Influence of Different Types of *Phyllostachys pubescens* (Poales: Poaceae) Leaves on Population Parameters of *Pantana phyllostachycae* (Lepidoptera: Lymantriidae) and Parasitic Effects of *Beauveria bassiana* (Moniliae: Moniliaceae)

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**ABSTRACT.** We found that *Pantana phyllostachycae*, a dangerous pest of moso bamboo (*Phyllostachys pubescens*), showed differences in growth and development after feeding on diverse types of moso bamboo leaves. The mortality rate of *Pa. phyllostachycae* due to *Beauveria bassiana*, an entomopathogenic fungus, was also affected by the varied larval diet. Larval and pupal developmental duration of *Pa. phyllostachycae* was longer when feeding on “off-year” bamboo leaves. Pupal weight and adult fertility were higher when feeding on “on-year” bamboo leaves. Mortality due to *B. bassiana* was significantly lower in larvae fed on on-year bamboo leaves than in larvae fed on off-year bamboo leaves. Larvae fed on new bamboo leaves had a shorter development period and higher survival rate than those fed on off-year bamboo leaves. However, mixed feed (mixture of new, on-year, and off-year bamboo leaves) decreased the egg production of *Pa. phyllostachycae*. After infection by the second generation of *B. bassiana*, the survival time of *Pa. phyllostachycae* fed on mixed feed increased significantly compared with the first generation. We also fed *Pa. phyllostachycae* different proportion of new bamboo leaves in mixed feed to simulate natural conditions. We found that increasing the proportion of new bamboo leaves in the food promoted pupal development and increased egg production; it also increased the resistance of larvae to the first generation of *B. bassiana*. The pathogenicity of the second generation of *B. bassiana* declined in all mixed feed treatments.

**Key Words:** tritrophic interaction, moso bamboo, on-and-off year, age distribution, different diet ingredient

Moso bamboo (*Phyllostachys pubescens* Mazel ex Houzeau de Lehaie) is an important economic crop that has spread from the tropical to the temperate zone owing to its fertile nature, rapid growth, and high economic value (Shi et al. 2005). The cultivated area of *P. pubescens* comprises approximately 56% of the bamboo forests in China (Zhou et al. 2006) and 90% of moso bamboo on Earth (Zhang 2003). Recent research on the ecological function of *P. pubescens* and its value as a carbon sink has made it a popular means to balance global carbon emissions (Song et al. 2011, Zhou and Jiang 2004, Zhou et al. 2006, Xiao et al. 2007, Lin et al. 2008).

Moso bamboo is a biennial: new bamboo leaves turn into “on-year bamboo leaves” after the first year of development from shoots, whereas on-year bamboo leaves turn into off-year leaves 2 yr later and then alternate between on- and off-year bamboo leaves in a 2-yr cycle. As on-year, off-year, and new moso bamboo leaves differ in their physi-ology (Zhen and Hong 1998), different leaf types have different effects on insect pests, their natural enemies, and arthropod communities (Chen 2006, Zhang et al. 2006). On-year, off-year, and new bamboo leaves exist in one moso bamboo forest simultaneously under natural conditions. This is known as the on-and-off year moso bamboo forest. Because of intensive management, most natural bamboo forests have been reforested into either an on-year (overwhelming proportion of new bamboo and on-year bamboo) or off-year bamboo forest (overwhelming proportion of new bamboo and off-year bamboo). These alterations to forest phenology produce yearly leaf shortages that last 2–3 mo (Zhen and Hong 1998), halve the economic benefit of moso bamboo (Chen 1983), and provide different feeding conditions for defoliators and their natural enemies (Qiu 1984).

*Pantana phyllostachycae* Chao (Lepidoptera: Lymantriidae) is one of the most dangerous pests of moso bamboo in China. It has three generations per year in China; the larvae feed on leaves from April to November until the temperature drops to 8°C (Chen and Dai 1997). *Pa. phyllostachycae* is found in the main moso bamboo production areas and can quickly destroy bamboo forests (Zhang et al. 2002). Intensive management of moso bamboo forest has increased the likelihood and severity of *Pa. phyllostachycae* damage (Chen and Dai 1997, Zhu 2002, Cai et al. 2003). Most of the research done on the biological and chemical control of *Pa. phyllostachycae* relates to its suppression in the field (Chen and Dai 1997, Huang 1977, Chen et al. 1999, Luo 2000, Zhu 2002, Cai et al. 2003). *Beauveria bassiana* (Balsamo Vuillemin (Ascomycota: Hypocreales) is an entomopathogenic fungus that has a wide range of hosts and is relatively harmless to natural enemies and beneficial insects (Kouassi et al. 2003, Shah and Pell 2003, Shipp 2003). As a result, it has become a major means of pest management for *Pa. phyllostachycae* and has been applied on a large scale (Chen and Dai 1997, Zhu 2002, Cai et al. 2003). Studies on the tritrophic interactions between moso bamboo, *Pa. phyllostachycae* and *B. bassiana*, are important for the development of effective IPM (Integrated Pest Management) strategies. Published research in this area does not distinguish, however, between the different food types eaten by moso bamboo defoliators. As a result, the relationship among different types of bamboo, leaf-eating pests, and their natural enemies is still unclear. Because it is important to develop new control strategies for moso bamboo pests, we studied how consuming different types of *P. pubescens* leaves affected *Pa. phyllostachycae* development and its mortality due to *B. bassiana*. The objectives were to quantify the effect of different types of moso bamboo leaves and different proportions of new bamboo leaves in the diet on 1) *Pa. phyllostachycae* growth and 2) mortality due to *B. bassiana*.

**Materials and Methods**

**Varieties of Moso Bamboo Leaves.** Three types of moso bamboo leaves were collected from Shaowu, Fujian Province, China (27° 11’ N; 117° 09’ E):
117° 26’ E) every month: new bamboo leaf (color of leaf is light green to dark green, white powder on the pole, with shoot shell on the bottom), off-year bamboo leaf (color of leaf is yellow to yellowish, leaf blade is narrower and sparse, easier to separate the leaves and branches, without shoot shell on the bottom), and on-year bamboo leaf (color of leaf is dark green, leaf blade is thick and bushy, hard to separate the leaves and branches, without shoot shell on the bottom) (Zhen and Hong 1998). All the leaf samples were transported to the laboratory in a vacuum bag and stored at 4°C. Insect Species. First-instar Pa. phyllostachysae larvae were collected from Shaowu, Fujian Province, China (27° 11’ N; 117° 26’ E), at the end of May 2013; we only collected healthy individuals. Pa. phyllostachysae were shipped back to the laboratory in insect cages. All experiments were carried out after the first molting of the larvae.

**B. bassiana Varieties.** The G1 (Generation 1) generation of B. bassiana was extracted from the infected larvae of Pa. phyllostachysae in the Shaowu. The G2 (Generation 2) generation for different treatments was extracted from the Pa. phyllostachysae (infected by G1 generation and died within 15 d), which share the same diet with the hosts of G2 generation treatments. Both G1 and G2 generations were reared on PDA medium for 10 d and kept at 4°C. The spore suspension (10⁹ conidia/ml) used for this experiment was diluted with Tween 80 (0.5%).

**Experimental Procedure.** All experiments were conducted in an environmental chamber maintained at 25 ± 2°C temperature, 70 ± 5% humidity, and a photoperiod of 16:8 (L:D) h. Larvae were reared in 60-mm diameter Petri dishes lined with moist filter paper. The diet for larvae was changed every 24 h until pupation. The freshly emerged adults were shifted to insect cages; each treatment was done in one cage.

**Effect of Different Moso Bamboo Leaves on Pa. phyllostachysae.** This experiment utilized fresh bamboo leaves as feed. We designed nine treatments: O (on-year bamboo leaf), F (off-year bamboo leaf), N (new bamboo leaf), M0 (50% O + 50% F), M1 (10% N + 45% O + 45% F), M2 (20% N + 40% O + 40% F), M3 (30% N + 35% O + 35% F), M4 (40% N + 30% O + 30% F), and M5 (50% N + 25% O + 25% F). According to our previous work (Su 2010), second-instar Pa. phyllostachysae had no selectivity on different type of bamboo leaves. Furthermore, the larvae were uniformly distributed in the forests and had strong movement ability (Ye 2005). Thus, the odds of Pa. phyllostachysae feeding on different types of bamboo leaves each day were the same. Only one type of moso bamboo leaf was used as diet each day for mixed feed treatment. At least 30 larvae were used for one treatment; the larval developmental duration, larvae survival, the pupal period, pupal weight, number of eggs per female, and egg hatching rate were represented as mean ± SE. Means followed by different letters within a column are significantly different (lower case letters, P ≤ 0.05) or show extremely significant differences (capital letters, P ≤ 0.01) in columns. O represents on-year bamboo leaf; F represents off-year bamboo leaf; N represents new bamboo leaf; and M represents mixed feed.

| Variety | Larval longevity (d) | Larval survival (%) | Pupal period (d) | Pupal weight (g) | Fecundity (number of eggs per female) | Hatching of eggs (%) |
|---------|---------------------|---------------------|-----------------|-----------------|--------------------------------------|---------------------|
| O       | 33.91 ± 0.51⁹       | 76.67               | 11.38 ± 0.61⁹  | 1.45 ± 0.03⁹    | 97.67 ± 5.13⁹                      | 90.11 ± 1.12⁹      |
| F       | 39.52 ± 0.61⁹       | 76.67               | 15.09 ± 0.71⁹  | 1.11 ± 0.01⁹    | 59.71 ± 7.32⁹                      | 72.36 ± 3.56⁹      |
| N       | 32.31 ± 0.80⁶       | 86.67               | 13.27 ± 0.89⁶  | 1.10 ± 0.01⁶    | 53.50 ± 5.53⁶                      | 81.23 ± 2.65⁶      |
| M       | 33.75 ± 0.40⁹       | 71.67               | 13.15 ± 0.26⁹  | 1.29 ± 0.01⁹    | 58.49 ± 1.16⁹                      | 67.68 ± 1.33⁹      |

Values are presented as mean ± SE. Means followed by different letters within a column are significantly different (lower case letters, P ≤ 0.05) or show extremely significant differences (capital letters, P ≤ 0.01) in columns.

**Results**

**Effect of Different Moso Bamboo Leaves on Pa. phyllostachysae.** We observed a direct effect of different moso bamboo leaves on the larval development of Pa. phyllostachysae (Table 1).

| Variety | Larval longevity (d) | Larval survival (%) | Pupal period (d) | Pupal weight (g) | Fecundity (number of eggs per female) | Hatching of eggs (%) |
|---------|---------------------|---------------------|-----------------|-----------------|--------------------------------------|---------------------|
| O       | 33.91 ± 0.51⁹       | 76.67               | 11.38 ± 0.61⁹  | 1.45 ± 0.03⁹    | 97.67 ± 5.13⁹                      | 90.11 ± 1.12⁹      |
| F       | 39.52 ± 0.61⁹       | 76.67               | 15.09 ± 0.71⁹  | 1.11 ± 0.01⁹    | 59.71 ± 7.32⁹                      | 72.36 ± 3.56⁹      |
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| M       | 33.75 ± 0.40⁹       | 71.67               | 13.15 ± 0.26⁹  | 1.29 ± 0.01⁹    | 58.49 ± 1.16⁹                      | 67.68 ± 1.33⁹      |

Values are presented as mean ± SE. Means followed by different letters within a column are significantly different (lower case letters, P ≤ 0.05) or show extremely significant differences (capital letters, P ≤ 0.01) in columns.

**Effect of Different Moso Bamboo Leaves on Pa. phyllostachysae.** Increases in the proportion of N in the diet prolonged larval developmental time (Fig. 1A); however, there were no statistically significant differences among the six mixed-feed treatments (F = 1.33, df = 5,120, P = 0.258). Survival rate of treatments M4 and M5 was much lower, while that of M0 and M3 was much higher than other treatments (Fig. 1B).

**Effect of Different Moso Bamboo Leaves on B. bassiana.** Diet-mediated changes in Pa. phyllostachysae influenced the parasitic rate of Pa. phyllostachysae were represented as mean ± SE. The data of M (mixed feed) treatment were the combination of M0, M1, M2, M3, M4, and M5. The data were subjected to statistical analysis such as analysis of variance (one-way analysis of variance, Tukey’s test for pairwise comparison, with P = 0.01 and P = 0.05). Life Span analysis of the survival time of the larvae under different treatments was used to show the different parasitic ability of B. bassiana on the larvae. Because there was virtually no larval mortality in the control group over 10 d, we do not show the control in the chart. SPSS software for WINDOWS version 16.0 (SPSS Inc, Chicago, IL) was used to perform the statistical analysis.

**Table 1. Effect of Pa. phyllostachysae larval diet on larval performance, pupal performance, and adult fecundity**

| Variety | Larval longevity (d) | Larval survival (%) | Pupal period (d) | Pupal weight (g) | Fecundity (number of eggs per female) | Hatching of eggs (%) |
|---------|---------------------|---------------------|-----------------|-----------------|--------------------------------------|---------------------|
| O       | 33.91 ± 0.51⁹       | 76.67               | 11.38 ± 0.61⁹  | 1.45 ± 0.03⁹    | 97.67 ± 5.13⁹                      | 90.11 ± 1.12⁹      |
| F       | 39.52 ± 0.61⁹       | 76.67               | 15.09 ± 0.71⁹  | 1.11 ± 0.01⁹    | 59.71 ± 7.32⁹                      | 72.36 ± 3.56⁹      |
| N       | 32.31 ± 0.80⁶       | 86.67               | 13.27 ± 0.89⁶  | 1.10 ± 0.01⁶    | 53.50 ± 5.53⁶                      | 81.23 ± 2.65⁶      |
| M       | 33.75 ± 0.40⁹       | 71.67               | 13.15 ± 0.26⁹  | 1.29 ± 0.01⁹    | 58.49 ± 1.16⁹                      | 67.68 ± 1.33⁹      |

Values are presented as mean ± SE. Means followed by different letters within a column are significantly different (lower case letters, P ≤ 0.05) or show extremely significant differences (capital letters, P ≤ 0.01) in columns.
ability of *B. bassiana*. Survival time of treatment F, in response to the infection of G1 generation, was significantly lower than treatment O \((P = 0.005)\) but was significantly higher than treatment M \((P = 0.048)\) (Fig. 2A). For G2 generation, the survival time of treatment O and M was significantly lower than treatments F and N \((P < 0.01)\) (Fig. 2B).

Differences between the parasitic ability of G1 and G2 generations within the same treatment were compared through “Life Span” analysis. No significant differences were found within treatments O, F, and N \((P > 0.05)\); the parasitic ability of G2 generation was significantly lower than G1 generation on treatment M \((P = 0.002)\).

**Effect of Mixed Feed on *B. bassiana***. The proportion of the new-year bamboo leaf in the diet also influenced the parasitic ability of G1 generation (Fig. 3A). The survival time of treatment M5 was significantly lower than that of M2 \((P = 0.003)\); treatment M2 was higher than M4 \((P = 0.049)\); and treatments M0 and M3 had no significant differences with others \((P > 0.05)\). The parasitic ability of G2 generation (Fig. 3B) declined significantly when compared with the G1 generation \((P = 0.002)\), and no significant difference in larval survival time was found among different treatments infected by the G2 generation \((P = 0.097)\). No significant differences were found between the parasitic ability of G1 and G2 generation within the same treatments \((P > 0.05)\).

**Discussion**

The results of this study suggest that consuming off-year bamboo leaves significantly prolongs both larval and pupal development time of *Pa. phyllostachysae*, whereas consuming on-year leaves significantly increases pupal weight and fecundity. Feeding larvae new bamboo leaves had a positive effect on larval developmental time and survival rate (Table 1). Host plant nutritional quality and secondary chemical compound composition greatly impact herbivore development and can increase developmental time and mortality. *Pa. phyllostachysae* feeding increases the secondary chemical compound content of moso bamboo leaf and negatively affects larval development (Zhang 2006). Because the differences in nutritional quality and secondary chemical compound composition of on-year, off-year, and new bamboo leaves are well documented (Zhen and Hong 1998), we speculated that a mixed feed would significantly affect *Pa. phyllostachysae*. Larvae fed on mixed feed only showed a decline in their fecundity, however, indicating that the costs associated with mixed feeding can accumulate over the lifetime of the insect.

A reasonable age distribution (balance between IPM and maximized yield) is the key to the fertility of moso bamboo forest (Yoshinaga 1994, 1996). Moreover, disorderly and unplanned deforestation makes it difficult to maximize the economic value and ecological functions of bamboo forests (Larsen 1995). Many studies (from a fiscal point of
view) put forward the hypothesis that different proportions of on-year, off-year, and new bamboo in the moso bamboo forests can alter yield. However, a reasonable age distribution of moso bamboo forest for the consideration of IPM has not yet been defined (Zhen and Hong, 1998, Lavee 2006). In this study, different varieties of mixed feed did not impact larval developmental time, and the proportion of new bamboo leaf in the diet did not affect pupal development and fecundity. Despite the lack of larval impact, 10% (M1) and 20% (M2) of new bamboo leaf in the diet prolonged the pupal duration and reduced the pupal weight and fecundity. The pupal duration and fecundity were shortened significantly with 50% new bamboo leaf in the diet (M5). Thus, 10–20% of new bamboo leaves in the diet was not suitable for the optimal development of *Pa. phyllostachysae* but 50% was suitable for its survival.

*B. bassiana* is widely used for biological control, though its effects are affected by many factors (Osborne et al. 2004). The disease resistance of larvae fed on-year bamboo leaves was significantly higher than those fed off-year bamboo leaves in both the G1 and G2 generations of *B. bassiana*. The parasitic ability of the G2 generation was significantly lower for larvae in the mixed feed treatments. Larval disease resistance increased with the proportion of new bamboo leaf in the diet in the G1 generation, but the pathogenicity of the G2 generation declined in all mixed-feed treatments. Like many other entomophagous fungi, the pathogenicity of *B. bassiana* is influenced by the biochemical composition of hosts (Hajek 1997, Vega et al. 2008). Although the biochemical composition of mixed feed was more complex than a single type of bamboo leaf, there were no significant among-treatment differences in larval mortality following infection by the G2 generation of *B. bassiana*. Increasing larval disease resistance linked to the biochemical composition of mixed feed are likely to be the reason why the parasitic ability of G2 generation declined on mixed feed treatments (Kuhn et al. 2004), further experimental proof may strengthen this hypothesis. By considering the effects of mixed feed on both *Pa. phyllostachysae* and *B. bassiana*, we suggest that about 20% new bamboo in the moso bamboo forest would help with the controlling of the pests.

*Pa. phyllostachysae* is not considered as damaging to on-and-off year bamboo forests because of the considerable inhibitory effects of secondary chemical compound within the host (Chen and Dai 1997). The occurrence and frequency of *Pa. phyllostachysae* damage to moso bamboo increases with the degree of intensive management (Chen and Dai 1997, Zhu 2002, Cai et al. 2003). The standard errors of the larval and pupal developmental time and fecundity of mixed feed treatment were smaller than others, suggesting that *Pa. phyllostachysae* fed on different moso bamboo leaves were similar in development and age. Also, the control efficiency of *B. bassiana* in on-and-off year bamboo forests was investigated in our study. Many studies showed both long- and short-term control efficiency of *B. bassiana* (Hajek 1997, Inglis et al. 2001). However, our study shows that it is hard for *B. bassiana* to maintain its control effect on *Pa. phyllostachysae* fed on the mixed feed. Therefore, the control efficiency of *B. bassiana* is hard to maintain in the on-and-off year bamboo forest. In previous studies, other insects were used as *B. bassiana* carriers for long-term control (Al-mazra‘awi

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**Fig. 2.** Survival function of *Pa. phyllostachysae* (feeding on different types of leaves and mixed feed) when infected with G1 generation (A) and G2 generation (B) of *B. bassiana*. Different letters within parentheses representing each treatment indicate significant differences (lower case letters, Life Tables analysis $P \leq 0.05$) or extremely significant differences (capital letters, Life Tables analysis $P \leq 0.01$). O represents on-year bamboo leaf; F represents off-year bamboo leaf; N represents new bamboo leaf; and M represents mixed feed.

**Fig. 3.** Survival of *Pa. phyllostachysae* (feeding on different components of mixed feed) when infected with G1 generation (A) and G2 generation (B) of *B. bassiana*. Different letters within parentheses representing each treatment indicate significant differences (lower case letters, Life Tables analysis $P \leq 0.05$) or extremely significant differences (capital letters, Life Tables analysis $P \leq 0.01$). M0: no new bamboo leaf; M1: 10% new bamboo leaf; M2: 20% new bamboo leaf; M3: 30% new bamboo leaf; M4: 40% new bamboo leaf; and M5: 50% new bamboo leaf.
et al. 2006, Quesada-Moraga et al. 2006). This may prove a useful strategy for long-term biological control of *Pa. phyllostachyces* in a bamboo forest.

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