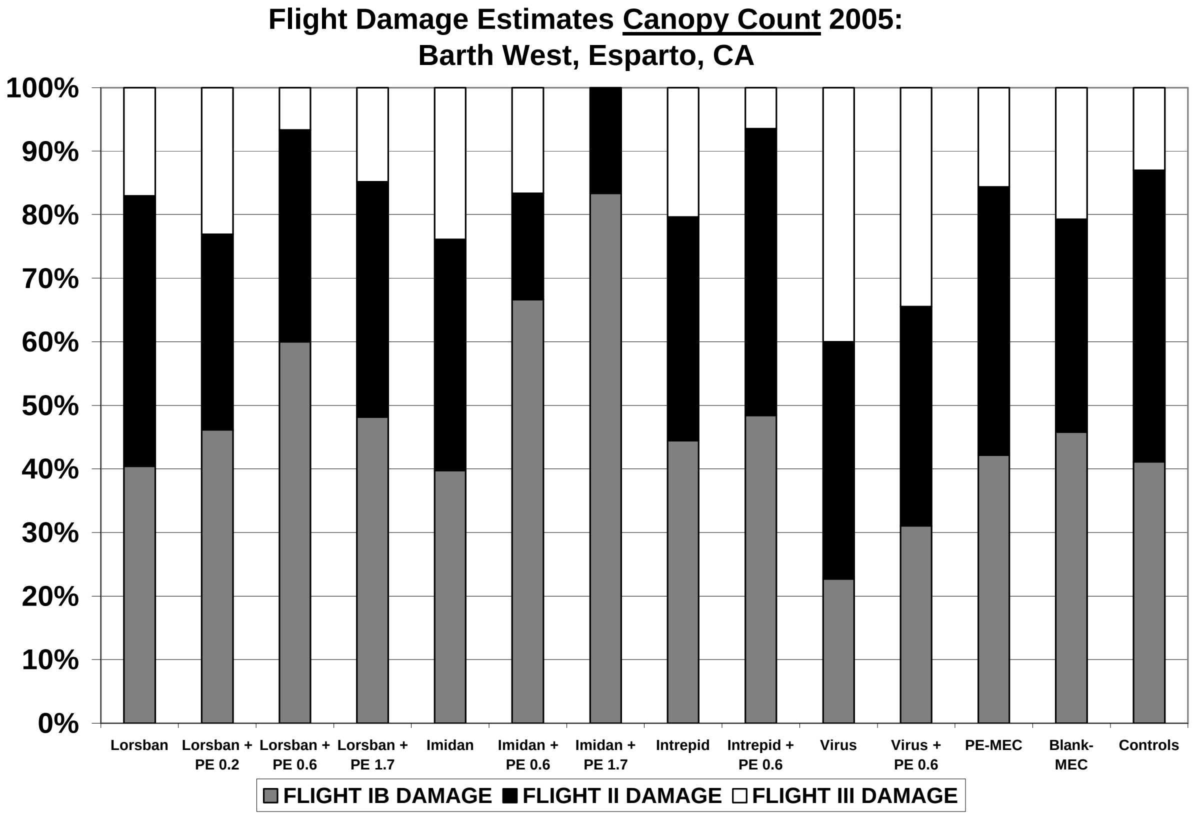
Analysis of the relative proportion of the total CM damage that occurred during the three flights is portrayed in Figures 8 and 9 for the 'knock-down' sampling and 'canopy-count' survey assessments. For all insecticide treatments, MEC standards, and un-sprayed controls a higher proportion of the season-long damage detected in the 'knock-down' sampling is attributed to having occurred during the 2nd flight, followed by the 1B flight damage and then the damage during the 3rd flight (Figure 8). One interesting observation is the lack of detected 3rd flight damage for Imidan + PE-MEC and Intrepid + PE-MEC relative to the 18% damage with these insecticides alone and up to 33% and an average of 19.7 ± 6.5% for the other treatments. Conversely, for the 'canopy-count' survey assessments a higher proportion of the season-long damage is attributed to having occurred during the 1B flight, followed by the 2nd and 3rd flight damage proportions (Figure 9). This could again be an effect of the basis of the damage survey used. For 'canopy-count' survey assessments, the higher proportion of damage found in the 'oldest' damage period, attributed to occurring during the 1B flight, could be due to the ease that the oldest damaged nuts that remain on the tree at harvest time have husks that are visually highly apparent and readily selected by the surveyor due to the darkened, shriveled and dried appearance of these husks.

Figure 8: Relative Percent proportions of the total damage that incurred during the three flight periods, 1B flight, 2nd flight and 3rd flight, for nut knock-down assessments.



% of Total damage	Lorsban				Imidan			Intrepid		Virus		PE-MEC	Blank-MEC	Controls
	alone	+ PE 0.2	+ PE 0.6	+ PE 1.7	alone	+ PE 0.6	+ PE 1.7	alone	+ PE 0.6	alone	+ PE 0.6			
FLIGHT IB	38%	28%	33%	40%	45%	63%	14%	28%	48%	14%	23%	29%	36%	27%
FLIGHT II	41%	52%	33%	50%	36%	38%	71%	54%	52%	53%	57%	51%	40%	49%
FLIGHT III	21%	20%	33%	10%	19%	0%	14%	19%	0%	33%	20%	20%	24%	23%

Figure 9. Relative Percent proportions of the total damage that incurred during the three flight periods, 1B flight, 2nd flight and 3rd flight, for canopy count assessments.



% of Total damage	Lorsban				Imidan			Intrepid		Virus		PE-MEC	Blank-MEC	Controls
	Alone	+ PE 0.2	+ PE 0.6	+ PE 1.7	alone	+ PE 0.6	+ PE 1.7	alone	+ PE 0.6	alone	+ PE 0.6			
FLIGHT IB	40%	46%	60%	48%	40%	67%	83%	44%	48%	23%	31%	42%	46%	41%
FLIGHT II	43%	31%	33%	37%	36%	17%	17%	35%	45%	37%	34%	42%	33%	46%
FLIGHT III	17%	23%	7%	15%	24%	17%	0%	20%	6%	40%	34%	16%	21%	13%

Impact and summary of the PE adjuvant's synergistic enhancement of the efficacy of the OP, IGR and microbial insecticides is portrayed in Table 7. The combination of the PK-MEC adjuvant with insecticide reduced codling moth damage over the reduced-rate insecticide alone by 77% for Lorsban, 88% for Imidan, 53% for Intrepid, and 49% for Cyd-X virus. Navel orangeworm damage was also significantly reduced. For all four insecticides, the mean (+ SEM) reduction in damage provided by the PE-MEC adjuvant in these particular experiments averaged 66.6% + 9.4% for reduction in CM damage and 63.9% + 23.7% for reduction in NOW damage. These studies show promise that the kairomone can improve insecticide efficacy and contribute to new IPM tactics for CM and NOW and the integrated reduction of aflatoxin incidence.

The pear-ester compound has the potential to be used to manage CM in an AK bait-spray by allowing for the death and removal of neonate larvae by promoting an increase in the temporal and spatial exposure to insecticides. The ability to selectively attract, prolong exposure and kill crawling CM larvae, could provide a critical and environmentally rationale means of direct and selective control using insecticides that will improve efficacy and resistance management (Chicon et al., unpublished; Sauphanor et al. in press). CM neonates are known to be more attracted to, prefer, and feed/penetrate more readily into pome-fruits over walnuts. CM larvae are relatively 'hesitant' to 'accept' and feed/penetrate into walnut husks (Mills Walnut Research Reports 2000). Our intent is to exploit this CM larval preference by presenting pear ester in AK bait-spray MEC formulation droplets to larvae in a walnut orchard environment to increase larval temporal and spatial exposure to and thereby lethality of insecticides. We have demonstrated in laboratory studies of various designs that the PE kairomone is the most effective, genetically-based attractant know for neonate CM (Knight & Light 2001). Moreover, three years of apple field trials in Argentina have demonstrated that the PE-MEC at very low rates (*ca*. 0.3 grams/acre) in full coverage bait-spray applications can reduce the effective rate of Guthion by 50 – 75% (Chicon et al., unpublished). Similarly, our results reported here, conducted in a well-replicated walnut field trial, demonstrated significant reductions in CM and NOW damage when a low rate of the PE-MEC was used as a spray adjuvant with reduced-rates of various types of insecticides.

CONCLUSIONS

This project's goal is to develop novel control methods using the pear-ester kairomone to directly attract and kill or disrupt the behavior of larval or adult female and male codling moths in walnut and pome-fruit orchards of California. This year's field trials have demonstrated some critical attributes and benefits for the use and potential integration of the pear ester into CM management practices. We have demonstrated that the slow-release MEC formulation of the kairomone, PE-MEC, can effectively enhance and synergize the control efficacy of various insecticides, ranging from contact-poison OPs to an ingestion-based IGR and a microbial virus. This bait-spray adjuvant enhancement effects were attained using modest, and thus eventually affordable, amounts of a.i. material, being effective at 0.6 grams/acre. That is merely 12 ml per acre (perhaps 1.5 fluid ounces per 500 gallon spray tank) of a dilute "concentrate." These results were revealed, and allowed to reach statistically significant proportions, through our experimental design to test reduced or lower than label rates of these established insecticides. Eventual the practical commercial use of this kairomone would be as an adjuvant to the Label Rates of insecticides, with the goal and benefit of the adjuvant being providing a degree of increased efficacy, but of a more subtle contribution nature, and moreover adding a higher dimension of assurance of control for the practitioner and grower.

But prior to such a leap to commercialization of this ‘attract and kill’ bait-spray tactic, a number of requisite steps through both research experiments and implementation demonstrations must be undertaken. For the next - 2006 season, specific challenging and critical experiments are proposed, including: 1) a continuation of single tree applications to resolve the active range and optimal dose for the PE-MEC adjuvant with lower – label-rates of various insecticides and 2) the expansion in scope, dimension, and practicality by testing the efficacy of this adjuvant technology using replicated orchard test plots of one acre or greater, conducted upon a number of walnut varieties and over a number of counties in both the Sacramento and San Joaquin Valleys. The research grant proposal submitted for 2006 – 2007 season is specifically to conduct efficacy studies using PE-MEC adjuvant bait-sprays in replicated commercial walnut orchards with the objectives of resolving: (1) the mode of action for the adjuvant with insecticides (possibly repeated-attraction, wandering, arrestment of larvae), (2) the placement and density of capsules required, (3) the residual activity of the PE-MEC capsules, and (4) the optimal application rate for adjuvant efficacy.

Figure 1. Randomized Block Design for the 13 Treatments and their 8 Replicate Trees for the Larvicidal PE-MEC Adjuvant – Insecticide trials, June – September 2005; Esparto, CA. Rows are West – East, Trees numbered from North Edge.
The location of the 20 randomly picked "Control" non-sprayed trees are designated by bark numbered circles or ovals.
20 acre Chandler Walnut orchard with 930 total trees, ca. 45 trees/acre; treatment trees spaced 3 trees X 3 trees apart in a grid.

	Col. 1	**Col. 2**	**Col. 3**	**Col. 4**	**Col. 5**	**Col. 6**	**Col. 7**	**Col. 8**	**Col. 9**	**Col. 10**
Row 1 North West	Intrepid	Blank-MEC#3	⑥	Intrepid. + PE-MEC 0.6 g/ac	Virus + PE-MEC 0.6 g/ac	Intrepid	Virus ⑭	Lorsban + PE-MEC 0.6 g/ac	Imidan + PE-MEC 1.7 g/ac	DA-MEC#8
Row 2	Lorsban + PE-MEC 1.7 g/ac	Lorsban + PE-MEC 0.2 g/ac	Virus + PE-MEC 0.6 g/ac	Imidan + PE-MEC 1.7 g/ac	Imidan	Lorsban	Intrepid. + PE-MEC 0.6 g/ac	Lorsban + PE-MEC 1.7 g/ac	Virus ⑱	Intrepid
Row 3 ②	Lorsban + PE-MEC 0.6 g/ac	Intrepid. + PE-MEC 0.6 g/ac	Imidan	Lorsban + PE-MEC 1.7 g/ac	⑩ Virus	Lorsban + PE-MEC 0.6 g/ac	*Imidan* + PE-MEC 1.7 g/ac	DA-MEC#6 Blank-MEC#7	Lorsban + PE-MEC 0.2 g/ac	Imidan + PE-MEC 0.6 g/ac
Row 4	Imidan	Virus ④	DA-MEC#2 Blank-MEC#4	Lorsban	Intrepid	Lorsban + PE-MEC 1.7 g/a ⑫	Lorsban + PE-MEC 0.2 g/ac	Virus + PE-MEC 0.6 ⑯	Intrepid. + PE-MEC 0.6 g/ac	Imidan + PE-MEC /ac ⑳
Row 5	DA-MEC#1 Blank-MEC#1	Imidan + PE-MEC 0.6 g/ac	Intrepid. + PE-MEC 0.6 g/ac	Imidan + PE-MEC 0.6 g/ac	Blank-MEC#6	Imidan	Lorsban	Virus	Intrepid	Lorsban + PE-MEC 1.7 g/ac
Row 6	Intrepid. + PE-MEC 0.6 g/ac	Lorsban	Imidan + PE-MEC 1.7 g/ac	Virus + PE-MEC 0.6 g/ac	Lorsban + PE-MEC 0.2 g/ac	DA-MEC#4 ⑨	Lorsban + PE-MEC 1.7 g/ac	Imidan + PE-MEC 1.7 g/ac	Lorsban + PE-MEC 0.6 g/ac	Imidan
Row 7	Lorsban	Intrepid	Lorsban + PE-MEC 1.7 g/ac	DA-MEC#3 Blank-MEC#5	Lorsban + PE-MEC 0.6 g/ac	Virus	Imidan ⑬	Imidan + PE-MEC 0.6 g/ac	Virus + PE-MEC 0.6 g/ac	Lorsban ⑲
Row 8	Virus	Lorsban + PE-MEC 1.7 g/ac	Lorsban	Lorsban + PE-MEC 0.2 g/ac	Imidan + PE-MEC 0.6 g/ac	Virus + PE-MEC 0.6 g/ac ⑪	Lorsba + PE-MEC .6 g/ac	Imidan	DA-MEC#7 Blank-MEC#8	Lorsban + PE-MEC 0.2 g/ac
Row 9 ①	Imidan + PE-MEC 0.6 g/ac	Imidan + PE-MEC 1.7 g/ac	Virus	Lorsban + PE-MEC 0.6 g/ ⑦	Intrepid + PE-MEC 0.6 g/ac	Lorsban + PE-MEC 0.2 g/ac	Virus + PE-MEC 0.6 g/ac	⑮ Intrepid	Lorsban	Lorsban + PE-MEC 0.6 g/ac
Row 10 South West	Virus + PE-MEC 0.6 g/ac	Blank-MEC# ③	Imidan + PE-MEC 0.6 g/ac	Intrepid	Imidan + PE-MEC 1.7 g/ac	Imidan + PE-MEC 0.6 g/ac	DA-MEC#5	Lorsban + PE-MEC 0.2 g/ac	Imidan ⑰	Intrepid + PE-MEC 0.6 g/ac

Table 3. Mean Percent **Codling Moth** Infestation Detected by **Nut Knock-Down Sampling** a Week Prior to Harvest, Esparto, CA.

Treatments:	**Percent Codling Moth Damage for each Replicate Tree:**								**Mean ± SEM**	**Statistical comparison of Insecticide Alone *vs.* Insecticide + PE-MEC rate**		
	#1	#2	#3	#4	#5	#6	#7	#8		**ANOVA F**	**LSD *P* value**	***t*-Test *P* value**
Lorsban	10	11	6	7	11	9	0	7	7.63 ± 1.28			
Lorsban + PE-MEC 0.2 g/ac	2	4	3	0	2	5	2	7	3.13 ± 0.77	9.09	< 0.01	< 0.02
Lorsban + PE-MEC 0.6 g/ac	2	2	2	2	0	3	0	1	1.50 ± 0.38	21.04	< 0.001	< 0.002
Lorsban + PE-MEC 1.7 g/ac	2	1	0	0	2	3	1	1	1.25 ± 0.13	22.90	< 0.001	< 0.002
Imidan	9	10	14	11	5	10	9	7	9.38 ± 0.94			
Imidan + PE-MEC 0.6 g/ac	1	4	3	2	2	2	0	2	2.00 ± 0.42	50.87	< 0.0001	< 0.0001
Imidan + PE-MEC 1.7 g/ac	2	0	2	0	1	0	2	0	0.88 ± 0.35	71.30	< 0.0001	< 0.0001
Intrepid	9	6	5	4	8	6	11	5	6.75 ± 0.84			
Intrepid + PE-MEC 0.6 g/ac	2	2	3	5	1	6	6	0	3.13 ± 0.81	9.64	< 0.01	< 0.01
Virus	8	10	5	7	4	12	9	11	8.25 ± 1.00			
Virus + PE-MEC 0.6 g/ac	3	2	3	8	3	7	6	3	4.38 ± 0.80	9.20	< 0.01	< 0.01
DA-MEC alone *	20	31	28	28	23	29	16	16	23.88 ± 2.12	1.62	NS	NS
Blank-MEC alone *	30	35	16	30	27	31	22	20	26.38 ± 2.28	1.88	NS	NS
Controls (no sprays)	32	29	40	38	28	20	31	21	29.40 ± 1.70			
	27	16	37	37	23	27	32	23				
	27	29	47	24								

* PE-MEC and Blank-MEC statistically compared with the no-spray Controls.

Table 4. Mean Percent **Codling Moth** Infestation Detected by **Canopy Count Sampling** Two Weeks Prior to Harvest, Esparto, CA.

Treatments:	**Percent Codling Moth Damage for each Replicate Tree:**								**Mean ± SEM**	**Statistical comparison of Insecticide Alone *vs.* Insecticide + PE-MEC rate**		
	#1	#2	#3	#4	#5	#6	#7	#8		**ANOVA F**	**LSD *P* value**	***t*-Test *P* value**
Lorsban	4	2	5	9	2	11	8	6	5.88 ± 1.16			
Lorsban + PE-MEC 0.2 g/ac	0	2	0	1	5	3	2	0	1.63 ± 0.63	10.45	< 0.01	< 0.01
Lorsban + PE-MEC 0.6 g/ac	4	5	3	2	0	1	0	0	1.88 ± 0.69	8.81	< 0.02	< 0.02
Lorsban + PE-MEC 1.7 g/ac	7	4	0	0	5	6	4	1	3.38 ± 0.96	2.76	NS (0.11)	NS (0.13)
Imidan	14	13	13	14	11	10	7	6	11.00 ± 1.10			
Imidan + PE-MEC 0.6 g/ac	2	0	0	1	1	2	0	0	0.75 ± 0.31	80.04	< 0.0001	< 0.0001
Imidan + PE-MEC 1.7 g/ac	2	1	1	0	0	0	0	2	0.75 ± 0.31	80.04	< 0.0001	< 0.0001
Intrepid	13	6	6	9	7	6	4	3	6.75 ± 1.10			
Intrepid + PE-MEC 0.6 g/ac	3	2	0	1	1	12	1	11	3.88 ± 1.69	2.03	NS (0.17)	NS (0.18)
Virus	8	11	6	7	11	14	15	3	9.38 ± 1.45			
Virus + PE-MEC 0.6 g/ac	5	1	2	9	1	3	7	1	3.63 ± 1.08	10.07	< 0.01	< 0.01
DA-MEC alone *	32	34	34	24	24	32	10	21	26.38 ± 2.95	6.35	< 0.05	< 0.05
Blank-MEC alone *	25	25	34	21	32	32	27	31	28.38 ± 1.60	7.20	< 0.05	< 0.05
Controls (no sprays)	11	10	10	29	21	15	25	16	18.85 ± 1.53			
	20	18	11	13	19	23	25	24				
	22	35	18	12								

* PE-MEC and Blank-MEC statistically compared with the no-spray Controls.

Table 5. Mean Percent **Navel Orangeworm** Infestation Detected by **Nut Knock-Down Sampling** Prior to Harvest, Esparto, CA.

Treatments:	**Percent Codling Moth Damage for each Replicate Tree:**								**Mean ± SEM**	**Statistical comparison of Insecticide Alone *vs.* Insecticide + PE-MEC rate**
	#1	#2	#3	#4	#5	#6	#7	#8		***t*-Test *P* value**
Lorsban	2	1	0	1	2	1	0	2	1.13 ± 0.30	
Lorsban + PE-MEC 0.2 g/ac	0	0	0	0	0	0	0	1	0.13 ± 0.13	< 0.05
Lorsban + PE-MEC 0.6 g/ac	0	0	0	0	0	0	0	0	0.00 ± 0.00	< 0.0001
Lorsban + PE-MEC 1.7 g/ac	0	0	0	0	0	0	0	0	0.00 ± 0.00	< 0.0001
Imidan	3	2	1	2	0	3	1	4	2.00 ± 0.46	
Imidan + PE-MEC 0.6 g/ac	0	1	1	1	0	0	0	1	0.50 ± 0.19	< 0.01
Imidan + PE-MEC 1.7 g/ac	0	0	0	0	0	0	0	0	0.00 ± 0.00	< 0.0001
Intrepid	0	2	1	1	0	1	1	1	0.88 ± 0.23	
Intrepid + PE-MEC 0.6 g/ac	1	0	0	0	0	1	1	0	0.38 ± 0.18	NS
Virus	0	1	0	0	0	0	0	0	0.13 ± 0.13	
Virus + PE-MEC 0.6 g/ac	0	0	1	1	0	0	0	0	0.25 ± 0.16	NS
DA-MEC alone *	6	11	7	6	2	6	4	0	5.25 ± 1.18	NS
Blank-MEC alone *	6	7	3	6	6	10	4	3	5.63 ± 0.83	NS
Controls (no sprays)	7	10	18	19	13	6	11	1	6.80 ± 1.13	
	7	1	14	7	5	4	8	5		
	2	7	17	6						

* PE-MEC and Blank-MEC statistically compared with the no-spray Controls.

Table 6. Mean Percent **Navel Orangeworm** Infestation Detected by **Canopy Count Sampling** Prior to Harvest, Esparto, CA.

Treatments:	**Percent Codling Moth Damage for each Replicate Tree:**								**Mean ± SEM**	**Statistical comparison of Insecticide Alone *vs.* Insecticide + PE-MEC rate**
	#1	#2	#3	#4	#5	#6	#7	#8		***t*-Test *P* value**
Lorsban	0	1	0	2	1	5	1	0	1.25 ± 0.59	
Lorsban + PE-MEC 0.2 g/ac	0	0	0	0	0	1	0	0	0.13 ± 0.13	< 0.01
Lorsban + PE-MEC 0.6 g/ac	1	1	0	0	0	0	0	0	0.25 ± 0.16	< 0.05
Lorsban + PE-MEC 1.7 g/ac	1	0	0	0	0	2	0	0	0.38 ± 0.26	< 0.05
Imidan	4	2	2	4	7	2	1	2	3.00 ± 0.68	
Imidan + PE-MEC 0.6 g/ac	0	0	0	0	0	0	0	0	0.00 ± 0.00	< 0.0001
Imidan + PE-MEC 1.7 g/ac	1	0	0	0	0	0	0	0	0.13 ± 0.13	< 0.0001
Intrepid	3	1	3	0	0	1	0	0	1.00 ± 0.46	
Intrepid + PE-MEC 0.6 g/ac	1	0	0	0	0	1	0	4	0.75 ± 0.49	NS
Virus	1	0	0	0	1	1	2	0	0.63 ± 0.26	
Virus + PE-MEC 0.6 g/ac	2	0	0	1	0	1	1	0	0.63 ± 0.26	NS
DA-MEC alone *	9	6	11	8	7	3	4	6	6.75 ± 0.92	NS
Blank-MEC alone *	4	6	11	7	8	1	6	10	6.63 ± 1.13	NS
Controls (no sprays)	3	4	0	12	8	2	13	4	5.80 ± 0.83	
	8	1	6	4	6	8	10	6		
	10	5	6	0						

* PE-MEC and Blank-MEC statistically compared with the no-spray Controls.

Table 7. Summary for the nut knock-down sampling data of the Mean Percent Codling Moth and Navel Orangeworm incurred damage for the various insecticide-alone treatments *verse* insecticide + PE-MEC adjuvant, and the percent reduction in damage observed due to the synergistic activity of the PE-MEC adjuvant. 2005 trials, Chandler walnut orchard, Esparto, CA.

Test Insecticide	**% CM Damage Insecticide Only**	**% CM Damage Insecticide + PE-MEC**	**% Reduction in CM Damage by PE-MEC Adjuvant**	**% NOW Damage Insecticide Only**	**% NOW Damage Insecticide + PE-MEC**	**% Reduction in NOW Damage by PE-MEC Adjuvant**
OP: **Lorsban-4E** **(Chlorpyrifos)**	6.5%	1.5%	76.9% (4.3X)	1.1%	0%	*ca*. 100%
OP: **Imidan 70-W** **(Phosmet)**	7.4%	0.9%	87.8% (8.2X)	2.0%	0%	*ca*. 100%
IGR: **Intrepid-2F** **(Methoxyfenozide)**	5.9%	2.8%	52.5% (2.1X)	0.9%	0.4%	55.6%
Biological: **Cyd-X Virus** **(CM Granulosis.)**	8.1%	4.1%	49.2% (2.0X)	0.1%	0.3%	0% (Not Reduced)
Mean Percentage:	7.0 ± 0.5%	2.3% ± 0.7%	66.6% ± 9.4% (3.0X)	1.0% ± 0.4%	0.2 % ± 0.1%	63.9% ± 23.7%
Controls- No Sprays	20.5%			6.8%		

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