Effect of Almond Insecticides, Fungicides, and Phytochemicals on Honey Bees

Project No.: **POLL18.Johnson**

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A. Summary

Beekeepers providing honey bees to pollinate almonds continue to experience unacceptable losses of adult and developing bees, sometimes resulting in colony failure. Many beekeepers attribute these losses to exposure to insecticides, fungicides, spray adjuvants and combinations of all three, that are applied during bloom. Results indicate that particular combinations of fungicides and insecticides, particularly Altacor and Tilt, have the potential to kill adult bees when sprayed directly and larvae when fed contaminated diet. Addition of the adjuvant Dyne-Amic makes this combination more toxic, causing adult mortality at levels below the maximum label rate. Dyne-Amic also increased the toxicity of other fungicides and insecticides to adult bees. Queens reared in the presence of the Altacor-Tilt-Dyne-Amic combination show reduced survival 6 weeks following adult emergence. The effects observed may explain some of the problems beekeepers are experiencing during and after almond bloom and provide support for recommendations made in the “Honey Bee Best Management Practices” to avoid insecticide and adjuvant use during bloom when bees are present.

B. Objectives

1. Test the potential for six fungicides, three insecticides and four spray adjuvants when applied alone, in two-way or three-way combinations (139 total treatments) to affect:
    a. Adult worker honey bee survival when sprayed with a formulated product or combinations of formulated products from 0.1X to 30X the maximum labeled application rate using a Potter spray tower
    Progress: Insecticides (Intrepid and Altacor) and fungicides (Pristine, Tilt, Luna Sensation and Vangard) tested with adjuvant Dyne-Amic in 2019 with noted increase in toxicity of some pesticides in the presence of the adjuvant. Testing with other adjuvant chemistries will proceed in 2020 to identify adjuvant components of concern.

    b. Larval worker honey bee survival when the constituents are applied alone or combined in field-relevant ratios at maximum sublethal concentrations for individual constituents
    Progress: Insecticides and fungicides were fed in diet to larvae reared in vitro in 2019. Pupal mortality was unacceptably high and trials will be repeated in 2020.

2. Using a semi-field queen rearing assay to determine whether a limited subset of insecticides, fungicides, adjuvants or their combinations can affect:
    a. Queen honey bee development and survival
    Progress: Trials feeding queen-rearing boxes with pollen contaminated with Intrepid, Altacor, Tilt and Pristine, in combination with Dyne-Amic, were performed in 2019. Additional replicates are needed in 2020.

    b. Translocation of active ingredients from pollen to nurse bees and in royal jelly for use in quantitative risk assessment
    Progress: Pollen, nurse bee and royal jelly samples have been submitted for pesticide residue analysis to determine the rate of translocation

C. Annual Results and Discussion

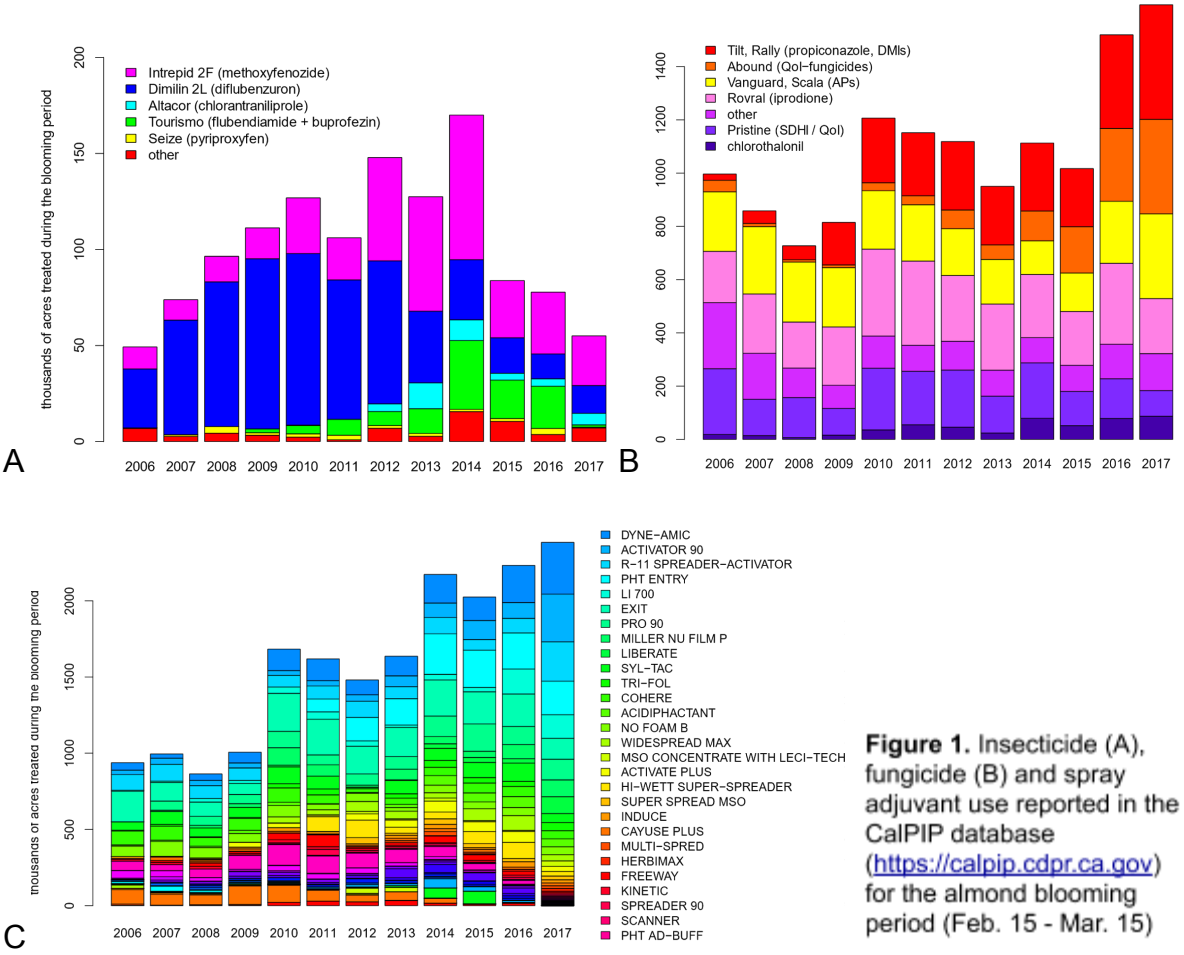

Figure 1. Insecticide (A), fungicide (B) and spray adjuvant use reported in the CalPIP database (https://calpip.cdpr.ca.gov) for the almond blooming period (Feb. 15 - Mar. 15)

Pesticides and adjuvants to which honey bees are most likely to be exposed alone, and in combination, were identified using data downloaded from the California Department of Pesticide Regulation's Pesticide Information Portal (**Figure 1**). These data, combined with

conversations with stakeholders were used to identify pesticides of current and future concern regarding bee health (insecticides Altacor and Intrepid; fungicides Tilt, Pristine, Luna Sensation and Vangard). The spray adjuvant Dyne-Amic was the most widely used, applied to ~200,000 acres in 2017, and was chosen as the representative adjuvant for combination testing.

Objective 1a: Effect of simulated field-rate spray application of insecticides, fungicides, and a spray adjuvant on adult honey bee workers

None of the formulated insecticides or fungicides, when sprayed on bees alone, resulted in elevated mortality at up to 30X the maximum label rate for almonds (**Table 1**). The toxic standard, Mustang Maxx, which is known to be harmful to bees, did cause a significant increase in mortality at the maximum label rate. The only insecticide-fungicide combination to cause significantly elevated mortality is the Altacor-Tilt combination, which is also known to be toxic to larvae (Wade et al. 2019).

	insecticide alone	Pristine (boscalid + pyraclostrobin)	Vanguard (cyprodinil)	Luna Sensation (fluopyram + trifloxystrobin)	Tilt (propiconazole)
with no spray adjuvant					
fungicide alone		> 30 X N=577	> 30 X N=400	> 30 X N=300	> 30 X N=1238
Altacor (chlorantraniliprole)	> 30 X N=1311	> 30 X N=760	> 30 X N=300	> 30 X N=296	10 X N=1575
Intrepid (methoxyfenozide)	> 30 X N=659	> 30 X N=339	> 30 X N=300	> 30 X N=300	> 30 X N=379
Mustang Maxx (zeta- cypermethrin)	1 X N=642				
with 2% Dyne-Amic spray adjuvant					
fungicide alone		30 X N=280	1 X N=499	> 30 X N=279	> 30 X N=440
Altacor (chlorantraniliprole)	1 X N=395	1 X N=600	1 X N=498	10 X N=293	0.1 X N=922
Intrepid (methoxyfenozide)	> 30 X N=201	10 X N=280	> 30 X N=299	10 X N=301	10 X N=295

Table 1. Multiples of the field application rate for insecticides, fungicides and combinations with and without addition of Dyne-Amic spray adjuvant causing a significant increase in adult bee mortality 48 h. relative to control after spray application (Fisher's Exact Test, $p < 0.05$ with Bonferroni correction for 157 comparisons). All combinations were tested at 1, 3, 10, and 30 X the field rate. All tests were repeated at least 3 times. The total number of bees tested (N) among all treatment levels is reported.

The addition of the adjuvant Dyne-Amic at 2% in the spray mixture resulted in significantly increased mortality of bees when combined with only Vanguard or only Altacor. All combinations involving the insecticide Altacor in the presence of Dyne-Amic resulted in

elevated bee mortality. The combination of Altacor, Tilt and Dyne-Amic cause elevated bee mortality at 1/10 the field application rate. Combinations involving the insecticid Intrepid, which were not previously toxic, became somewhat toxic in the presence of the adjuvant.

These data indicate that adjuvants, in combination with commonly used insecticides and fungicides, may be responsible for adult bee kill events reported by some beekeepers. They also strongly support the recommendation in the Almond Board's Bee BMPs to avoid using adjuvants during bloom. Work in 2020 will test the potential of different adjuvants, containing different active ingredients, to increase pesticide toxicity to bees with the goal of identifying an adjuvant class (e.g. organosilicones) with high synergistic potential.

Objective 1b: Determine the effect of insecticides, fungicides, and combinations on larval honey bees during development.

	insecticide alone	**Pristine (2.09 µg boscalid + 1.06 µg pyraclostrobin)**	**Vangard (4.28 µg cyprodinil)**	**LunaSensation (1.14 µg fluopyram + 1.14 µg trifloxystrobin)**	**Tilt (0.90 µg propiconazole)**	**Rovral (4.56 µg iprodione)**
larval mortality with no spray adjuvant						
fungicide alone		34% (96)	30% (86)	10% (79)	29% (170)	18% (96)
Altacor (2.05 µg chlorantraniliprole)	16% (64)	31% (16)	31% (32)	44% (16)	55% (64)	28% (32)
Intrepid (2.28 µg methoxyfenozide)	29% (143)	27% (128)	44% (45)	22% (63)	41% (95)	24% (46)
Dimilin (2.28 µg diflubenzuron)	29% (112)	31% (16)	69% (16)	56% (16)	66% (47)	
5.1 µg dimethoate (toxic standard)	58% (240)					
solvent control	22% (346)					
larval mortality with 0.33% Dyne-Amic in larval diet						
fungicide alone		37% (95)	22% (67)	27% (94)	31% (172)	32% (96)
Altacor (2.05 µg chlorantraniliprole)	24% (96)	56% (16)	44% (32)	56% (32)	70% (64)	75% (32)
Intrepid (2.28 µg methoxyfenozide)	31% (128)	30% (128)	10% (48)	30% (64)	28% (80)	19% (64)
Dimilin (2.28 µg diflubenzuron)	63% (110)	61% (31)	56% (16)	56% (16)	50% (48)	
5.1 µg dimethoate (toxic standard)	72% (64)					
solvent control	34% (207)					

Table 2. Percent of *in vitro* reared larvae dying prior to pupation. Total number of larvae treated are in parentheses.

Larvae were reared *in vitro* and fed pesticide combinations with or without addition of Dyne-Amic to the larval diet. Overall, mortality of larvae in control treatments was unacceptably high so results should be interpreted with caution (**Table 2**). However, dimethoate, the toxic standard, did kill more larvae than the negative control treatment. The combination of chlorantraniliprole, iprodione and Dyne-Amic resulted in the highest level of

mortality observed (75%). While initial results are suggestive, further trials, with improved overall survival, will be conducted to determine if the addition of a spray adjuvant does indeed increase pesticide toxicity to developing bees.

Objective 2a. Effect of selected insecticides, fungicides, adjuvants, and their combinations on the development and survival of queen honey bee

Queen honey bees were reared from 24-hr old larvae by worker bees provisioned with pesticide-free pollen or pollen artificially contaminated with formulated insecticides, fungicides, or spray adjuvants. Three replicated trials were completed in 2019 to evaluate the effect of Altacor, applied alone or in combinations with Tilt, Dyne-Amic or Tilt and Dyne-Amic at maximum labeled application rates on queen development. A subset of newly emerged queens were installed separately in queenless colonies and followed for 6 weeks, the typical timeframe for a virgin queen to mate and produce a new cohort of adult worker bees. All queens survived similarly to emergence as adults regardless of treatments. When pollen was contaminated with the Altacor-Tilt or Altcarot-Tilt plus Dyne-Amic combinations, fewer adult queens survived more than 6 weeks compared to the negative control (**Figure 2**). Brood frames produced by queens surviving to the end of the trials were photographed at 24-hr intervals for 72 hours. These photos will be processed with automated image analysis using the program MIPAR to determine egg-laying rates as a measure of queen productivity. Egg-laying data will be used as an input for pesticide risk assessment in BeePOP.

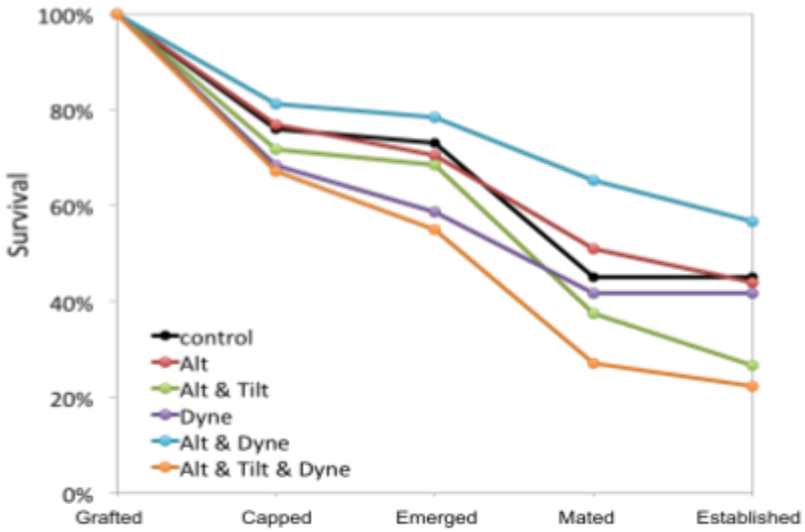

Figure 2. Mean percentages of queen larvae surviving to cell capping, adult emergence, mating, and colony establishment (producing a new cohort of worker bees). The percentages were calculated based on 30 grafted larvae per treatment in three replicated trials (90 larvae total per treatment).

Two replicated queen-rearing trials were performed with pesticide-free pollen (negative control) or pollen artificially contaminated with Intrepid, Pristine, or a combination of Intrepid and Pristine at maximum application rates according to product labels. A total of 60 larvae per treatment were grafted and followed through adult emergence. Fewer adult queens emerged with the Intrepid treatment (averaging 16%) compared to the Pristine (44%) and control (54%) treatments. Survival in the Intrepid-Pristine combination treatment was inconsistent, with 79% adult emergence in one trial and 28% in the other (54% average). Because both of the replicates were performed in late season (September), we did not tract queen survival through

mating and colony estashmiment. More replicates will be performed in 2020 to evaluate queen survival post-emergence and verify the effect of the Intrepid-Pristine combination.

Objective 2b. Translocation of pesticide active ingredients from pollen to nurse bees and in royal jelly.
Pollen, nurse bee, and royal jelly samples from this year's queen-rearing trials have been submitted to the Agriculture & Food Laboratory at the University of Guelph for pesticide residue analysis. Residue data are anticipated in February 2020.

D. **Outreach Activities**

1. Johnson, R.M. Interactions Between Insecticides and Fungicides Applied to Almonds During Bloom. Keynote presentation at the American Beekeeping Federation annual meeting, Myrtle Beach, SC. January 10, 2019. Approximately 500 beekeepers
2. Johnson, R.M. Pesticides Colloquium Series, Bee Informed Project Webinar. November 8, 2019. 15 members of the BIP tech-transfer team
3. Lin, C.-H and Johnson, R.M. Translocation of pesticides applied during almond bloom and their effects on honey bee queen development. American Bee Research Conference (in conjunction with the American Honey Producers Association Convention), Tempe, AZ. January 12, 2019. Approximately 60 beekeepers and honey bee researchers.

E. Materials and Methods:

1a. A Potter spray tower (Burkard Scientific Ltd.; **Figure 3**) was used to simulate in-field exposure of adult worker bees to a foliar spray (Potter 1952). Groups of 20 bees each received a treatment of a formulated fungicide, insecticide, or a combination, with and without a spray adjuvant, in multiples of the maximum label rate (1X, 3X, 10X, and 30X). Water was applied as a negative control and Mustang Maxx (zeta-cypermethrin) as a positive control or toxic standard. Bees were then placed in cups, fed with sugar water (1:1 w/w sucrose in water) and mortality was recorded daily for 3 days.

Figure 3. Potter spray tower for application of formulated pesticides to bees at multiples of the maximum application rate

1b. Honey bee larvae were reared using an in vitro method (Schmehl et al. 2016, Wade et al. 2019) and active ingredients and Dyne-Amic (0.25%) were added in 2% acetone to the semi-artificial diet provided on Day 3 of the feeding protocol. Larval mortality was assessed daily during feeding for larvae and after transfer of pupae to the pupation plate. Unexpectedly high pupal mortality was observed throughout the summer. Therefore additional replicates, using a fresh batch of royal jelly from an alternative supplier, will be performed in 2020.

2a & b. Queens were reared from 24-hr old larvae by approximately 1 kg (2.4 lb) of young worker bees enclosed in swarm boxes (**Figure 4**) (Spivak and Reuter 1994, Johnson and

Percel 2013). The boxes were provisioned with clean sucrose solution (50% w/w) and pollen artificially contaminated with formulated pesticides in two separate combination designs:

(a) water (negative control), Altacor alone, Altacor combined with Tilt, Dyne-Amic alone, Dyne-Amic combined with Altacor, Dyne-Amic with Altacor and Tilt

(b) water, Intrepid alone, Pristine alone, and Intrepid combined with Pristine.

These pesticides and spray adjuvant were chosen based on spray simulation and larval rearing studies previously conducted by our lab group, and were added to pesticide-free pollen at maximum application rates according to product labels. Pollen, nurse bees, and royal jelly were collected and submitted to the Agriculture & Food Laboratory (University of Guelph) for pesticide residue analysis. Capped queen cells were transferred to a healthy colony to be incubated until all adult queens emerged. Ten virgin queens per treatment from the Altacor, Tilt and DyneAmic trials were installed separately in small queenless colonies to assess queen survival to mating and colony establishment (defined as the emergence of a new adult cohort produced by the queen). After the 6-week assessment, brood frames produced by established queens were photographed at 24 hour intervals for 72 hours to determine queen egg laying rates, which will be used as an input to a version of the BeePOP colony model updated for pesticide risk assessment by USEPA (DeGrandi-Hoffman et al. 1989).

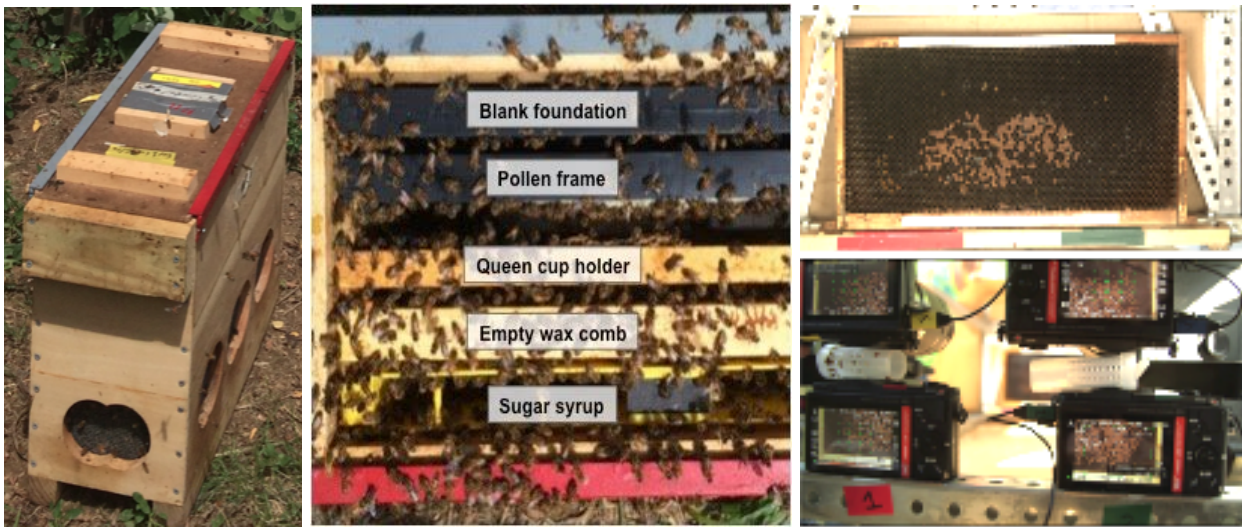

Figure 4. Images of the swarm box (left), frame arrangement in the box (center) and the camera system setup (right) to capture high-resolution images of each quarter of the frame, which can be analyzed in the laboratory for cell contents.

F. **Publications that emerged from this work**

Wade, A., C.-H. Lin, C. Kurkul, E. R. Regan, and R. M. Johnson. 2019. Combined Toxicity of Insecticides and Fungicides Applied to California Almond Orchards to Honey Bee Larvae and Adults. Insects. 10.

G. **Literature Cited**

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Johnson, R. M., and E. G. Percel. **2013**. Effect of a fungicide and spray adjuvant on queen-rearing success in honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 106: 1952–1957.

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