FINAL PROJECT REPORT

Year 3 of 3

Project Title: Models to assess pesticide impacts on CM, OBLR and C. nigricornis

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Other funding sources

We have submitted and received a new grant (\$21,438, one year) to the Washington State Commission on Pesticide Registration to leverage some of the work being done on this grant. That grant is "Evaluating low dose insecticide residues on codling moth flight and behavior".

Total Project Funding: Year 1: \$74,266 Year 2: \$79,287 Year 3: \$82,378

WTFRC Collaborative expenses:

Item	2012	2013	2014
Miscellaneous ¹	2300	2392	2488
Total	2300	2392	2488

Budget 1

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Item	2012	2013	2014
Salaries	46,783	49,233	51,889
Benefits	18,429	19,317	19,494
Wages	3,200	4,500	4,680
Benefits	554	437	454
Equipment	0	0	0
Supplies	2,500	2,600	2,704
Travel	800	1,200	1,248
Miscellaneous (plot charges at WSU			
Sunrise: 2 acres@\$1,000/acre)	2,000	2,000	0
Total	74,266	79,287	80,468

Objectives:

- 1. Develop life history information needed for the *Chrysopa nigricornis* model.
- 2. Develop mortality versus residue age curves for the three species to six commonly used pesticides.
- 3. Develop demographic models that will estimate the pesticide effects on *C. nigricornis*, OBLR, and CM.

Significant Findings:

- Life history information was reported on last year and was used to develop the demographic models in Objective 3.
- OBLR bioassays from two seasons found larval mortality >80% with residues up to 60-days old for Altacor, Delegate and Warrior. Residue activity was shorter for Proclaim and Entrust with OBLR larval mortality <60% by day 20 and for Assail where mortality was below 30% by residue day 7. The data was used in developing the demographic models for this species.
- Lacewing residue mortality bioassays are completed for five of the six pesticides for leaves from 2012. Bioassays using leaves from 2013 are in progress for all six pesticides.
- Lacewing larval mortality was ≤20% for Altacor, Assail, Delegate, Entrust and Proclaim with residues that were four-days old. Twelve-day old residues for these five materials were virtually no different from the control. Warrior four-day old residues had >70% lacewing larval mortality but was <30% by residue day 25.
- Extra leaves from field applications were collected, sealed and frozen for use in residue mortality bioassays in 2012, 2013 and 2014. This has allowed us to continue testing residue effects beyond the field season.
- The models for Chrysopa nigricornis, OBLR, CM, and Chrysoperla carnea were completed.
- Simulations looking at the greatest period of susceptibility to pesticides were run for all four models.
- Optimal treatment programs for codling moth and OBLR were evaluated and compared to currently recommended programs.
- Lacewing populations were relatively unaffected by organic treatment options for either codling moth or OBLR, but tend to be susceptible to conventionally based programs even with the short residual activity found in our residual assays.

Results & Discussion:

Objective 1. Note: This objective was completed last year, so this narrative is the same as last year's. Newly emerged adult *C. nigricornis* were selected from the colony and used in experiments to gather life history information such as adult female longevity and oviposition rates. A single male and female lacewing were paired together in a large plastic deli container covered with mesh netting for aeration and containing a 2-ounce deli cup with 10% honey water. Each pair was fed weekly with Mediterranean flour moth eggs and three times per week with frozen wooly apple aphids collected from low input or non-sprayed orchards. Each pair was held in a growth chamber with 16L:8D photoperiod, at 77°F and 50-60% relative humidity. Containers were checked daily for egg clusters, which were counted and removed once recorded. A subset from the removed eggs were placed in a 4-ounce deli cup, given *E. kuehniella* eggs and placed in a growth chamber to evaluate progeny survival rates.

Results: Life history data was analyzed and used as the basis for the demographic models (Obj. 3). The data showed that this species (which is predaceous as an adult) is extremely long lived. They also have an extremely long oviposition period and high reproductive rate that results in the mortality corrected female egg production being 739 female eggs/female and 95% egg production does not occur until 79 days (2120 DD). Comparing these results to *Chrysoperla carnea* (that is not

predaceous as an adult), the adult longevity is much longer (nearly 2x as long) and 95% of the total egg production occurs nearly 1.5 times later. Even without the initial demographic model, it is clear that overlapping generations are the rule (even if we assume the mortality rate in the field is 2-3 x what happens in the lab), and that the phenology models are primarily telling us when the next generation's reproduction starts to occur. If adult lacewings do have the sort of mortality rates found in the lab (very unlikely), some of the overwintering generation of adults could last until late July or early August.

Objective 2. OBLR mortality was obtained for the aged residues of six pesticides over two seasons. In 2012, the late summer trials (August 4 application) were successful, while in 2013 residue mortality bioassays were completed for both an early season (May 31 application) and summer season (July 19 application). In general, Altacor, Delegate and Warrior had activity that resulted in >80% larval mortality at older residue ages compared to Entrust, Proclaim and Assail. The timing of the application (early vs. later season) also appeared to affect the longevity of the residue activity for most pesticides tested with summer applications lasting significantly longer. Additional bioassays were also conducted in 2014 to look at the effects of horticultural oils on OBLR 1st instar larval mortality. Both organic and conventional orchards use horticultural oils in their pest management programs and it was important to get a good estimate of mortality rates for the demographic models. For these assays, clean leaves were dipped into a 1% oil solution and set under a fume hood to dry. Five larvae were then confined to 2 cm leaf discs from treated leaves, similar to method used for the pesticide residue assays. Mortality was 64% higher on oil treated leaves compared to the control. This information and the residue mortality data have been incorporated into the demographic models (Obj. 3) for OBLR.

The first round of lacewing residue bioassays were finished for Altacor, Assail, Delegate, Entrust and Proclaim and almost complete for Warrior using frozen leaves from 2012. Results indicate that all of the pesticides, with the exception of Warrior, have low mortality ($\leq 20\%$) on 1st instar lacewing larvae for residues between 4 and 12 days old (Table 1). The corrected mortality for Warrior with 4 day old residues was >70% and does not decline below 20% until residue day 47. Bioassays for Warrior residues between 7 and 25 days old are in progress and should be finished by the end of the year. We are also in the process of running the second round of bioassays for all six pesticides using previously frozen leaves from 2013 to confirm our results.

We focused much of our efforts during the spring and summer of 2014 on CM bioassays using apples with field-aged residues for the 6 pesticide treatments. We obtained unsprayed, non-infested apples for these experiments at the Columbia View research orchard where mating disruption was applied over two 1-acre blocks in late April. Pesticide treatments were applied in one block on June 6 (early season) and on July 18 (summer season) for the second block. The summer season block was also treated with several applications of codling moth virus in May and June to reduce infestation during the first generation flight. Apples were collected twice a week for the first two weeks after spray applications and then every week thereafter (40 day old residue early season; 55 day old residue summer season). For the assays, CM neonate larvae <24h old (five in early season; two in summer season) or 80 (summer) CM larvae were evaluated for each pesticide and residue sample day. Larvae were checked for mortality at 7 days after exposure to each residue sample.

CM mortality for a majority of the control treatments was highly variable and ranged between 40-80% over the season. We attempted to address this issue by running simultaneous second control tests during the early season where only two larvae were confined to a single apple and replicates included more apples. In comparison, this alternative appeared moderately better with 10% lower mortality rates on average, thus this methodology was used during the summer bioassays. Unfortunately, these assays continued to yield control mortality that was inconsistent and above the acceptable 20% threshold to conduct a meaningful analysis. The data shows activity for all pesticide materials for residues 30 days and older, however, since we are unable to correct the data for natural mortality it is difficult to determine the actual treatment mortality and its relevance to pest management decisions. We are continuing to look into other natural mortality correction factors that could be applied to this data set.

Residue	% Corrected mortality					
age (days)*	Altacor	Assail	Delegate	Entrust	Proclaim	Warrior
4	18.2	9.2	14.0	7.2	20.5	72.8
7	6.5	7.7	7.1	11.7	5.3	In progress
12	1.3	4.2	8.5	11.8	2.0	In progress
25	-	-	-	-	-	20.0
32	-	-	-	-	-	26.8
47	-	-	-	-	-	18.0

Table 1. Corrected lacewing mortality (%) for six pesticides testing activity of field aged residues.

* Not all treatments were tested for all residue ages. When mortality rates were within control levels (< 15% mortality; Altacor, Assail, Delegate, Entrust, Proclaim) pesticide residue effects are low to none.

Objective 3. We completed four models this year (codling moth, oblique-banded leafroller, and the lacewings *Chrysopa nigricornis* and *Chrysoperla carnea*). These models are unique because they not only provide the phenology, they also allow estimation of pesticide effects when treatments are applied at different times, have different activities (e.g., ovicide, larvicide) on the target population, and last different amounts of time. In addition, the obliquebanded leafroller accounts for the fact that the number of instars is variable, allowing individuals in any generation to go through either five or six instars. The codling moth model was radically modified to allow better estimates of the effect of mating disruption, and uses seasonal environmental conditions to estimate how mating disruption effects vary over the season. All the models now use real weather data to drive longevity of residues and population effects; for this report we used data from WSU-TFREC.

Each of the models has a control population (no treatments), and allows the user to specify eight different treatment programs, each of which can have up to 18 different sprays applied. The treatment programs can be specified based on degree-days, and the residue length is specified on a calendar date basis. Each of the type of treatments (Table 2) is based on either literature data or data collected in our lab. Combination treatments are also possible (e.g., CM granulosis virus + oil, pesticide + oil, Bt + oil); if oil or an oil combination is specified, the oil effect only acts on those individuals present at the time of application; there is no residual effect. The user needs to specify which AWN station and which year is used to drive the model. The models also allow the user to specify a particular codling moth treatment program and evaluate the effect of that program not only on codling moth, but also leafrollers and both lacewing species (and for OBLR treatments assess effects on CM and the two lacewings).

We evaluated the models in several ways: (1) we applied a single simulated pesticide application every 90 DD whose residue lasted only 25 DD throughout the season to evaluate the timing of susceptibility of the target species; (2) we evaluated several different codling moth treatments (using mating disruption or not) and OBLR treatments using the normal and optimal recommended timings **Table 2.** Sensitivity of codling moth, obliquebanded leafroller, and two species of lacewings to pesticides used in simulations. "Pesticide" is a combination of several different efficacious compounds and uses the average efficacy of those materials.

	Treatment						
Stage Affected	CM virus	Bt	Pesticide	oil**	virus +oil**	<i>Bt</i> + oil**	Pesticide + oil**
CM egg	_	_	_	80%	80%	80%	80%
CM larva (neonate only)	75%/6d*	_	90%/14d	30%	82.5%	30%	93%
OBLR egg	_	_	_	56%	56%	56%	56%
OBLR larva	_	85%/7d	90%/20d	71%	71%	84%	93%
lacewing eggs	_	_	0%	18%			18%
lacewing larvae	_	_	67%/7d	0%			67%
lacewing adults	_	_	99%***	0%			99%

* % mortality/longevity of residue

**all oil treatments only affect the susceptible stages at the time of application, they have no residue combination treatments immediately revert back to the activity/residue of the other component or either Bt + oil on CM or virus + oil on OBLR, effects default to oil alone; for both lacewings, Bt and virus have no activity, so combinations default to oil alone

***caused 99% mortality in 108 DD; adults normally live ≈1440 DD

(Tables 3 & 4) and evaluated the non-target effects the other three species; (3) we used temperature data from 2014 (very warm year) and 2011 (cool year).

General Results:

- 1. Pesticide effects operate on a calendar date basis (primarily from UV light degradation or by being partially washed off by rainfall). There is also a plant growth issue over longer periods of time (e.g., new leaf production or growth in the fruit diameter); these effects are predictable on a degree-day basis but are not included in the model (the project to evaluate this was rejected last year by the technology committee).
- 2. Pesticide effects on either pests or natural enemies are greater during warmer years, warmer times of the year, or at warmer locations. This is because for a given length of the residue (e.g. 7 days), in warmer situations more of the population will go through the sensitive stage (because more DD are accumulated) and thus more of the population is exposed to the pesticide in the susceptible stage (assuming the pesticide is put on at the correct time). The corollary of this is that during colder years normal spray programs (e.g., two codling moth sprays per generation) may leave the later part of the generation untreated. The greater suppression during the warm weather needs to be balanced with the longer season and more generations that might come through.
- 3. Mating disruption also is more effective when it is warm. This is because for a given number of days before mating occurs (e.g., 3 days), in warmer years the delay on degree-day basis is greater which reduces the reproductive rate compared to individuals not exposed to a delay in mating.
- 4. The best conventional treatment (with no MD) for CM for the first two generations is not as good as a relatively weak treatment only in the first generation when mating disruption is used.

Times of greatest susceptibility to sprays: CM – The effect of a single spray of oil and a larvicide effects are similar in respect to time of maximum effect with the larvicide effect offset slightly to account for egg hatch (Fig. 1). Oil acts as an ovicide and kills 80% of the eggs present at the time of application and 30% of the neonates present, and thus has a greater effect than the larvicides, which only affect the neonate larvae present at the time of application and 25 DD later. The larvicide effect would be larger if we had a longer residue, but for this simulation, we were investigating times of peak susceptibility and not which treatment was better.

OBLR – The use of oil has only a minor effect until between 400–550 DD when the adults of the overwintering generation begin to lay eggs (Fig. 2). Similarly, the larvicide treatment has a strong effect on the overwintering larvae (after diapause is broken), which quickly is lost after ≈ 270 DDF (Fig. 2); then increases as the next generation of larvae starts to increase between 1080-1350 DD. The new overwintering larvae are never well controlled by sprays, since they feed only briefly and then enter diapause in the 1st through early 3rd instars. As with the codling moth, remember that these simulations have only a very short residual so that we can evaluate the timing of the sprays. Another thing to consider is that the larval effects are at the maximum level (i.e., there is no correction for larvae feeding within the rolled leaf). In addition, the long residual effect of the sprays after leaf growth decreases in the summer would increase the efficacy of the larvicide compared to the oil (ovicide) treatment.

Lacewings – Although both species of lacewings share the same responses to pesticides in each of the different stages (Table 2), that does not mean that they share the same sensitivity to application timing. *C. nigricornis* overwinters in a silken pupal case, and emerges later in the season than *C. carnea*, which overwinters as a diapausing adult. Thus, even before any models are made, it is apparent that *C. nigricornis* would not be greatly

Fig. 1. Seasonal effect of a single pesticide application with a 25 DD residual activity for codling moth.

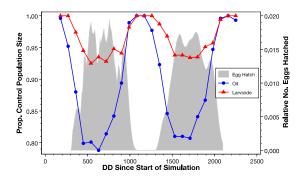


Fig. 2. Seasonal effect of a single pesticide application with a 25 DD residual activity for obliquebanded leafroller.

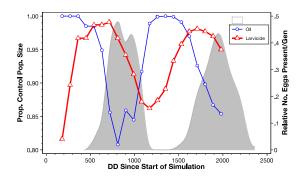
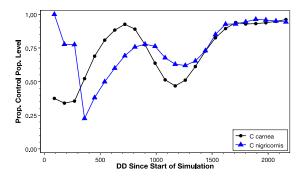


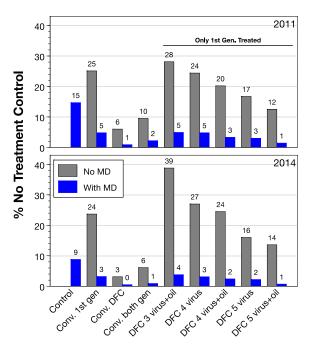
Fig. 3. Seasonal effect of a single pesticide application with a 25 DD residual activity for the lacewings *Chrysopa nigricornis* and *Chrysoperla carnea*.



affected by the earliest spring sprays, whereas *C. carnea* would be more likely to be suppressed. Simulations showed the expected trend, with *C. carnea* heavily impacted by the initial spray (at 90 DD), whereas *C. nigricornis* was not affected at all until emergence started around 180 DD (Fig. 3). The sensitivity of the two species was offset and is related to the early season emergence times. The latter part of the season (after 1200 DD) should not be taken as showing that there is no effect with sprays at that time – the low effect there is caused by the fact that two generations of both species had already occurred, so the full effect sprays at that time would not be reflected until the next (overwintering) generation occurs.

Optimal Codling Moth Timing: Conventional treatments for codling moth generally call for two sprays in the first two generations. The first spray is put on about 250 DD after first emergence (175 DD) or at 425 DD. The second spray in the first generation is then put on 14-16 days later, depending on the residual effectiveness for the spray used. The second-generation sprays start at 1425 DD and the second is also put on 14-16 days later. Another treatment program is called the delayed first cover (DFC) program, where and ovicide (generally oil) can be applied at 375 DD this kills most of the unhatched eggs, and allows the first cover spray to be delayed until 525 DD, the second cover is then again delayed 14-16 days depending on residual of the material used. The second generations are treated the same, with the first oil spray applied at 1375 DD and the first cover spray delayed until 1525 DD and the second applied at 14-16 days later.

Fig. 4. Comparison of different treatment programs under mating disruption or not in 2011 (cold year) and 2014 (warm year). Numbers represent the population size as a percentage of the no MD control.



Organic management must be done in conjunction with mating disruption because of the short

residual of all the materials (other than Entrust) registered. We can use the same tactics of delayed first cover (an oil applied at 375 DD), then either virus + oil or virus alone for subsequent treatments. Table 3 has the timings investigated.

Results:

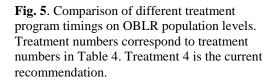
All the efficacy information is based on the percentage of the control (no treatments applied) population level. In the no-mating disruption treatments, the comparison between the conventional treatment (#3) and the delayed first cover (#2) treatment showed that the delayed first cover decreased the CM population level to 3.1% of the control compared to 6.1% in 2014 and in 2011 to 6.0% compared to 9.5% of the control (Fig. 4). The difference between the two programs is a result of the residues dissipating at the end of the generations earlier in the conventional treatment program.

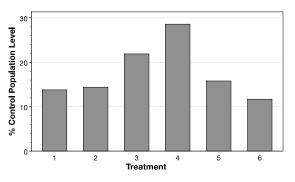
atments.
5. Same as #4, using 4 virus sprays
6. Same as #5, but using virus + oil sprays
7. Same as #4, but using 5 virus sprays
8. Same as #7, but 5 virus + oil sprays

Table 3. Pesticide t	timings for	codling moth	treatments
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The organic treatments (without mating disruption) generally performed equivalent to the conventional treatment restricted to the first generation (#1), with several performing better, particularly when five treatments were applied (Fig. 4). However, when mating disruption was used, all the treatments (except #4) performed better than the best no mating disruption treatment (#2); treatment 4 was similar to the delayed first cover conventional treatment (#2) and better than the traditional timing with just two conventional sprays per generation. Thus, even a very soft organic program (#4) is roughly equivalent to the best non-MD program.

The simulations clearly show that MD makes any





treatment program better than in its absence and even in situations with an out of control population, MD makes a conventional program more effective and cheaper than excessive treatments. The addition of oil to the virus treatments reduced the population about 1-2% compared to the no oil treatments when mating disruption was used. Mating disruption by itself resulted in population levels that were 8.8 (2014) vs 14.7% (2011) of the no MD control.

OBLR optimal timing

The timing of leafroller sprays has always been more vague than codling moth sprays, partially because fruit damage is a result of feeding on leaves that touch the fruit, rather than the insect requiring fruit resources to complete development. In addition, the phenology of OBLR is more complicated with some individuals completing five instars and some requiring six instars. The exact targets for control are not so narrow as with CM, essentially the eggs and larvae are targets, with some larvae protected by feeding within the feeding shelters and not being exposed to pesticides. The simulations we ran did not take this into account, thus the treatment efficacy is optimistic compared to what would be found in the field.

The current guidelines state control of the overwintering larvae should be done by 370 DD when less than 10% of the population has entered the pupal stage (which is unaffected by pesticides). If the population is high in the spring, the summer generation control is recommended to occur between 700-750 DD, and if populations remain high, treatments should occur between 1800-1880 DD so that overwintering larvae don't damage the fruit. It is important to realize that the programs listed are only needed when the population sampling indicates things are out of control, and can cease if populations are adequately reduced – the intensive programs listed are not always needed. Simulations showed that the timings in the previous paragraph are suboptimal for control of OLBR (see Fig. 5, treatment 4), with the 370 DD figure treatment catching only a portion of the sixth instar and the latter half of the fifth instar. Treatment in the overwintering generation needs to be on by

Table 4. OBLR treatments corresponding to Figure 5.

- 1. 90 DD Pesticide, 720 DD oil, 900 DD 2 Bt + oil sprays 7 days apart, Bt spray 7 days later
- 2. 90 DD 2 Bt sprays, 900 DD 2 Bt + oil sprays 7 days apart, Bt spray 7 days later
- 3. 90 DD Pesticide, 900 DD pesticide + oil
- 4. 370 DD Pesticide, 1350 DD pesticide + oil, 1800 DD Pesticide + oil
- 5. 90 DD Pesticide, 900 DD 2 Bt + oil sprays 7 days apart, Bt spray 7 days later
- 6. 90 DD 2 *Bt* sprays, 900 DD 2 *Bt* + oil sprays 7 days apart, *Bt* spray 7 days later, 950 DD pesticide + oil

about 100 DD to affect the earlier instars and to prevent leafrollers that skip the sixth instar from getting to the pupal stage. The summer generation larval treatments between 700-750 DD occur too early for maximum efficacy, essentially wasting any residual effect of larvicides on only the first part of the first instar, although ovicides at this point in time are efficacious and all sprays applied between 900-1145 DD should include oil to increase efficacy by killing eggs. We found the optimal timings were 90 DD (pesticide only or two Bt sprays 7 days apart), and either a pesticide + oil combination at 900 DD or two Bt + oil sprays 7 days apart followed by a *Bt* spray 7 days later. Simulations treating the new overwintering generation showed additional population suppression, which might be useful if populations are not under control later in the season. Simulations not in Figure 5 showed that treating the (spring) overwintering generation resulted in an additional reduction in the population size, compared to treatments that only target the second generation.

Non-target effects

The timings for codling moth can be tested against the two lacewings as well as OBLR. Conversely, the effects of the OBLR treatments can be run against the two lacewings and CM. **Fig. 6**. Effect of CM treatments (Table 3) on OBLR, *C. carnea*, and *C. nigricornis* population levels.

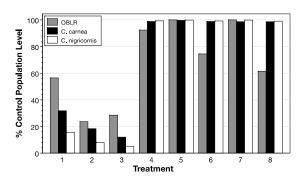
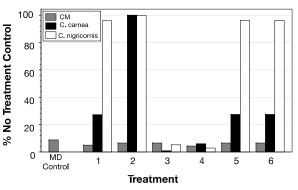


Fig. 7. Effect of OBLR treatments (Table 4) on CM, *C. carnea* and *C. nigricornis* population levels.



The three conventional codling moth treatments (#'s 1-3) suppress both lacewing species to between 10 and 27% of the control population size for *C. carnea* and 9-17% for *C. nigricornis* (Fig. 6). In contrast, the organic treatments (#'s 4-8) had virtually no effect on either lacewing species. OBLR population suppression from codling moth sprays was minimal for most treatments although the delayed first cover caused a 72.2% reduction and the conventional two treatments per generation caused a 68.5% reduction. The organic treatments with oil included did reduce the populations, but generally by only 25-35%.

OBLR sprays caused little additional suppression of CM if mating disruption was not used, except for the current recommendations (e.g., treatment 4 above), which reduced the population \approx 80%. When mating disruption was used, the OBLR treatments generally caused less than 5% change from the mating disruption treatment with no pesticides applied (Fig. 7). The lacewings showed differences between the treatments with *C. nigricornis* surviving better than *C. carnea* in treatments 1, 5, and 6 and no differences in treatment 2. Survival was very low in treatments 3 and 5 which were combinations of pesticide + oil treatments. Not all the treatments using conventional pesticides were toxic; treatment 1, 5 and 6 had pesticide either very early or very late, so that *C. nigricornis* was not yet emerged (treatments 1 and 5) or most had already emerged by the time of the last application (treatment 6).

Executive Summary:

This project developed the basic life history information needed to model pesticide effects on the most abundant lacewing predator (*Chrysopa nigricornis*) and also synthesized the information for codling moth (CM), obliquebanded leafroller (OBLR), and the second most abundant lacewing predator in tree fruits in Washington (*Chrysoperla carnea*) and developed models for all four species. The residual assays provided us with good estimation of the longevity of residues for lacewings and OBLR. The work on CM residual assays was not useful because control mortalities were too high and inconsistent.

The models for the four insects are incredibly valuable as a way to evaluate different pesticide treatment programs on both pests and two key natural enemies. The CM simulations clearly showed that the new delayed first cover treatment program recommended the past 3-5 years is significantly better (\approx 1.5 fold better) than the old conventional treatment program using only two sprays per generation. The simulations also showed that even very soft organic programs applied only during the first generation when used in conjunction with mating disruption reduced the population levels as much or more than the best conventional treatment programs spanning multiple generations when mating disruption was not being used. Several of the organic treatment programs with mating disruption show that even high populations can be reduced quickly as long as external, uncontrolled populations are not migrating into the orchard. Any situation where CM populations are considered to be out of control would be controlled better using mating disruption along with a suitable spray program.

The OBLR simulations suggest that the current recommendations for control of OBLR populations are not optimal and need to be adjusted. Early season applications need to be moved earlier (to ≈ 100 DD), and larvicide treatments for the summer generation should start at ≈ 900 DD and protect the crop during the 900-1260 DD period. OBLR field-aged residue studies showed many of the materials lasted much longer than most people realize, although the growth of new foliage reduces the potential for control as the larvae often move to younger (and untreated) leaves.

The non-target effects of both OBLR and CM treatments could be broadly characterized as organic treatments (excluding Entrust, which would behave similarly to a conventional pesticide) having minor effects on the two lacewings. In general, OBLR treatments (both conventional and organic) made little difference for the CM populations, especially if mating disruption was used. However, in the two conventional codling moth treatments that were applied in both CM generations did reduce OBLR population levels approximately 80%. Two of the organic CM treatments that used heavy doses of oil reduced population levels about 25-30%.