Title: **Evaluating effects of agrochemicals on honey bees colonies pollinating Oregon crops**

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Summary

Honey bee pollination is worth more than $ 600 million in Oregon. Healthy and strong bee colonies and beekeeping industry are critical for Oregon's agricultural economy. Recent annual honey bee colony losses (averaging 30%) are alarming to both beekeepers and growers, who are interdependent for their economic viability. The effects of pesticides on bee populations has garnered significant attention of the public and scientific community since the reporting of significant colony declines, and especially since the recent mass bumble bee kills in Oregon. However, research on the effects of pesticides on honey bees is very complex. About 121 different chemicals and their byproducts have been detected in North American pollen, wax, and bees (Mullin et al. 2010).

The impacts of pesticides on honey bee colony health are becoming clearer as more research is being conducted; however, we still lack knowledge on a number of fronts. Much of the data on the effects of these chemicals on bees focuses on a single chemical and does not capture effects of combined exposure, and since honey bees are exposed to so many chemicals simultaneously, single-chemical studies may not be sufficient for determining negative impact of a chemical. Studies conducted in the lab are also not often supplemented by field studies, which raises questions about whether laboratory results are true under field conditions. In this study we attempted to quantify different pesticides accumulating in honey bee hive matrices (nectar, pollen, wax) during crop pollination (blueberry, cherry and carrot seed crop) and evaluate their impacts on honey bee colony health. We used a controlled field study to investigate the potential of predominant agrochemicals detected in bee hive matrices in these cropping systems.

Objectives

1) Quantify concentrations of pesticides in bees, nectar, pollen during blueberry, cherry and carrot seed crop pollination.

2) Evaluate effects of the pesticides quantified above on honey bees using field studies.

Procedures / Materials and Methods

Objective 1: Evaluating Pesticide Concentrations: In co-operation with participating beekeepers, we evaluated honey bee colonies during pollination of each of the three target crops, and collected samples of bees, nectar / honey, pollen, and wax for chemical analysis. We also collected samples of nectar and pollen directly from each of the target crop fields to validate the presence of specific pesticides in the bee hive matrices.

Objective 2: Pesticide toxicity analysis: For controlled field experiment we selected field-relevant concentrations of predominant chemicals detected in the bee hive matrices placed in the three target crops. All experimental colonies for this study were established at an Oregon State University apiary. The experiment consisted of the following four treatments: control, imidacloprid, chlorothalonil and a combination of imidacloprid and chlorothalonil. A full-factorial (2 x 2 x 9), completely blocked experimental design was used for this study and each treatment was replicated nine times. Nine blocks of four colonies (36 colonies total) were used in the experiment. Experimental colonies were exposed to imidacloprid and/or chlorothalonil via a pollen patty diet. Pollen patties were made fresh every week for 4 weeks. Imidacloprid and chlorothalonil (pure active ingredients, Sigma Aldrich®, MO, USA) were infused to pollen through acetone solutions. The target concentrations of the two chemicals in the pollen patties were chosen based on the mean concentrations of these chemicals in stored pollen, as reported by Mullin et al.: 39 ppb imidacloprid and 3014.8 ppb chlorothalonil. The concentrations used here represent field-realistic concentrations in bee bread, and provide a balance between the lowest and worst-case scenario concentrations that may occur in the field.

Pollen patties were removed and replaced weekly for four weeks. During this time, pollen traps were placed on all colonies during the exposure period to prevent the consumption of incoming pollen and induce feeding on the pollen patty diet. Patties were placed between hive bodies for

each colony using a 4.5-cm spacer. Pollen consumption for each week was calculated as the change in pollen mass, accounting for the weight of the pie tin.

At approximately monthly intervals, colony evaluations were conducted to determine adult bee population and honey stores. The percentage of the area covered by bees, brood, and honey were visually estimated for each frame of each colony. Estimates for each were totaled for all frames in a colony, and recorded as total frames of bees, brood, and honey.

Weekly, we counted the number of bees returning to each hive over a 3-minute period. We also quantified hypopharyngeal gland protein content using a standard BCA assay (Pierce Biotech BCA Assay Kit, Thermo Scientific, IL, USA). Hemolymph was extracted from 10-15 live nurse bees per experimental colony for immunocompetence assays.

Significant Accomplishments (Results):

Following are pesticide residue analysis (ppb) results pertaining to pollen collected from colonies in blueberry and cherry:

Blueberry		
Azoxystrobin	Fungicide	7.5
Bifenthrin	Pyrethroid	18.5
Captan	Fungicide	58
Chlorpyrifos	Organophosphate	12.8
Chlorothalonil	Fungicide	2840
Imidacloprid	Neonicotinoid	24
Pendimethalin	Herbicide	28
Pyraclostrobin	Fungicide	25.4
Trifluralin	Herbicide	4.3

Cherry		
Azoxystrobin	Fungicide	302
Chlorpyrifos	Organophosphate	35.2
Fluvalinate	Pyrethroid	7.4
Myclobutanil	Fungicide	72
Pendimethalin	Herbicide	11.8
Tebuconazole	Fungicide	42

Pollen Patty Consumption: Total pollen patty consumption for all four weeks of treatment did not depend on imidacloprid exposure ($F_{1,22} = 0.776$, $p= 0.388$), chlorothalonil exposure ($F_{1,22} = 0.0003$, $p = 0.986$), or the combination of both chemicals ($F_{1,22} = 0.085$, $p = 0.774$).

Adult Bee Population: Adult bee population was not significantly different between levels of imidacloprid ($F_{1, 24} = 0.090$, $p = 0.767$), chlorothalonil ($F_{1, 24} = 1.806$, $p = 0.193$), or the combination of both chemicals ($F_{1, 24} = 0.295$, $p = 0.592$). Adult bee population was significantly affected by time ($F_{3, 96} = 99.351$, $p < 0.0001$). Neither the effect of imidacloprid ($F_{3, 96} = 0.517$, $p = 0.672$), chlorothalonil ($F_{3, 96} = 0.368$, $p = 0.777$) nor was there an interaction between both chemicals and time ($F_{3, 96} = 0.337$, $p = 0.798$).

Percent Change in Adult Bee Population: The percent change in adult bee population was not significantly affected by imidacloprid exposure ($F_{1, 24} = 0.564$, $p = 0.460$), chlorothalonil exposure ($F_{1, 24} = 0.572$, $p = 0.457$), or the combination of both chemicals ($F_{1, 24} = 0.313$, $p = 0.581$). There were no significant interactions between any of the chemicals and time.

Brood Area: In the model containing all data points, brood area was not affected by imidacloprid ($F_{1,24} = 0.281$, $p = 0.601$) or chlorothalonil ($F_{1,24} = 0.0008$, $p = 0.9775$). The interaction between chlorothalonil and imidacloprid was nearly significant at the 0.10 alpha level ($F_{1,24} = 2.886$, $p = 0.102$). Brood area was also significantly different between months for all groups ($F_{3,96} = 269.021$, $p < 0.0001$). There were no significant interactions between any of the treatments and time. The interaction between these chemicals appeared to be one in which the colonies treated with both chemicals had more total brood than colonies treated with either chemical alone, but neither group was significantly different from the control. However, the interaction between the two chemicals became insignificant between groups after the Tukey adjustment.

Percent change in Brood Area: Percent change in brood area was not significantly affected by imidacloprid exposure ($F_{1, 24} = 0.030$, $p = 0.863$) or chlorothalonil exposure ($F_{1, 24} = 0.128$, $p = 0.723$). The interaction between both chemicals also did not significantly affect change in brood area ($F_{1, 24} = 1.012$, $p = 0.325$). Neither the effect of imidacloprid ($F_{2, 64} = 0.1091$, $p = 0.897$) nor the effect of chlorothalonil ($F_{2, 64} = 1.272$, $p = 0.287$) had a significant interaction with time. There was also no significant interaction between imidacloprid, chlorothalonil, and time ($F_{2, 64} = 0.384$, $p = 0.683$).

Pollen Foragers: The median number of pollen foragers in the colonies was not affected by imidacloprid ($F_{1, 24} = 1.288$, $p = 0.268$) or chlorothalonil ($F_{1, 24} = 0.334$, $p = 0.569$). There was no interaction between the chemicals ($F_{12, 371} < 0.0001$, $p = 0.993$). The median number of pollen foragers in the colonies was different between different weeks ($F_{12, 371} = 14.866$, $p < 0.0001$). However, neither the effect of imidacloprid ($F_{12, 371} = 0.991$, $p = 0.457$) nor chlorothalonil ($F_{12, 371} = 0.967$, $p = 0.480$) interacted with time. There was also no interaction between both chemicals and time ($F_{12, 371} = 0.918$, $p = 0.529$).

Non-Pollen Foragers: Results were drastically different between the model containing outliers and the model excluding them. The model containing outliers detected no significant effect of imidacloprid ($F_{1, 24} = 1.944$, $p = 0.176$), chlorothalonil ($F_{1, 24} = 1.866$, $p = 0.185$), or their interaction ($F_{1, 24} = 0.940$, $p = 0.342$). There were no interactions between the chemicals and time. Time was the only factor that had a significant effect on non-pollen foragers in this model ($F_{12, 384} = 40.799$, $p < 0.0001$). In the model that excluded outliers from the previous model, the effect of both imidacloprid ($F_{1, 24} = 0.580$, $p = 0.454$) and chlorothalonil ($F_{1,24} = 1.680$, $p = 0.207$) remained insignificant, as did their interaction ($F_{1,24} = 0.285$, $p = 0.598$). The effect of time remained significant ($F_{12, 364} = 55.226$, $p < 0.0001$). However, when outliers were removed, the interaction between imidacloprid and time became significant ($F_{12, 364} = 2.058$, $p = 0.019$).

Total Foragers: In the model that contains all data points, there was no significant effect of imidacloprid ($F_{1, 24} = 0.960$, $p = 0.337$) nor chlorothalonil ($F_{1, 24} = 0.719$, $p = 0.405$) on the total number of foragers returning to the colonies. There was also no additional effect of the combination of both chemicals on total returning foragers ($F_{1, 24} = 0.047$, $p = 0.830$). The number of total foragers returning to the colony differed significantly between weeks ($F_{12, 384} = 27.541$, $p < 0.0001$). However, the interactions between the chemicals and time were not significant. When outliers were excluded, the total number of returning foragers still differed significantly between weeks ($F_{12, 365} = 31.825$, $p < 0.0001$). The effect of imidacloprid ($F_{12, 365} = 3.368$, $p = 0.079$) and chlorothalonil ($F_{12, 365} = 2.987$, $p = 0.097$) became significant at the 0.10 alpha level. There was still no interaction between the two chemicals ($F_{1, 24} = 0.604$, $p = 0.445$), nor were there any interactions between the chemicals and time.

Hypopharyngeal Gland Protein Content: Hypopharyngeal gland protein content did not differ significantly between levels of imidacloprid ($F_{1, 24} = 1.050$, $p = 0.316$) or chlorothalonil ($F_{1, 24} =$

2.066, p = 0.164). It was also unaffected by the combination of both chemicals ($F_{1, 24}$ = 0.071, p = 0.792). Furthermore, there were no interactions between either of the chemicals, their combination, and time. Furthermore, hypopharyngeal gland protein content was not significantly different between months ($F_{2, 64}$ = 0.372, p = 0.691).

Prophenoloxidase Activity: The model containing outliers showed that prophenoloxidase activity was not significantly affected by imidacloprid ($F_{1,24}$ = 1.879, p = 0.183) chlorothalonil ($F_{1,24}$ = 0.017, p = 0.897), or their interaction ($F_{1,24}$ = 0.666, p = 0.423). When the two outliers were removed, the interaction between chlorothalonil and time became significant ($F_{2,61}$ = 6.401, p = 0.003). All other main effects and interactions remained insignificant, except for the main effect of time, which remained significant ($F_{2,61}$ = 34.963, p < 0.0001).

Benefits & Impact: The results from the above study provide valuable insights on potential effects of agrochemicals on colony health. Information obtained from this study will enable stakeholders (growers and beekeepers) to predict and avoid risks to bee colonies which will potentially strengthen the economic sustainability of both beekeepers and producers.

Additional funding received during project term: We received additional funding from the National Honey Board.

Future funding possibilities: We may be able to seek funding from couple of funding agencies to expand this research.

Dissemination of results: Results from this study were presented at regional beekeepers association. Manuscript pertaining to this work will be published in peer reviewed journal and will be summarized in stakeholder publications such as Oregon State Beekeepers Association newsletter.