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Dynamic Changes in the Number of Entomopathogenic Fungal Spores Carried by *Pyemotes zhonghuajia*

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Abstract [**Objectives**] This study aimed to quantitatively investigate the dynamic patterns of spore powder of *Metarhizium anisopliae* and *Beauveria bassiana* carried on the body surface of *Pyemotes zhonghuajia*, providing a theoretical basis for optimizing synergistic biological control strategies utilizing natural enemies and pathogens. [**Methods**] Under laboratory conditions, the spore load on mites after crawling on a spore-powder-coated surface for different durations (0 – 4 h), and the spore shedding after crawling on a clean surface for different durations (0 – 4 h) post-loading were measured. [**Results**] The spore load of mites for both fungi increased with crawling time, but exhibited distinct kinetic patterns. The load of *M. anisopliae* spores peaked at 2 h (6.86×10^4 spores/mite) and then decreased significantly; whereas the load of *B. bassiana* spores continued to increase to a maximum (12.83×10^4 spores/mite) within 4 h, with the rate of increase slowing significantly after 2 h. During the shedding phase, the number of both types of spores decreased with crawling time. After 4 h, the residual amount of *B. bassiana* spores (3.18×10^4 spores/mite) was significantly higher than that of *M. anisopliae* (2.51×10^4 spores/mite). [**Conclusions**] The process of *P. zhonghuajia* carrying entomopathogenic fungal spores exhibits significant temporal dynamics and species specificity. The findings identify key time points for spore loading and shedding, providing crucial parameters for determining the optimal pre-release "loading" duration and assessing the mites' sustained dispersal capacity in the field, which holds significant importance for advancing the application of synergistic biological control technology.

Key words *Pyemotes zhonghuajia*, Entomopathogenic fungi, Vector behavior, Biological control, Dynamic changes

0 Introduction

Biological control is a core strategy in Integrated Pest Management (IPM), highly valued for its environmental friendliness and strong target specificity. *Pyemotes zhonghuajia* is a widely distributed ectoparasitic natural enemy in China with considerable application potential[1]. Despite the small size of its adult females, measuring only 200 – 300 μm in length[1], this mite possesses remarkable lethality, capable of killing hosts hundreds of thousands of times its own body weight[2 – 3], with a host range spanning several important orders including *Coleoptera*, *Lepidoptera*, *Hymenoptera*, and *Hemiptera*[4]. Field release practices have demonstrated that *P. zhonghuajia* achieves control efficacy exceeding 90% against various wood-boring pests such as *Anoplophora glabripennis*, *Cryptorrhynchus lapathi*, *Laspeyresia* sp., and *Dioryctria* sp., showcasing its outstanding pest control capabilities[5 – 6]. Concurrently, representative entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* also play significant roles in controlling agricultural and forestry pests[7].

In recent years, synergistic biological control strategies combining natural enemies with entomopathogenic fungi have gained considerable favor[8]. Studies have reported that combining *P. zhonghuajia* with entomopathogenic fungi can significantly enhance pest control efficiency. Utilizing *P. zhonghuajia* as a vector to disseminate these fungi leverages its host-searching behavior to deliver spores precisely to concealed pest locations that are difficult for spray applications to reach[9], and can also disrupt the host cuticle or immune system during parasitism, thereby significantly enhancing fungal infection efficiency[10 – 12]. However, for the successful application of this synergistic strategy in large-scale field practice, it is essential to clarify the core biological processes of the mite acting as a vector. A key question pertains to the dynamic patterns of entomopathogenic fungal spore acquisition and shedding by the mite.

This study addresses this gap by aiming to investigate the dynamic process of interaction between *P. zhonghuajia* and spore powders of *M. anisopliae* and *B. bassiana*. By elucidating the relationship between the number of entomopathogenic fungal spores carried by the mite and time, we provide crucial theoretical foundations and data support for evaluating its field dispersal efficiency and optimizing release strategies, such as determining the optimal pre-release "carrying" time. This will facilitate the innovation and development of synergistic natural enemy-pathogen biological control technologies.

1 Materials and methods

1.1 Experimental materials *Pyemotes zhonghuajia*, collected in 2020 from persimmon trees infested by *Sinoxylon japonicum* Lesne (Coleoptera: Bostrichidae) in Jizhou, Tianjin, China, and subsequently reared in the laboratory using larvae of the *Angoumois* grain moth (*Sitotroga cerealella*) as hosts; spore powder (80 billion spores/g) of *M. anisopliae* strain CQMa421 was purchased from Chongqing Julixin Biological Engineering Co., Ltd.; *B. bassiana* strain Bb-1 was isolated and identified from field-

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collected larvae of the peach longhorn beetle (*Aromia bungii*) killed by the fungus in Hebei Province, and its spore powder was produced by culturing on 1/4 SDAY medium in the laboratory.

1.2 Experimental methods

1.2.1 Detection of spore loading dynamics on *Pyemotes*. First, 0.01 g of *M. anisopliae* or *B. bassiana* spore powder was sprinkled into separate 12 cm diameter Petri dishes. The powder was shaken and then evenly spread using a fine, soft brush. Crawling female mites were then transferred onto the spore-coated surfaces. At intervals of 10 min, 30 min, 1 h, 2 h, 3 h, and 4 h post-transfer, crawling mites were collected and placed into 200 μL microcentrifuge tubes. Each tube, pre-filled with 100 μL of 0.1% Tween-80 solution, received 10 mites. Each treatment was replicated three times. The number of spores was observed and recorded under a microscope using a hemocytometer, and the number of spores carried per mite was calculated.

1.2.2 Detection of spore shedding dynamics from *Pyemotes* body surfaces. Based on the loading test results, a crawling duration of 2 h on the spore-coated surface was selected as the initial spore loading time for *Pyemotes*. Mites loaded with spores were then transferred onto six clean 9 cm Petri dishes (50 mites per dish) and allowed to crawl freely. Samples were taken at 10 min, 30 min, 1 h, 2 h, 3 h, and 4 h after transfer onto the clean surfaces. Each sample consisted of 10 mites placed into 100 μL of 0.1% Tween-80 solution. Mites sampled immediately after loading (0 h) served as the control. Each time point was replicated three times. The number of residual spores was similarly quantified using a hemocytometer to analyze the relationship between crawling time on the clean surface and spore shedding.

1.3 Data processing Experimental data were organized and charts were generated using Microsoft Excel 2019. One-way analysis of variance (ANOVA) was performed using DPS 7.05 software, and significant differences among group means were assessed using Duncan's new multiple range test at a significance level of $P < 0.05$.

2 Results

2.1 Dynamics of spore load on mites crawling on entomopathogenic fungal spore powder surfaces The dynamic changes in the spore load on the body surface of *P. zhonghuajia* crawling on surfaces coated with spore powders of the two entomopathogenic fungi over time are shown in Fig. 1. Overall, the spore load increased significantly with prolonged crawling time for both fungi, but distinct kinetic patterns were observed between the two.

For *M. anisopliae*, the spore load on mites increased with crawling time, reaching a peak of $(6.86 \pm 0.36) \times 10^4$ spores/mite at 2 h. Subsequently, as crawling time extended to 4 h, the load not only failed to increase but showed a significant decline, eventually stabilizing.

In contrast, for *B. bassiana*, the spore load on mites increased continuously throughout the experimental period (0 – 4 h), reaching a maximum of $(12.83 \pm 0.60) \times 10^4$ spores/mite, although it is noteworthy that the rate of increase slowed markedly after crawling exceeded 1 h, and the load differences among 1, 2, 3, and 4 h were not statistically significant ($P > 0.05$).

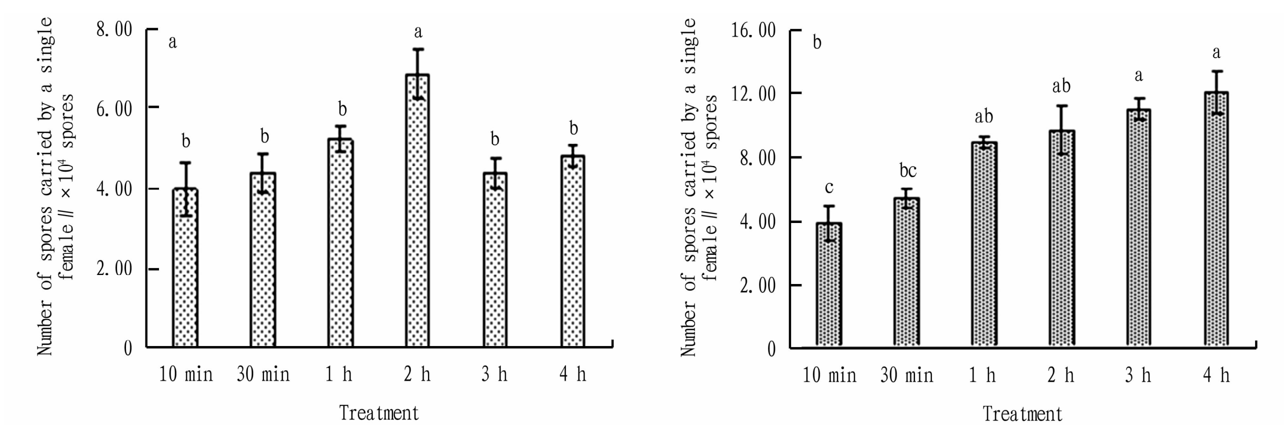

Fig.1 Dynamic changes in spore load on mites over crawling time on entomopathogenic fungal spore powder surfaces: (a) *Metarhizium anisopliae* CQMa421, (b) *Beauveria bassiana* Bb-1

2.2 Dynamics of spore shedding from *Pyemotes* carrying spore powder crawling on clean surfaces The shedding dynamics of spores from the body surface of mites carrying spore powder while crawling on clean surfaces are shown in Fig. 2. The results indicate that the number of spores for both entomopathogenic fungi decreased significantly with increasing crawling time on the clean surface.

After 4 h of crawling, the number of *M. anisopliae* spores carried on the mite body surface decreased from the initial $(6.15 \pm 0.38) \times 10^4$ spores/mite to $(2.51 \pm 0.35) \times 10^4$ spores/mite, representing an approximate loss rate of 59.19%. Similarly, the number of *B. bassiana* spores decreased from $(10.78 \pm 0.76) \times 10^4$ spores/mite to $(3.18 \pm 0.29) \times 10^4$ spores/mite, representing an approximate loss rate of 70.50%. Throughout the observation period, the residual amount of *B. bassiana* spores on the mites was consistently higher than that of *M. anisopliae* spores.

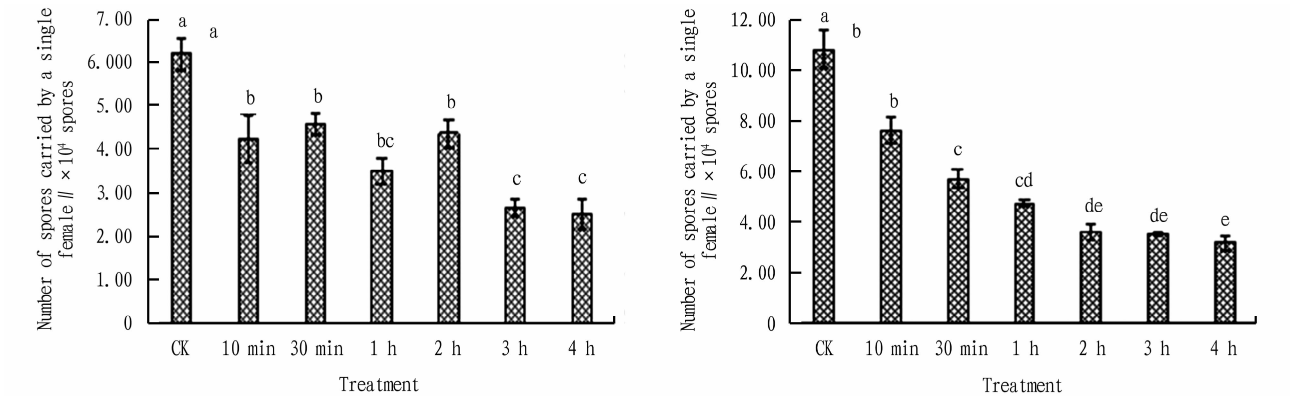

Fig. 2 Dynamic changes in spore load on mites carrying spore powder over crawling time on clean surfaces: (a) *Metarhizium anisopliae* CQ-Ma421, (b) *Beauveria bassiana* Bb-1

3 Discussion

This study systematically quantified the dynamic process of *P. zhonghuajia* carrying spores of *M. anisopliae* and *B. bassiana* on its body surface. The results demonstrate that the efficacy of the mite as a vector for entomopathogenic fungi is significantly influenced by time and the physical characteristics of the spores.

Distinct differences were observed in the loading kinetics of the two fungal spores. The decline in *M. anisopliae* load after peaking at 2 h may be associated with the detachment of spore clumps. In contrast, the *B. bassiana* spore load continued to increase within 4 h (albeit at a reduced rate after 2 h), resulting in a significantly higher final load. During the shedding phase, the number of both spore types decreased over time, with *B. bassiana* exhibiting a higher shedding rate. Nevertheless, the absolute residual amount of *B. bassiana* spores remained consistently higher. This discrepancy likely stems from differences in physical properties such as spore size, shape, and surface hydrophobicity. While the trends in our findings align with those reported by Wu *et al.* [13], the absolute carrying capacity observed here was lower. This discrepancy may be attributed to variations in spore powder purity or dispersion, suggesting that the physical quality of the spore formulation is also a critical factor influencing vector efficiency.

Conducted under laboratory conditions, this study establishes a foundation for understanding vector behavior. Future research should investigate under more field-realistic conditions: (i) the patterns of loading and shedding on different substrates (*e. g.*, bark, leaves); (ii) the efficiency of spore transfer onto pest body surfaces; (iii) the impact of spore carriage on mite behavior and survival fitness. Such investigations will be crucial for screening optimal fungal strains, determining the optimal pre-release loading duration, and evaluating the overall pest control efficacy.

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