Effect of Insecticides, Fungicides and Combinations Applied to Almonds During Bloom on Survival of Honey Bee Larvae

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Introduction

- Almond orchards in California's Central Valley are also home to nearly 2 million honey bee colonies during bloom
- Almond flowers are a rich foraging resource for bees, but are commonly treated with fungicides that may be tank-mixed with insecticides
- Pollinating beekeepers have reported observing dead adult bees and dead and malformed immature worker bees in 80,000 colonies during and after almond bloom (Flottum, 2014)
- To determine the role that almond pesticides and combinations play in the observed bee kills we sought to answer these questions:
  1. **What pesticides and pesticide combinations are bees most likely to be exposed to during almond bloom?**
  2. **What effect does exposure to common insecticides, fungicides or tank mix combinations have on larval honey bee development?**

Methods: Combinations During Bloom

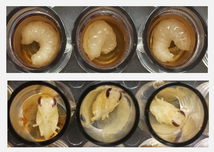
1. Pesticide use data for almonds were downloaded from the California Pesticide Information Portal (http://calpip.cdpr.ca.gov/main.cfm) for the years 2007-2015
2. Insecticide and fungicide application data were summarized for the blooming period, defined as February 15 – March 15, using the statistical package R (**Figure 1A and 1B**)
3. Tank mix events were identified when pesticide use was reported for the same section (COMTRS) applied to the same number of acres and on the same date **(Figure 1C)**
4. Ratios of combinations of pesticides fed to larval bees were calculated based on the ratio of the maximum label rates for both active ingredients (**Table 1**)

	Fungicide	boscalid : pyraclostrobin (Pristine)	iprodione (Rovral)	propiconazole (Tilt)
Insecticide	**µg/larva**	4.68 : 2.37	5.04	2.24
chlorantraniliprole (Altacor)	1.02	1 : 4.6 : 2.3	1 : 4.9	1 : 2.2
diflubenzuron (Dimilin)	2.28	1 : 2 : 1	1 : 2.2	1 : 1
methoxyfenozide (Intrepid)	2.25	1 : 2 : 1	1 : 2.1	1 : 1

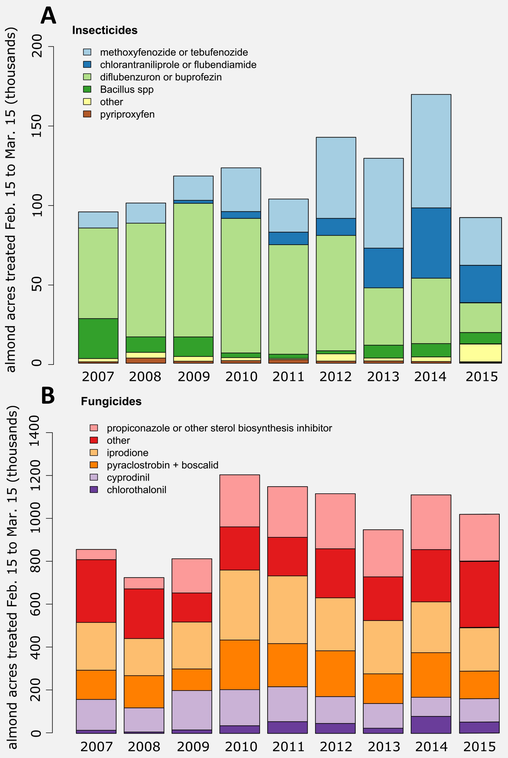
Table 1. Dosage and ratios (highlighted) of technical grade insecticide : fungicide active ingredients fed to larvae in a single dose in formulated royal-jelly diet.

Methods:

- Worker bee larvae were reared *in vitro* and fed with royal jelly-sugar-yeast extract diets following protocols by Schmehl et al. (2016)
- Newly hatched, ~24 hr old larvae produced by the same queens were grafted into individual cells of 48-well culture plates



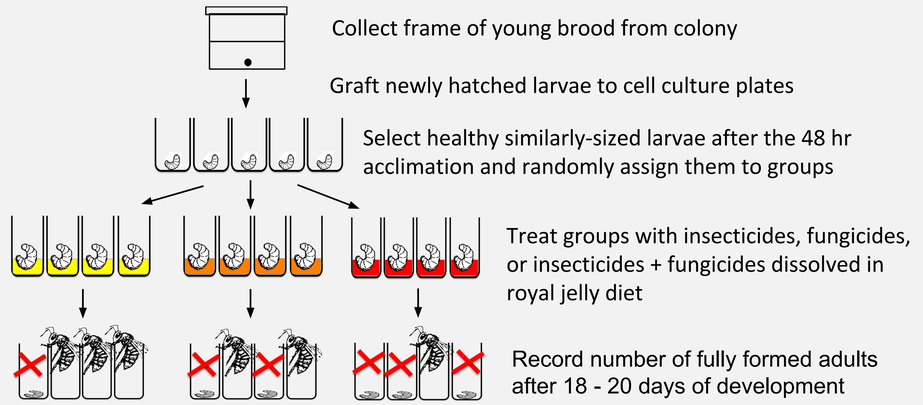
Developing honey bee larvae (top) and pupae (bottom) in cell culture plates.



Active ingredient	Trade Name	Mode of Action	Group	
Diflubenzuron	Dimilin 2L	Inhibitor of chitin synthesis	15	Insecticides
Buprofezin	Tourismo	Inhibitor of chitin synthesis	15	
Pyriproxyfen	Seize 35 WP	Juvenile hormone mimic	7	
Methoxyfenozide	Intrepid 2F	Ecdysone receptor agonist	18	
Flubendiamide	Tourismo	Ryanodine receptor modulator	28	
Chlorantraniliprole	Altacor	Ryanodine receptor modulator	28	
Iprodione	Rovral 50 SP	MAP/Histidine-Kinase osmotic signal transduction	2	Fungicides
Propiconazole	Tilt	Sterol biosynthesis inhibitor	3	
Fenbuconazole	Indar 2F	Sterol biosynthesis inhibitor	3	
Metconazole	Quash	Sterol biosynthesis inhibitor	3	
Tebuconazole	Tebuzol	Sterol biosynthesis inhibitor	3	
Pyraclostrobin + Boscalid	Pristine	Inhibitor of cellular respiration	7 / 11	
Cyprodinil	Vangard WG	Methionine biosynthesis	9	

Figure 1. Area to which insecticides (A), fungicicides (B), or the ryanodine receptor modulators (C) mixed with fungicides were applied to almonds in California during the blooming period.

- After 72 h acclimation, groups of 16 healthy larvae were selected to receive treatments
- On the 4th day after grafting, each larva was fed with 30 µl of diet contaminated with technical grade insecticide and/or fungicide active ingredients dissolved in acetone. Diets with 2% acetone were used as the negative control and dimethoate (5.1 µg/larva) as the positive control
- The percentage of individuals developing into adults was recorded
- 12 replicates were performed for each insecticide, fungicide or combination



Results:

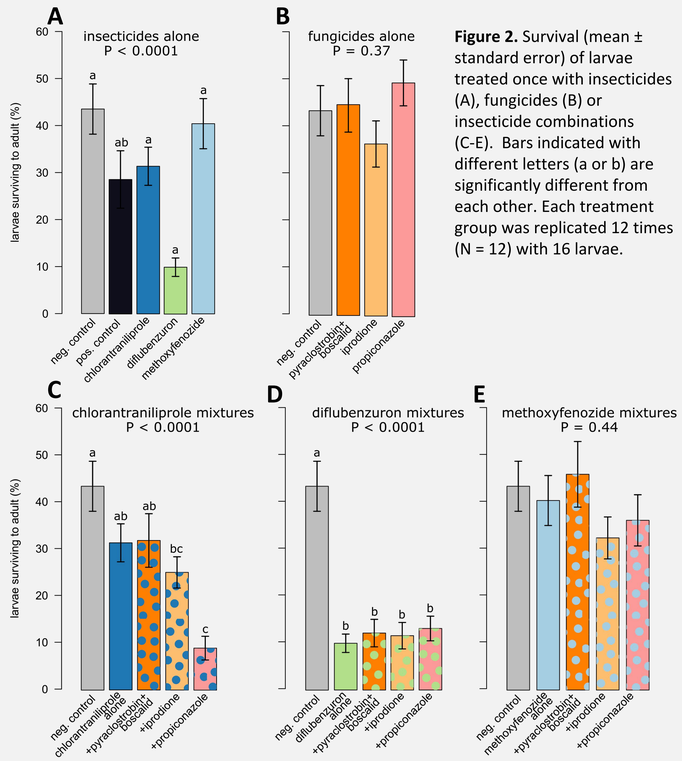


Figure 2. Survival (mean ± standard error) of larvae treated once with insecticides (A), fungicides (B) or insecticide combinations (C-E). Bars indicated with different letters (a or b) are significantly different from each other. Each treatment group was replicated 12 times (N = 12) with 16 larvae.

Conclusions

- Application of fungicides during bloom is widespread, but application of insecticides occurred on only 178,367 acres in 2014 and dropped by nearly half in 2015 to 90,706 acres
- The active ingredients in the fungicides Pristine, Rovral and Tilt showed no significant effect on survival of honey bee larvae **(Figure 2B)**. However, the fungicides propiconazole and iprodione significantly increased the toxicity of the insecticide chlorantraniliprole when applied a tank-mix ratio **(Figure 2C)**
- Diflubenzuron reduces larval survival whether applied alone or in combination with fungicides **(Figure 2D)**
- In 2015 approximately 50,000 honey bee colonies placed on 26,551 acres of almonds were likely exposed to pesticides that were found to affect larval development in this study
- Future work will focus on generating dose response curves and testing the effect of spray adjuvants on pesticide toxicity to larvae

Acknowledgements

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