ACTIVE YEASTS IMPROVE SELECTIVE INSECTICIDES FOR CODLING MOTH CONTROL IN PEARS

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ABSTRACT

Studies conducted in 2014 examined the use of bread yeast (RedStar's, *Saccharomyces cerevisiae* [BY]) and sugar (S), corn steeped liquor (Brandt's Monterey Insect Bait [MIB]), and pear ester (Trécé's, DAMEC [PE]) as additives to improve the efficacy of conventional and organic insecticides. Laboratory-based fruit-dip bioassays found that MIB was only effective with Altacor or Entrust when larvae were initially placed on adjoining foliage. In contrast adding BY/S to water increased the level of larval damage but also improved Delegate and Exirel and showed a general improvement across all insecticide classes. Field trials conducted in both pear and apple found that PE can be used in pear and apple to enhance insecticides such as the spinosyn, Entrust and the diamide, Altacor. Field data from apple suggested that MIB is helpful only early in the season when the majority of eggs are laid off of the fruit. Laboratory bioassays have found that dry formulations of yeasts isolated from codling moth larvae can increase the kill of codling moth larvae by Cyd-X 6-fold and are twice as effective as adding BY/S.

OBJECTIVES

1. Evaluate the efficacy of adding either yeast and sugar or corn steep liquor insect bait alone or in combination with microencapsulated pear ester to several classes of insecticides in field-lab bioassays.
2. Conduct field trials to validate the use of these adjuvants in seasonal management programs in replicated research plots.
3. Facilitate and monitor field trials in growers' orchards with moderate to high codling moth pressure using these adjuvants in various seasonal spray programs.

PROCEDURES

Laboratory Assays: Laboratory studies were conducted with adding MIB or BY/S to various insecticides to see if fruit injury from codling moth could be reduced. Studies were conducted with organic 'Red Delicious' apples kept in cold storage at 2 ^{o}C. Apples were washed in bleach and then rinsed in water prior to the assays. Apples were dipped in various insecticides or water alone and with either MIB (2 qts per 100 GPA) or BY/S (3 and 1 lb per 100 GPA) added. Altacor (4 oz), Entrust (6 oz), Exirel (13.4 oz), Delegate (6 oz), Intrepid (16 oz), and Assail (3.4 oz) were tested. Five fruits were considered to be a replicate and 3-37 replicates were conducted with each insecticide. Insectary-reared codling moth larvae were placed with a fine paint brush to either the upper rim of the fruit or to the adjoining foliage. Five neonates were placed on each treatment replicate. Cups were kept in a room at 25 ^{o}C and fruits were inspected for codling moth injury after 14 d.

Studies were conducted with fruit and adjoining foliage. Apples were washed in bleach and then rinsed in water prior to the assays. Shoots were collected from an unsprayed ‘Fuji’ block. Shoots were placed in a plastic cup of water and a single fruit was placed on the lid of the cup touching the foliage. Apples were dipped and foliage was sprayed with each of the three treatments: water alone, insecticide alone and insecticide plus MIB (2 qts per 100 GPA). Twenty replicates were conducted with Altacor (4 oz rate per 100 GPA) and 10 replicates with Entrust (6 oz rate per 100 GPA). Insectary-reared codling moth larvae were placed with a fine paint brush to either the upper rim of the fruit or to the adjoining foliage. Five neonates were placed on each treatment replicate. Cups were kept in a room at 25 ^{o}C and fruits were inspected for codling moth injury after 14 d.

Laboratory studies were conducted with the granulosis virus of codling moth (CpGV) and the additions of several yeasts. Apples were dipped in various 500 ml solutions including: water alone, Cyd-X (3 oz per 100 gallons), and Cyd-X with 1 lb white cane sugar per 100 gallon, plus either RedStar cake yeast (*Saccharomyces cerevisiae*), at 1 lb or dried yeast formulations (0.3 lb rate) of RedStar, *Cryptococcus tephrensis*, or *Metschnikowia pulcherrima*. The latter two yeasts were provided by Dr. Peter Witzgall (Alnarp, Sweden) and were originally collected by our laboratory from codling moth larvae. Five fruits were considered to be a replicate and 15-46 replicates were conducted with each treatment. Apples were allowed to dry and were then placed in a plastic cup with a lid. Five insectary-reared codling moth neonate larvae were placed with a fine paint brush to the upper rim of each fruit. Cups were kept in a room at 25 ^{o}C and fruits were inspected for codling moth injury after 14 d. Injury was scored as shallow ‘stings’ where larvae were dead and ‘entries’ where larvae reached the seed cavity of the fruit and were alive.

Field Trials 2014: Studies were conducted at the USDA Farm with both Altacor and Entrust applied to apple and pears. Both insecticides were applied in different areas of the Bartlett orchard. Altacor was tested on Golden Delicious and Entrust was tested on Red Delicious. Seven treatments were evaluated in each of these three studies: water only, insecticide alone, insecticide with DAMEC or MIB added, insecticide with both MIB and DAMEC added, and DAMEC and MIB alone. Seven replicates were included in the Entrust pear study but only 5 replicates were useable in the pear Altacor study due to low crop load in the block. Treatments in the two apple studies had either 8 or 9 replicates. All replicates were assigned randomly as single trees in the orchard and were flagged. Altacor was always sprayed first and Entrust was applied one day later. Pears were sprayed with Altacor on 4 and 19 June, 7 and 22 July and 8 August. Reds were sprayed first on 2 and 18 June, 1, 17, and 31 July, 15 August, and 4 September, and Goldens were sprayed one day later. All trees were sprayed with ½ gallon using a handgun sprayer at 100 psi and equipped with a D5 nozzle. Pears were sampled by inspecting all fruit per tree on 25

August. Apples were sampled during three periods including late June, mid-August, and mid-September. The first two apple samples were intended to thin the crop load over the entire tree and the number of fruit sampled varied from 30 – 125. The September sample removed and inspected 50 apples from each tree from throughout the canopy. The proportion of fruit injured by codling moth was subjected to an angular transformation and differences among treatments were tested with ANOVA. Means were separated at $P < 0.05$.

RESULTS

Laboratory bioassays. No significant differences were found for water or the six insecticides tested when MIB was added (Table 1). In fact, no clear pattern could be seen to discern if the addition of MIB improved or reduced the efficacy of insecticides in these bioassays. In comparison, the addition of BY/S in general increased the proportion of uninjured fruits except when added to water (Table 2). With only water the addition of BY/S clearly improved the ability of codling moth larvae to injure fruits. The addition of BY/S to the diamides and spinosyns resulted in some significant improvements, such as reduced proportions of 'stings', total injury, and increased proportion of uninjured fruits (Table 2).

A considerable drop in the proportion of larvae causing fruit injury was found in the water controls when larvae were placed off of the fruit (Fig. 1). This was not seen when either Entrust or Altacor were applied alone. However, when MIB was added to either insecticide we found a large drop in the success of larvae being able to injure the fruit (Fig. 1).

Significant differences in efficacy were found among yeasts added to CpGV (Table 3). In general most of the fruit injuries in the water control were classified as 'entries. In comparison, the great majority of injuries when the virus was used were classified as 'stings'. Significant differences were found among treatments for the proportion of larvae causing 'entries' and were still alive after 14 days. The two dry yeast formulations from Europe had significantly fewer 'entries' than Cyd-X alone, but did not differ. Results with *S. cerevisiae* differed in relation to their efficacy with Cyd-X alone. The wet formulation reduced the proportion of entries similarly to the European dry yeasts. The dry formulation of *S. cerevisiae* had an intermediate response among treatments (Table 2).

Field trials. The trend was similar in both apples and pears with the addition of DAMEC reducing fruit injury with the use of Entrust by 30-60% in the various samples (Table 4). The addition of DAMEC appeared to improve Altacor only slightly in apple and no injury was found with DAMEC added to Altacor in the pear study. The addition of MIB appeared to improve Entrust in both apple and pear. However, the benefit in apple was only seen early in the season (late June). No benefit was found by combining the two behavior modifying chemicals in either crop.

DISCUSSION

Progress has been achieved during this two year project to evaluate the potential use of attractants to improve codling moth management. Field data has suggested that pear ester (DAMEC) can be added to improve some insecticides used in pear. DAMEC was registered in 2014 and was used by growers to target problem areas in orchards and also periods of peak larval densities. Pear ester affects larval host searching behaviors and has been shown to cause female moths to lay eggs farther from pear fruits requiring larvae to traverse longer routes across insecticide residues. MIB is an unstable sugary formulation that is likely a feeding stimulant for codling moth. We found that larvae placed directly on fruits dipped with MIB and insecticides were likely to damage the fruits despite the toxicity of the insecticide. However, MIB appeared to have some effectiveness early in the season when more eggs are laid on foliage than fruits. Laboratory bioassays showed that MIB worked much better when larvae were placed on adjoining foliage. This suggests that MIB could be used during the first half of the season. Interestingly, we did not see any benefit from combining MIB and PE.

Dry formulations of yeasts were prepared from the yeasts isolated from codling moth larvae in Washington and these appeared to further improve the efficacy of adding yeast and sugar to CpGV. Our laboratory assays when larvae are placed on the fruit show that CpGV is not able to prevent shallow stings though larvae soon die. Our results support previous lab and field trials showing that the yeast and sugar can significantly increase the kill of codling moth larvae which would be effective in dampening the population growth of codling moth in orchards between generations and years. Unlike in apples, ‘stings’ on pears early in the season is not likely a problem for growers. The availability of dry formulations of yeasts is a big improvement in terms of transport and storage of yeasts for pest control. Our data have been shared with a few manufacturers of either microbial insecticides or fermentation yeasts to evaluate whether there is any commercial interest. I believe there is interest and of course money is always the issue to develop new products. Certis provided free production of a wet formulation of the *Cryptococcus* yeast and the dry yeasts were produced for free by a facility in Sweden. It is not clear whether we can go forward with the use of these specialized yeasts without some level of commercial support. It is more likely that we will need to continue to use the available formulations of *Saccharomyces* yeast.

It is important to consider what has not yet been done in this project. After two years we have not gotten to the larger grower trials that were our third objective. Prior to this milestone we still need further laboratory and field research. In the laboratory we need to conduct more studies with the dry yeast with both virus and synthetic insecticides with larvae placed on the fruit versus on adjoining foliage. This can help show whether use of the additives should be restricted to the first generation when eggs are mostly laid on foliage or over the entire season. In the field, we should conduct another season with the dry yeasts for both CpGV and selected insecticides.

Table 1. Laboratory fruit-dip bioassays with water and six insecticides alone or with MIB.

Treatment [a]	No. reps	Mean (SE) proportion larvae causing fruit injury: Stings	Entries	Total	Mean (SE) proportion clean fruit
Water	18	0.007 (0.005)	0.451 (0.029)	0.458 (0.030)	0.089 (0.024)
Water + MIB	6	0.000 (0.000)	0.367 (0.045)	0.367 (0.045)	0.200 (0.089)
	ANOVA	$F_{1, 22} = 0.66$	$F_{1, 22} = 2.22$	$F_{1, 22} = 2.43$	$F_{1, 22} = 2.77$
		$P = 0.42$	$P = 0.15$	$P = 0.14$	$P = 0.11$
Altacor	18	0.033 (0.010)	0.038 (0.011)	0.071 (0.016)	0.700 (0.057)
Altacor + MIB	18	0.042 (0.011)	0.064 (0.013)	0.107 (0.020)	0.600 (0.067)
	ANOVA	$F_{1, 34} = 0.30$	$F_{1, 34} = 2.12$	$F_{1, 34} = 1.06$	$F_{1, 34} = 1.25$
		$P = 0.59$	$P = 0.15$	$P = 0.31$	$P = 0.27$
Entrust	8	0.095 (0.043)	0.110 (0.026)	0.205 (0.037)	0.375 (0.096)
Entrust + MIB	10	0.104 (0.036)	0.092 (0.022)	0.196 (0.029)	0.340 (0.060)
	ANOVA	$F_{1, 16} = 0.01$	$F_{1, 16} = 0.18$	$F_{1, 16} = 0.05$	$F_{1, 16} = 0.06$
		$P = 0.91$	$P = 0.68$	$P = 0.82$	$P = 0.80$
Exirel	6	0.053 (0.013)	0.020 (0.009)	0.073 (0.012)	0.633 (0.062)
Exirel + MIB	6	0.053 (0.020)	0.073 (0.019)	0.127 (0.033)	0.533 (0.099)
	ANOVA	$F_{1, 10} = 0.08$	$F_{1, 10} = 4.29$	$F_{1, 10} = 0.61$	$F_{1, 10} = 0.60$
		$P = 0.78$	$P = 0.07$	$P = 0.45$	$P = 0.46$
Delegate	3	0.040 (0.000)	0.000 (0.000)	0.004 (0.000)	0.800 (0.000)
Delegate + MIB	3	0.013 (0.013)	0.000 (0.000)	0.013 (0.013)	0.933 (0.067)
	ANOVA	$F_{1, 4} = 4.00$	-	$F_{1, 4} = 4.00$	$F_{1, 4} = 4.00$
		$P = 0.12$		$P = 0.12$	$P = 0.12$
Intrepid	3	0.120 (0.023)	0.120 (0.046)	0.240 (0.040)	0.133 (0.067)
Intrepid + MIB	3	0.107 (0.071)	0.107 (0.013)	0.213 (0.074)	0.400 (0.116)
	ANOVA	$F_{1, 4} = 0.31$	$F_{1, 4} = 0.01$	$F_{1, 4} = 0.16$	$F_{1, 4} = 4.08$
		$P = 0.61$	$P = 0.92$	$P = 0.71$	$P = 0.11$
Assail	3	0.013 (0.013)	0.133 (0.013)	0.147 (0.013)	0.600 (0.000)
Assail + MIB	3	0.013 (0.013)	0.107 (0.035)	0.120 (0.023)	0.467 (0.133)
	ANOVA	$F_{1, 4} = 0.00$	$F_{1, 4} = 0.60$	$F_{1, 4} = 1.04$	$F_{1, 4} = 1.00$
		$P = 1.00$	$P = 0.48$	$P = 0.37$	$P = 0.37$

[a] Insecticides were used at 1% of their recommended field rate for codling moth. Bread yeast and sugar were formulated at a 3 and 1 lb per 100 gallon rate, respectively.

Table 2. Laboratory fruit-dip bioassays with water and six insecticides alone or with bread yeast and sugar (BY/S).

Treatment [a]	No. reps	Mean (SE) proportion larvae causing fruit injury: Stings	Entries	Total	Mean (SE) proportion clean fruit
Water	37	0.008 (0.003)	0.370 (0.029)	0.377 (0.029)	0.103 (0.023)
Water + BY/S	16	0.013 (0.005)	0.543 (0.030)	0.555 (0.031)	0.000 (0.000)
	ANOVA	$F_{1, 51} = 1.18$	$F_{1, 51} = 11.90$	$F_{1, 51} = 12.33$	$F_{1, 51} = 8.88$
		$P = 0.28$	**$P < 0.01$**	**$P < 0.001$**	**$P < 0.01$**
Altacor	21	0.067 (0.016)	0.057 (0.011)	0.124 (0.019)	0.533 (0.059)
Altacor + BY/S	21	0.040 (0.014)	0.050 (0.011)	0.090 (0.016)	0.648 (0.058)
	ANOVA	$F_{1, 40} = 3.32$	$F_{1, 40} = 0.12$	$F_{1, 40} = 1.55$	$F_{1, 40} = 1.81$
		$P = 0.08$	$P = 0.73$	$P = 0.22$	$P = 0.19$
Entrust	17	0.071 (0.017)	0.108 (0.020)	0.179 (0.027)	0.377 (0.071)
Entrust + BY/S	21	0.057 (0.013)	0.078 (0.018)	0.135 (0.018)	0.495 (0.051)
	ANOVA	$F_{1, 36} = 0.23$	$F_{1, 36} = 1.02$	$F_{1, 36} = 1.35$	$F_{1, 36} = 2.07$
		$P = 0.63$	$P = 0.32$	$P = 0.25$	$P = 0.16$
Exirel	16	0.023 (0.007)	0.080 (0.019)	0.103 (0.021)	0.638 (0.058)
Exirel + BY/S	20	0.006 (0.003)	0.066 (0.018)	0.072 (0.018)	0.700 (0.057)
	ANOVA	$F_{1, 34} = 4.52$	$F_{1, 34} = 0.62$	$F_{1, 34} = 1.47$	$F_{1, 34} = 0.55$
		$P < 0.05$	$P = 0.44$	$P = 0.23$	$P = 0.46$
Delegate	14	0.040 (0.008)	0.006 (0.004)	0.046 (0.008)	0.771 (0.041)
Delegate + BY/S	15	0.013 (0.006)	0.003 (0.003)	0.016 (0.007)	0.920 (0.038)
	ANOVA	$F_{1, 27} = 7.08$	$F_{1, 27} = 0.43$	$F_{1, 27} = 8.84$	$F_{1, 27} = 7.24$
		$P < 0.05$	$P = 0.52$	**$P < 0.01$**	**$P < 0.05$**
Intrepid	6	0.140 (0.040)	0.020 (0.014)	0.160 (0.036)	0.367 (0.131)
Intrepid + BY/S	6	0.093 (0.017)	0.027 (0.020)	0.120 (0.023)	0.433 (0.101)
	ANOVA	$F_{1, 10} = 0.93$	$F_{1, 10} = 0.02$	$F_{1, 10} = 0.69$	$F_{1, 10} = 0.17$
		$P = 0.36$	$P = 0.89$	$P = 0.43$	$P = 0.69$
Assail	17	0.019 (0.006)	0.240 (0.026)	0.259 (0.027)	0.200 (0.045)
Assail + BY/S	14	0.031 (0.010)	0.180 (0.032)	0.211 (0.031)	0.300 (0.066)
	ANOVA	$F_{1, 29} = 0.66$	$F_{1, 29} = 2.47$	$F_{1, 29} = 1.40$	$F_{1, 29} = 1.54$
		$P = 0.42$	$P = 0.13$	$P = 0.25$	$P = 0.22$

[a] Insecticides were used at 1% of their recommended field rate for codling moth. Bread yeast and sugar were formulated at a 3 and 1 lb per 100 gallon rate, respectively.

Table 3. Laboratory apple fruit-dip bioassays with CpGV (Cyd-X) plus the yeasts; *Saccharomyces cerevisiae (Sc), Cryptococcus tephrensis (Ct)*, and *Metschnikowia pulcherrima (Mp)* plus sugar.

Treatment	No. reps	Mean (SE) proportion larvae causing fruit injury: Stings (dead)	Entries (live)	Total	Mean (SE) proportion clean fruit
Water	23	0.052 (0.023)b	0.454 (0.037)a	0.497 (0.031)a	0.052 (0.023)
Cyd-X	32	0.308 (0.021)a	0.058 (0.007)b	0.365 (0.022)b	0.081 (0.022)
Cyd-X + S	10	0.392 (0.042)a	0.024 (0.014)bc	0.416 (0.044)ab	0.100 (0.033)
Cyd-X + *Sc*/S dry yeast	20	0.372 (0.024)a	0.024 (0.009)bc	0.396 (0.026)ab	0.100 (0.027)
Cyd-X + *Sc*/S cake yeast	23	0.383 (0.027)a	0.014 (0.006)c	0.397 (0.025)ab	0.130 (0.032)
Cyd-X + *Ct*/S	54	0.374 (0.014)a	0.013 (0.003)c	0.387 (0.014)b	0.089 (0.019)
Cyd-X + *Mp*/S	42	0.369 (0.019)a	0.011 (0.003)c	0.380 (0.020)b	0.076 (0.024)
ANOVA [a] df = 6, 197		$F = 14.04$ **$P < 0.0001$**	$F = 29.20$ **$P < 0.0001$**	$F = 3.08$ **$P < 0.01$**	$F = 1.07$ $P = 0.38$

[a] Data for the proportion of stings, entries, and clean fruit could not be normalized and were analyzed with a non-parametric Kruskal-Wallis ANOVA of ranks. Data for the total injury were normalized and a one-way ANOVA was used

Table 4. Summary of the effect of adding DA-MEC to the insecticides Altacor or Entrust in apple and pear, 2014.

	Mean (SE) percentage of fruit injury		
Crop - Treatment	June	August	September
Gold Apples – UTC	15.2 (2.3)a	50.2 (9.9)a	79.0 (4.2)a
Altacor	3.8 (1.0)c	3.1 (0.9)b	3.3 (1.1)c
Altacor + DAMEC	3.1 (1.0)c	3.4 (1.2)b	2.7 (0.7)c
Altacor + MIB	5.0 (1.0)bc	0.9 (0.3)b	2.9 (0.9)c
Altacor + Both	4.2 (1.1)c	3.9 (0.4)b	3.8 (1.0)c
DAMEC alone	13.2 (2.2)ab	30.1 (3.5)a	51.1 (4.5)b
MIB alone	15.2 (3.1)a	31.5 (5.1)a	44.2 (3.0)b
	$F_{6, 53} = 8.45$	$F_{6, 41} = 30.89$	$F_{6, 53} = 95.75$
ANOVAs	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Red Apples – UTC	16.3 (4.0)a	77.9 (6.2)a	81.8 (3.9)a
Entrust	6.3 (2.4)abc	9.1 (3.0)b	18.9 (2.8)c
Entrust + DAMEC	4.3 (1.9)bc	5.3 (2.0)b	10.4 (1.1)c
Entrust + MIB	1.7 (0.8)c	7.3 (1.4)b	18.4 (1.6)c
Entrust + Both	4.0 (1.2)abc	13.6 (4.0)b	20.3 (4.0)c
DAMEC alone	9.8 (2.3)ab	59.7 (10.0)a	71.3 (5.1)ab
MIB alone	8.4 (2.1)abc	59.9 (7.0)a	60.9 (4.4)b
	$F_{6, 54} = 4.47$	$F_{6, 53} = 27.28$	$F_{6, 53} = 55.79$
ANOVAs	$P < 0.001$	$P < 0.0001$	$P < 0.0001$
Pears – UTC	-	22.3 (3.9)a	
Altacor	-	0.4 (0.4)b	-
Altacor + DAMEC	-	0.0 (0.0)b	-
Altacor + MIB	-	1.2 (0.9)ab	-
Altacor + Both	-	0.5 (0.3)b	-
DAMEC alone	-	9.5 (2.0)ab	-
MIB alone	-	5.0 (2.5)ab	-
	-	$F_{6,28} = 14.51$	-
ANOVA		$P < 0.0001$	
Pears – UTC	-	24.6 (4.5)a	-
Entrust	-	2.8 (1.2)ab	-
Entrust + DAMEC	-	0.9 (0.6)b	-
Entrust + MIB	-	1.3 (0.7)b	-
Entrust + Both	-	1.3 (0.6)b	-
DAMEC alone	-	22.8 (4.5)a	-
MIB alone	-	27.1 (4.9)	-
ANOVA	-	$F_{6, 42} = 24.26$ $P < 0.0001$	-

Figure 1. Comparison of larval success when larvae were placed initially on fruit versus surrounding foliage