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## Synergistic action of *Pyemotes zhonghuajia* and *Beauveria bassiana* against *Aromia bungii*

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### Abstract

The red-necked longhorn beetle *Aromia bungii* is a destructive wood-borer difficult to control with insecticides. We evaluated a combined biocontrol strategy using the ectoparasitic mite *Pyemotes zhonghuajia* and the entomopathogenic fungus *Beauveria bassiana*. Fungal virulence was dose- and stage-dependent, causing up to 100% mortality at  $10^8$  conidia mL<sup>-1</sup> within 5–7 days. Mites alone induced limited mortality (~44% in young larvae, ≤7% in older ones), whereas conidia-carrying mites achieved complete kill within 7–8 days, comparable to direct fungal application. The combination significantly accelerated mortality ( $P < 0.05$ ), reflecting synergistic interaction between mite venom and fungal infection. These findings highlight the potential of *P. zhonghuajia* as both a parasitoid and a vector for *B. bassiana*, offering an innovative and eco-friendly approach to manage *A. bungii* and related xylophagous pests.

**Key words:** *Aromia bungii*; *Pyemotes zhonghuajia*; *Beauveria bassiana*; synergy; biological control

### Introduction

The red-necked longhorn beetle, *Aromia bungii* (Faldermann), is a destructive xylophagous pest of stone fruit trees (*Prunus* spp.) and an emerging invasive threat in Asia and Europe (Horrocks *et al.* 2024). Its cryptic larval development under bark hinders detection and renders conventional control strategies costly and inefficient. Chemical insecticides face constraints in controlling *A. bungii* due to its concealed life stages and growing concerns over resistance and non-target risks. Hence, developing environmentally sustainable alternatives is imperative. Entomopathogenic fungi are promising microbial agents for boring pests. *Beauveria bassiana* is widely applied against diverse insects, with spores that penetrate the cuticle and proliferate within hosts (Li *et al.* 2023; Zou 2020). Laboratory assays confirm its pathogenicity against *A. bungii*, achieving up to 70% larval mortality under optimal conditions (Shi *et al.* 2009). However, fungal infections often require several days to kill, and efficacy declines against older instars.

The ectoparasitic mite *Pyemotes zhonghuajia* Yu, Zhang & He represents a promising complementary biocontrol agent. This ovoviparous idiobiont native to China (Li *et al.* 2019, 2025), exhibits extraordinary lethality: a single female (~24 ng) can immobilize caterpillars weighing >680,000 times its body mass within hours (Chen *et al.* 2021). It has a broad host range across Lepidoptera and Coleoptera, including *Spodoptera frugiperda* and *Mythimna separata* (Tian *et al.* 2020a; Feng *et al.* 2022). Moreover, field deployments have successfully suppressed pests such as the fall armyworm in sorghum (Feng *et al.* 2022) and the potato tuber moth in storage (Feng *et al.* 2025; Liu *et al.* 2023). Beyond acute mortality, *P. zhonghuajia* imposes sublethal and transgenerational costs on survivors, reducing development, reproduction, and fitness (Liu *et al.* 2023; Song *et al.* 2022, 2024). Furthermore, recent molecular analyses reveal venom-induced immune and metabolic disruption, increasing host susceptibility to secondary pathogens (Song *et al.* 2024; Wang *et al.* 2025).

The integration of *P. zhonghuajia* with *B. bassiana* offers a novel route for managing *A. bungii*, combining rapid host paralysis with systemic fungal infection. Unlike systems involving predatory mites, which risk intraguild conflict (Cen *et al.* 2025), fungi represent compatible partners, and synergistic interactions between pathogens and parasitoids are increasingly recognized (Koller *et al.* 2023). Recent studies confirm that *P. zhonghuajia* can vector viable *B. bassiana* conidia, delivering lethal infections to wood-boring beetles with high efficacy (Wu *et al.* 2025). Moreover, its compatibility with entomopathogenic fungi has been demonstrated in storage systems without compromising mite performance (Song *et al.* 2024). Building on this foundation, we tested the hypothesis that conidia-carrying mites accelerate mortality of *A. bungii* larvae compared to either agent alone, providing the first evaluation of a mite–fungus complex for longhorn beetle management.

## Material and methods

### *Fungal isolate and insect colonies*

The entomopathogenic fungus *Beauveria bassiana* used in this study was isolated from *Aromia bungii* larvae naturally killed by white muscardine disease and collected in orchards of the Changli Institute of Pomology, Hebei Academy of Agriculture and Forestry Sciences (Hebei, China). The isolate was purified and identified by sequencing the ITS region prior to assays, and the sequence was assigned the GenBank accession number PX597163.

Larvae of *A. bungii* used in this study were collected from peach orchards in Shunping County, Hebei, China, in April 2024. Each larva was individually housed in a plastic centrifuge tube and maintained in an artificial climate chamber (PRX-250C, Ningbo Saifu Experimental Instrument Co., Ltd., China) at  $10 \pm 1$  °C and  $60 \pm 5\%$  relative humidity in darkness until use. For bioassays we used two larval size/age classes: “young larvae” (body length 1.0–1.5 cm) and “mid-to-late instars” (3.0–3.5 cm).

The colony of *P. zhonghuajia* was established in July 2020 from individuals collected in Ji Zhou, Tianjin, China, which were parasitizing adults of *Sinoxylon japonicum* on persimmon trees. The mites were maintained in climate chambers at  $25 \pm 1$  °C,  $60 \pm 5\%$  RH in darkness, and reared on mature 5th-instar larvae of *Sitotroga cerealella*.

## Bioassay procedures

### *Pathogenicity of B. bassiana conidial suspensions against A. bungii larvae*

A 200 µL aliquot of a  $1.0 \times 10^7$  conidia mL<sup>-1</sup> stock was evenly spread on quarter-strength SDAY plates and incubated at 27 °C for 15 days. Conidia were gently scraped and suspended in sterile 0.5% (v/v) Tween-80. The suspension was passed through a 200-mesh cell strainer to remove mycelial fragments. The filtrate was then adjusted to  $1.0 \times 10^8$ ,  $1.0 \times 10^7$ ,  $1.0 \times 10^6$ ,  $1.0 \times 10^5$  and  $1.0 \times 10^4$  conidia mL<sup>-1</sup> using 0.5% Tween-80 as diluent for dose–response bioassays.

Bioassays were performed separately on two distinct developmental stages of *A. bungii* larvae: young larvae and mid-to-late instars. For each stage, individual larvae were grasped with sterile forceps and immersed for 5 s in a given conidial suspension. Excess liquid was blotted off on sterile filter paper, and larvae were transferred one per well into disinfected 24-compartment plastic rearing boxes (single-well inner size 4.2 cm × 2.2 cm × 3.5 cm). Boxes were held in an climate chambers at  $25 \pm 1$  °C and  $60 \pm 5\%$  RH in the dark. Sterile 0.5% Tween-80 served as the negative control. Each treatment consisted of 20 larvae with three independent replicates (n = 60 per dose). Larval death, defined as no response to gentle probing with an insect pin, was recorded daily between 09:00 and 11:00 for 10 consecutive days. To confirm *B. bassiana* as the cause of death, larvae recorded as dead were subjected to observation for an additional 7 days to check for the emergence of typical *B. bassiana* mycelium.

### *Lethality of P. zhonghuajia carrying B. bassiana to A. bungii larvae*

Approximately 0.01 g of dry *B. bassiana* conidial powder was dusted evenly onto the bottom of a 12-cm Petri dish using a No. 0 camel-hair brush. Actively walking female *P. zhonghuajia* were transferred into the dish and allowed to walk for 1 h. Females whose bodies were uniformly dusted yet showed normal locomotion were selected for bioassays. The mean conidial load per female was  $\sim 1.03 \times 10^4$  conidia (estimated microscopically). One *A. bungii* larva was placed in a glass finger tube (17 × 80 mm). Depending on larval size class, either 2 or 5 female mites (young larvae) or 6 or 10 female mites (mid-to-late instars) were introduced per tube. Treatments included (i) mites without conidia, (ii) mites carrying conidia, plus two controls: (iii) blank control (no mites), and (iv) a positive fungal control in which larvae were treated with a  $1.0 \times 10^8$  conidia mL<sup>-1</sup> suspension (by 5-s immersion as above). Each treatment had 15 tubes and was replicated three times (n = 45 larvae per treatment). Tubes were stoppered with cotton and incubated at 25

$\pm 1$  °C and  $60 \pm 5\%$  RH in darkness. Mortality and confirmation for fungal outgrowth followed the same protocol as described above.

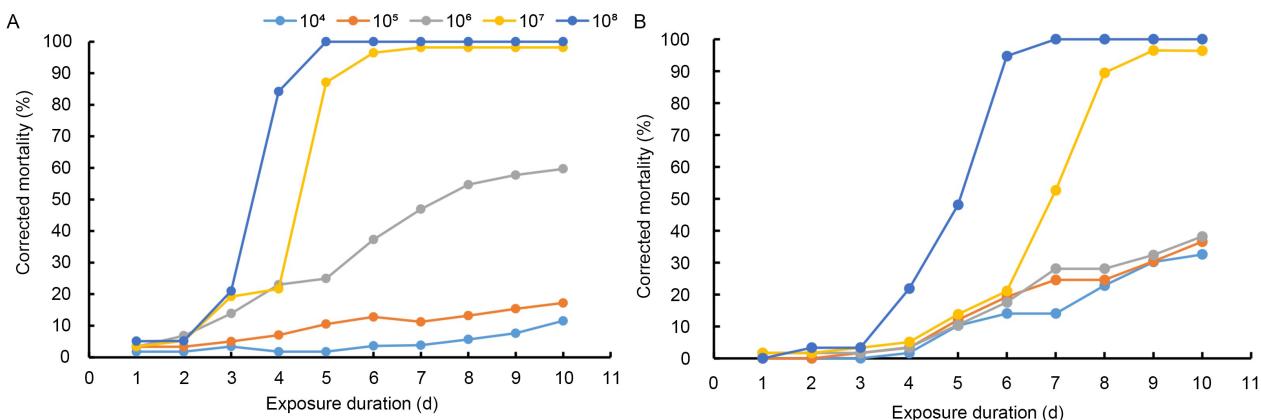
#### Data analysis

Raw data were collated in Microsoft Excel. The mortality rate ( $MR$ ) for each replicate was calculated as  $MR(\%) = (N_D/N_T) \times 100$ , where  $N_D$  is the number of dead larvae and  $N_T$  is the total number of larvae inoculated. The corrected mortality rates ( $CMR$ ) were calculated as  $CMR(\%) = [(MR_T - MR_C)/(100 - MR_C)] \times 100$ , where  $MR_T$  is the mortality rate of treatment group and  $MR_C$  is the mortality rate of control group. Data were then analyzed in SPSS v22.0 (IBM Corp., Armonk, NY, USA). Treatment means were compared using Duncan's new multiple range test (DMRT) at  $\alpha = 0.05$ . Percentage data were checked for assumptions prior to analysis; if needed, arcsine square-root transformation was applied, while untransformed means are reported.

## Result

### Mortality of *Aromia bungii* larvae exposed to *Beauveria bassiana*

Across five doses ( $10^4$ – $10^8$  conidia  $\text{mL}^{-1}$ ), Abbott-corrected cumulative mortality increased with both exposure time and conidial concentration, with clear instar-specific differences (Fig. 1A). For young larvae, the highest dose ( $10^8$  conidia  $\text{mL}^{-1}$ ) produced a rapid rise in mortality: 20.99% on day 3, 84.21% on day 4, and 100% by day 5. At  $10^7$  conidia  $\text{mL}^{-1}$ , mortality reached 87.10% on day 5 and plateaued at ~98% by days 7–10. Doses  $\leq 10^6$  conidia  $\text{mL}^{-1}$  remained  $\leq 60\%$  by day 10. For mid-to-late instars, mortality rose more slowly at the same doses (Fig. 1B). At  $10^8$  conidia  $\text{mL}^{-1}$ , cumulative mortality was 3.33% on day 3, 21.84% on day 4, 48.07% on day 5, 94.74% on day 6, and 100% on day 7. At  $10^7$  conidia  $\text{mL}^{-1}$ , mortality did not exceed ~53% until day 7 and approached ~96% by days 9–10. Doses  $\leq 10^6$  conidia  $\text{mL}^{-1}$  stayed  $\leq 38\%$  by day 10.



**Figure 1.** Cumulative corrected mortality of *Aromia bungii* larvae exposed to different concentrations of *Beauveria bassiana* conidial suspensions. **A**, young larvae (body length 1.0–1.5 cm); **B**, mid-to-late instars (3.0–3.5 cm).

#### Median lethal time

Probit regressions showed a strong, monotonic dose effect on killing speed (Table 1). For young larvae,  $LT_{50}$  values (95% CI) shortened from 8.08 d (7.22–9.04) at  $10^6$  to 3.40 d (2.57–4.49) at  $10^7$  and 2.33 d (1.39–3.91) at  $10^8$  conidia  $\text{mL}^{-1}$ . However, at the two lowest concentrations, efficacy dropped markedly. The  $LT_{50}$  was 105.52 days (53.16–209.44) at  $10^5$  conidia  $\text{mL}^{-1}$ , and at  $10^4$  conidia  $\text{mL}^{-1}$ , the model extrapolated an impractically high  $LT_{50}$  of 1061.48 days (39.06–28847.00) with an extremely wide confidence interval, indicating that median mortality was not achievable within the experimental period. For mid-to-late instars,  $LT_{50}$  similarly decreased from 16.84 d (10.45–27.14) at  $10^6$  to 5.83 d (4.27–7.95) at  $10^7$  and 3.73 d (2.88–4.83) at  $10^8$  conidia  $\text{mL}^{-1}$ . Notably, at the lower concentrations ( $10^4$  and  $10^5$  conidia  $\text{mL}^{-1}$ ), the  $LT_{50}$  values were 11.17 days (7.94–15.72) and 10.05 days (7.88–12.80), respectively, which were substantially shorter than those for young larvae at the same doses, suggesting a different dose-sensitivity relationship between the two stages at sub-lethal concentrations.

All probit regressions showed acceptable fits ( $r = 0.76$ – $0.98$ ). Collectively, the results demonstrate that higher dose and younger instar both substantially accelerated mortality, while the dose-response relationship at very low concentrations appears more complex and stage-specific.

**Table 1.** Median lethal time ( $LT_{50}$ ) of *Beauveria bassiana* conidial suspensions against larvae of *Aromia bungii* at different concentrations.

Treatment stage	Conidial concentration (conidia $mL^{-1}$ )	Probit regression equation	Correlation coefficient ( $r$ )	$LT_{50}$ (days)	95% confidence interval (days)
Young larvae	$10^4$	$Y=0.76X+2.70$	0.7558	1061.48	39.06-28847.00
	$10^5$	$Y=0.99X+3.01$	0.9560	105.52	53.16-209.44
	$10^6$	$Y=2.25X+2.96$	0.9844	8.08	7.22-9.04
	$10^7$	$Y=4.95X+2.37$	0.8942	3.40	2.57-4.49
	$10^8$	$Y=7.94X+2.08$	0.7845	2.33	1.39-3.91
Mid-to-late larvae	$10^4$	$Y=5.68X-0.95$	0.9179	11.17	7.94-15.72
	$10^5$	$Y=5.27X-0.28$	0.9494	10.05	7.88-12.80
	$10^6$	$Y=2.16X+2.35$	0.9074	16.84	10.45-27.14
	$10^7$	$Y=4.28X+1.72$	0.8555	5.83	4.27-7.95
	$10^8$	$Y=9.57X-0.47$	0.9090	3.73	2.88-4.83

#### *Lethality of Pyemotes zhonghuajia carrying B. bassiana conidia*

Dusting female *P. zhonghuajia* for 1 h yielded an average load of  $\sim 1.03 \times 10^4$  conidia per mite. Mites alone caused modest mortality. In young larvae, peaks were  $\sim 44\%$  for both 2-mite (day 5) and 5-mite (day 6) treatments (Table 2). In mid-to-late instars, mortality stayed  $\leq 7.14\%$  across 10 days even at 10 mites per tube. Conidia-carrying mites markedly improved kill relative to mites alone. In young larvae, “2 mites + conidia” rose from negligible levels on days 1–3 to 68.89% on day 5, 93.33% on day 6, and 100% on day 7; “5 mites + conidia” reached 80.00% on day 4 and 100% on day 7. In mid-to-late instars, “6 mites + conidia” reached 46.67% on day 5 and 100% on day 7, while “10 mites + conidia” climbed to 95.56% on day 7 and 100% on day 8. Compared to the positive fungal control ( $10^8$  conidia  $mL^{-1}$  immersion), conidia-bearing mites achieved comparable terminal mortality in mid-to-late instars (100% by day 7–8), but for young larvae the immersion treatment reached 100% one–two days earlier (day 5 vs. day 7). Thus, mite-mediated delivery narrows the performance gap relative to direct immersion, especially for older larvae, while still outperforming mites alone by a wide margin.

## Discussion

The potential of entomopathogenic fungi and parasitic mites has been reported for controlling various boring and defoliating pests, including *Aromia bungii* and other coleopteran or lepidopteran species (Chen *et al.* 2021; Feng *et al.* 2022; Shi *et al.* 2009; Song *et al.* 2024; Wang *et al.* 2025). However, this study is the first to evaluate the compatibility of the ectoparasitic mite *P. zhonghuajia* with the entomopathogenic fungus *B. bassiana* against larvae of *A. bungii*. Although both agents alone suppressed larval survival, only the combined use of conidia-carrying mites produced accelerated mortality across larval stages and achieved control levels comparable to high-dose fungal immersion. These findings indicate that mite-mediated fungal delivery can enhance fungal infection without compromising mite performance, highlighting a novel avenue for integrated management of longhorn beetles.

Entomopathogenic fungi such as *B. bassiana* are well-established biocontrol agents against coleopteran borers, with conidia penetrate the cuticle and cause systemic mycosis (Inglis *et al.* 2001; Zou 2020). Previous bioassays with *A. bungii* demonstrated that fungal infection can achieve up to 70% mortality, although killing typically requires several days and efficacy decreases against older larvae (Shi *et al.* 2009; Horrocks *et al.* 2024). Our results align with and extend these findings: mortality was dose-dependent and significantly higher in younger instars, with  $LT_{50}$  values decreasing from over eight days at  $10^6$  conidia  $mL^{-1}$  to just over two days at  $10^8$  conidia  $mL^{-1}$ . In contrast, mid-to-late instars were more tolerant, requiring higher doses and longer exposure to reach comparable levels of mortality. These findings confirm that *B. bassiana* alone can provide substantial control, but also underline the need for complementary tactics to overcome stage-specific limitations.

**Table 2.** Corrected mortality (%) of *Aromia bungii* larvae under treatments with *Pyemotes zhonghuaijia, Beauveria bassiana*, and their combination.

Treatment day	Young larvae		Mid-to-late larvae						
	2 mites/tube	2 mites + fungus	5 mites/tube	5 mites + fungus	Fungus only	6mites/tube	6mites + fungus	10 mites/ tube	10 mites + fungus
Day 1	2.22±2.22Bc	4.44±2.22ABd	17.78±5.88Ac	8.89±5.88ABd	4.44±2.22ABd	0.00±0.00Ba	0.00±0.00Bd	0.00±0.00Bb	4.44±2.22Ad
Day 2	15.56±2.22Bbc	4.44±2.22Cd	24.44±2.22Abc	15.56±2.22Bcd	11.11±2.22BCd	0.00±0.00Ba	0.00±0.00Bd	0.00±0.00Bb	6.67±3.85Ad
Day 3	28.89±4.44Aab	6.67±3.85Bd	33.33±3.85Aabc	24.44±2.22Ac	22.22±2.22Ac	0.00±0.00Aa	0.00±0.00Ad	0.00±0.00Ab	6.67±3.85Ad
Day 4	37.78±4.45Bab	35.56±8.01Bc	33.33±3.85Babc	80.00±10.18Ab	84.44±8.01Ab	0.00±0.00Ba	4.44±2.22Bd	0.00±0.00Bb	26.67±7.70Ac
Day 5	44.44±8.01Ca	68.89±9.69Bb	33.33±3.85Cabc	93.33±3.85Aa	100±0.00Aa	0.00±0.00Ba	46.67±19.25Ac	0.00±0.00Bb	53.33±7.70Ab
Day 6	44.44±8.01Ba	93.33±3.85Aa	44.44±5.88Ba	93.33±3.85Aa		6.67±3.85Ca	68.89±9.69Bb	0.00±0.00Cb	60.00±3.85Bb
Day 7	41.43±9.51Ba	100±0.00Aa	42.06±4.83Bab	100±0.00Aa		6.67±3.85Ba	100±0.00Aa	0.00±0.00Cb	95.56±2.22Aa
Day 8	38.64±9.54Aab		39.19±5.40Aab			6.67±3.85Ba		6.67±3.85Ba	100±0.00Aa
Day 9	37.55±8.93Aab		37.36±7.20Aab			6.83±3.85Aa		6.83±3.85Aa	
Day 10	37.55±8.93Aab		37.36±7.20Aab			7.14±4.13Aa		7.14±4.13Aa	
								0.00±0.00Bb	

Values are mean  $\pm$  SE. Different uppercase letters within a row indicate significant differences among treatments on the same day ( $P < 0.05$ ). Different lowercase letters within a column denote significant differences across days for the same treatment ( $P < 0.05$ ). Control group mortality remained low: young larvae, 11.11% at day 10 (first death at day 7); mid-to-late instar larvae, 8.89% at day 10 (first death at day 9).

The ectoparasitic mite *P. zhonghuajia* represents a distinctive biocontrol agent distinguished by its extraordinary killing efficiency and broad host range. Previous studies demonstrated that a single female mite can immobilize and kill lepidopteran larvae many orders of magnitude larger than itself within hours (Chen *et al.* 2021; Tian *et al.* 2020a). Field and storage trials further confirmed its practical utility, with significant suppression of fall armyworm in sorghum fields and potato tuber moth populations in stored tubers (Feng *et al.* 2022; Liu *et al.* 2023). In our assays with *A. bungii*, mites alone caused modest mortality in young larvae (~44% by day 5), but were substantially less effective against mid-to-late instars ( $\leq 7\%$  mortality). This pattern highlights both the strength and the limitation of mite parasitism: rapid host paralysis through venom injection ensures immediate suppression of vulnerable instars, yet older larvae enclosed within woody tissues appear less susceptible. These findings are consistent with recent work showing stage-specific differences in host susceptibility and emphasize the need to pair *P. zhonghuajia* with complementary agents to extend its control potential (Song *et al.* 2024; Wang *et al.* 2025).

When *B. bassiana* and *P. zhonghuajia* were applied in combination, mortality of *A. bungii* larvae increased substantially compared to either agent alone, with near-complete kill achieved within 7–8 days across instars. This additive to synergistic interaction likely reflects their complementary modes of action: mites rapidly paralyze hosts, thereby reducing defensive responses and creating opportunities for fungal penetration, while fungal infection compensates for the mites' reduced efficacy against larger or more protected larvae. Such functional complementarity has been documented in other systems where entomopathogenic fungi act synergistically with arthropod natural enemies to enhance suppression of concealed pests (Koller *et al.* 2023; Roy & Pell 2000; Zimmermann 2007). Critically, unlike intraguild interactions between predatory insects and *P. zhonghuajia* that may result in antagonism (Cen *et al.* 2025), fungi do not represent hosts or competitors, reducing the risk of interference. Our findings align with recent evidence that *P. zhonghuajia* can vector viable *B. bassiana* conidia (Wu *et al.* 2025), positioning the mite as both a direct parasitoid and a delivery vehicle for microbial inoculum. This dual role broadens the ecological niche of the mite–fungus complex and underscores its potential as an innovative tool for integrated management of invasive wood-boring beetles.

This study demonstrates that combining the ectoparasitic mite *Pyemotes zhonghuajia* with the entomopathogenic fungus *Beauveria bassiana* offers a powerful new strategy for managing the red-necked longhorn beetle *Aromia bungii*. While *B. bassiana* provided dose-dependent mortality and *P. zhonghuajia* caused rapid but stage-limited suppression, their combination achieved near-complete larval mortality within 7–8 days, underscoring functional synergy between fast-acting venom and systemic infection. These results, though obtained under laboratory conditions, highlight the promise of cross-guild biocontrol integration. Future work should validate efficacy in orchards, assess ecological safety, and clarify mechanistic interactions to guide scalable integrated pest management.

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