FINAL PROJECT REPORT

Project Title: Identification of resistance to codling moth and leafroller in *Malus*

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Other funding sources: NONE

Total Project Funding: **Year 1:** $37,904 **Year 2:** 53,399 **Year 3:** $53,348

Budget History

Item	2011	2012	2013
Salaries (grad student)	22,901	23,817	26,055
Benefits	1,895	1,970	2,207
Wages	10,094	15,840	15,120
Benefits	514	2,772	1,466
Equipment	0	0	0
Supplies	1,500	6,500	6,000
Plot fees	0	2,000	2,000
Travel	1,000	500	500
Miscellaneous	0	0	0
Total	37,904	53,399	53,348

Objectives:

1. Identify and characterize resistance in *Malus* accessions growing at the Sunrise Research Orchard to codling moth and leafroller.
2. Localize the genes that confer resistance to codling moth (CM) and leafrollers (OBLR).
3. Develop predictive genetic markers to identify codling moth and leafroller resistance in potential parents and seeding populations of the breeding program.

Significant Findings:

1. Leaf bioassays for OBLR were developed that revealed differences in larval survivorship, development time, and pupal and adult weight. A 21-day leaf bioassay will provide sufficient data on larval survivorship and development rate allowing a screen of more varieties.
2. Bioassays for CM using whole fruit appear to provide good data on larval survival and development time.
3. Twenty percent, 5 of 20 apple varieties evaluated, showed some degree of negative impact on OBLR suggesting resistance is present to some degree.
4. Some apple varieties showed signs of abnormal OBLR larval development expected from exposure to juvenile hormones.
5. A whole-leaf bioassay for OBLR, developed in year one (2011) was utilized to assess differences in larval survivorship, development time, pupal weight, and adult fecundity for different genotypes of *Malus*.
6. For some genotypes (e.g., Cox's Orange Pippen), resistance was expressed as a function of the plants' phenology (spring, summer, fall).
7. Some apple genotypes (e.g., Antonovka 1.5) appeared to disrupt normal hormone development in OBLR larvae, which inhibited the completion of pupal development, suggesting plant-produced juvenile hormone analogs might be involved.
8. Other mid-eastern genotypes (i.e., KAZ 96-07-06) expressed high mortality to OBLR larvae. No larvae survived to pupate. More importantly, prior to death, most larvae showed signs of hemorrhaging, suggesting the action of plant proteases on the digestive system of OBLR.
9. Oil as a residue on foliage was shown to be highly toxic to young OBLR larvae while the codling moth virus had no effects.
10. One genotypes, KAZ-181, showed almost no impact on OBLR in all bioassays. This genotype could therefore function as a susceptible model for comparison with other genotypes instead of using artificial diet.
11. We were not able to replicate the production of larval-pupal intermediates when only later instar larvae (5^{th} and 6^{th}) were exposed to leaves of genotypes that had produced larval-pupal intermediates in previous bioassays.
12. The use of a matrix population model provided a good method of synthesizing all parameters derived from the leaf bioassay for OBLR. This model provided an estimate of the intrinsic rate of increase and the net reproductive rate and replaces the index method used previously.
13. Using new versus old leaves in OBLR bioassays was demonstrated as critical to avoid overestimating resistance.
14. The variation of resistance of *Malus* genotypes to OBLR across different periods of the growing season has made it difficult to interpret results.

Results and Discussion:

Characterization of Malus resistance to OBLR.

Data on the development time and mortality of OBLR larvae reared on an artificial diet was collected as an independent internal standard to affirm the viability of OBLR in the colony and to compare with OBLR larvae reared on leaves of various *Malus* genotypes. (Fig. 1) Previously reported work showed that under controlled temperature conditions (22% RF; 23°C; 16:8 LD), OBLR larvae were primarily in the second instar after seven days, in the fourth instar after 14 days, in the fifth and sixth instars after 21 days and in the pupal stage after 28 days. These development data provided a timeline on which to evaluate OBLR development on leaves, and when to change leaves with minimal disturbance to larvae. Since transferring an insect larva during the sensitive molt period can increase mortality, checking leaves every seven days when most larvae were not in the process of molting was an attempt to introduce artificial mortality into the bioassay.

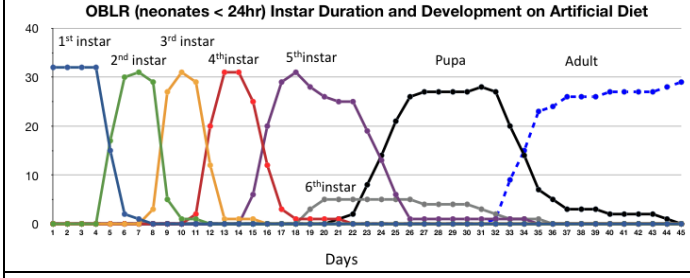

Figure 1. Number of OBLR larvae in a life stage when reared on artificial diet.

The bioassay method developed in 2011 provided leaf quality over time to measure key developmental parameters in 2012. The bioassay involved using a whole leaf placed in a large Petri dish (94 mm X 16 mm) with the leaf petiole placed inside an Eppendorf vial (2.0 ml) that contained water. The Eppendorf vial was inserted through a hole in the side of the plastic Petri dish and sealed with Teflon tape to prevent larval escapes (Fig. 2).

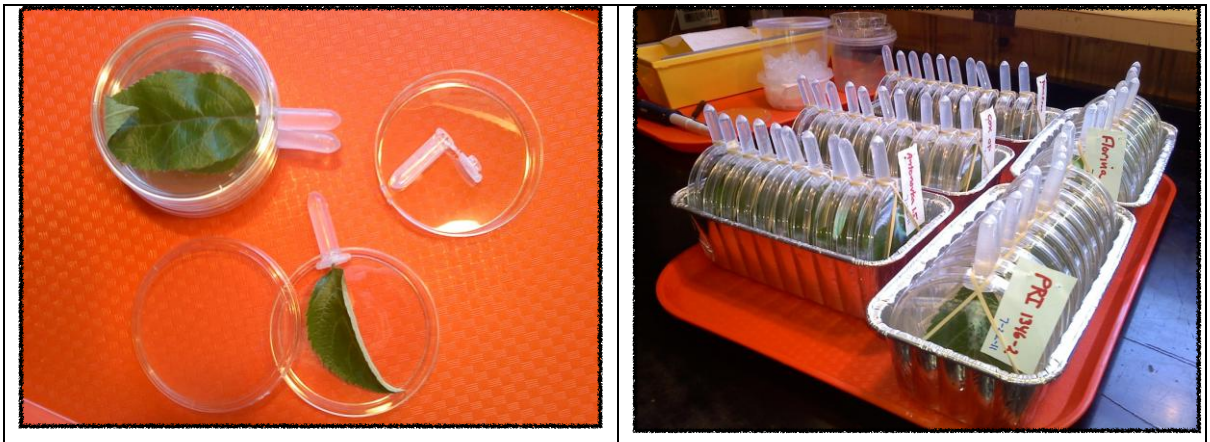

Figure 2. Whole leaf bioassay method developed for OBLR.

In 2011 twenty apple genotypes were evaluated for possible OBLR resistance. The genotypes were: Antonovka 1.5, PRI 1346-2, Redfree, Florina, Cox's Orange Pippin, Northern Spy, Liberty, Russian Seedling, Jonafree, Cortland, Yellow Transparent, Viking, Lady, Jonathan, Virginiagold, Trent, Delicious, Poeltsamaa Winter Apple, Haralson and Granny Smith. These varieties were only evaluated during the summer of 2011, thus did not represent possible resistance traits that could be expressed at other times of the growing season.

Figure 3 shows three types of survivorship curves for a select group of apple varieties, which represented patterns of survivorship curves for other apple varieties evaluated in 2011. There was high survival over 63 days for larvae reared on pinto bean diet (Type I). Florina (Type I) represents a variety where OBLR survival was high. Granny Smith (Type II) represents a group of other varieties, Group A (see Fig. 3). Lady and Trent (Type III) are varieties where there was high larval mortality in young instars. Northern Spy (Type II) also resulted in high larval mortality but most of the mortality occurred on later larval instars. Antonovka (Type II) is a variety in which mortality was expressed mostly in later instars. Larvae that fed on Antonovka showed developmental abnormalities that were similar to larvae that have been exposed to juvenile hormone at the wrong time in the life cycles.

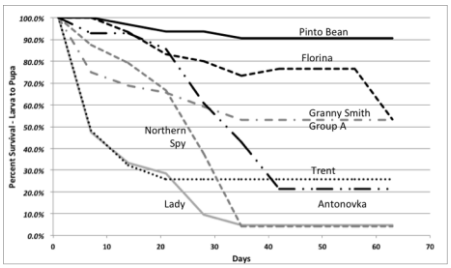

Figure 3. Average percent survival of OBLR larvae reared on different apple varieties. Type I – Florina, Delicious, Haralson, Poeltsamaa, PRI 1346-2, Russian Seedling, and Jonathan; Type II – Granny Smith, Northern Spy, Antonovka 1.5; Type III – Lady and Trent.

When mortality was examined for just the early instars (1-4 → Day 21), three genotypes had highest mortality, Viking (52%), Yellow Transparent (73%) and Lady (71%) (Fig 4). If induction of resistance is stimulated by feeding of OBLR larvae this early mortality might signal a particular mechanism of resistance that might be more important than others.

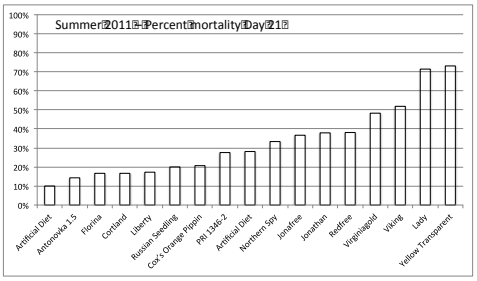

Fig. 4. Percent OBLR larval mortality after 21 days, 2011.

Other life history parameters that were measured in 2011 were development time (days to pupation) and pupal weight. A slower development time can be an indicator or plant resistance as can the weight, which is directly correlated with size, of pupae. The development time for OBLR reared on artificial diet was 25-26 days for males and 29-30 days for females. Development time on apple leaves was generally longer than on diet, but some varieties like Yellow Transparent, Viking and Liberty had exceptionally long development times of 40+ days. Pupal weight of OBLR reared on diet was 83-102 grams for males and 138-178 grams for females. Pupal weight for OBLR fed on leaves was almost always lower than when fed on diet, ranging from 80 to as low as 47 grams for males and 133 to as low as 58 grams for females. Liberty and Viking had very low pupal weights to go along with their slow development times.

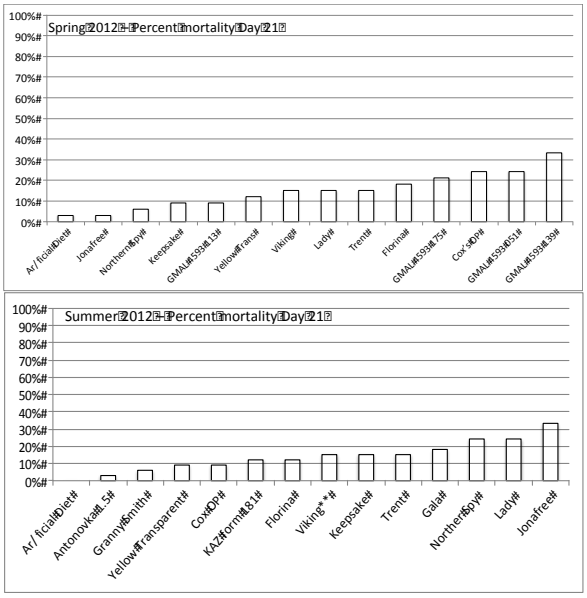

Fig. 5. Percent OBLR larval mortality after 21 days, 2012.

In 2012, 25 *Malus* genotypes were evaluated for possible OBLR resistance, eleven from the "diversity map set"; Antonovka 1.5, Yellow Transparent, Northern Spy, Viking, Keepsake, Cox's Orange Pippin, Jonafree, Trent, Lady,

and Florina, and Granny Smith and fourteen from the "core diversity set" which originated from the middle east; KAZ form 181, Sieversii UZB GMAL 3265, KAZ 95-05-01P-22, KAZ 96-09-05, KAZ 96-07-06, KAZ 95-08-06, Sieversii TAJ GMAL 3244, Sieversii TUR GMAL 2251, Sieversii KYR GMAL 3158, KAZ 96-05-05, Sieversii KAZ GMAL 3310, Sieversii KYR GMAL 1750, KAZ 96-09-02, and KAZ 96-07-03. To determine if resistance was expressed differentially over the season for a specific *Malus* genotype, OBLR development was observed in three distinct periods (spring, summer and fall). Here we report on mortality of OBLR at Day 21 for thirteen *Malus* genotypes for the spring and summer periods, nine that showed variability across both time periods. The snapshot view at Day 21 showed relatively low larval mortality, however, there was differential mortality expressed in different genotypes in different periods. For instance Jonafree and Northern Spy showed low mortality in spring but high mortality in summer where as Cox's Orange Pippen (OP) showed high mortality in the spring but lower mortality in the summer.

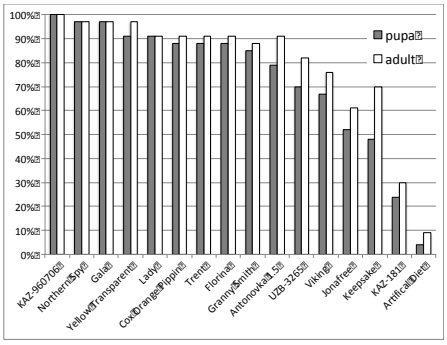

Fig. 6. Percent mortality of OBLR at the pupal and adult stages, summer 2012.

We know that this snapshot of mortality is not the total picture of developmental effects of *Malus* genotypes on OBLR development but it provides a common reference of comparison of larval mortality prior to pupal formation. Some of the larvae surviving at Day 21 did not complete normal development to the pupal or adult stage. Figure 6 shows the percent mortality of these *Malus* genotypes in the summer period to the pupal and adult stages. Clearly additional mortality occurs from larvae dying after Day 21, abnormally formed pupae or pupae that never emerge as adult.

We do not show data for the 14 middle eastern genotypes, however, one, KAZ form 181 had very low mortality, equal to the artificial diet while several KAZ and UZB genotypes had mortality at Day 49 (all adults or deformed/dead pupae) greater than 70%.

In 2013 we again followed *Malus* genotypes in the diversity map set for two periods, spring and summer. We intended to follow these genotypes through the fall but a hailstorm that severely damage foliage and fruit made it impossible to get good data for this period. Here we report on mortality of OBLR at Day 21 for twelve (spring) and eleven (summer) *Malus* genotypes, with nine that showed variability across both time periods. The snapshot view at Day 21 showed high relatively larval mortality in the spring and very low mortality in the summer (Fig. 7). Granny Smith, Golden Delicious and Viking had very high mortality in the spring but low mortality in the summer, along with other genotypes. These results suggest that induction of resistance in the spring could be different than in summer.

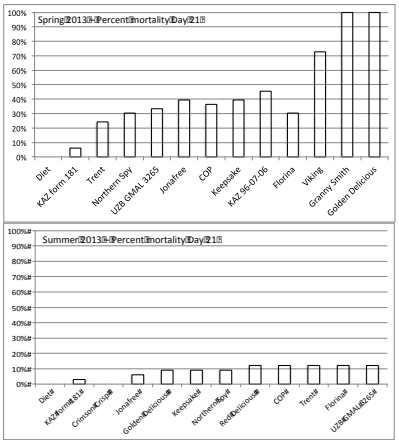

Fig. 7. Percent OBLR larval mortality on Day 21, 2013.

In 2013 we also evaluated for the first time without being compromised by insecticide treatments *Malus* genotypes in the Parent mapping set (Red Delicous, Arlet, Braeburn, Splendour, Cripps Pink, WSU-2, Coop-15, Honeycrisp, Sundowner, Crimson Crisp, Fuji, Cameo, Aurora, Enterprise and Jazz). We evaluated sixteen genotypes with the hope that these data allow us to move to Objective 2 - localization of genes that confer resistance to OBLR. However, results from this effort were highly unexpected and do not provide information we can use to identify a genetic basis for resistance. Larval mortality in the spring and summer exceeded 80% for each genotype. In fact, most of the

mortality occurred in late instar larvae with very little mortality during the first four instars (Day 21 or less). Virtually no adults were produced in the bioassays in either spring or summer periods.

Example of genotype variation across seasonal periods – Exposing OBLR larvae to *Malus* genotypes was limited due to the lack of availability of leaves on some genotypes and to human resources required to follow OBLR through development to the adult stage. However some genotypes in the *Malus* diversity set were followed through two years and two seasons, spring and summer. Figure 8 shows survival to the adult stage in the summer period for 2012 and 2013. Survival was high in 2013 for OBLR reared on the artificial diet, KAZ-181 and some of the other KAZ and UZB genotypes. There were much lower levels of survival to the adult stage in 2012 for Cox's Orange Pippen, Trent, Florina, Northern Spy and Gala than in 2013. Of all the Malus genotypes Gala, Northern Spy and Keepsake showed the highest level of resistance.

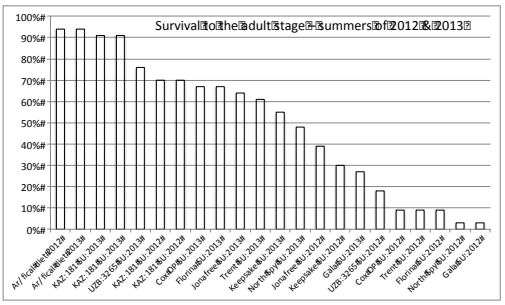

Fig 8. Percent survival to the adult stage of OBLR in the summers of 2012 and 2013.

Population Growth as an Assessment Tool – Synthesis of bioassay data for leaffrollers is a challenge as there are several factors measured, each of which could contribute to what might be called resistance in *Malus* genotypes. In 2012 we developed and index to incorporate multiple factors as way of synthesizing the development data collected. While this approach was useful it was not as robust as we desired. We therefore turned to a modeling approach using a PopTools matrix program that allowed us to make projections of population growth using the development data collected from our bioassays (development time for each stage, mortality, fecundity and sex ratio). Figure 9 shows results of running developmental data through the population matrix model. The intrinsic rate of increase is a measure of population growth. The artificial diet, KAZ-181, Trent and UZB along with Cox's Orange Pippen had high intrinsic rate of increase values for both periods of 2013. Many of the genotypes had intrinsic rate of increase equal to zero, indicating there would be no population growth due either to high mortality or no reproduction by adults that were produced. Of course these data are confounded to some degree by the low number of individual OBLR larvae we were able to rear (max of 33 per bioassay period). We have similar data for the 2012 season but there is not sufficient room to report it here.

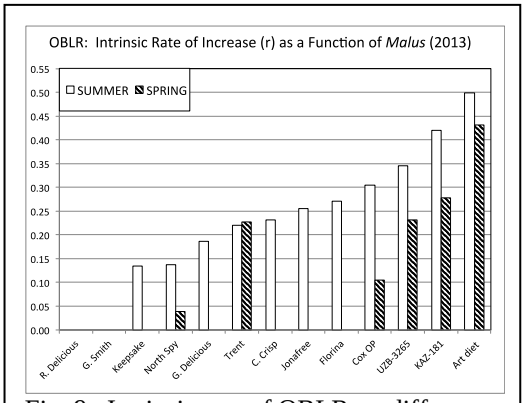

Fig. 9. Intrinsic rate of OBLR on different Malus genotypes based on 2013 bioassays.

Effect of leaf age on OBLR development – In 2013 we conducted a research project comparing the development of OBLR on three Malus genotypes (Red Delicious (RD), Golden Delicious (GD) and Crimson Crisp) where we collected old and new leaves from each to run our bioassay. Figure 10 shows that OBLR larvae exposed to old leaves of Crimson Crisp, RD and GD had much higher mortality at Day 21 compared to those exposed to

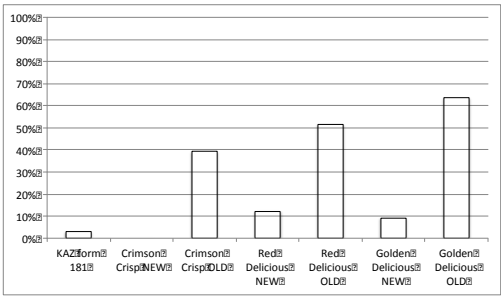

Fig. 10. Percent mortality of OBLR larvae on Day 21.

young leaves. Since OBLR larvae can readily move and choose a leaf that is a better food source, one that is not producing chemicals that inhibit development or increase mortality, it is important when conducting leaf bioassays to use as young of leaves as possible. While these results do not represent novel information regarding leaf age and impact on insect development they are instructive of how we might interpret some variations in our data from year to year when leaf availability in the diversity map set were limited in the summer relative to spring.

Other development parameters informing OBLR bioassay results – Several factors other than mortality inform the impact of Malus genotypes on OBLR. These include larval development rate (LDR), pupal weight (PW), adult mortality rates and adult longevity. We analyzed all our data looking at these parameters for Malus genotypes where we had data across two or more seasonal periods. Figure 11 shows data for LDR and PW. LDR was high in the artificial diet, due in part to a lack of disturbance of larvae and the high level of nutrition provided by the diet (Fig. 11 top). The LDR for KAZ-181, UZB3265, and Florina were close to that of the artificial diet. The LDR was lowest on Cox's Orange Pippen, Jonafree, Viking and Yellow Transparent. Pupal weight was highest for larvae reared on the artificial diet for reasons mentioned above (Fig. 11 bottom). The pupal weight for KAZ-181 was the closest to pupae from the artificial diet. Pupal weights for the other Malus genotypes were similar but about 50% lower compared to those from the artificial diet. There was a high degree of variability between AL and AMR with little difference in these parameters between the artificial diet and *Malus* genotypes (data not shown).

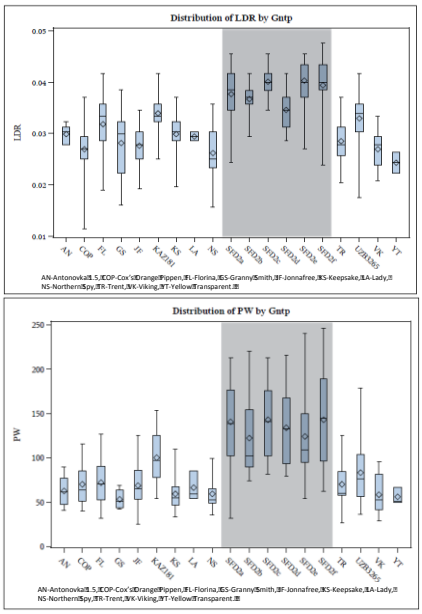

Fig. 11. Larval development rate (top) and pupal weight (bottom) for OBLR reared on different *Malus* genotypes (Gntp).

Hormonal effect on OBLR development – In 2011 and 2012 we observed that when OBLR larvae were reared on certain *Malus* genotypes, incomplete pupation or larval-pupal intermediates – see image at right - were produced that resembled exposure to a juvenile hormone (JH). Since high JH levels have an abnormal effect if they are present at the last larvae stage we conducted a study in an attempt to confirm whether certain *Malus* genotypes could consistently produce the larval-pupal intermediates. We reared OBLR larvae until the fifth instar on artificial diet and then transferred them to apple leaves using our bioassay method and follow them through to the adult stage. The genotypes evaluated were KAZ 95-05-01-P22, Antonovka 1.5, UZB GMAIL 3265, KAZ 96-09-05 and Red Delicious. While a few larval-pupal intermediates were produced the percent was far below what we had observed previously. It is possible that the cause of larval-pupal intermediates is driven by a lack of adequate nutrition when OBLR larvae are reared in foliage over their entire life and is not specifically related to the induction of a chemical that mimicked JH in the *Malus* genotypes.

Effect of oil and CM virus on OBLR – In 2012 we started using the parent block at Sunrise to assess the *Malus* genotypes impact on OBLR development. However, a mix up in the general pest control program resulted in Intrepid being applied in the spring to trees in the parent set causing high mortality in our bioassays. The intent was to shift the use of CM virus plus oil in the summer to conduct leafroller bioassays so we set out to test whether the CM virus and oil would have negative impacts on OBLR larvae. We found that there was no impact of CM virus

on OBLR larvae, however, we did discover that when oil was applied by an airblast sprayer at 1% concentration to apple trees, and leaves were then collected and ran through our bioassay, all neonate larvae died within the first 7 days.

Effect of fungicides on OBLR – The impact of mildew control on OBLR was evaluated as part of this project since fungicides were applied as part of a maintenance program to apple blocks where leaves were collected for bioassays. There is some literature that has shown negative effects of fungicides on insect or mites so to ensure our bioassay results were not compromised by fungicides. We conducted a bioassay using the diet incorporation of two fungicides, Procure and Rally, and evaluated the development of OBLR larvae. There was no difference in mortality of OBLR larvae reared on diet with concentrations of each fungicide ranging from 0 (untreated) to 300 ppm.

Codling moth resistance 2013 – At the end of the first codling moth generation, selected trees (16) in the GMAIL mapping set were evaluated for fruit injury. No insecticide sprays had been applied to the block and fruit injury was readily observed on most trees. The total number of fruit per tree was counted with the number injured by codling moth recorded. There was an average of 106 (42-175) fruit per tree and the average percent fruit injury was 7.8 (0.0-27.7). These trees were close together so it is doubtful spatial distribution of the codling moth population accounted for the observed differences. These results point to variation in the resistance of genotypes in the GMAIL mapping set to codling moth injury. Plans were to complete the codling moth survey at the end of the second generation; however, two hailstorms in the latter half of summer injured the fruit so that sampling was not possible.

Plans for 2014 – Leafrollers and Codling Moth. We are proposing with a new one year project to continue exploring genotypes with highest levels of resistance and those with high levels of susceptibility. We are proposing to work with Dr. Dhingra's laboratory to identify genes that are up-regulated when OBLR feeds on selected genotypes. We will not have access to the Parent map set in 2014 due to needs for these trees for other research projects, therefore, pursing additional data from these genotypes seems unlikely. We will complete assessment of the GMAIL mapping set and the HCxCP mapping set to codling moth injury in the field, through rearing of injury to fruit and through bioassay.

Localize genes for resistance. We intended to be at a point where we might be able to research the apple genome for expressions of resistance to leafroller by year three. Because we were not able to get data from the Parent set at Sunrise we likely do not have a robust enough data set to explore this objective. The confounding of multiple needs for the same mapping sets associated with the apple breeding program suggests that the genotypes in the parent and possibly the Pedigree set should be replicated by grafting to trees in the pest control plantings at Sunrise.

Develop predictive markers for resistance. This aspect of the project will not be completed within the time frame of the project unless some unexpected results are found in the search for localized genes in Objective 2.

Executive Summary:

This project was originally designed to explore possible resistance in *Malus* genotypes to a leaf feeding insect, OBLR, and a fruit feeding insect, codling moth. We focused our initial efforts on developing a bioassay that allowed us to rear OBLR through their entire life cycle with minimal disturbance and while preserving as best we could the integrity of the excised leaf. The bioassay seemed to work well, at least with regard to maintaining good leaf quality for seven days, which was determined as the best time interval for changing leaves. The first year of the study we evaluated 19 genotypes from the diversity map set at Sunrise. There were only two trees of each genotype and this restricted the population of leaves, which could be used in bioassays. In 2012 and 2013 we evaluated some of the same genotypes from the diversity map set with the objective of determining seasonal variation in resistance. There was much more seasonal variation in resistance expressed in the different genotypes than expected. We were able to evaluate genotypes from the Parent map set in 2013 and found that most of these caused high mortality in OBLR with few or no adults produced and with most of the mortality occurring in late instars. These results have made it difficult to interpret data and identify genotypes that show consistent levels of resistance to OBLR. We did identify one variety, KAZ-181 that caused almost no negative impact on OBLR. KAZ-181 could be a good susceptible genotype to use in future field studies to gage resistance of other genotypes. We showed that old leaves have a definite negative impact on OBLR compared to new leaves. Because OBLR can and does moves from one feeding site to another during its life history, always moving to newer leaves when the opportunity affords itself, we knew we needed to always use newer leaves in our bioassay. The number on trees in the diversity set most likely impacted our bioassay results in summer and fall periods by restricting the availability of new leaves.

We utilized a simple matrix population model to synthesize the life history parameters, stage specific mortality, development time, fecundity and sex ratio, derived from out OBLR bioassays. This method generated a couple of values, the intrinsic rate of increase and net reproductive rate, which were easy to understand was a better method than the indexing method we used in 2012.

We did demonstrate as part of this project that oil residues on foliage have a high negative impact on OBLR larval survival. We have not observed this before with larger larvae but when neonate larvae were exposed to oil residues we consistently observed high mortality. No negative effects on OBLR were observed when exposed to CM virus or the fungicides Rally and Procure.

Preliminary samples of codling moth injury to trees in the GMAIL mapping set showed a high degree of variability following the first generation in 2013. However, due to two hailstorms that injured foliage and fruit in late summer, additional surveys of possible codling moth resistance in the GMAIL and Honey Crips X Crips Pink mapping set could not be completed.

There is a great deal of research that needs to be done to explore insect and disease resistance in the *Malus* genome, however, the problem of using the same trees for horticultural and pest studies arose as a barrier in this project. A long-term plan should be developed to plant *Malus* genotypes of highest interest in areas of Sunrise where pests are not or marginally controlled. This would allow for more trees from which to harvest foliage or fruit without the risk of pesticide contamination and to challenge trees in the field with pests like OBLR and codling moth.