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Research

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Pollen Treated with a Combination of Agrochemicals Commonly Applied During Almond Bloom Reduces the Emergence Rate and Longevity of Honey Bee (Hymenoptera: Apidae) Queens

Dylan F. Ricke,[1,3,©] Chia-Hua Lin,[2] and Reed M. Johnson[1]

[1]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691, USA, [2]Department of Entomology, The Ohio State University, Rothenbuhler Honey Bee Research Laboratory, 2501 Carmack Rd., Columbus, OH 43210, USA, and [3]Corresponding author, e-mail: ricke.10@osu.edu

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Abstract

Honey bee (*Apis mellifera* L.) colonies that pollinate California's almond orchards are often exposed to mixtures of agrochemicals. Although agrochemicals applied during almond bloom are typically considered bee-safe when applied alone, their combined effects to honey bees are largely untested. In recent years, beekeepers providing pollination services to California's almond orchards have reported reductions in queen quality during and immediately after bloom, raising concerns that pesticide exposure may be involved. Previous research identified a synergistic effect between the insecticide active ingredient chlorantraniliprole and the fungicide active ingredient propiconazole to lab-reared worker brood, but their effects to developing queens are unknown. To test the individual and combined effects of these pesticides on the survival and emergence of developing queens, we fed worker honey bees in closed queen rearing boxes with pollen artificially contaminated with formulated pesticides containing these active ingredients as well as the spray adjuvant Dyne-Amic, which contains both organosilicone and alkyphenol ethoxylate. The translocation of pesticides from pesticide-treated pollen into the royal jelly secretions of nurse bees was also measured. Despite consistently low levels of all pesticide active ingredients in royal jelly, the survival of queens from pupation to 7 d post-emergence were reduced in queens reared by worker bees fed pollen containing a combination of formulated chlorantraniliprole (Altacor), propiconazole (Tilt), and Dyne-Amic, as well as the toxic standard, diflubenzuron (Dimilin 2L), applied in isolation. These results support recommendations to protect honey bee health by avoiding application of pesticide tank-mixes containing insecticides and adjuvants during almond bloom.

Key words: mixture toxicity, Royal Jelly, spray adjuvant, translocation

As pollinators, honey bees (*Apis mellifera* L.) are essential for the large scale production of many crops (Reilly et al. 2020). Commercial beekeepers in the United States now generate a majority of their revenue through pollination contracts (Goodrich 2019), which they fulfill by moving their colonies between crop blooms. Of these, California's almond crop utilizes the most honey bee colonies every year, representing over 80% of managed colonies in the United States (Goodrich and Durant 2020). The values of the resulting pollination services were recently estimated to exceed $4 billion per year in almonds alone (Reilly et al. 2020).

Although almond pollination provides revenue for the beekeeping industry, beekeepers have reported colony health issues during and immediately after bloom that may be related to pesticide exposure. Reports include sudden bee die-offs, which are typical of acute pesticide exposure, as well as symptoms of chronic exposure, including reduced queen quality and survival in the weeks following bloom (Pollinator Stewardship Council 2014). Like migratory beekeepers, queen producers located in California's almond-growing region have also reported bee health issues in the weeks following bloom (Oliver 2013). The effects of pesticides used during almond bloom on queen development is a potentially major issue because reductions to queen quality often precipitate the death of colonies (vanEngelsdorp et al. 2013, Kulhanek et al. 2017) and pesticide residues have been correlated with increased rates of queen events (supersedure or death) in commercial colonies across the United States (Traynor et al. 2016). A high concentration of queen-rearing operations are located in the almond-growing region of California (Cobey et al. 2011), which produce queens during or shortly after almond bloom (Oliver 2013).

A variety of pesticides are often applied to almonds simultaneously in the form of tank mixtures (Mullin et al. 2015). Previous studies have found that mixtures of common agrochemicals can cause lethal and sublethal effects to developing queens. For example, DeGrandi-Hoffman et al. (2013) found that queens reared on diets containing the insecticide chlorpyrifos and the common fungicide Pristine (pyraclostrobin and boscalid) demonstrated increased viral titers as larvae and emerged from pupation at reduced rates. In colonies fed pollen treated with field-relevant levels of the fungicide active ingredients propiconazole (Pro) and chlorothalonil, rates of queen events and brood loss increased (Traynor et al. 2021). Studies on honey bee workers provide additional evidence that agrochemical mixtures pose high risk to developing queens. Wade et al. (2019) found that the combination of Pro and the insecticide chlorantraniliprole (Chl) had a pronounced synergistic effect on the mortality of lab-reared worker brood. This finding contrasts with the results of an earlier study on the toxicity of Chl in isolation on adult honey bees, which supported its labeling as a "bee safe" product (Dinter et al. 2010). Similarly, Pro and most other fungicides are considered safe to apply when bees are active, though some fungicides have been shown to cause adverse effects on honey bees in combination with other pesticides (Fisher et al. 2017, Carnesecchi et al. 2019).

In addition to pesticides, tank mixtures often contain spray adjuvants, which are used to improve aspects of pesticide performance (wetting, particle size, etc.) during application. Research on spray adjuvants containing organosilicone and ethoxylate compounds as principal functioning agents indicate that these compounds are toxic to honey bees when combined with pesticides (Mullin et al. 2015) and may be more toxic individually than certain pesticides (Mesnage and Antoniou 2017). Although the adjuvant Break-Thru was not found to affect queen survival during development (Johnson and Percel 2013), there are a wide variety of adjuvants used in almond fields for which the effects on bees is unknown. As with studies on agrochemical mixtures, most evidence of adjuvant toxicity in honey bees is derived from studies with workers. For example, a study on lab-reared workers found that larval exposure to 10 ppm of a common organosilicone synergized the pathogenicity of Black Queen Cell Virus (Fine et al. 2017). This has clear implications for queens because this virus infects and kills developing queens and was found to be prevalent in colonies contracted to pollinate almonds (Glenny et al. 2017). Despite growing evidence that some common adjuvants are toxic to honey bees, they are widely considered to be toxicologically "inert" and undergo little testing for bee safety (but see USEPA 2021).This issue is especially relevant to California's almond orchards, where the usage of organosilicone adjuvants increased by more than 5-fold from 2001 to 2013 (Mullin et al. 2016). Of these, the adjuvant Dyne-Amic (Dyn), which contains both an organosilicone and an alkyphenol ethoxylate, is among the most widely used (CDPR 2021).

In addition to the pesticides and adjuvants identified above, almonds are regularly sprayed with insecticides during bloom, despite recommendations against this practice (Almond Board of California 2020). These insecticides are not acutely toxic to bees and include the previously mentioned chlorantraniliprole, which acts on ryanodine receptors in insect muscle, as well as insect growth regulators (IGRs), including diflubenzuron (Dif) and methoxyfenozide, that affect insect development. IGRs were shown to reduce the feeding ability of nurses as well as the emergence rate of queen-laid eggs (Fine 2020). Dif was previously found to reduce the survival of developing queens (Johnson and Percel 2013) and lab-reared worker brood (Wade et al. 2019).

In the present study, we investigated the individual and combined effects of the formulated products Altacor (35% Chl), Tilt (41.8% Pro), and Dyn on the survival of developing queens. Dimilin 2L (10% Dif) was included as a positive control. Queens were grafted into enclosed queen-rearing boxes (Spivak 1994, Johnson and Percel 2013) and were provided with nurse bees, syrup, and pollen. Pollen diets were either untreated (negative control) or treated with formulated agrochemicals. The translocation of each pesticide active ingredient from treated pollen into nurse bees and their royal jelly secretions was measured as well as queen survival throughout pupation, adult emergence, and to 7 d post-emergence.

Methods

Queen-rearing Trials

Experiments were conducted at Waterman Agricultural Research and Natural Resources Laboratory (WANRL) at the Ohio State University in Columbus, OH, from 2016-2018. Queen rearing trials were performed using a modified swarm box method (Johnson and Percel 2013, Spivak et al. 1994; Fig. 1). This approach limits the exposure of developing queens and their nurses to confounding variables associated with free-flying colonies (outside sources of pollen, weather events, etc.). Briefly, each swarm box was provisioned with 180 g of pollen and 2 liters of 50% (w/w) sucrose solution. Each box received thirty 24–48-h-old worker larvae, which were grafted into base mount JZ-BZ queen cups on a queen cell bar frame (Mann Lake Ltd., Hackensak, MN). Finally, each box received 3.15 liters of nurse bees (approximately 1.12 kg), which were shaken from multiple healthy colonies. Nurses did not receive any treatment prior to the start of each trial and were therefore only exposed to the treated pollen during the 96-h queen-rearing phase of each trial.

Experimental treatments were prepared by dissolving formulated products, alone or in combination, in distilled water to make a stock solution. The negative control contained only distilled water. Solutions were then blended with dried bee-collected pollen (Betterbee, Greenwich, NY) at a liquid: pollen ratio of 1:4 (w:w) using a food processor (Ninja Express Chop, SharkNinja Operating LLC, Chino, CA) to achieve target concentrations. The bulk pollen was thoroughly mixed prior to being portioned among trials. The target concentrations of the chemicals in pollen treatments were 40 ppm for Chl, 90 ppm for Pro, and 100 ppm for Dif. Diets with the adjuvant were treated to contain 0.8% Dyn by weight. Concentrations were chosen based on the maximum field application rates for each product in almonds (Supp Table 1 [online only]). These rates were chosen to simulate a high-exposure scenario immediately following a single pesticide application event. Five grams of treated pollen was sampled for pesticide analysis (described below) before the pollen was fed to each swarm box to determine the concentrations of each pesticide in treated pollen.

Two separate experiments were conducted. The first experiment included treatments of Altacor (Chl), Tilt (Pro), and a combination of Altacor + Tilt (Chl+Pro). The second experiment also included treatments of Dyn, Altacor + Dyn (Chl+Dyn), and Altacor + Tilt + Dyn (Chl+Pro+Dyn). An additional treatment with the insecticide Dimilin 2L (Dif) was included in the first experiment as a positive control. Each experiment was performed in three replicated trials. A detailed protocol for setting up the swarm boxes and conducting the rest of the experiment is provided (Supp File 2 [online only]).

Prepared swarm boxes, with grafted larvae, were stored in a dark room at 20–28°C for 96 h. At this time, 5 g of nurse bees (found clustering on the queen cell frame) and 5–7 capped queen cells were

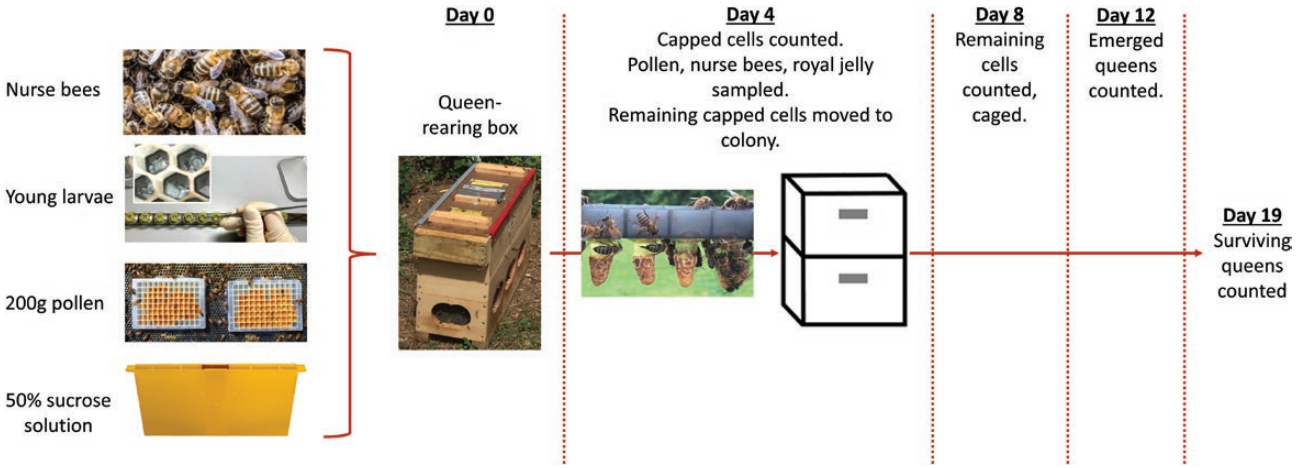

Fig. 1. The queen-rearing approach used for this study. Queen-rearing boxes were prepared on day 0. On day 4 (96 h later), samples of pollen, nurse bees, and the royal jelly from a subset of capped queen cells were taken for chemical analysis. Capped cells were counted and moved to a strong incubating colony. On day 8, the remaining cells were counted and caged. On day 12 through day 19, living and dead emerged queens were counted every 2-3 d. Any queens not emerging by day 19 were counted as dead. Detailed methods are presented in Supp File 2 [online only].

removed from each swarm box for pesticide residue analyses (Fig. 1). The number of queen cells that were sampled varied between treatments as different numbers were needed to yield at least 1 g of royal jelly for chemical analysis. In trials receiving the Dif treatment, queen cells were not sampled for chemical analysis if survival was already low by day 4. This ensured that Dif trials could still serve as a positive control for all timepoints during survival analysis. Royal jelly from the sampled queen cells was manually extracted using a microspatula and stored in airtight microcentrifuge tubes at –20°. The remaining queen cells were moved to a strong colony where they were incubated until adult queens emerged. On the eighth day of the trial, all capped queen cells were counted and individually caged to protect the cells and confine the adult queens once they emerged. The individually caged cells were checked every 2–3 d to record the number of queens that had emerged. Queen survival following emergence was recorded until 7 d after the first queen emergence was noted.

Pesticide Residue Analysis

Pollen, nurse bees, and royal jelly samples were stored at –20°C prior to being sent to The University of Guelph's Agricultural and Food Laboratory for analysis by LC/MS/MS. Concentrations of each pesticide active ingredient (Chl, Dif, and Pro) were determined for each sample. Five grams of the untreated commercial pollen was also submitted for comprehensive pesticide screening to estimate the background level of pesticides in the pollen.

For each trial, the translocation of each active ingredient into nurse bees and royal jelly secretions was calculated as the concentration of the active ingredient in each divided by its concentration in the previous hive component (pollen or nurse bees, respectively). Prior to running statistical tests, the distributions of translocation rates for each chemical were tested for normality with a Shapiro–Wilk test (R Core Team 2020). To test whether the spray adjuvant Dyn affected the translocation rates of pesticide active ingredients, a nonparametric Kruskal–Wallis rank sums test was performed across mixtures. Differences between the total translocation of each active ingredient from pollen into royal jelly were also tested for significance with a Kruskal–Wallis rank sums test, followed by a post-hoc Dunn's test with a Bonferroni correction, using the R package dunn.test (Dinno 2017). For all tests, adjusted P values < 0.05 were considered statistically significant.

Survival Analysis

Counts of living and dead queens at 4, 8, 12 (emergence), and 19 d post-grafting (7 d post-emergence) were used to calculate the probability of queens surviving to each timepoint for each trial. Trials were omitted from the analysis according to two criteria: (1) trials with (negative) control mortality greater than 50% on day 12, or (2) trials with positive control (Dif) survival on day 12 greater than the corresponding survival of queens in the negative control group. A comparison of the overall survival between treatment groups was performed with a pairwise log-ratio test with a Bonferroni correction using the pairwise_survdiff function in the R package survival (Therneau 2021). This test is suitable for analyses in which some number of subjects are censored from the study prior to the conclusion of the study. Censored queens in our study included those that were removed on day 4 in order to sample the royal jelly in their cells. On day 12, another subset of queens were removed for a companion study on the reproductive effects of the agrochemicals used in the present study. Finally, the survival of a subset of queens were measured up to day 19, the rest of which were censored from the study on day 12 (Supp Table 4 [online only]). The R code for all analyses and the associated datasheets can be found at https://doi.org/10.6084/m9.figshare.14541918.v2.

Results

Pesticide Residue Analysis

The median concentrations of Chl, Pro, and Dif in treated pollen were 26, 88.5, and 66 ppm, respectively (Fig. 2, Supp Table 2 [online only]). The concentrations of each active ingredient were 1–2 orders of magnitude lower between successive hive components (pollen > bees > jelly, Fig. 2). Residues of pesticides that were not applied as experimental treatments (contaminants) were either not detected or only detected at a fraction of the concentration of chemicals that were applied as treatments. The concentrations detected and the limits of detection for Chl, Dif, and Pro from experimental samples are provided in Supp Tables 3 and 4. None of the pesticide active ingredients used for this study (Chl, Pro, Dif) were detected in the untreated commercial pollen that was used.

A Shapiro–Wilk test found that the translocation rates of Chl (n = 27, w = 0.869), Dif (n = 7, w = 0.738), and Pro (n = 20,

$w = 0.655$) from pollen into royal jelly were not normally distributed ($P = 0.003$, 0.009, and $P < 0.001$, respectively). A Kruskal–Wallis rank sums test did not find a statistically significant difference between the translocation rates of Chl (df = 3, $\chi^2 = 0.943$, $P = 0.815$) or Pro (df = 2, $\chi^2 = 0.208$, $P = 0.901$) when applied in different chemical mixtures. The same results were found after removing datapoints from trials receiving Chl+Dyn or Chl+Pro+Dyn, which had the lowest number of replicates (Supp Table 5 [online only], Supp Fig. 1 [online only]), for both Chl (df = 1, $\chi^2 = 3.158$, $P = 0.0755$) and Pro (df = 1, $\chi^2 = 0.610$, $P = 0.435$). When comparing the translocation rates of each active ingredient from pollen into royal jelly, a Dunn's test with a Bonferroni correction found a statistically significant difference between Pro and Dif ($\chi^2 = 14.733$, $Z = 3.5734$, $P < 0.001$) and Pro and Chl ($\chi^2 = 14.733$, $Z = 2.6719$, $P = 0.011$), but not between Chl and Dif ($\chi^2 = 14.733$, $Z = -1.841$, $P = 0.098$). A statistically significant difference between the translocation rates of Chl and Pro was still found if Dif, which had the lowest number of samples and served primarily as a positive control for survival analysis, was omitted from the test ($\chi^2 = 8.439$, $Z = 2.905$, $P < 0.002$).

Survival Analysis

For each treatment group, 89–180 queens from 3–6 queen boxes were included in the survival analysis (Table 1). Raw survival data is presented in Supp Table 6 [online only]. By day 12, the mean survival rates of all experimental groups were less than that of the control group, except for the Pro group (Table 1, Supp Fig. 2 [online only]). Differences with the control group became more pronounced on day 19. A pairwise log-rank test found significant differences in the overall survival curves of the control group and the Chl+Pro+Dyn ($P = 0.006$) and Dif ($P < 0.001$) groups (Fig. 4). In addition, the survival of the positive control group, which was treated with Dif, was significantly different from all other groups ($P < 0.05$). Differences in survival for all other pairwise comparisons were non-significant ($P > 0.05$, Supp Table 7 [online only]).

Discussion

In agreement with previous studies (DeGrandi-Hoffman et al. 2013, Johnson and Percel 2013, Dively et al. 2015, Böhme et al. 2018, 2019, Milone et al. 2021), we found that the translocation rates of chemicals into royal jelly were quite low and never exceeded 1% of the concentrations in treated pollen (Fig. 3). Despite the low levels of chemicals detected in royal jelly, we found that the average probability of emergence was reduced by about 75% in groups reared on pollen containing the positive control Dimilin 2L (Dif) and by nearly 30% in groups reared on pollen containing a combination of Altacor (Chl), Tilt (Pro), and Dyne-Amic (Dyn), relative to the negative control group (Table 1, Supp Fig. 2 [online only]). Concentrations of pesticide active ingredients were 2–3 orders of magnitude greater in treated pollen relative to the royal jelly secretions of nurse bees, supporting a filtering role of nurses against the exposure of brood to food-borne toxicants. Notably, chemical concentrations were 1–2 orders of magnitude greater in samples of nurses relative to the royal jelly we collected from queen cells.

Our results indicate that nurses can effectively mitigate queen exposure to pesticides, but their protective function can be

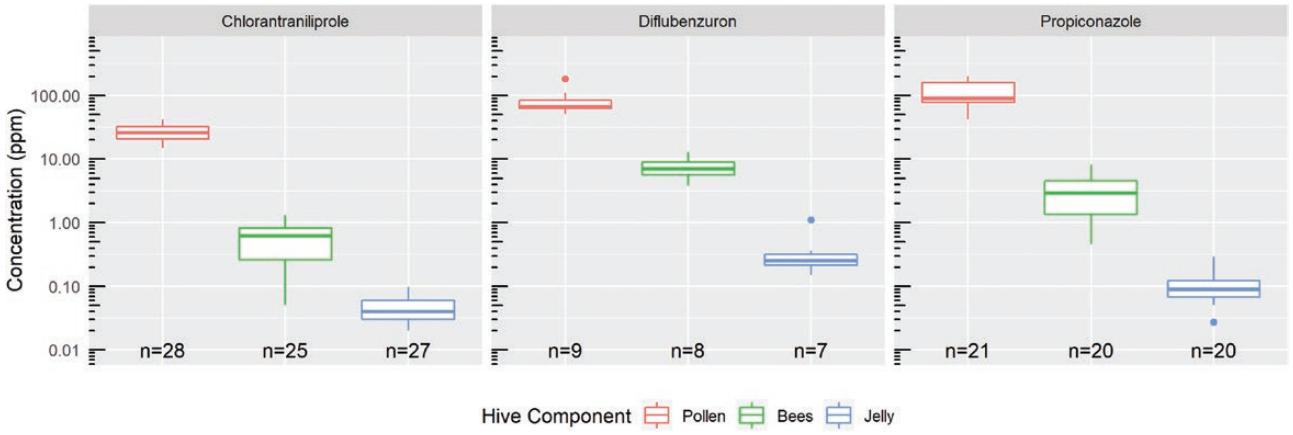

Fig. 2. Concentrations of pesticide active ingredients detected from each hive component (pollen, nurse bees, or royal jelly). Data were pooled across all trials. Pesticide residue data and limits of detection are presented in Supp Tables 2 and 3 [online only].

Table 1. The number of trials, number of queens (omitting queens that were removed for chemical analysis), and mean probabilities of survival (± 1 standard deviation) for each treatment group at each timepoint

		Day 0–4		Day 0–8		Day 0–12		Day 0–19	
Treatment	Trials (*n*)	Queens (*n*)	Mean ± SD	Queens (*n*)	Mean ± SD	Queens (*n*)	Mean ± SD	Queens (*n*)	Mean ± SD
Chl	6	180	0.85 ± 0.10	143	0.81 ± 0.12	143	0.75 ± 0.13	105	0.58 ± 0.19
ChlDyn	3	90	0.86 ± 0.11	73	0.81 ± 0.15	73	0.70 ± 0.20	64	0.70 ± 0.20
ChlPro	3	89	0.80 ± 0.15	73	0.80 ± 0.16	73	0.75 ± 0.18	54	0.56 ± 0.26
ChlProDyn	3	90	0.67 ± 0.23	74	0.67 ± 0.23	74	0.53 ± 0.30	59	0.42 ± 0.40
Control	3	90	0.88 ± 0.13	71	0.86 ± 0.14	71	0.76 ± 0.16	45	0.65 ± 0.21
Dif	6	179	0.81 ± 0.12	143	0.45 ± 0.05	143	0.20 ± 0.06	106	0.03 ± 0.06
Dyn	3	89	0.68 ± 0.16	70	0.68 ± 0.16	70	0.59 ± 0.29	53	0.46 ± 0.44
Pro	6	180	0.91 ± 0.05	145	0.91 ± 0.05	145	0.90 ± 0.06	96	0.52 ± 0.25

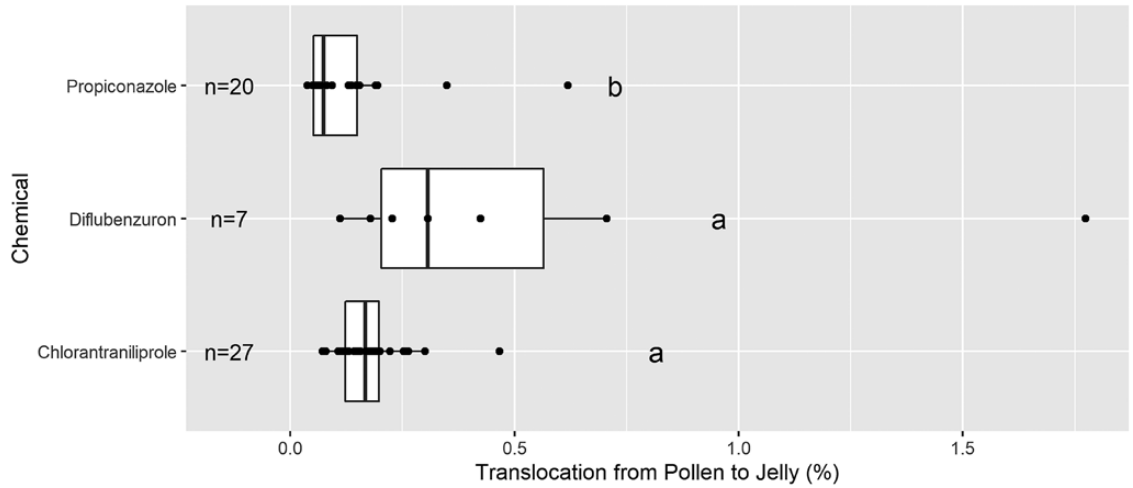

Fig. 3. The translocation rates of each chemical from treated pollen into royal jelly. Each point represents the translocation rate of the given chemical measured from a single queen-rearing box trial. Rates were calculated as the proportion of the concentration of each chemical in jelly over the concentration measured from treated pollen. Significant differences were found between Pro/Dif and Pro/Chl ($P < 0.05$).

overwhelmed when exposed to toxic mixtures. This has evolutionary significance: a reliance upon nurses and other socially mediated means of detoxification may explain the paucity of detoxification genes in the honey bee genome (Claudianos et al. 2006). In support of this, Lucchetti et al. (2018) found that nurses buffered brood from exposure to the naturally occurring pollen phytotoxin echimidine. Although nurses may protect brood from dietary toxicants, nurse exposure to pesticides can cause developmental effects to their hypopharyngeal glands that can ultimately impair their ability to tend to brood (Heylen et al. 2011, Hatjina et al. 2013, Zaluski et al. 2017). In queen-rearing experiments, this has been directly linked to reductions in both the quantity and metabolomic profile of royal jelly (Degrandi-Hoffman et al. 2015, Milone et al. 2021). We did not measure the effects of our treatments on nurses, which may include effects to their hypopharyngeal glands as well as their nursing behavior. This remains an interesting avenue for future studies.

In a previous study, Chl and Pro were found to have a synergistic effect on larval mortality (Wade et al. 2019). The present study extends this work to developing queens. Like other sterol biosynthesis inhibiting (SBI) fungicides, Pro is designed to inhibit enzymes that are closely related to key detoxification enzymes, the cytochrome P450 monooxygenases, in honey bees (Johnson 2015). Several studies have found synergistic toxicity between SBI fungicides such as Pro and insecticides in the pyrethroid and neonicotinoid classes (Johnson et al. 2013, Robinson et al. 2017, Carnesecchi et al. 2019), as well as quercetin, a naturally-occurring phytochemical common in pollen (Mao et al. 2017). In a large-scale survey of commercial bee colonies across the United States, SBI residues in beeswax were a significant predictor of both colony collapse and queen mortality (Traynor et al. 2016).

Although we did not find that the combination of Altacor (Chl) and Tilt (Pro) reduced queen survival relative to treatments receiving just Altacor, Tilt, or the negative control, these differences may have become evident if queen health had been tracked over a longer timeframe, or if additional measures of queen fitness were included. For example, Milone and Tarpy (2021) found that queens reared on wax and pollen treated with a combination of pesticides at field-relevant levels had reduced sperm viability in their spermathecae. This was observed despite negligible levels of direct oral exposure via royal jelly. Walsh et al. (2020) found that queens reared on wax treated with common pesticides, including common miticides used in beekeeping, produced fewer eggs as adults, had smaller worker retinues, and produced profiles of mandibular pheromones that were less attractive to worker bees in behavioral assays. Importantly, the effects of agrochemical mixtures on queens will likely be exacerbated by their effects on other members of the colony. For example, the viability of drone sperm was found to be reduced in drones reared on wax contaminated with pesticides, which may have long-term effects to the productivity of mated queens (Fisher and Rangel 2018). Finally, there are many other agrochemicals applied in almonds whose combined effects may have been more or less severe than those included in the present study. Fisher et al. (2017) found that combinations of the dicarboximide fungicide Iprodione 2SE Select reduced the survival of foragers following spray exposure when combined with certain strobilurin-containing fungicides (Pristine or Quadris). This is notable given that these mixtures lacked any insecticides. Subsequently, Fisher et al. (2021) found that the growth of field colonies was reduced when fed pollen containing just Pristine at field-relevant concentrations.

Our trials with Dimilin 2L, which served as a positive control for survival analysis, reinforce past studies indicating that it poses unacceptable risk to honey bee brood. Assuming that queens consume 380 µg of jelly over their development (Dietz and Lambremont 1970), queen larvae in our study consumed up to ~ 0.152 µg of Dif. This is an order of magnitude lower than the acute oral dose used in a previous study (2.28 µg) (Wade et al. 2019). It is also well below acute contact LD50s observed in worker larvae exposed between the third and sixth instar (2.42–6.02 µg/larva) (Gupta and Chandel 1995). Although we found Dif residues in jelly were below these acutely toxic levels, queens reared with Dif-treated pollen had significantly lower survival relative to all other treatments (Fig. 4, Supp Fig. 2 [online only]). These results corroborate the findings of Thompson et al. (2005), who observed half as many new eggs per day and a 6-fold increase in the rate of brood replacement in colonies treated with sucrose solution containing Dimilin Flo at a concentration mimicking its maximum field application rate in the United Kingdom.

We found a significant difference in the translocation rates of Pro and Chl (Fig. 3), but the mechanisms underlying the relative translocation of these and other food-borne contaminants of honey

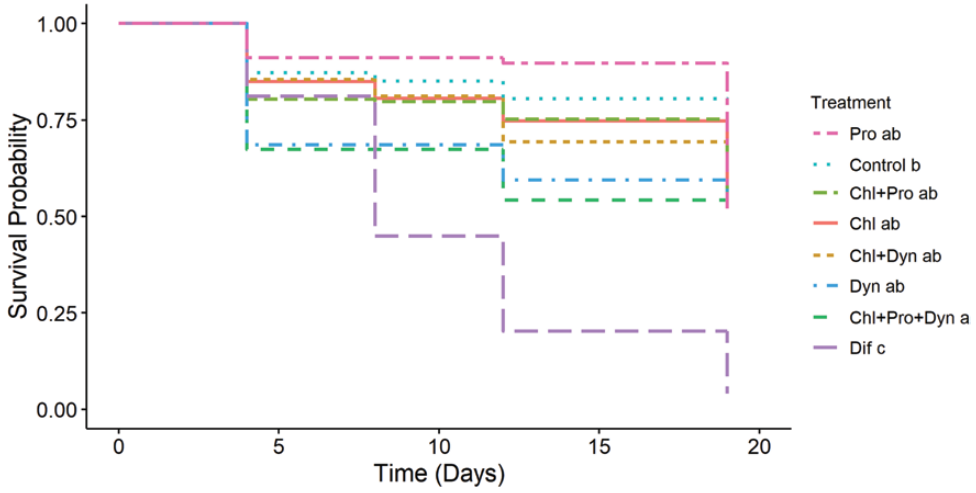

Fig. 4. Kaplan–Meier survival curves for queens reared with each pollen treatment. Data for each chemical were pooled across all trials. Counts of living and dead queens were taken on days 4 (capping), 8, 12 (emergence), and up to 7 d post-emergence (day 19). Letters in the legend indicate significant differences ($P < 0.05$). Exact P values are presented in SuppTable 5 [online only]. Items in the legend are ordered by their mean rate of survival on day 19.

bee colonies remains unknown. It is possible that the pesticides we detected in royal jelly originated from traces of treated pollen carried on the outside surfaces (legs, mandibles) of bees and not from the interior of nurses during jelly synthesis or secretion. The former will occur at some rate for any pesticide, whereas the latter may be attenuated by a pesticide's chemical behavior within nurse bees (Davis and Shuel 1988). In either case, the effective translocation of pesticides from pollen into royal jelly in this study was found to be quite low. Furthermore, no significant difference was found between the translocation rates of each pesticide active ingredient when applied in isolation or when combined with Dyn. It is possible that most food-borne pesticides are filtered out or metabolized prior to translocation into royal jelly, but this area warrants further attention. In terms of apicultural pesticide treatments for the control *Varroa destructor,* which are either applied in strips or through fumigation, lipophilicity was found to correlate with their accumulation into hive wax (Bonzini et al. 2011) and may explain their relative accumulation into royal jelly (Tananaki et al. 2009). In terms of food-borne pesticides, however, a recent study found that the relative translocation of 13 pesticides into royal jelly did not correlate with lipophilicity (Böhme et al. 2018). Although we have focused on queens in the present study, it is worth noting that exposure to food-borne chemicals is likely to be especially intense for developing workers, whose diet contains a greater admixture of honey and pollen, including any residual chemicals, following their third day of feeding by nurse bees (Böhme et al. 2019).

The effects of agrochemicals on brood can interact with other stressors associated with the long-distance movement of colonies between crop blooms, such as increased rates of viral transmission (Cavigli et al. 2016). This is important, in part, because the combination of stressors faced by migratory colonies may undercut the profitability of almond pollination for beekeepers (DeGrandi-Hoffman et al. 2019). DeGrandi-Hoffman et al. (2013) found increased virus titers in queen larvae exposed to the insecticide chlorpyrifos and the fungicide Pristine, which has been commonly used in almonds outside the blooming period. A similar result was found in adult workers exposed to pollen treated with the fungicides boscalid and pyraclostrobin (DeGrandi-Hoffman et al. 2015). Fine et al. (2017) found that the exposure of larvae reared in vitro to an organosilicone adjuvant synergized the pathogenicity of common honey bee viruses. The interaction of almond agrochemicals with stressors other than pathogens has received relatively less attention. The interaction of stressors encountered by contracted colonies in almond fields warrants further investigation, especially as it pertains to queen health.

Conclusion

Agrochemical mixtures remain a plausible cause of queen health issues occurring around almond bloom, particularly in combination with the other stressors involved in the annual migration of honey bees for pollination (vanEngelsdorp et al. 2013). Given the low levels of pesticide active ingredients detected in royal jelly, the effects of agrochemical mixtures on developing queens likely resulted from indirect effects on nurses in addition to direct toxicity to queens. These findings support current best management practices recommending that neither insecticides nor adjuvants be combined with fungicides applied to almonds during bloom when honey bees are present for pollination (Almond Board of California 2020).

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Author Contributions

R.M.J. and C.H.L. conceptualized and designed the study. C.H.L. developed the methodology and conducted the experiments. D.F.R. analyzed data and wrote the initial draft of the manuscript. All authors contributed equally to editing and reviewing of the manuscript.

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