Effect of Application Time on Honey Bee Exposure to Fungicide

Project No.: **14-POLL16-Pettis/Johnson**

Project Leader: Dr. Jeff Pettis
Research Entomologist
USDA-ARS Bee Research Laboratory
Bldg. 306 BARC-E
10300 Baltimore Ave.
Beltsville, MD 20705 USA
301.504.7299
301.504.8736 (fax)
jeff.pettis@ars.usda.gov

Project Cooperators and Personnel:
Dr. Jody Johnson, Cullaborate
Sarah Bluher, USDA-ARS Bee Research Laboratory
Gordon Wardell, Wonderful Orchards

Objectives:

1) Determine if spraying iprodione fungicide at different times of day (AM vs. PM) leads to differences in contamination of almond pollen sampled from anthers.
2) Determine if spraying time affects exposure levels of honey bees through bee-collected pollen sampled from hive-entrance traps.
3) Assess potential repellency effects of iprodione on foraging activity following morning and evening spray.

Interpretive Summary:

Although fungicides have not been traditionally viewed as a threat to honey bee health, recent work has revealed synergistic toxic effects of field relevant combinations of insecticides and fungicides (Johnson et al 2013). Furthermore, fungicide loads in bee-collected pollen have been correlated to the prevalence of the common honey bee parasite *Nosema* (Pettis et al 2013). It is therefore important to reduce honey bee exposure to fungicides.

Our objective in this study was to determine if spraying fungicide at different times of day (AM vs. PM) would lead to differences in the exposure levels of honey bees. We hypothesized that AM spray, applied while anthers were exposed and during prime foraging hours, would result in higher concentrations of iprodione found in pollen sampled both directly from anthers and from hive entrance traps.

In order to capture potential honey bee exposure to fungicide before and after AM and PM fungicide application, we measured: (1) Forager counts of: (a) bees visiting flowers within a given area and (b) pollen-bearing bees returning to the hive. (2) Fungicide levels in: (a) pollen sampled directly from anthers and (b) pollen collected in traps at the hive.

As expected, anther pollen collected immediately following AM spray had significantly higher concentrations of iprodione than anther pollen collected the morning after PM spray. However, counter to our expectations, the concentrations detected in forager-collected pollen were significantly higher following PM spray than following AM spray. The simultaneously high loads of iprodione in anther pollen and low loads in bee-collected pollen following AM spray suggest that the difference in hive contamination stems from a difference in forager activity within the contaminated sites, rather than a difference in the levels of iprodione in available forage. Indeed, there was a decrease recorded in both pollen foragers and flower visitors from Day 1 PM spray to Day 3 AM spray.

Materials and Methods:

Set-up

Two pallets of four hives each were set up at a distance of 570m apart in the northwest and northeast corners of the almond orchard at Ranch 3470 of Wonderful Orchards in S. California (Sites 1 & 2, **Map**). Two spray zones were designated for PM and AM treatments, each consisting of ten adjacent rows of trees covering 1.5 hectares: PM Spray Zone 1 in the northwest corner and AM Spray Zone 2 in the northeast corner. Two hives on each of the pallets were used to trap pollen and three hives per pallet were used to monitor foraging activity. Anthers were sampled from clusters of trees in Zones 1 and 2. The study spanned three days. PM spray took place at 6pm on Day 1, AM spray at 11am on Day 3. Day 1 yielded pre-treatment baseline data, Day 2 post-PM treatment, Day 3 post-AM treatment.

The fungicide iprodione (Rovral 4F) was sprayed according to label instructions, applied using an air blast ground rig at a uniform rate across the treated areas. There were two separate fungicide applications, the first conducted in Zone 1 at 6pm on Day 1 of the study, the second in Zone 2 at 11am on Day 3.

Objective 1. Anther pollen

Anthers were sampled on Days 2 and 3. Each anther sampling took place over a ten-minute interval during which exposed anthers with visible pollen were clipped from trees within the spray zone of interest.

Objective 2. Bee-collected pollen

Pollen traps were set up at 5pm prior to each day of data collection, and removed at the same time on the following day for analysis. Fungicide residues were measured in bee-collected pollen and from hand-collected anthers in the sprayed and unsprayed zones.
Pollen and anthers were analyzed at the USDA-AMS laboratory in Gastonia NC using established methods for the fungicide.

Objective 3. Forager activity

Forager counts were taken at the hives and at flowers within the treated and untreated areas. At the hives, foragers were counted returning to the hive entrance over a three-minute interval, and categorized based on the presence or absence of corbicular pollen loads as either pollen or nectar foragers. In trees, a 1 m^2 area was designated with visual markers and the number of unique individuals foraging within that area was counted over a three-minute interval. The flowers in each area were also counted to provide a measure of bloom density.

Results:

Objectives 1 & 2. Iprodione Loads

Samples taken from Site 1 colonies seven hours after PM spray (Day 2, 13:00) had the highest levels of iprodione found in forager-collected pollen, 2410 ppb (**Figure 1**). The iprodione concentration in Site 1 forager-collected pollen decreased over time, dropping to 961 ppb by 6:00 on Day 2 and returning to 0 ppb by Day 3. There was no iprodione detected in Site 2 anthers or forager-collected pollen following PM spray, indicating that the PM spray from Zone 1 did not extend to Site 2.

While anther pollen sampled after AM spray had the highest overall concentrations of iprodione in this study, the forager-collected pollen sampled after AM spray had significantly lower levels of iprodione than that sampled after PM spray. In contrast to the PM spray, AM spray resulted in contamination of both sites. In fact, the concentration of iprodione found in Site 1 anther pollen following AM spray in Zone 2 was more than twice that found in Site 2.

Objective 3. Foraging Counts

The mean number of pollen foragers returning to hives decreased as the study progressed, from 39 on Day 1 to 13 on Day 3. Similarly, forager counts in trees decreased from Day 1 to Day 3, from 6 to 2.8 (**Table 1, Supplemental Figure**).

Table 1. Mean foraging counts across study period

	Day 1	Day 2	Day 3
Pollen Foragers	39	27	13
Nectar Foragers	114	105	219
Foragers in Trees	6	3.6	2.8

Mean foraging counts across the three days of study. Pollen and nectar foragers counted returning to hive entrance during three-minute interval, differentiated by presence/absence of corbicular load.

Discussion:

As expected, anther pollen collected immediately following AM spray had significantly higher concentrations of iprodione than anther pollen collected the morning after PM spray. However, counter to our expectations, the concentrations detected in forager-collected pollen were significantly higher following PM spray than following AM spray.

The higher concentration of iprodione in Site 1 vs Site 2 following Zone 2 spray indicates that AM spray spread from Zone 2 to Zone 1. This dispersal may have been caused by increased volatilization of iprodione and/or reduced performance of the adjuvant. The difference in application efficiency could be caused by differing wind, temperature and humidity conditions during or immediately following application.

The simultaneously high loads of iprodione in anther pollen and low loads in bee-collected pollen following AM spray suggest that the difference in hive contamination stems from a difference in forager activity within the contaminated sites, rather than a difference in the levels of iprodione in available forage. Indeed, there was a decrease recorded in both pollen foragers and flower visitors from Day 1 to Day 3, lending further support to this explanation. It is possible that foraging activity was further reduced on Day 3 due to repellency of iprodione following AM spray; however any potential repellency effects were masked by the dominant trend of a steady overall decrease in foraging across the study period.

Conclusion:

While the actual exposure to iprodione in the hives was lower following AM spray vs PM spray, the potential for exposure through anther pollen was higher following AM spray. The fact that anther pollen contained higher loads of iprodione following AM spray suggests that there is a greater potential for exposure during foraging hours following morning vs. evening fungicide application. It is unclear whether the increased dispersal following AM vs. PM spray was caused by time-of-day effects or conditions specific to the days of this study.

The difference in the iprodione levels of pollen found in the hives between the two treatments may have been caused by an overall reduction in pollen foraging and/or reduction in visitation to almond flowers across the study. As a consequence of the unexpectedly early and shortened period of almond bloom in the spring of 2015, the study period coincided with the final week of bloom. The number of available blooms progressively decreased from Day 1 to Day 3 (personal observation) and could account for the differences in foraging activity, leading to differences in contamination. In future studies, these questions should be re-addressed during a time of consistent bloom.

Acknowledgements:

We would like to thank the Almond Board of California for funding this study, and Wonderful Orchards for providing the study site.

References:

Johnson RM, Dahlgren L, Siegfried BD, Ellis MD (2013) Acaricide, Fungicide and Drug Interactions in Honey Bees (*Apis mellifera*). PLoS ONE 8(1): e54092. doi:10.1371/journal.pone.0054092

Pettis, Jeffery S., et al. "Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen Nosema ceranae." *PLoS One* 8.7 (2013): e70182.

Iprodion Loads in Pollen

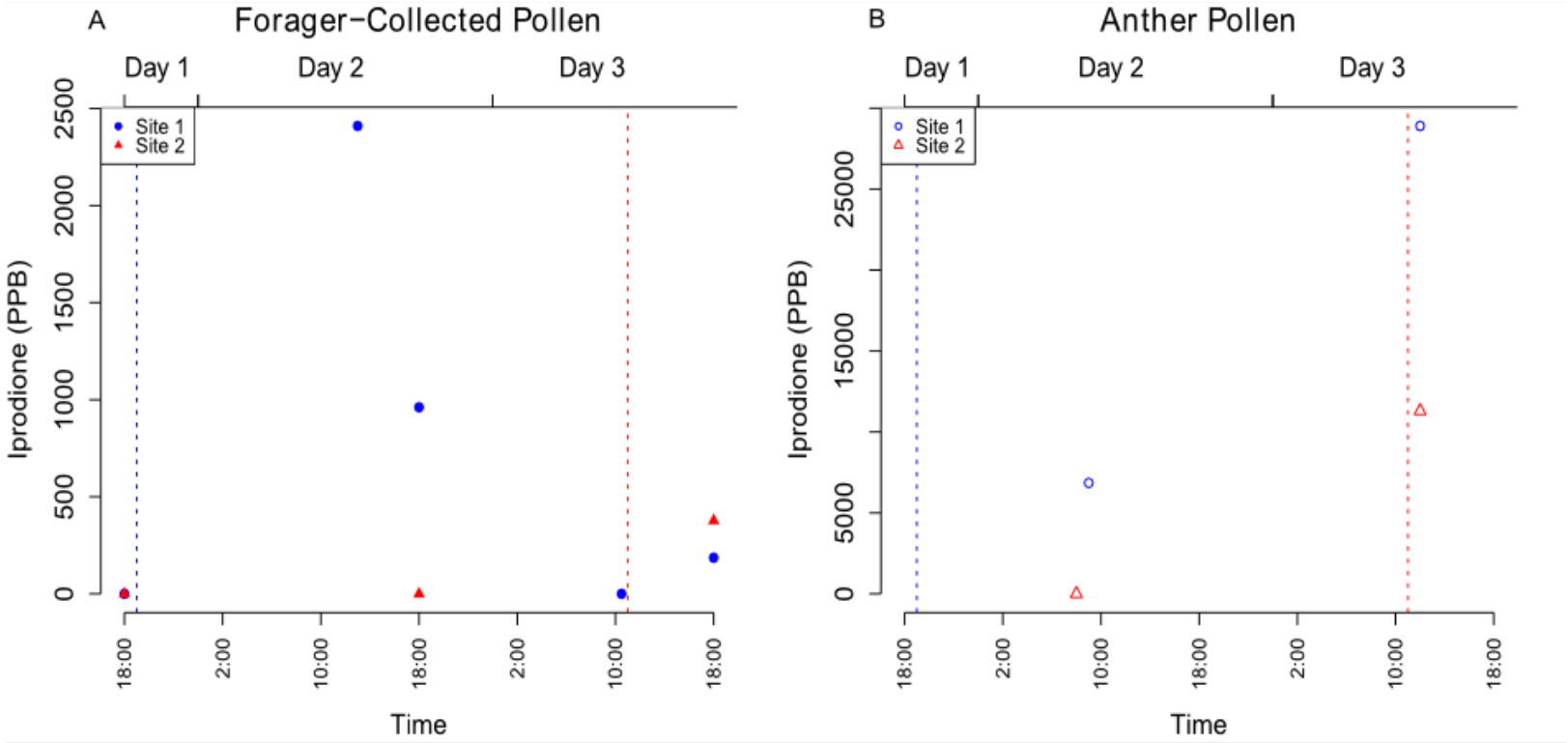
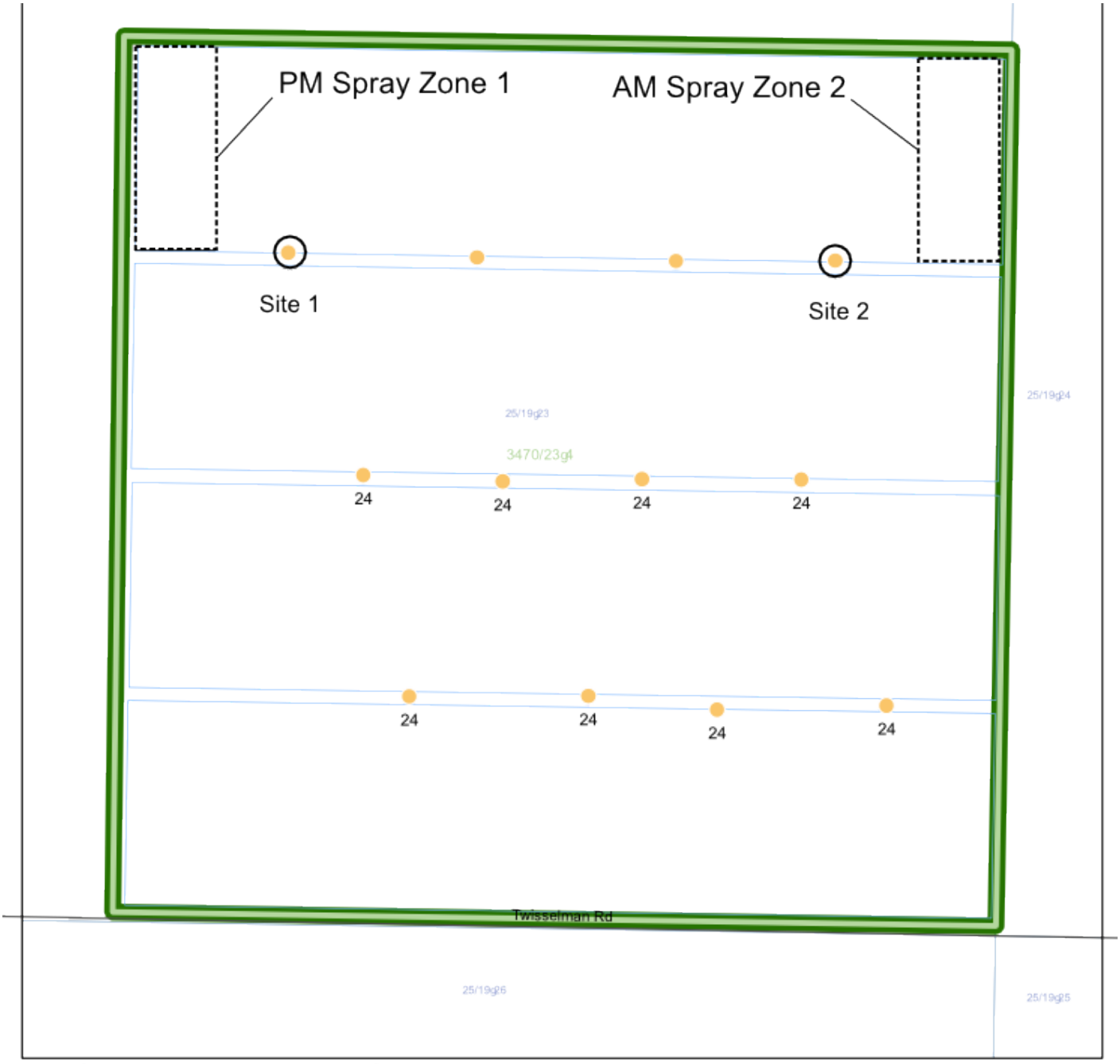
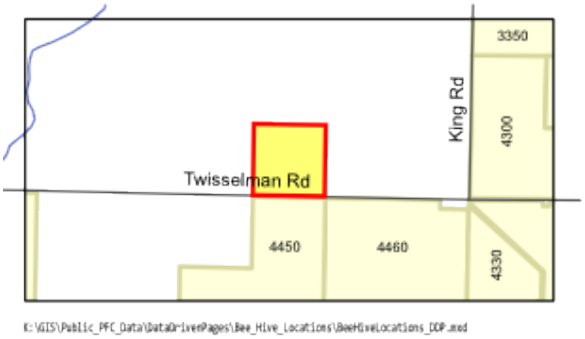


Figure 1. Iprodione loads (ppb) detected in (A) Forager-collected ollen and (B) Anther pollen from Site 1 (blue circles) and Site 2 (red triangles) across the three days of study.





E:\GIS\Public_PFC_Data\DataDrivenPages\Bee_Hive_Locations\BeeHiveLocations_DDP.mxd

Beekeeper Name
Marquette Apiaries West Valley

Ranch3470
West Valley

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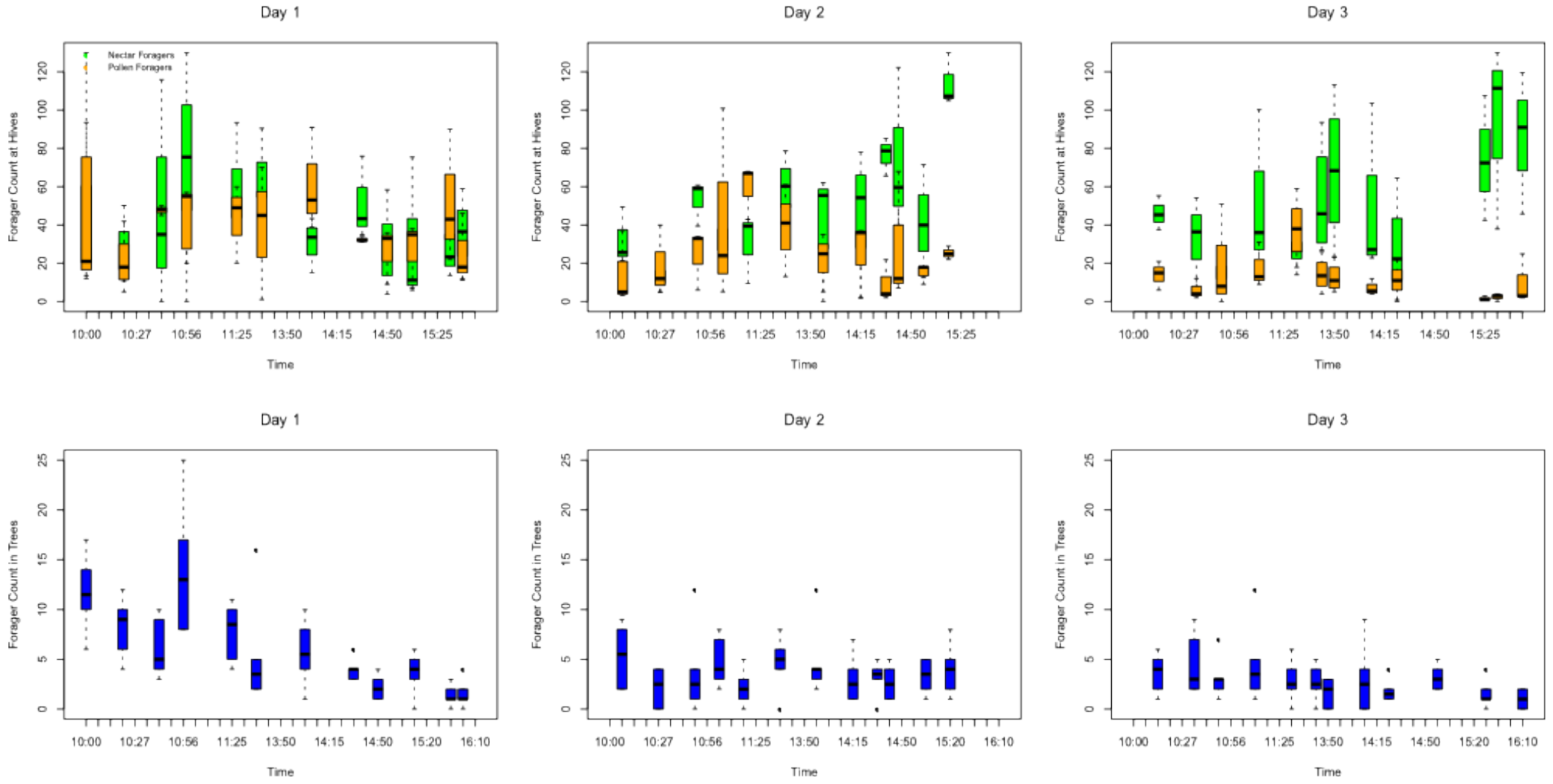
PARAMOUNT FARMING

1/19/2015d11:09:35dAM

Note: Paramount Farming changed name to Wonderful Orchards, Spring 2015

Forager Counts



Supplemental Figure: Forage counts observed at hives and trees across study area through the course of the 3-day study.