Science Bulletin xxx (xxxx) xxx

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Short Communication

Diamide insecticides impair honey bee queen reproduction and colony development

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A R T I C L E I N F O

Article history:
Received 8 November 2025
Received in revised form 29 November 2025
Accepted 9 December 2025
Available online xxxx

Bees are crucial pollinators that sustain natural ecosystems and agriculture [1]. Their global decline, driven by pathogens, climate change, habitat loss, and agrochemicals, threatens ecological stability and crop production [2,3]. In intensive farming systems, chemical pesticides can cause acute and chronic effects on bee physiology, behavior, and reproduction [4,5]. Although most studies focus on adult bee mortality, this metric alone does not account for colony collapse. The precise colony-level consequences of field-realistic pesticide exposure remain contentious. Diamide insecticides—such as chlorantraniliprole (Chl) and cyantraniliprole (Cya)—rank among the world's top-selling insecticides [6]. They act by activating the ryanodine receptor (RyR), a calcium-release channel on the endoplasmic reticulum membrane, leading to feeding cessation, muscle paralysis, and insect death [7]. While generally regarded as safe for bees and used to control hive pests, emerging evidences indicate that chronic Chl exposure can impair honey bee locomotion and queen development [8,9]. However, the colony-level impact of sublethal diamide exposure—particularly on queen reproduction—remains poorly understood. Here, we analyzed Chl and Cya residues in bee-related matrices across China and investigated how low-dose exposure affects *Apis mellifera ligustica* Spinola queen fertility. Through flow cytometry and field experiments, we uncovered a previously overlooked risk: diamide insecticides can impair queen reproduction and drive colony failure, providing critical insights into pesticide-bee interactions and for pollinator protection.

Samples from bees' colonies were collected and analyzed by mass spectrometry for their content in diamide insecticides (Table S1 online). From 2020 to 2022, Chl was frequently detected across various bee-associated matrices, with detection rates ranging from 20.8% to 50.0% at or above the limit of quantification (LOQ) (Fig. S1 online). In contrast, Cya was rarely detected (less than 1% above the LOQ, Fig. S1 online), although its increasing use in China raises growing concern regarding its potential effects on bees. This aligns with previous reports of diamide residues in pollen, wax, and honey [10], raising concerns about their long-term colony-level impacts. Acute toxicity assays revealed that Chl exhibited low toxicity to bee larvae (LD_{50} = 5.87 μg/larva), whereas Cya was significantly more toxic (LD_{50} = 0.22 μg/larva) (Table S2 online). Although the detected environmental concentrations of both insecticides were well below their respective LD_{50} values, their sublethal effects on bee development and reproduction remain unknown.

Following our established protocol (Fig. 1a), sublethal effects were assessed with queens reared from larvae fed diets containing environmentally relevant doses of Chl or Cya. Based on residue levels detected in royal jelly, two dose groups were selected: 7 and 70 ng per larva for Chl, and 0.2 and 2 ng per larva for Cya. No significant differences were observed in the initial body weight or thorax width of emerging queens between insecticide-treated and control groups (Table S3 online). Upon emergence, the reared queens were introduced into new colonies under field conditions, where they initiated matting flights and began oviposition within

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https://doi.org/10.1016/j.scib.2025.12.013

Please cite this article as: Y. Huang, Q. Gao, Q. He et al., Diamide insecticides impair honey bee queen reproduction and colony development, Science Bulletin, https://doi.org/10.1016/j.scib.2025.12.013

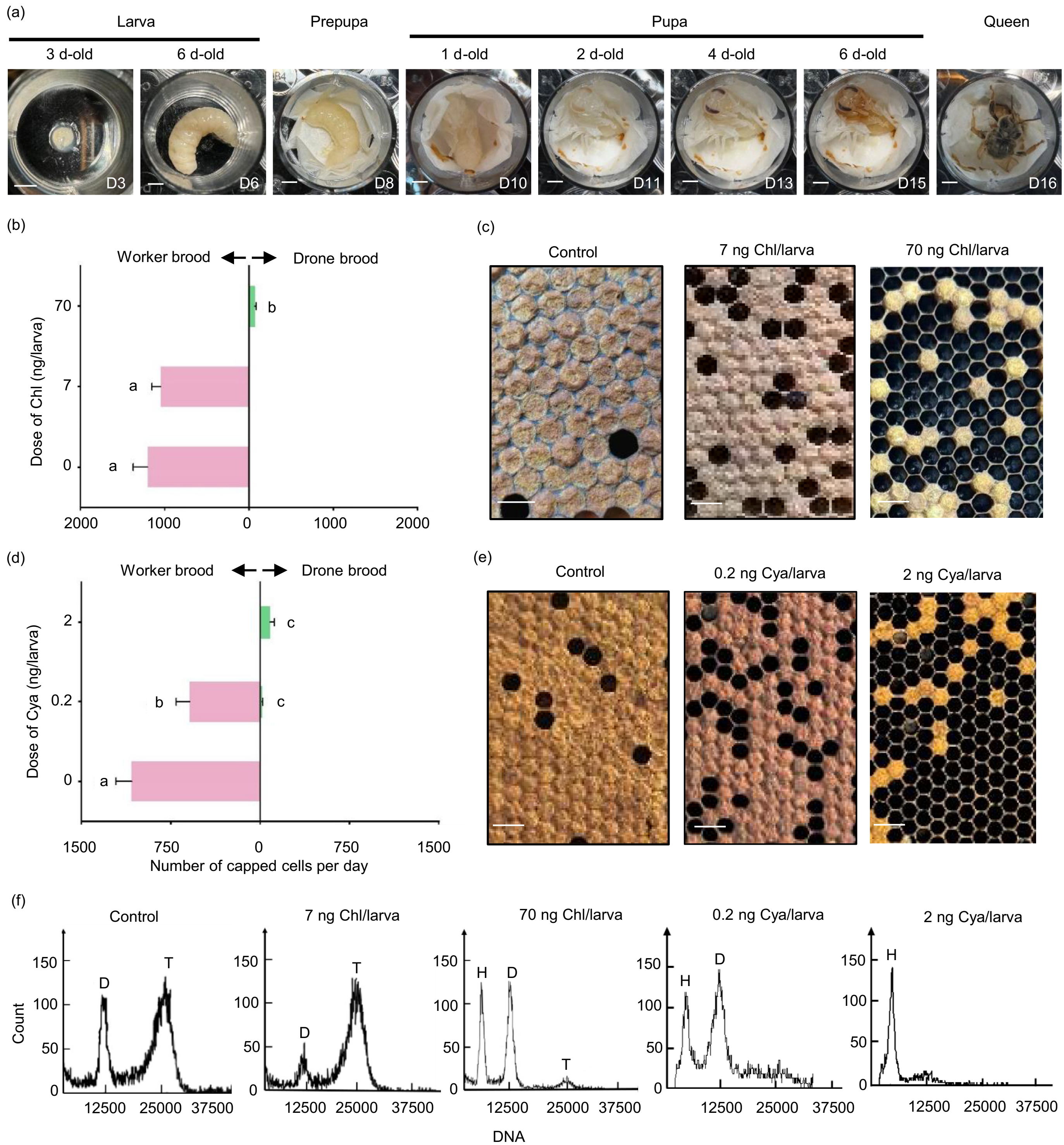
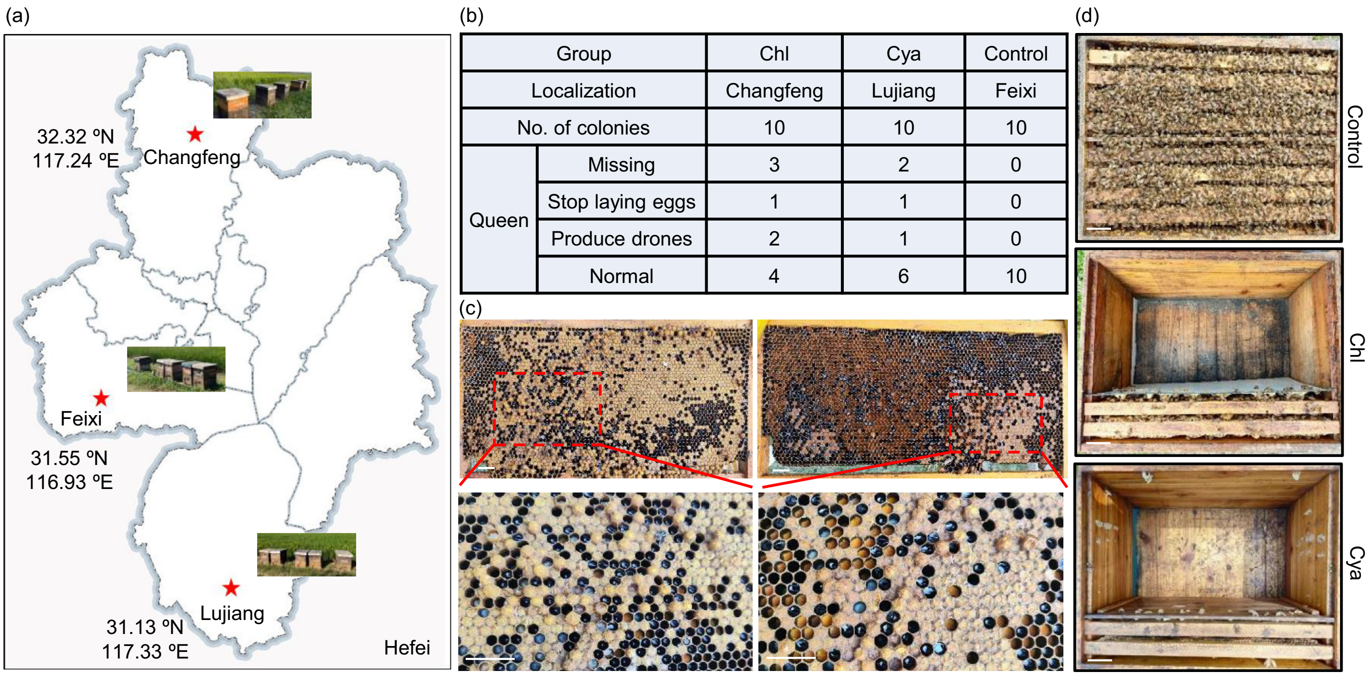


Fig. 1. Effects of diamide insecticides on honey bee queen reproductive output. (a) Schematic of the laboratory queen-rearing protocol. Scale bar: 2 mm. (b) Worker and drone brood production in colonies exposed to different doses of chlorantraniliprole (Chl). (c) Representative capped-brood patterns from colonies treated with acetone (control), 7 and 70 ng Chl/larva, respectively. Scale bar: 1 cm. (d) Worker and drone brood production in colonies exposed to cyantraniliprole (Cya). (e) Representive capped-brood patterns from colonies treated with acetone control, 0.2 and 2 ng Cya/larva, respectively. Scale bar: 1 cm. (f) Flow cytometric analysis of egg ploidy from colonies treated with acetone control, 7 ng Chl/larva, 70 ng Chl/larva, 0.2 ng Cya/larva, and 2 ng Cya/larva. Ploidy classifications: H, haploid; D, diploid; T, tetraploid. Data are represented as the mean ± SE (standard error) of three replicates, with different letters above bars indicating the significant differences. Statistical analysis was performed using one-way ANOVA followed by Tukey's test ($P < 0.05$).

seven days, similar to the control. However, queen fertility was significantly and dose-dependently reduced following exposure to Chl (Fig. 1b, c, $F = 27.8$, df = 8, $P = 0.001$) and Cya (Fig. 1d, e, $F = 29.4$, df = 11, $P < 0.001$). Unexpectedly, queens developed from larvae exposed to high doses (70 ng Chl/larva or 2 ng Cya/larva) produced only drone brood in worker combs—averaging 66.7 ± 20.5 and 80.3 ± 22.4 drones per day, respectively—whereas queens in the low-dose and control groups produced exclusively worker off-



Group		Chl	Cya	Control
Localization		Changfeng	Lujiang	Feixi
No. of colonies		10	10	10
Queen	Missing	3	2	0
	Stop laying eggs	1	1	0
	Produce drones	2	1	0
	Normal	4	6	10

Fig. 2. Field evaluation of diamide insecticide exposure on honey bee colonies. (a) Experimental locations (asterisks) in paddy fields near Hefei, Anhui Province, China. Five hives, each housing two colonies, were placed at different experimental sites. (b) Queen performance metrics under diamide exposure. (c) Drone brood production in worker cells by queens exposed to diamides. Scale bar: 2 cm. (d) Representative colony status across treatment groups at 60 d post-exposure. Scale bar: 4 cm.

spring (Fig. 1b–e). The sex of the brood was confirmed by analyzing the morphology of capped cells and identifying emerging adult bees (Fig. S2 online).

The possibility that the observed drone-only brood resulted from failed mating or sperm defects was excluded by conducting artificial insemination with healthy sperm collected from drones in insecticide-free colonies. Consistent with the results from naturally mated queens, artificially inseminated queens that had been exposed to diamide insecticides during larval development also produced only drones in worker combs (Fig. S3 online). These findings confirm that the reproductive impairment is not attributable to mating failure, but rather to the effects of diamide insecticide exposure.

Freshly laid eggs from treated and control queens were analyzed by flow cytometry to determine whether diamide insecticide exposure affects egg ploidy. Queens reared from larvae exposed to 7 ng Chl/larva produced diploid eggs that developed into worker bees, similar to the control group (Fig. 1f). In contrast, queens exposed to 70 ng Chl/larva produced only haploid eggs, which developed into drones. A similar dose-dependent effect was observed in queens exposed to Cya (Fig. 1f). These drone broods were confirmed to be laid by queens (Fig. S4a online), not by workers, as dissection revealed normal ovarian development in workers from both treated and control colonies (Fig. S4b online). To our knowledge, this is the first report linking diamide insecticides to drone-only egg-laying in honey bee queens. As social insects, bees rely on queens' single mating event, where sperm is stored in the spermatheca. Since fertilized eggs give rise to diploid females and unfertilized eggs develop into haploid males, the production of drone-only brood indicates that sperm was not successfully utilized during egg-laying. While the underlying mechanism remains to be fully elucidated, one plausible explanation is that RyR activation impairs neuromuscular control in the queen's reproductive system. Queens actively regulate whether an egg is fertilized prior to deposition into drone or worker cells [11]; disruption of this muscular control could prevent sperm release from the spermatheca, leading to exclusive production of unfertilized, haploid eggs. Given that diamides induce muscle paralysis via sustained calcium release from the sarcoplasmic reticulum [12,13], this mode of action is consistent with the observed reproductive failure.

Rice is a globally cultivated crop, and diamide insecticides are commonly applied in rice paddies. Honey bees forage on rice plants to collect pollen, and are realistically exposed to these insecticides [14,15]. To assess the colony-level impact of diamide insecticides under field conditions, Chl and Cya were applied to rice paddies at the recommended agricultural dosage, while untreated paddies served as controls (Fig. 2a). In each area, ten honey bee colonies were established, and bees were allowed to forage freely, collect pollen from treated or untreated rice plants. As expected, worker bee mortality remained low across all groups. However, within two weeks of exposure, insecticide-treated colonies exhibited a loss of queens, cessation of egg laying, or drone-only brood production (Fig. 2b, c). By the 60th day post-treatment, the treated colonies had collapsed and become vacant, while control colonies continued to develop normally (Fig. 2d). These results indicate that even field-relevant application rates of diamide insecticides can compromise queen reproductive capacity and ultimately lead to colony failure. While our findings may evoke comparisons to Colony Collapse Disorder (CCD), the diamide-induced effects constitute a separate and distinct phenomenon. These findings underscore that even insecticides deemed "low-risk" can pose hidden threats to pollinator health by compromising reproductive success rather than causing direct mortality.

In summary, our study reveals that exposure to Chl or Cya can impair queen reproductive function, leading to reduced worker production or complete drone-only broods and subsequent colony failure. Given the widespread use of diamide insecticides and their detection in bee-related matrices, their indiscriminate application may threaten both managed and wild bee populations. Although queen replacement may offer a potential mitigation strategy, our findings highlight the urgent need to reassess the ecological safety of diamide insecticides. This work suggests that insecticide development should incorporate the assessment of chronic effects on bees, and establishes a foundation for evaluating their broader ecological impacts on pollinator populations.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2023YFD1702100 and 2023YFD1701300), the National Natural Science Foundation of China (32572905, 32172460, 32230015, and 32021001), Earmarked Fund for China Agriculture Research System (CARS-44-KXJ10), and the New Cornerstone Science Foundation (NCI202328). We thank staff in the comprehensive stations of China Agriculture Research System (Bee) for assistance with the sample collection for pesticides residue analysis; Yifan Wu, Jiawei Huang, and Xiaoshi He for the help with maintaining bee colonies; Fengcheng Liang and Zhiping Yin for providing the facilities to perform field test.

Author contributions

Haiqun Cao and Sibao Wang conceived the study. Yong Huang, Quan Gao, Sibao Wang, and Haiqun Cao designed the research. Qibao He, Qiongqiong Liu, Tengfei Shi, Linsheng Yu, and Qing Yang collected samples from different comprehensive stations. Yong Huang, Quan Gao, Qibao He, Jinjing Xiao, and Min Liao assayed the field test. Qibao He, Jinjing Xiao, Qiongqiong Liu, Yanhong Shi, and Qing Yang performed the research in the laboratory. Yong Huang, Quan Gao, Qibao He, Jinjing Xiao, Yuying Liu, Yanhong Shi, and Yancan Wu analyzed the data. Yong Huang, Quan Gao, Qibao He, and Jinjing Xiao wrote the manuscript. Sibao Wang, Bruce D. Hammock, and Haiqun Cao revised the manuscript. All authors commented on the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2025.12.013.

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