FINAL PROJECT REPORT

Project Title: Integrating codling moth granulovirus into conventional orchards

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Total Project Funding: $42,000

Budget History:

Item	**Year 1: 2013**	**Year 2: 2013**
Salaries		
Benefits		
Wages	17,454	17,454
Benefits	1,546	1,546
Equipment		
Supplies		
Travel	2,000	2,000
Plot Fees		
Miscellaneous		
Total	21,000	21,000

OBJECTIVES

The overall objective of this project was to develop effective feeding stimulants for codling moth larvae that can significantly improve the performance of selective insecticides. Selective insecticides are materials that are toxic for codling moth and whose use will not disrupt the biological control of mites, aphids, mealybugs, or other secondary pests. These include the microbial insecticides, CpGV and Bt's, and synthetic compounds such as Intrepid and Altacor. Specific objectives of the project included conducting laboratory bioassays with possible feeding stimulants such as, naturally occurring yeasts isolated from codling moth larvae, commercial bread yeast, monosodium glutamate, L-aspartate, and Monterey Insect Bait. The second specific objective of this project was to evaluate a promising subset of the most effective feeding stimulants characterized in the laboratory bioassays with CpGV in field trials at the USDA Research Farm.

SIGNIFICANT FINDNGS

2012

- The addition of a*Metschnikowia sp.* Yeas, collected from codling moth larvae, with cane sugar significantly improved the efficacy of a CpGV insecticide (Cyd-X) in laboratory bioassays.
- Three additional species of yeast were isolated from codling moth field-collected larvae and were found to also improve the performance of CpGV in laboratory bioassays..
- Other materials, such as active bread yeast with sugar, the amino acid, L-aspartate with sugar, and the Monterey Insect Bait were found to be very effective in improving the activity of the CpGV in laboratory bioassays. MSG with or without sugar was not very effective in similar bioassays.
- Yeast adjuvants are not compatible with Bt insecticides.
- Field trials with *Metschnikowia* sp. yeast and bread yeast both combined with sugar and added with a low rate of Cyd-X both significantly increased larval mortality and reduced fruit injury in a season-long virus spray program. The addition of MSG or L-aspartate without sugar enhanced the kill of larvae, but did not add any protection of the fruit from codling moth injury.
- The addition of the microencapsulated pear ester formulation did not improve the efficacy of CpGV with or without bread yeast and sugar added.

2013

- Laboratory assays found that the addition of bread yeast and sugar improved the effectiveness of Altacor but not Entrust, Delegate, or Intrepid.
- The use of Cyd-X HP at a low rate alone with the addition of several adjuvants did not prevent codling moth injury on apples despite the use of 11 sprays timed every 5 - 12 d during the season.
- The addition of bread yeast plus sugar or Monterey Insect Bait significantly increased the proportion of dead larvae compared with the virus alone.
- The addition of the yeast *Cryptococcus tephrensis* (isolated from codling moth larvae) with sugar to the virus reduced the numbers of codling moth larvae overwintering in bands on tree trunks by 80% compared with the virus alone.

RESULTS & DISCUSSION

2012: Yeasts isolated from field-collected codling moth larvae were found to significantly increase the effectiveness of CpGV (Table 1). The addition of sugar alone had minimal effect but adding sugar to the yeasts significantly increased the toxicity of CpGV. Larval mortality was significantly increased with the addition of bread yeast with or without sugar to CpGV, but levels of mortality were not quite as high as with the field-collected yeasts. Rates of yeast higher than 3 lbs per 100 gallons have not been tested due to consideration of the economics of adding an adjuvant. The optimal level of brown cane sugar is also unknown but the effect of adding 1 or 3 lb seems to vary among tests with different materials. The higher rate was selected for field trials due to the possible effect of weathering. MSG was found not to be an effective additive. The amino acid, L-aspartate with sugar was effective. The Monterey Insect Bait provided the highest level of larval mortality with CpGV in 2012 bioassays.

The addition of the yeasts with sugar significantly reduced codling moth fruit injury compared with the virus alone. The virus applied at the low rate of 1 oz per 100 gal was not very effective in preventing fruit injury under these high pressure conditions. The use of PE MEC with CpGV provided no additional control. Both MSG and L-aspartate significantly increased larval morality but levels of fruit injury were not reduced compare with the virus alone. The addition of all adjuvants except PE MEC significantly increased the proportion of dead larvae and decreased the proportion of live larvae in or exiting the fruits in fruits (Table 2).

2013: Results clearly showed that the use of Cyd-X HP at such a low rate does not prevent codling moth injury on apples despite the use of 11 sprays timed every 5 -12 d during the season (Table 2). However, the use of the virus did significantly increase the proportion of dead larvae. The majority of dead larvae caused small ‘stings’ on the fruits but other larvae were found dead deep inside the fruit. Both the addition of bread yeast and sugar or MIB significantly increased the proportion of dead larvae compared with the water control or with the unsprayed trees. The proportion of dead larvae with both the bread yeast and sugar and MIB was also significantly higher than with the virus alone. The density of larvae found in bands placed on the trunk of trees varied among treatments (Table 2). The addition of the either of the two yeasts plus sugar or MIB resulted in a significant decrease in the number of larvae found in bands compared with the untreated trees. However, only the use of *C. tephrensis* and sugar significantly reduced the number of larvae in bands compared with the water control. No difference was found in the number of larvae in bands among all of the treatments that included the virus though the mean densities varied by 4-fold.

The addition of bread yeast and cane sugar only significantly improved the performance of Altacor at a 5% of the field rate (Table 3). These bioassays are continuing in order to get more replicates and studies with Assail will be completed. Also, we will run similar assays with 0.05% Delegate. Following the completion of the bread yeast and sugar bioassays these tests will be repeated with the addition of Monterey Insect Bait at 2.0 qts per 100 gallons.

Table 1. Summary of laboratory bioassays with various materials added to CpGV (Cyd-X).

Active material / rate per 100 gallons		Brown cane sugar (lb)	Mean proportion dead larvae
Untreated		0	0.08
CpGV / 1 oz		0	0.30
CpGV / 1 oz	+	3	0.34
CpGV / 1 oz + Inactive torula yeast / 3lb		0	0.38
CpGV / 1 oz + Active bread yeast / 3 lb		0	0.49
CpGV / 1 oz +		1	0.50
CpGV / 1 oz + MSG / 1lb	+	3	0.51
CpGV / 1 oz + MSG / 1lb		0	0.53
CpGV / 1 oz + Active bread yeast / 1lb		0	0.57
CpGV / 1 oz + Inactive torula yeast/ 3lb	+	3	0.65
CpGV / 1 oz + L-aspartate / 1lb		0	0.65
CpGV / 1 oz + *Metschnikowia* spp. / 1 lb		0	0.66
CpGV / 1 oz + Active bread yeast / 3lb		1	0.68
CpGV / 1 oz + Active bread yeast / 3 lb	+	3	0.73
CpGV / 1 oz + Blossom Protect / 1.25 lb		0	0.73
CpGV / 1 oz + Active bread yeast / 1lb	+	1	0.74
CpGV / 1 oz + *Cryptococcus tephrensis* / 3lb		0	0.74
CpGV / 1 oz + *Metschnikowia* spp. / 3 lb		0	0.75
CpGV / 1 oz + *Metschnikowia* spp. / 3 lb	+	1	0.75
CpGV / 1 oz + Blossom Protect / 1.25 lb	+	3	0.78
CpGV / 1 oz + L-aspartate / 1 lb	+	3	0.80
CpGV / 1 oz + *Aureobasidium pullulans* 3 lb		0	0.81
CpGV / 1 oz + *Metschnikowia* spp. / 1 lb	+	1	0.84
CpGV / 1 oz + *Cryptococcus* sp. n / 3lb		0	0.86
CpGV / 1 oz + *Aureobasidium pullulans* 3 lb	+	1	0.89
CpGV / 1 oz + *Metschnikowia* spp. / 3 lb	+	3	0.90
CpGV / 1 oz + Monterey Insect Bait / 2 qts		0	0.92
CpGV / 1 oz + *Cryptococcus tephrensis* / 3lb	+	1	1.00

Materials in shaded rows were tested in field trials.

Table 2. Field evaluations with the granulosis codling moth virus (CpGV) during 2012-13 adding wild yeasts (WY) isolated from codling moth larvae, bread yeast (BY), monosodium glutamate (MSG), L-aspartate (L-Asp), and Monterey Insect Bait (MIB) alone or plus sugar (S) or pear ester (PE), N = 10.

Year / #	Treatment [a]	Mean (SE) %		
		% larval mortality	% uninjured fruit	No. larvae per band
2012 / 1	UTC	13.8 (3.2)b	60.3 (7.1)c	-
	CpGV	43.0 (4.1)b	66.7 (3.4)c	-
	CpGV + WY1 + S	80.8 (2.4)a	78.9 (2.7)b	-
	Insecticides [b]	48.0 (17.6)ab	98.5 (0.4)a	-
	ANOVA	$F_{3, 26} = 10.98$ $P < 0.001$	$F_{3, 26} = 90.39$ $P < 0.0001$	-
2012 / 2	UTC	17.8 (3.1)b	51.1 (5.1)c	32.8 (4.0)a
	CpGV	41.2 (3.9)b	61.7 (3.1)bc	17.6 (5.4)ab
	CpGV + PE	42.3 (2.1)b	66.9 (3.8)ab	14.1 (5.1)b
	CpGV + BY + S	81.4 (2.4)a	78.3 (2.4)a	9.5 (2.6)b
	CpGV + PE + BY + S	73.8 (2.2)a	74.3 (2.4)ab	13.6 (5.1)b
	ANOVA	$F_{4, 45} = 18.83$ $P < 0.0001$	$F_{4, 45} = 38.54$ $P < 0.0001$	$F_{4, 45} = 4.21$ $P < 0.01$
2012 / 3	UTC	9.8 (2.4)c	71.9 (2.0)b	-
	CpGV	47.0 (3.6)b	80.8 (2.7)a	-
	CpGV + MSG	81.8 (2.0)a	77.0 (2.8)ab	-
	CpGV + L-Asp	80.6 (1.8)a	83.4 (1.3)a	-
	ANOVA	$F_{3, 36} = 176.95$ $P < 0.0001$	$F_{3, 36} = 45.24$ $P < 0.0001$	-
2013 / 4	UTC	21.2 (2.1)c	54.3 (2.5)	10.6 (1.7)a
	Water only	16.0 (3.0)c	57.5 (2.6)	9.1 (2.3)ab
	CpGV	68.0 (5.0)b	60.9 (2.7)	6.8 (3.2)ab
	CpGV + BY + S	86.1 (2.4)a	58.4 (3.5)	2.9 (0.9)abc
	CpGV + WY2 + S	76.0 (5.9)ab	66.0 (5.2)	1.4 (0.5)c
	CpGV + L-Asp + S	81.7 (2.7)ab	61.2 (3.4)	5.1 (1.7)abc
	CpGV + MIB	89.1 (1.5)a	64.9 (2.1)	2.1 (0.7)bc
	ANOVA	$F_{6, 58} = 68.09$ $P < 0.0001$	$F_{6, 58} = 1.61$ $P = 0.16$	$F_{6, 58} = 5.77$ $P < 0.0001$

Column means for each orchard followed by a different letter were significantly different, $P < 0.05$.

[a] Wild yeasts include *Metschnikowia* sp. (WY1) and *Cryptococcus tephrensis* (WY2) .

Table 3. Summary of laboratory fruit assays comparing the effectiveness of several standard insecticides at 1 and 5% of the labeled field rate applied alone versus with the addition of bread yeast and sugar.

Insecticide	Rate	Mean (SE) proportion of injured fruits		Fisher's Exact Test
		Alone	W' Bread yeast and sugar	
Water	-	1.00	1.00	*P* = 1.00
Intrepid	1%	0.63	0.57	*P* = 0.79
	5%	0.40	0.50	*P* = 0.60
Entrust	1%	0.60	0.60	*P* = 1.00
	5%	0.33	0.27	*P* = 0.78
Altacor	1%	0.40	0.27	*P* = 0.41
	5%	**0.33**	**0.07**	***P* = 0.02***
Delegate	1%	0.19	0.09	*P* = 0.66
	5%	0.00	0.00	*P* = 1.00

N = 30, five neonate larvae were placed on the upper portion of each fruit. Fruit injury was scored after 14 days.

EXECUTIVE SUMMARY

Tremendous progress was made in the two years of this project in developing feeding adjuvants to enhance the toxicity of insecticides for codling moth. This is the first study to use live yeasts to enhance insecticides. The activity of several wild yeasts isolated from codling moth was characterized as high in combination with CpGV. However, an inexpensive and readily available yeast, *Saccharomyces cerevisiae* (bread yeast) was also found to exhibit significant activity. Previous studies reporting minimal activity from adding sugar alone to CpGV were confirmed. The addition of PE MEC with the virus provided no additional activity. The level of activity from adding MSG was shown to be low, but the amino acid L-aspartate looked promising when used with sugar. Trials also suggest that Monterey Insect Bait is a promising adjuvant for codling moth and should be tested with Bt insecticides.

Studies investigating the use of adjuvants with conventional insecticides have started and to date the use of bread yeast and sugar improves the activity of Altacor but not the spinosyns or the IGR, Intrepid. Studies will be conducted with Assail and each insecticide with Monterey Insect Bait during January. Differences in the chemical properties of insecticide formulations and their relative systemic penetration of plant surfaces are likely key factors influencing the benefit of adding a feeding stimulant for enhanced toxicity to codling moth. Altacor is known to be long-lived and has excellent systemic absorption into plant parts. Dupont personnel have shared with us that they believe that the diamide insecticides have an important oral route of entry for fruit feeding insects. They also expressed that the spinosyns are more active through dermal penetration than diamides. Our bioassays would support these general comments concerning the relative importance of different routes of entry into the target pest. Further laboratory bioassays with bread yeast and sugar as well as Monterey Insect Bait with the diamide insecticides are continuing.

A recently-registered microencapsulated formulation of pear ester has been effective in improving conventional insecticides used in apple, including organophosphates, neonicotinoids, diacylhydrazines, spinosyns, and diamides. Pear ester is not a feeding stimulant but increases larval wandering (host searching behavior). The enhanced wandering stimulated by pear ester increases larval exposure to insecticides. Thus, a cocktail made with a feeding stimulant and a wandering stimulant could provide significantly improved levels of control of codling moth with a range of insecticide classes. No previous studies of codling moth have combined the use of additives that can stimulate both host searching and larval feeding to improve conventional insecticide-based control.

The goal and potential benefit of this research to apple growers is the development and validation of pest management programs that avoid disruption of biological control and prevent problems with some secondary pests. Minimizing this impact through substitution of less disruptive materials, the use of fewer sprays, or the application of reduced rates are options which would also help to reduce the typically higher costs of the new chemistries. The use of behaviorally-active, inexpensive additives is a rational approach which may allow this integrated program to be implemented.