



Greater wax moth control in apiaries can be improved by combining *Bacillus thuringiensis* and entrapments

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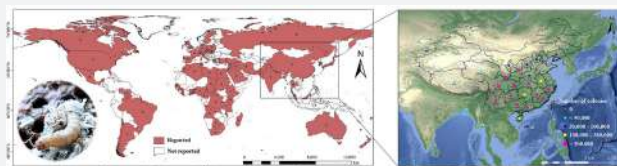
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Introduction

The GWM is a pervasive pest of honey bee colonies worldwide, especially *Apis cerana cerana*, a bee species indigenous to China, which is vulnerable to GWM. The larvae of GWM have destructive feeding habit and can feed on combs, causing significant damage to beehives and resulting in economic losses. At present, various methods have been proposed as alternatives for GWM control, and the use of biological agents, particularly toxins from the bacterium Bt, has emerged as a potential solution. Although the potential of Bt against GWM has been acknowledged, several issues hinder its widespread adoption. Thus, the application of Bt-based biological control of GWM in beehives is still rare.



Current damage status investigation of *Galleria mellonella*. a Worldwide distribution of the GWM. b Distribution of *A. cerana cerana* rearing colonies in China. Different colors or sizes indicated different population sizes.

Methods

Bioassay of Bt against GWM larvae: Using the self-established bioassay method, and spraying on or pressing into the comb to determine insecticidal activity of Bt to the GWM larvae. **Screening lures for the GWM larvae:** In the Y-type olfactometer, six choice tests were performed with the 1st - 2nd instar GWM larvae, including (i) dark comb vs. beeswax; (ii) dark comb vs. honey; (iii) dark comb vs. pollen; (iv) beeswax vs. honey; (v) beeswax vs. pollen; and (vi) pollen vs. honey.

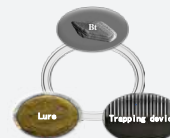
Indoor and field trapping experiments: Indoor experiments, two entrapment A (trapping device containing lure A + BiotGm) and entrapment B (trapping device containing lure B + BiotGm) were tested in pollination boxes, and GWM larval survival and comb loss were measured. Field tests used entrapment A in three *Apis cerana cerana* apiaries, to assess GWM damage for colonies and calculate the control efficacy of entrapment A against GWM.



Using the self-established bioassay method to determine acute oral toxicities of Bt to the GWM larvae.



Trapping device that prevents bees from contacting the lure.

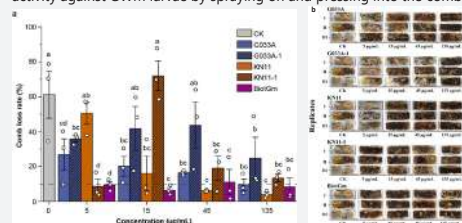


The materials and fabrication process for the preparation of biocontrol entrapment.

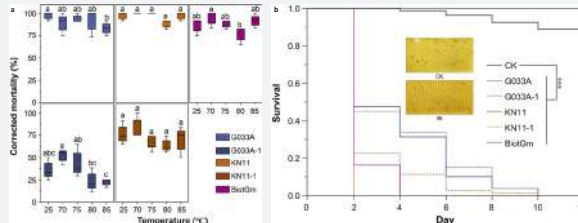
Results

BiotGm exhibits high insecticidal activity against GWM larvae

A novel Bt strain (designated BiotGm) exhibits high insecticidal activity against GWM larvae with a $LC_{50} = 1.772 \mu\text{g/g}$. Furthermore, BiotGm always showed high insecticidal activity against GWM larvae by spraying on and pressing into the comb.

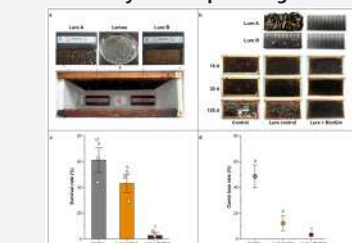


Activity of spraying Bt on comb against the GWM. a The loss rates (a) and actual destruction situation (b) of combs sprayed with 5, 15, 45, and 135 $\mu\text{g/mL}$ Bt solution and invaded for 4 weeks by 2nd instar GWM larvae.



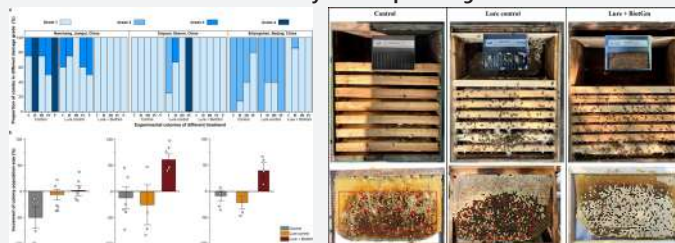
Activity of pressing Bt into the foundation against the GWM. a The effect of insecticidal activity of five Bt products after continuous heating at 25, 70, 75, 80, and 85 $^{\circ}\text{C}$ for 6 h. b The survival of 2nd instar GWM larvae exposed to the comb foundation containing Bt (4000 $\mu\text{g/g}$) for 12 days.

Indoor activity of entrapment against GWM



Indoor activity of the entrapment against GWM. a Setup for trapping device containing lure and schematic of the indoor trapping experiment. b Representative images of the attraction and control effects of entrapment after 120 days. c Survival of GWM larvae after 30 days. d The comb loss rate after 120 days employing entrapment.

Field biocontrol efficacy of entrapment against GWM



Field biocontrol efficacy of entrapment against GWM. a Proportion of comb in different GWM damage grades after finishing the trial in different test groups for three field sites. b Changes of population size before and after the trial in different test groups from three field sites. c Representative images of the attractive effect and control efficacy of entrapment in field conditions.

A new entrapment (lure for GWM larvae + BiotGm and matching a trapping device that prevents bees from contacting the lure) effectively controlled GWM larvae in both laboratory and field trials.

Conclusion

We design an entrapment method consisting of a lure for GWM larvae, a novel Bt strain BiotGm (with high insecticidal activity), and a trapping device that prevents bees from contacting the lure. All results find that this method reduces the population of GWM larvae in both laboratory and field trials, and the field control effectiveness of the entrapment against GWM was over than 93%.

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The greater wax moth (GWM), *Galleria mellonella* (Lepidoptera: Pyralidae), is a major bee pest that causes significant damage to beehives and results in economic losses. *Bacillus thuringiensis* (Bt) appears as a potential sustainable solution to control this pest. Here, we develop a novel Bt strain (designated BiotGm) that exhibits insecticidal activity against GWM larvae with a LC50 value lower than 2 µg/g, and low toxicity levels to honey bee with a LC50 = 20598.78µg/mL for larvae and no observed adverse effect concentration = 100 µg/mL for adults. We design an entrapment method consisting of a lure for GWM larvae, BiotGm, and a trapping device that prevents bees from contacting the lure. We find that this method reduces the population of GWM larvae in both laboratory and field trials. Overall, these results provide a promising direction for the application of Bt-based biological control of GWM in beehives, although further optimization remain necessary.