FINAL PROJECT REPORT

Project Title: Developing new natural enemy and pest models for WSU-DAS

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Percentage time per crop: Apple: 40% Pear: 25% Cherry: 25% Stone Fruit: 10%

Other funding sources: None

WTFRC Collaborative expenses: None

Total Project Funding: $151,887

Budget History:

Item	2013
Salaries	90,378
Benefits	33,252
Wages	20,016
Benefits	1,941
Equipment	0
Supplies	3,500
Travel	2,800
Plot Fees	0
Miscellaneous	0
Total	$151,887

Objectives:

1. Validate phenology models for *C. plorabunda*, *E. fummipennis* and *D. brevis* in non-bearing blocks.
2. Develop and implement new models for aphids, mites, and natural enemies.
3. Develop a molecular test to differentiate the different *Chrysoperla* species present in the SCRI grant collections to improve the phenology and lure information for that species complex.

Significant Findings:

- Phenology models for *C. plorabunda* and *E. fumipennis* predict limited first-year emergence data from non-bearing apple blocks fairly well. Spray records and rain events account for some observed deviations from the models. More NE data is needed, in particular for *D. brevis*.
- Models based on literature data for the European red mite (ERM) and the two-spotted spider mite that predict population growth rate have been completed.
- Further evaluation of the ERM model suggest that we can get a more complete picture of phenology than previously thought (at least until mid-June), which may simplify management.
- We have obtained five members of the *Chrysoperla plorabunda* species complex that have been identified as separate species using song analysis by Dr. Charles Henry for evaluation of genetic methods to separate the different species.
- Of the five members of the *C. plorabunda* species complex, *C. downesi* can be readily distinguished from the other four species using mitochondrial genes.
- New PCR primers have been developed and purchased, but have not yet been evaluated because of significant health issues in two of Unruh's technicians that process PCR samples. A further progress report will be available when the new primers have been tested.

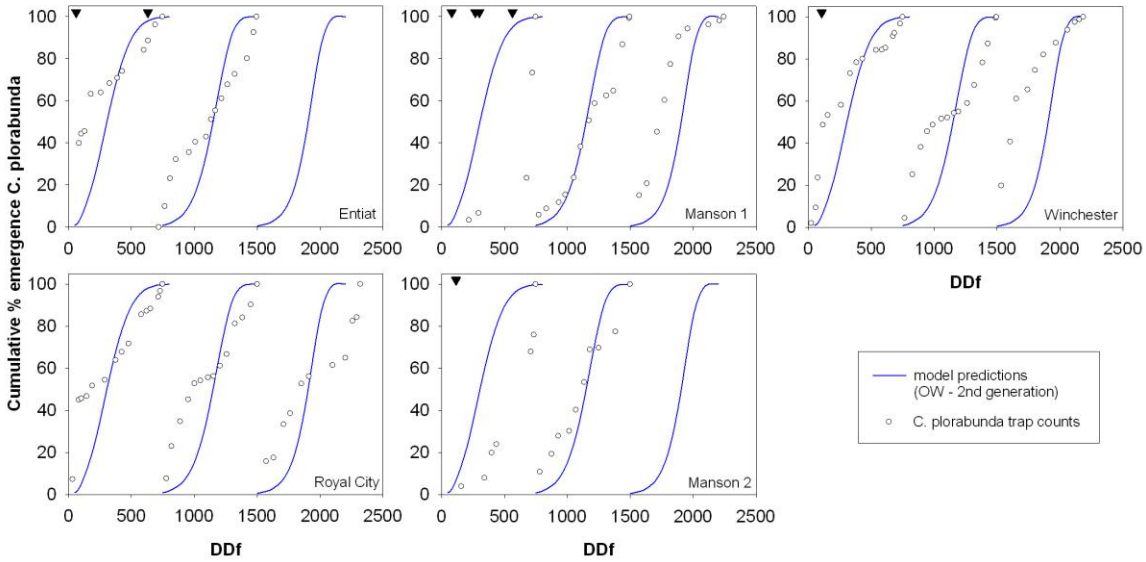
Results and Discussion:

Objective 1. Five non-bearing apple orchards were monitored for natural enemies 1-2 times a week using HIPV lures as well as beat samples. These trap catches were then analyzed in combination with in-orchard temperature records and compared to model predictions. Spray records were received for all orchards, except for Royal City.

Overall, model predictions for the lacewing *C. plorabunda* and the syrphid fly *E. fumipennis* matched HIPV trap captures quite well (Figs. 1, 2). The last emergence of *C. plorabunda* (3rd flight) and *E. fumipennis* (4th flight) were only partial in two orchards (Entiat and Manson 2) and all five orchards, respectively, and hence not plotted. These locations did not accumulate enough heat units by the end of the season to complete the last generation. In addition, cumulative trap counts were not plotted when less than 20 individuals were caught within a generation, which occurred frequently with *E. fumipennis* at all our sites (Fig. 2).

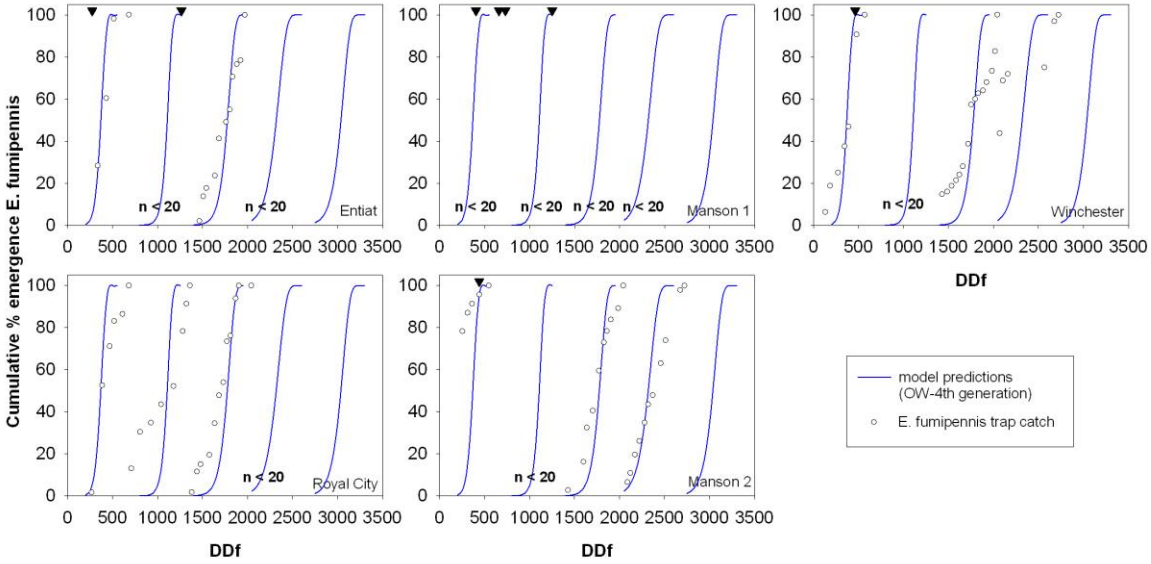
The model comparison graphs also nicely show where the trap captures deviated from the model predictions and can provide a clue as to what might have caused these deviations. Pesticide applications, for example, can delay the emergence of natural enemies as seen during the first emergence of *C. plorabunda* in Manson 1 and Manson 2, where fungicides + insecticides and a fungicide were applied, respectively. Pesticide applications also possibly reduced the number of syrphid flies in some orchards, in particular during the second flight (Fig. 2). In addition, rain and other spray applications can explain reduced natural enemy activity and, consequently, trap catch as observed at the Winchester orchard during the second *C. plorabunda* flight in July (Fig. 1). A second and third year's monitoring data (including additional locations) are important to finalize the model validation.

Fig. 1. Comparison of observed and predicted lacewing (*Chrysoperla plorabunda*) emergence in five non-bearing apple orchards in 2013. Generations not plotted if DD for last generation not reached. Triangles indicate insecticide and/or fungicide application.



Regarding additional natural enemies that we monitored, *Deraeocoris brevis* was not caught in sufficient numbers to validate the model with this year's data. However, we found the highest numbers of *D. brevis* in the older grafted orchard (Royal City). Therefore, future data collection will need to focus on older non-bearing grafted orchards to achieve the necessary natural enemy numbers for model validation. The woolly apple aphid parasitoid *Aphelinus mali* also only occurred in larger numbers in the grafted orchard that was notably infested with woolly apple aphid. The minute pirate bug *Orius* is another predator that was abundant in the two medium-sized non-bearing orchards (Entiat and Winchester).

Fig. 2. Comparison of observed and predicted syrphid fly (*Eupeodes fumipennis*) emergence in five non-bearing apple orchards in 2013. Generations not plotted if less than 20 individuals caught or DD for last generation not reached. Triangles indicate insecticide and/or fungicide application.



We also conducted beat samples to supplement lure trap catches with numbers of juvenile natural enemies. Except for the grafted orchard, the beat samples yielded very low numbers of natural enemies throughout the year, indicating that natural enemies were not established and did not reproduce in those young orchards. Except for the grafted orchard, which had a high number of woolly apple aphid colonies, all other orchards showed little signs of aphid or mite infestations that would sustain predacious syrphid fly, lacewing, or *Deraeocoris* larvae. The observed HIPV trap catches in the younger orchards then likely reflect an influx of adults from surrounding orchards looking for mates and/or food sources for their offspring.

Objective 2. The models for aphids and mites are based on extensive literature searches and compilation and analysis of the resulting data. We have focused on two pest mites, the two-spotted spider mite (TSSM), *Tetranychus urticae* and the European red mite (ERM), *Panonychus ulmi*, both of which can be serious pests in all of our orchard crops in Washington State (and throughout the world). Because both species are worldwide pests, the literature data was extensive and analysis generally showed good agreement on all the different parts of the life history needed for model development.

European red mite:

ERM overwinters in the egg stage on spurs and cracks in the bark. Data in the literature include the development time for overwintering eggs, effect of low temperatures on survival of overwintering eggs, and the development rates of all the other (non-overwintering) stages. In addition, we found complete life tables by five authors that were run at 10 different temperatures, so that we could synthesize developmental rates (including upper and lower thresholds for development), population growth, and other parameters that would be useful in predicting risk over time.

Overwintering Eggs: The overwintering eggs showed the greatest variability in developmental time between studies. Our data set was composed of six studies that together tested development times at 24 temperatures. The studies were published from 1961 to 2000 and the locations of the studies varied from Greece, Japan, Canada, and Great Britain. Normally, we put all the data together on a common axis for the analysis, but when we did that, the fit was relatively poor (for this sort of analysis) (Fig. 3). Analysis of each study separately gave somewhat similar results for threshold and development times, but visual inspection showed basically two groups of data. The first group came from two studies (one of the Canadian studies and the study from Great Britain (open circles)) and the other group came from four different studies (Greece, two from Canada, and Japan). The group I studies estimated the lower threshold for development was 40.6°F the egg stage duration was 278 DDF, while group II studies provided the estimate of 43.5 °F and 394 DDF as the duration. If the data are corrected for the different thresholds, there is little difference in duration on a DD scale (about 24 DDF). The differences between the different studies mean that before we start to use the egg hatch predictions that we need to collect overwintering eggs and see which threshold appears to work best in Washington State – this should be a relatively simple study.

Fig. 3. Development rate of ERM overwintering eggs at different temperatures from literature data.

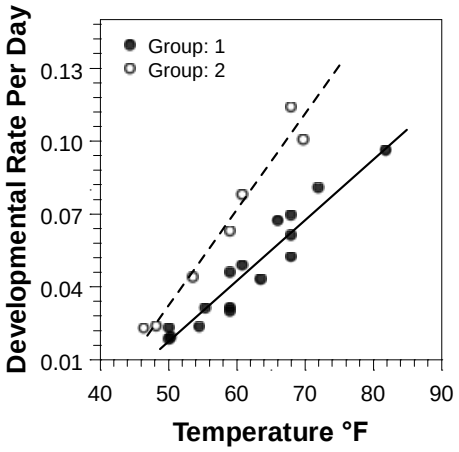
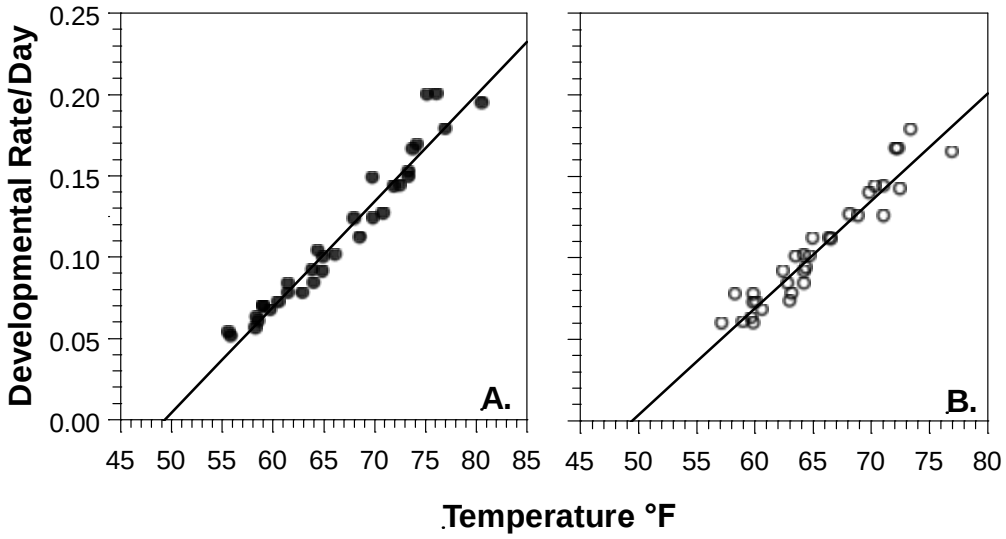


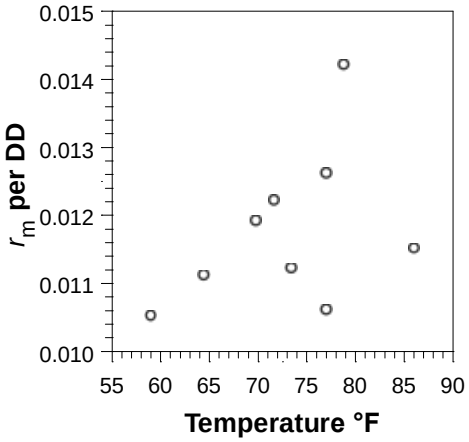
Fig. 4. Development rates versus temperatures from literature data for ERM. A. non-overwintering eggs B. immature stages.



Other stages: In contrast to the overwintering egg data, the studies for all other stages showed good agreement between the different studies (Fig. 4A,B). The non-overwintering eggs and the immature stages both showed the lower threshold for development was about 49.2°F and the duration required for both were very similar (158 and 152 DDF for eggs and immatures, respectively).

Population growth rate: The population growth rate was estimated from life tables done at nine different temperatures. The growth rate number is called r_m and indicates the change in population size per female on a daily basis. However, we can't use the daily growth rate to estimate change in population directly because temperature in the field varies greatly over time and mites (like insects) develop based on temperature units (DD). However, by dividing the raw r_m values by the number of DD accumulated per day at the temperatures at which the study was done, we can estimate the population growth rate on a DD basis, which allows us to estimate population growth in the field based on DD accumulations. This can then be combined with our knowledge of the length of time required for completion of each stage and forecast weather to see how much the population grows in the next week (or more). One of the assumptions of this model is that there is no particular pattern of the growth rate with temperature; thus for this to work, we need to plot the corrected r_m data versus temperature and we should see no statistical relationship. If this is true, then we estimate the corrected growth rate by just taking the average growth rate over the different temperatures.

Fig. 5. The growth rate of the population on a DD scale versus temperature. The lack of any statistical trend means we can use the average growth rate to predict population growth.



Our corrected r_m data showed no statistical relationship with temperature (Fig. 5) and the average corrected r_m values was 0.0062 individuals per DD. By using the weather forecast for the next week-10 days, we can then project how fast the population grows. Our data shows that generation time (T) is 462 DD and the population doubling time is 109 DD. The population doubling time is

the time required for the population to increase by a factor of two, but it is important to realize that this is primarily how long the females take to lay enough eggs to double the population. Those eggs then have to hatch and the immature stages need to complete development before the next generation of individuals occurs. Thus 109 DD from any point in time, all of those "new" individuals are in the egg stage (since it takes 158 DD for non-overwintering eggs to hatch). By evaluating the temperature predictions, we can estimate what stage the new individuals are in and how long before the next generation of adults appear and begin reproduction.

While the discussion above is somewhat technical, what is important is that the details of the population growth can be summarized both graphically and in a simple table to give the consultants a good idea of how quickly the population could potentially grow in the next period of time and when management might be required. We can also project a sample estimate (e.g., we have 0.5 adult females/leaf) into the near future to help understand when the next sample should be taken. There is also further information that can be gleaned from the underlying heat-driven development, including potentially a much more complete picture of phenology than previously thought. Evaluating this data shows that it may be possible to predict phenology relatively accurately at least until mid-June. Combined with longer-ranged forecasts, the phenology should simplify management tactics.

Two spotted spider mite:
Unlike the ERM, the two spotted spider mite (TSSM) overwinters as an adult female in diapause under the bark or in at the base of the trees. Data in the literature was extensive and provided good information on duration of each stage (4 studies at 12 temperatures) and population growth studies (r_m values from six studies at 11 temperatures).

Duration and thresholds: The lower developmental threshold for TSSM was nearly identical to the ERM, at 49.3°F and the duration required was 97 and 275 DD for the eggs and immature stages. Like the ERM temperature-development data, there was little variation between studies for either eggs or immatures.

Population growth rate: Similar to the ERM, life table studies showed the population growth rate on a DD scale (r_m corrected) was independent of temperature, and the average growth rate was 0.0078 individuals per DD. This value is ≈25% higher than that of ERM and is one of the reasons that TSSM are considered one of the most important mite pests on a wide range of crops. The population doubling time is 82 DDF, or about 30% faster than ERM and the generation time is ≈ 504 DDF or slightly longer than ERM. The population projections can be easily done with forecast data, and like the ERM, we can also give how long is required for the different stages to be completed and provide the information on management in both graphic and tabular form.

We have not evaluated the TSSM model for phenology predictions as we have the ERM model. It is possible that the phenology predictions are possible, but it is likely they are more difficult to implement because the overwintering stage is a mated female in diapause, so we would need to evaluate the breaking of diapause, which is likely much more variable than emergence from the overwintering eggs.

Objective 3. Specimens of five different populations of lacewings in the *Chrysoperla plorabunda* species complex that were differentiated by song analysis were received from Prof. Charles Henry of the University of Connecticut in mid-April 2013. These were identified by Dr. Henry as *C. plorabunda, C. adamsi, C. johnsoni, C. mohave and C. downesi.*

Studies in this grant initially focused on evaluation of Genbank accessions of Western *Chrysoperla* "species" sequences to determine if there were unique variations that would allow the development of

diagnostic PCR primer sets. The Genbank data showed that there were very few nuclear DNA sequences for *Chrysoperla*, but there were 823 accessions available from the mitochondrial genome with 90% of those focused on four mitochondrial genes. The four gene regions combine to yield 4,630 bases in length for each species to evaluate differences. Multiple single base substitutions were found to distinguish *C. downesi* from the other four species. Similarly, multiple unique single base substitutions in a subgroup of *C. plorabunda* can distinguish them from other populations of *C. plorabunda* as well as *C. adamsi*, *C. johnsoni*, *C. mojave* and *C. downsei*. New PCR primers have been designed and purchased for the nuclear ribosomal gene complex and the spaces between these genes (ITS), but these have not been tested. Delays in the laboratory evaluation of these primers occurred because the two technicians in the Unruh laboratory have required significant medical leave (4 months total) from late summer to the fall. No funds from the WTFRC have yet been spent on this objective and a further progress report will be made available as the work is finished up.

Executive Summary:

This grant was initially submitted as a three-year grant, but reduced to a one-year project. In view of that, the work we performed is commensurate with progress that would be expected in one year. We collected and processed a single year's data from five non-bearing blocks that can be used to help validate the models for *Chrysoperla plorabunda* and *Eupeodes fumipennis*. Examination of that data suggests that we need to focus more on older non-bearing blocks or grafted non-bearing blocks where more pest pressure and natural enemy activity is occurring.

The development of models for secondary pests (aphids and mites) this year focused on European red mite (ERM) and two-spotted spider mite. For both species, literature data from numerous sources showed good agreement in terms of threshold and duration for the different stages. Further, population growth was shown to be predictable on a degree-day scale, which allows us to provide a relatively simple population projection using degree-days that can be obtained from our NOAA forecasts. In addition, because we know the duration of each stage on a DD scale, we can predict life history information important for management such as the time of egg hatch or next adult generation. Our current 7-10 day forecasts are useful, but for such a quick growing pest, management is still difficult to implement – longer-range forecasts should greatly expand the importance of the models for ERM and possibly TSSM. The literature analysis showed that some simple lab data will be required to help clarify the hatch of overwintering eggs for ERM (two choose between the two groups shown in figure 3). Work on the programming of the models into DAS has not yet started (the models were just finished), but we are in the process of designing an interface that could be shared by a number of these secondary pest models.

The molecular work to detect which species of *Chrysoperla plorabunda* species complex has shown that one member of the complex can be readily separated out of from the others. The primers to separate the others based on nuclear ribosomal genes have been designed and purchased, but not yet tested.

Overall, while good progress was made, we did not and cannot finish all three objectives in a project designed as a three-year project in a single year. Our work to date shows the validity of our approaches and has suggested some changes that might be required (as outlined in our new proposal).