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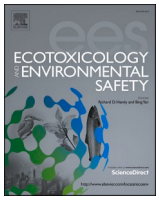

The active ingredients of a mitotoxic fungicide negatively affect pollen consumption and worker survival in laboratory-reared honey bees (*Apis mellifera*)

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ABSTRACT

Recent observations of many sublethal effects of pesticides on pollinators have raised questions about whether standard short-term laboratory tests of pesticide effects on survival are sufficient for pollinator protection. The fungicide Pristine® and its active ingredients (25.2% boscalid, 12.8% pyraclostrobin) have been reported to have low acute toxicity to caged honey bee workers, but many sublethal effects at field-relevant doses have been reported and Pristine® was recently found to increase worker pollen consumption, reduce worker longevity and colony populations at field relevant concentrations (Fisher et al. 2021). To directly compare these whole-colony field results to more standard laboratory toxicology tests, the effects of Pristine®, at a range of field-relevant concentrations, were assessed on the survival and pollen consumption of honey bee workers 0–14 days of age. Also, to separate the effects of the inert and two active ingredients, bees were fed pollen containing boscalid, pyraclostrobin, or pyraclostrobin plus boscalid, at concentrations matching those in the Pristine® treatments. Pyraclostrobin significantly reduced pollen consumption across the duration of the experiment, and dose-dependently reduced pollen consumption on days 12–14. Pristine® and boscalid significantly reduced pollen feeding rate on days 12–14. Boscalid reduced survival in a dose-dependent manner. Consumption of Pristine® or pyraclostrobin plus boscalid did not affect survival, providing evidence against strong negative effects of the inert ingredients in Pristine® and against negative synergistic effects of boscalid and pyraclostrobin. The stronger toxic effects of Pristine® observed in field colonies compared to this laboratory test, and the opposite responses of pollen consumption in the laboratory and field to Pristine®, show that standard laboratory toxicology tests can fail to predict responses of pollinators to pesticides and to provide protection.

1. Introduction

Honey bees (*Apis mellifera*) are essential to agriculture worldwide (Calderone, 2012; Garibaldi et al., 2013) but continue to face challenges to their health from a variety of environmental stressors (Le Conte et al., 2010; Johnson et al., 2010; Smith et al., 2013; Simon-Delso et al., 2014; Dolezal and Toth, 2018; Sponsler et al., 2019). Pesticides constitute a major stressor for pollinators (Johnson et al., 2010) and have been observed to induce numerous negative effects on honey bees (Mao et al., 2017; Tsvetkov et al., 2017; Farina et al., 2019; Sponsler et al., 2019). Fungicides may be of particular concern due to their prevalence in the foraging environment (Cullen et al., 2019) and as in-hive contaminants (Mullin et al., 2010; Pettis et al., 2013). Additionally, a number of fungicides are applied in bee pollinated crops systems during bloom (Janousek and Gubler, 2010), when honey bees are foraging, thus increasing the risk of exposure.

An obstacle in assessing pesticide risk is devising tests that examine effects of pesticide exposure that will apply to field conditions. Current testing standards prescribe maintenance of honey bees in caged laboratory conditions (OECD, 1998, 2017) over a maximum of ten days (OECD, 2017), with most Tier 1 studies focusing only on survival. The focus on survival may be problematic as many sublethal effects of pesticides have been documented, and these can have negative fitness effects on colonies (Desneux et al., 2007). Caging bees provides the benefit

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of protecting them from other potential environmental stressors, precise regulation of food composition and amount, and ease of observation (Table 1). However, caged bees are separated from the context of the hive and natural social interactions which may alter how sublethal effects influence fitness. Also, pesticides may interact with or exacerbate other environmental stressors in field realistic scenarios (Table 1). Colony-level, Tier 2, tests have revealed situations in which laboratory Tier 1 tests have not predicted serious negative effects, but the opposite result has also been found (Pilling et al., 2013; Henry et al., 2015; Wood and Goulson, 2017). Thus, testing procedures conducted in laboratory settings or field settings have different advantages and disadvantages (Table 1), and there have been insufficient comparisons to conclude whether standard laboratory toxicology testing focused on survival of caged bees generally provide good predictions of results in field colonies.

Pristine® fungicide is licensed for use on numerous bee pollinated crops during bloom (Janousek and Gubler, 2010; Rucker et al., 2019). The active ingredients of Pristine®, boscalid and pyraclostrobin, both act on fungal targets by inhibiting mitochondrial respiration (Avenot and Michailides, 2007). Laboratory testing procedures focused on short-term survival predict minimal adverse effects of Pristine® on honey bees as the oral LD_{50} value for boscalid is 1660,000 ppb, while that for pyraclostrobin is 731,000 ppb (EPA, 2013), compared to reported values in pollen collected from honey bee colonies of 1–5 and 1–12 ppb respectively, (Ostiguy et al., 2019), and values of 2000–24,000 ppb (2–24 ppm) in pollen collected from bees foraging on Pristine®--sprayed almonds (Fisher et al., 2021).

However, numerous laboratory studies have now found negative sublethal effects of Pristine® or its components at field-realistic levels. Consumption of pollen containing 2.3 ppm Pristine® by laboratory-caged honey bees reduced protein digestion and pollen consumption (DeGrandi-Hoffman et al., 2015). Pristine® inhibited isolated honey bee mitochondria at concentrations of 5 ppm and higher (Campbell et al., 2016). Consumption of boscalid reduced worker long-term survival (LC_{50} 1174 ppm, Simon-Delso et al., 2018) and flight performance (10 ppm in nectar, Liao et al., 2019). Pyraclostrobin consumption negatively affected bee midgut epithelial integrity and adult walking distance when fed to honey bee larvae at about 5 ppm (Tadei et al., 2019, 2020), damaged midgut epithelia and reduced lifespan when fed to adult stingless bees at 5 ppm and above (da Costa Domingues et al., 2020; Tadei et al., 2020) and reduced the height of mandibular gland secretory cells when fed to colonies of honey bees in pollen at 0.85 ppm (Zaluski et al., 2017). These findings suggest that there are numerous sublethal negative effects of exposure to Pristine® or its active ingredients.

There are currently no published data testing the effect of field-relevant doses of Pristine® on survival or pollen consumption of caged adult workers, and a key question for regulatory agencies is the applicability of short-term laboratory tests of toxicity real-world conditions, here the effect of Pristine® consumption across a range of field-relevant concentrations was measured using laboratory-caged adult workers.

2. Materials and methods

2.1. Worker collection and maintenance

Six brood frames were collected from three large, queen-right colonies at the Arizona State University Polytechnic Campus honey bee laboratory and placed in an incubator set at 34 °C and 90% relative humidity (rh). After 24 h, newly-emerged workers (1260) were collected and evenly distributed among 126 plastic mesh cylindrical cages (Fig. 1) in groups of 10 individuals. Each cage was supplied with ad libitum 30% sucrose solution in a 5 mL Eppendorf feeder tube inserted into a precut hole in a rubber stopper that closed off one end of a cage (Fig. 1). The sucrose solution was replaced every other day corresponding to the collection of data for pollen consumption and mortality.

2.2. Fungicide treatment

Each cage received 250 mg of a pollen (Bulk Foods, Toledo, OH) mixture incorporating one of five different concentrations of one of four treatment groups: the fungicide formulation Pristine® (BASF Corporation, Research Triangle Park, NC); the combined active ingredients of Pristine® (boscalid and pyraclostrobin) isolated from inert components; or each active ingredient as an individual treatment (Table 2). The fungicide concentrations ranged from an order of magnitude lower to mid-range of those measured in corbicular pollen for bees foraging in California almond orchards (Fisher et al., 2021). To test whether effects of Pristine® were due to individual, additive or synergistic effects of

Table 1
Differences in conditions and what can be measured between tests of chemical toxicity between lab tests with caged workers and colony field tests.

Treatment	Concentration (ppm)			Outcome comparisons		
	Boscalid	Pyraclostrobin	Inert ingredients	Average pollen consumption (g bee^{-1} day^{-1})	Survival (%)	Fungicide consumed (ng bee^{-1} day^{-1})
Pristine 0.575	0.15	0.07	0.365	8.8	94.7	5.06
Pristine 1.15	0.3	0.15	0.71	8.1	90	9.315
Pristine 2.3	0.6	0.3	1.4	8.5	93.4	19.55
Pristine 4.6	1.15	0.6	2.85	8.3	90	38.18
Pristine 9.2	2.3	1.15	5.75	8	88.5	73.6
Boscalid 0.15	0.15	0	0	9.1	89.7	1.365
Boscalid 0.3	0.3	0	0	7.9	91.8	2.37
Boscalid 0.6	0.6	0	0	8.8	83.6	5.28
Boscalid 1.15	1.15	0	0	9.7	89.6	11.155
Boscalid 2.3	2.3	0	0	9.3	**82**	21.39
Pyraclostrobin 0.07	0	0.07	0	10.5	93.2	0.735
Pyraclostrobin 0.15	0	0.15	0	8.7	98.3	1.305
Pyraclostrobin 0.3	0	0.3	0	**6.6**	98.3	1.98
Pyraclostrobin 0.6	0	0.6	0	**7.3**	94.9	4.38
Pyraclostrobin 1.15	0	1.15	0	**6.5**	93.3	7.475
Pyraclostrobin + Boscalid (P + B) 0.22	0.15	0.07	0	7.9	98.3	1.738
P + B 0.45	0.3	0.15	0	9.5	87.5	4.275
P + B 0.9	0.6	0.3	0	8.9	92.8	8.01
P + B 1.75	1.15	0.6	0	7.4	87.9	12.95
P + B 3.45	2.3	1.15	0	8	91	27.6
Control	0	0	0	9.4	96.6	N/A

Bolded numbers indicate pollen consumption or survival rates that differed significantly from the control.

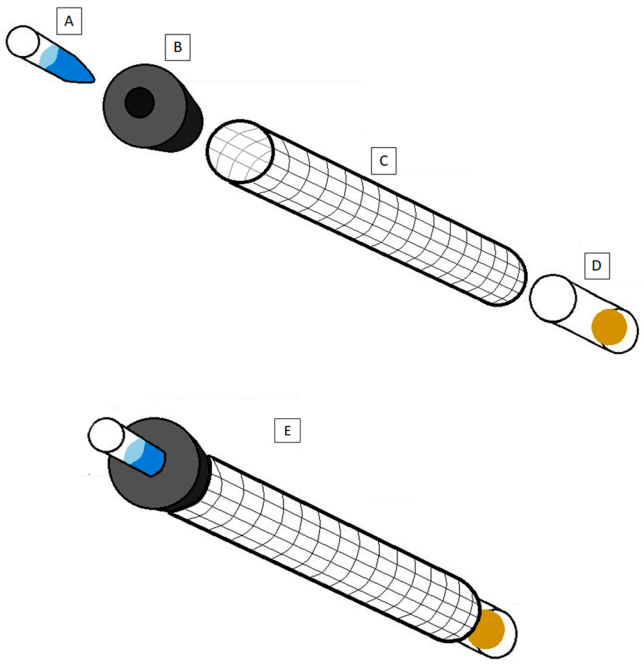

Fig. 1. Cage unit used to contain honey bees in vitro. (**A**) Bees could drink 30% sucrose solution through a couple of small holes drilled into a 5 mL Eppendorf tube. (B) Rubber stopper. (C) Plastic mesh cylindrical cage (3.8 cm diameter x 24.1 cm length). (D) Cut Falcon tube loaded with a pollen mixture containing a known concentration of Pristine®, boscalid, pyraclostrobin or pyraclostrobin + boscalid for treatment groups. Control cages were supplied with untainted pollen. (E) Fully assembled cage unit.

Table 2
Treatment concentrations used in this study.

	Testing conditions	What can be measured
Lab-rearing	workers, larvae, reproductives tested separately	individual survival, consumption, activity, behavior, physiology
	complete dietary control	
	control of temperature, humidity, bee age	
	easy to calculate dose from consumption of nectar or pollen	
Colony field test	simultaneous larval, adult and reproductive exposure	individual survival, consumption, activity, behavior, physiology
	exposure to colonial microbes	colonial growth, survival
	exposure to colonial parasites and pathogens (mites, viruses)	
	variable temperatures and humidities, at least for foragers	
	variable food resources	
	altered social interactions and behavior (e.g. trophallaxis)	
	biotic and abiotic stress synergisms	
	bees must perform taks in challenging conditions that may reveal sublethal weaknesses	
	colony-level stress responses possible	
	calculation of individual dosages more complex and less accurate	

For outcome comparisons, generalized linear models were used to assess pollen consumption and survival. Bolded numbers indicate pollen consumption or survival rates that differed significantly from the control.

boscalid and pyraclostrobin, the effects of Pristine® were compared to treatments with the identical concentration of just boscalid, just pyraclostrobin, or both (Table 2).

2.3. Experimental design

Thirty total cages (about 300 bees) were allocated to each treatment group which were further divided into five groups of six cages corresponding to different concentrations of a treatment group (about 60 bees in six cages consumed pollen containing a single concentration of fungicide). Six separate cages (60 bees) were controls that were also stocked with 250 mg of pollen containing no fungicide. The pollen mixture consisted of equal parts pollen, sucrose and dry baking fondant (each 30.6% of the mixture) added to deionized water (8.2%). For fungicide-treated groups the designated fungicide was dissolved in deionized water at the select concentration for incorporation into the pollen mixture. The pollen mixture was held in pre-weighed cut 50 mL Falcon tubes that were inserted into the end of a cage opposite the 5 mL Eppendorf feeder. Once stocked with bees, each cage was randomly placed in an incubator (34 °C, 75% relative humidity). Each container was resupplied with a fresh 250 mg load of its designated pollen mixture once a week to maintain freshness.

Cages were assessed for pollen consumption and mortality every other day for two weeks (7 total assessments). Pollen consumption rate of the cage was calculated by the change in mass of the tube+pollen (g), correcting for the empty weight of the container. Total pollen consumption was calculated as the sum of all pollen consumed by that cage over the 14 days of the experiment, so this parameter was affected by both individual pollen consumption and survival of bees in the cage. To assess individual pollen consumption rates, daily per worker pollen consumption was calculated approximately every two days from the mass of pollen consumed, divided by the number of live bees present in the cage, and by the number of days elapsed. Bees were considered deceased if they did not move and had their proboscis extended. Deceased individuals were removed from their cages to reduce the likelihood of disease-spread.

2.4. Statistical analysis

For testing the statistical significance of effects of the different treatments and concentrations on total pollen consumption, a Generalized Linear Model (GLM) was utilized with pollen consumption as a continuous parametric parameter. After running the full model, significant effects of treatment vs. control, and treatment concentration on pollen feeding within each treatment group were tested. Daily per worker pollen consumption was evaluated using repeated measures ANOVA, first testing a full model with all treatments, testing effects of treatment, day and concentration. Then significant effects of concentration for each treatment on daily pollen consumption at each time point were tested. For testing the statistical significance of effects of the different treatments and concentrations on survival, a GLM was utilized with individual survival as binomial data, testing the effect of treatment and concentration. After testing the full model, significant effects of treatment vs. control, and treatment concentration on survival within each treatment group were tested. In all cases when the effects of individual treatments (on pollen feeding or survival) were tested, a Bonferroni correction (0.05/5 treatments = 0.01 P value required for significance) was applied to reduce the chances of spurious significance. All data analyses were performed using JMP Pro 13 (SAS Inc., Cary, NC). Statistical significance was set at $\alpha = 0.05$ for all tests performed.

3. Results

3.1. Effect of fungicide treatments on total pollen consumption

Fungicide exposure significantly affected total pollen consumption (full model: $\chi^2 = 105.44$, $P < 0.0001$) with treatment ($\chi^2 = 104.77$, $P < 0.0001$) and concentration ($\chi^2 = 11.74$, $P = 0.0006$) exerting significant effects (Fig. 2A). Bees fed higher concentrations of pyraclostrobin had lower total pollen consumption ($\chi^2 = 24.73$, $P = 0.0002$)

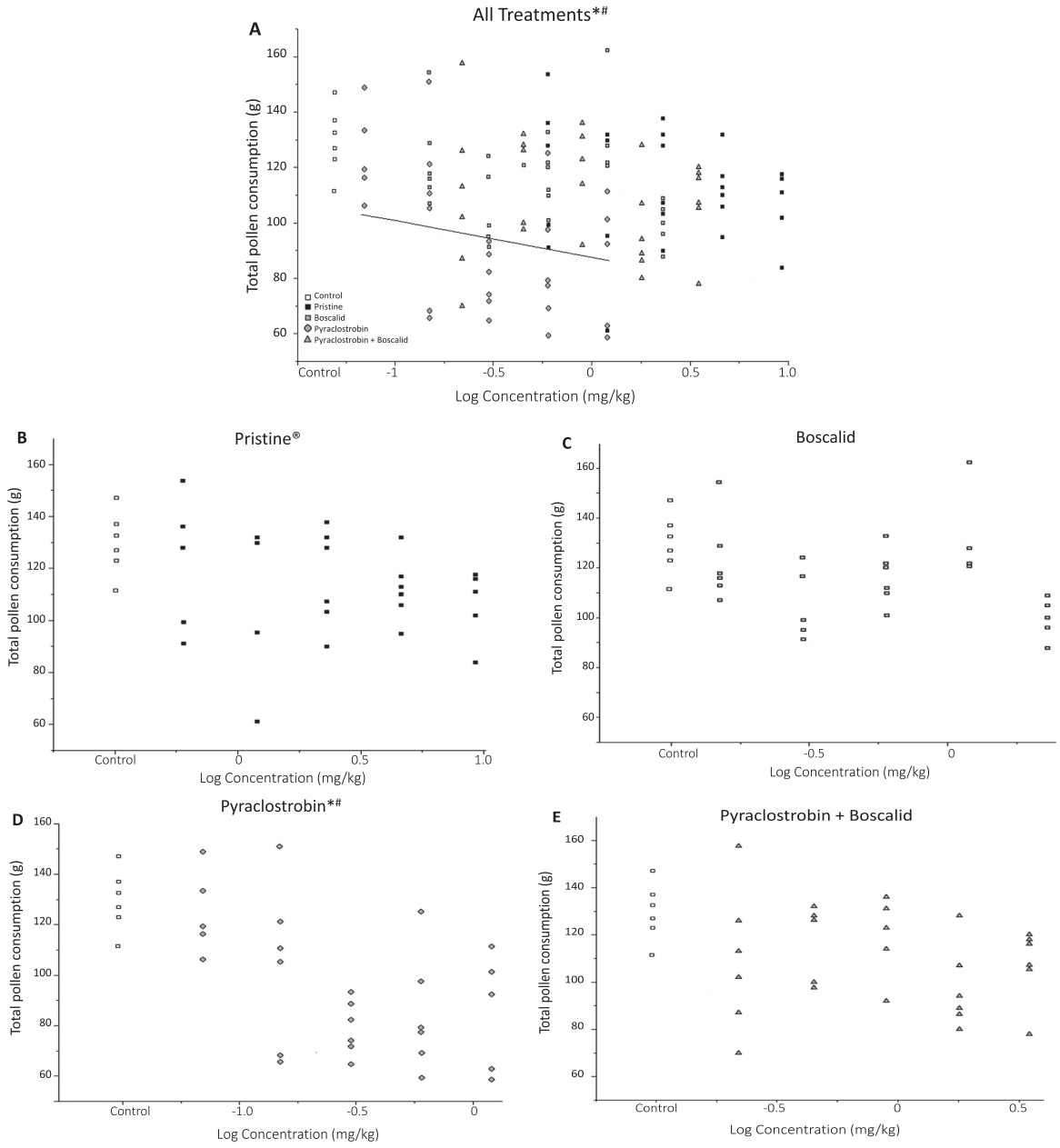

Fig. 2. Total pollen consumption for treatment groups assessed over a 14-day period. Where relevant "*" indicates a significant treatment effect and "#" indicates a significant concentration effect. (**A**) Overall fungicide exposure significantly affected pollen consumption ($\chi^2 = 105.44$, $P < 0.0001$) with concentration exerting a significant impact ($\chi^2 = 11.74$, $P = 0.0006$). Among the treatment groups only pyraclostrobin differed significantly from the control ($\chi^2 = 24.73$, $P = 0.0002$). (**B**) Pristine® treated groups did not differ significantly from the control; (**C**) boscalid treated groups did not differ significantly from the control; (**D**) pyraclostrobin treated groups differed significantly from the control; (**E**) pyraclostrobin + boscalid (P + B) treated groups did not differ significantly from the control.

(Fig. 2D). Pristine®, boscalid, and pyraclostrobin + boscalid (P + B) treated groups did not differ significantly from the control (Fig. 2B, C, E).

3.2. Effect of fungicide treatments on daily pollen consumption

Fungicide exposure (F = 2.25, $P = 0.004$) and day (F = 10.25, $P < 0.0001$) significantly impacted daily per worker pollen consumption with all experimental groups combined (Fig. 3A). Across the full experiment, bees fed pyraclostrobin had significantly lower daily per worker pollen consumption than did control bees (F = 17.20, $P < 0.0001$) (Fig. 3D). The Pristine®, boscalid, and P + B treatments did not significantly affect pollen consumption over the duration of the experiment (Fig. 3B, C, E). However, for several of the treatment groups (but not the controls), daily pollen feeding rate fell dramatically during days 12–14 (Fig. 3B–D). This drop in daily pollen feeding rate was significant for the Pristine® (treatment effect: $X^2 = 22.25$, $P < 0.0001$), boscalid (treatment effect: $X^2 = 30.41$, $P < 0.0001$) and pyraclostrobin (concentration effect: $X^2 = 8.18$, $P = 0.004$) treatment groups relative to the control.

3.3. Effect of fungicide treatments on honey bee worker survival

With all treatment groups combined, fungicide exposure also significantly affected honey bee survival (Fig. 4A, $\chi^2 = 17.83$, $P = 0.003$), with treatment exerting a significant effect ($\chi^2 = 14.80$, $P = 0.005$). Though there was a trend toward higher mortality at higher fungicide concentrations overall (Fig. 4A), this was not significant

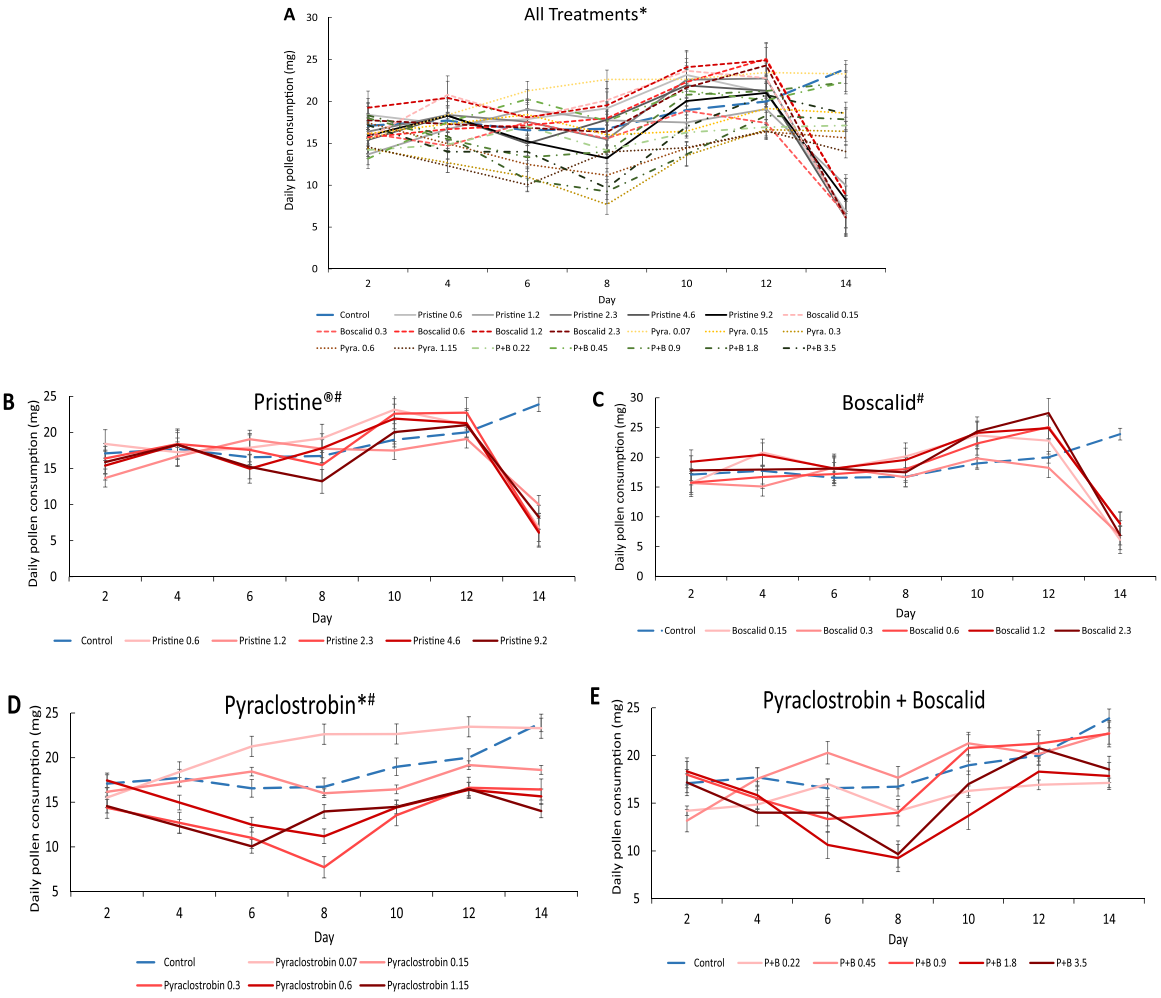

Fig. 3. Daily pollen consumption for treatment groups assessed over a 14-day period. For all graphs, means and standard errors are shown. Where relevant "*" indicates a significant treatment effect and "#" indicates treatment groups that underwent a significant reduction in pollen feeding between days 12 and 14 relative to the control. (**A**) Overall fungicide treatment ($F = 2.25$, $P = 0.004$) and day ($F = 10.25$, $P < 0.0001$) significantly affected daily pollen consumption. Among the treatment groups only pyraclostrobin differed significantly from the control ($F = 17.20$, $P < 0.0001$). (**B**) Pristine® treated groups did not differ significantly from the control; (**C**) boscalid treated groups did not differ significantly from the control; (**D**) pyraclostrobin treated groups differed significantly from the control; (**E**) pyraclostrobin + boscalid (P + B) treated groups did not differ significantly from the control.

overall ($\chi^2 = 2.50$, $P = 0.11$), or within any of the specific treatments (Fig. B-E). Bees fed boscalid-tainted pollen experienced significant increases in mortality ($\chi^2 = 10.56$, $P = 0.001$) (Fig. 4C). However, the Pristine®, pyraclostrobin, and P + B treatment groups did not significantly affect mortality (Fig. 4B, D, E).

4. Discussion

The most important finding is that effects of fungicides measured in the laboratory with caged adults differ from effects measured in intact colonies. Caged bees experienced reduced pollen consumption with fungicide treatment and concentration (Fig. 2), but with intact field colonies there were dose-dependent increases in pollen consumption (Fisher et al., 2021). With caged bees no effect of consuming pollen containing 2.3 ppm Pristine® was found on mortality, but strong effects in intact field colonies (Fisher et al., 2021). The opposing findings of this laboratory study and a previous field study may be accounted for by the social dynamics within the hive. Impairment of protein processing resulting from Pristine® consumption (DeGrandi-Hoffman et al., 2015) in larvae and adults may induce similar effects to food deprivation in larvae. Larvae deprived of food are known to release more of the pheromone E-β-ocimene stimulating more visits from nurse bees (He et al., 2016) and a higher ratio of feeding events (Huang and Otis, 1991). Such a perceived deficit in adequate larval provisioning may in turn result in greater adult consumption and collection of protein resources to compensate. This feedback loop is absent in laboratory conditions, potentially contributing to reduced pollen feeding among Pristine®-exposed bees. Inconsistencies between laboratory and field studies (Fisher et al., 2021) may necessitate approaches to toxicity assessments that account for the dynamics of social insect society.

Young honey bee workers consuming one of the isolated active ingredients of Pristine®, pyraclostrobin, reduced their pollen consumption over the experimental duration, and boscalid and Pristine® reduced daily per worker pollen consumption on days 12–14 (Table 2, Figs. 2D, 3D). Only boscalid increased mortality (Table 2, Fig. 4C). However, consumption of pollen containing Pristine® or a combination of pyraclostrobin plus boscalid did not significantly impact mortality. These outcomes suggest that the toxicity of boscalid and pyraclostrobin are tempered by inert ingredients in Pristine® and/or by interactions between the active ingredients. Possibly the inert ingredients of Pristine® confer some degree of protection by slowing the release of the active ingredients, or affecting rates of transport of the active ingredients into honey bee tissues. Several recent studies have reported toxic effects of the inert ingredients found in pesticides (Mullin et al., 2015; Fine et al., 2017). Because the inert ingredients in Pristine® are proprietary and unknown, the Pristine® and pyraclostrobin plus boscalid treatment

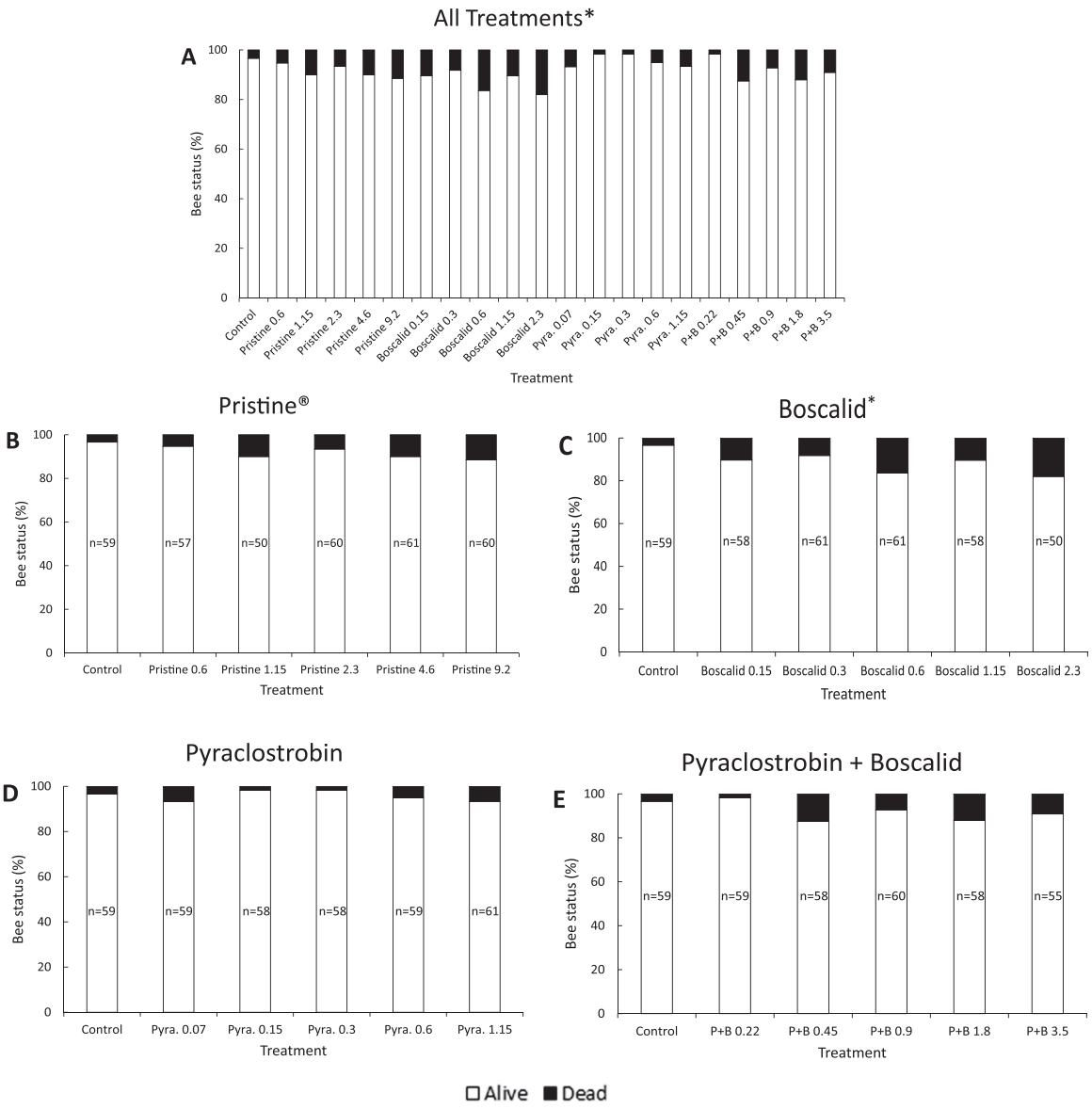

Fig. 4. Proportion of bees alive or dead for treatment groups assessed over 14 days. Where relevant "*" indicates a significant treatment effect. (**A**) Fungicide exposure significantly affected honey bee survival ($\chi^2 = 17.83$, $P = 0.003$). Among the treatment groups only boscalid differed significantly from the control ($\chi^2 = 10.56$, $P = 0.001$). (**B**) Pristine® treated groups did not differ significantly from the control; (**C**) boscalid treated groups differed significantly from the control; (**D**) pyraclostrobin treated groups did not differ significantly from the control; (**E**) pyraclostrobin + boscalid (P + B) treated groups did not differ significantly from the control.

groups were compared to assess if the inert ingredients of Pristine® are toxic, but the lack of great negative effects resulting from Pristine® consumption suggests that the inert ingredients did not enhance toxicity. A possible synergistic effect of the pyraclostrobin plus boscalid (P + B) treatment was expected, but no toxicity was detected (Figs. 2E, 3E, 4E). One possible explanation is that the combined active ingredients may have induced a hormetic physiological response in exposed bees that counteracted the impact of individual active ingredients. In rodents, mitochondrial inhibition has been observed to induce mitochondrial growth, reversing negative impacts on mitochondrial respiration (Tavallaie et al., 2020). Both pyraclostrobin and boscalid inhibit mitochondrial function in honey bees, and possibly increase mitochondrial proliferation (Campbell et al., 2016). Conceivably the combined active ingredients stimulated mitochondrial proliferation or other hormetic response that either active ingredient did not trigger individually, causing these more complex formulations to show less toxicity over the time period measured.

The finding that pyraclostrobin reduced pollen consumption (Table 2, Figs. 2D, 3D) may have resulted from midgut damage and altered consumption behavior. Pyraclostrobin damages midgut epithelia in honey bees (Tadei et al., 2020), which may affect feeding tendencies (Gregorc et al., 2018) and pollen-digesting capabilities (DeGrandi-Hoffman, 2015). Since pyraclostrobin is a mitochondrial poison, it is plausible that it reduces ATP production by epithelial tissue, impairing gut function and repair processes. It is also plausible that pyraclostrobin affects the concentration or composition of yeasts in the gut, with consequent effects on digestion. Reduced digestive rates can reduce feeding rates due to negative inhibition by gut stretch receptors in insects (Simpson and Bernays, 1983).

The finding that consumption of pollen containing boscalid reduced survival of workers (Table 2, Fig. 4C) differs somewhat from other studies, as some earlier reports indicated that LD_{50} doses for young bees in the laboratory were greater than 11 micrograms/bee (European Commission, 2008; Aubee and Lieu, 2010). Simon-Delso et al. (2018)

calculated LD_{50}s for boscalid consumed in sugar syrup of 300 micrograms bee^{-1} day^{-1} for 8 days of consumption, and 30 micrograms bee^{-1} day^{-1} for 25 days of consumption. Simon-Delso et al. (2018) examined boscalid exposure over a longer timeframe, and found that toxicity increased with bee age, suggesting that effects may accumulate over time. Data in this study did not permit calculation of LD50s, as there was no significant concentration effect ($\chi^2 = 2.50$, $P = 0.11$), likely because the range of concentrations used was relatively small. Bees consuming 2.3 ppm boscalid experienced a 15% drop in survival, corresponding to a dose of 21.4 ng (Table 2), much lower than previously reported. This could be due to the consumption of boscalid in pollen rather than nectar, the age of bees tested, or bee strain.

5. Conclusion

Current pesticide toxicity testing standards prescribe the maintenance of honey bee larvae or adult workers in laboratory conditions (OECD, 1998, 2013, 2016, 2017) to assess the effects of a chemical of interest, with colony-level tests only required when significant evidence of toxicity is found. Concentrations of Pristine® that reduce worker longevity and colony population levels in the field (Fisher et al., 2021) do not reduce survival in laboratory-caged workers, also concentrations that increased pollen consumption in the field (Fisher et al., 2021) reduced pollen consumption for caged bees in the laboratory. These differing outcomes indicate the importance of assessing toxicity and sublethal effects in field conditions in addition to laboratory assessments, even if laboratory tests do not reveal a significant hazard. Field-based assays allow for examination of aspects of pollinator biology not replicable in laboratory settings including colony fitness outcomes, which are the relevant fitness outcomes for social insects (Table 1). While laboratory tests with caged bees provide important benefits such as the ability to separately assess life stages and castes, and to carefully control experimental conditions, field, whole-colony assessments provide the critical benefits of factoring in other co-occurring environmental stressors and intra-colony dynamics (Table 1). With the demonstrated importance of larval health and signaling on adult behavior (He et al., 2016, Wang et al., 2016) these results suggest that toxicity assessments using intact colonies that allow occurrence of social interactions are required to more accurately characterize the impact of agrochemicals on honey bee health even when laboratory studies with caged bees indicate no effects on survival.

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CRediT authorship contribution statement

Adrian Fisher: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Teddy Cogley**: Investigation, Methodology, Data curation, Writing – review & editing. **Cahit Ozturk**: Investigation, Methodology, Data curation. **Gloria DeGrandi-Hoffman**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing. **Brian Smith**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing. **Osman Kaftanoglu**: Conceptualization, Funding acquisition, Methodology. **Jennifer H. Fewell**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing. **Jon F. Harrison**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All datasets generated for this study will be uploaded to Mendeley Data.

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