Arbuscular Mycorrhiza Fungi Strengthen the Beneficial Effects of Warming on the Growth of *Gynaephora Menyuanensis* Larvae

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Abstract: Herbivores experience an unprecedented variously impacts of climate warming. Besides, arbuscular mycorrhiza fungi(AMF) also is influence on herbivores through their common host plants. Surprisingly, there are no reports about how AMF affect the responses of herbivores to warming. To close this gap, we conducted a two factors experiment to research the effects of warming, fungicide (AMF suppression), and their interaction on the development of *Gynaephora menyuanensis* larvae, an endemic generalist herbivore species in northeastern Tibetan Plateau, and nitrogen content of *Elymus nutans*, which was the main food of *G. menyuanensis*. Warming significantly advanced the pupation time (PT), expanded the phenomena of protandry and increased the growth rate (GR) of *G. menyuanensis* larvae. Fungicide not affected the development of *G. menyuanensis* larvae, despite their negative effects on the content of *E. nutans*. Warming with fungicide decreased the GR of *G. menyuanensis* compared with warming treatment. In other words, AMF strengthen the beneficial effects of warming to *G. menyuanensis*. This study provides the first evidence of the impacts of AMF on the response of herbivore to warming.

Keywords: Climate warming, Fungicide benomyl, AMF, *Gynaephora menyuanensis.*

1. INTRODUCTION

Since 1850s, the global surface temperature has increased by about 0.76 °C and is recently predicted to increase 1.1-6.4°C within this century (IPCC 2007). Recent studies have shown that elevated temperatures are beneficial to herbivores by strengthening their predation [2], but these effects depended highly upon herbivores species [3] and the optimum temperature [4]. In addition, other abiotic and biotic factors, such as soil fertility [5, 6], precipitation [7], wind strengthens [8], the belowground insect [9], enemies of herbivores [10], and arbuscular mycorrhiza fungi (AMF) [11-13] can also affect the growth of herbivores through changing interspecific interactions between predations. Among all these factors, AMF is one of the most widely distributed soil organisms, which can form mutualistic associations with the roots of land plant species [14]. AMF can facilitate host plant to uptake mineral elements (N, P etc) in exchange for photosynthesis from host plant [15]. Most of studies have been showed that AMF affect indirectly the growth of insect herbivores through changing plant growth [16-18]. However, individual effects of elevated temperature or AMF on growth of the insect herbivores have been well reported, but it is little that the interactive effects of elevated temperature and AMF on growth of the insect herbivores. Based on these, we studied the effects of warming and AMF on larvae of grassland caterpillar *G. menyuanensis* by a field controlled experimental system consisting on the Qinghai-Tibetan Plateau (QTP). This is because that the Qinghai-Tibetan Plateau (QTP) covers 2.5 million km^2 in China, where alpine meadow is the dominant vegetation, and is very sensitive to the climate warming [19].. In this system, *Gynaephora menyuanensis* is an endemic species in the northeastern on QTP, and uses *Elymus nutans* for its food [19]. We predicted that AMF strengthen the beneficial effects of warming on the growth and develpment of *Gynaephora menyuanensis* larvae.

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2. MATERIALS AND METHODS

2.1. Filed Site and Experimental Design

This study was conducted at the Haibei Alpine Meadow Ecosystem Research Station, which is located

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in the northeastern Qinghai-Tibet Plateau of China (37°37′N, 101°12′E, 3,200m a.s.l.). The climate is characterized with short-cool summer, and long-severely cold winter. The annual mean temperature is -2℃. The annual mean precipitation is 500mm, and > 80% of which occurs during summer. The vegetation in this region is dominated by *Kobresia humilis, Festuca ovina, Elymus nutans, Poa pratensis, Carex scabrirostris, Scirpus distigmaticus, Gentiana straminea, Gentiana farreri, Blysmus sinocompressus*, and *Potentilla nivea*. A detailed information refer to the description of Zhao *et al*. [22].

We designed a two factors experiment consisting of four treatments in March, 2016: no warming and no fungicide (NWNF), no warming and fungicide (NWF), warming and no fungicide (WNF), warming and fungicide (WF). Each treatment has five replicates. The open top chambers (OTCs) had been used to simulate climate warming [23]. We manipulated AMF colonization through applying the fungicide benomyl, which have been used widely to suppress the AMF colonization with minimal effects on other soil microflora in the filed system [24, 25]. In order to suppress the AMF colonization in short-term, we transplanted the plant community into flowerpots (32cm diameter×23cm height) in situ, and used syringes (50ml) to inject 1L benomyl (20g/L) in each pot every 5 days from March until October, 2016.

At 3 April, we caught 1st instar larval *Gynaephora menyuanensis*, and then set *G. menyuanensis* with equal body mass into each pot. Synchronize with this, two circular steel rings were installed at bottom and top of flowerpots, and covered by steel screen with mesh size of 0.2mm×0.2mm, which can prevent the caterpilars from escaping during the experiment.

2.2. Measurement of AMF Colonization and *G. Menyuanensis*

Three soil cores (2.5cm diameter×14cm deep) were sampled in October 2016 from each pot. Then, we washed the roots free of soil and estimated the AMF colonization according to Liu and Chen [26]. Development situation of *G. menyuanensis* was recorded every day since the mid of March, 2016. Both weight of the larvae and number of pupation were recorded at 3 July before their pupation. The growth rate (GR) was calculated using the function: GR= (M_f-M_i)/d, where M_f and M_i were final weight and initial weight, and d represented the days between two weighting. Then, we record the pupation time (PT) and weight the pupae according to Cao *et al*. [27]. After pupation of all caterpillars, we collected the leaves of *Elymus nutans*, a representative graminoid plant in each treatment. They were sun-dried in the field, and oven-dried at 60℃ on returning to our laboratory.

2.3. Statistical Analyses

All data met the normal distribution and homogeneity of variance. Two-way analyses of variance (ANOVA) were used to test the effects of warming, benomyl and their interaction on AMF colonization, PT and GR of *G. menyuanensis*. The post hoc multiple comparison was used to illustrate the differences of each variable between treatments at the 95% confidence level ($P \leq 0.05$). All analyses were conducted using IBM SPSS Statistics 22.0 (SPSS Inc).

3. RESULTS

3.1. AMF Colonization Rate

Fungicide significantly reduced mycorrhiza colonization (F = 57.373, $P < 0.001$, Table **1**). Colonization rate of AMF in all fungicide pot was 29% lower than that in no fungicide pot (Figure **1**).

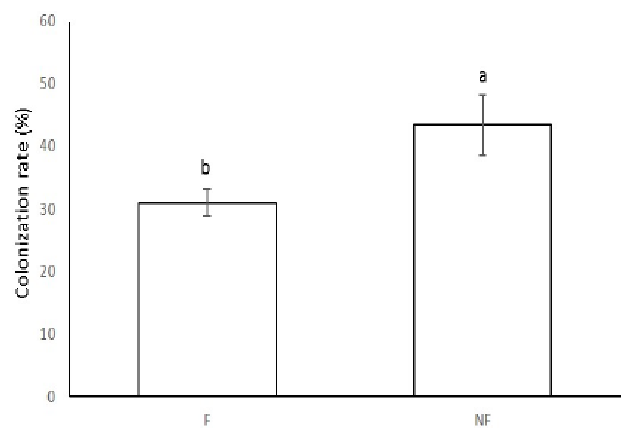

Figure 1: Colonization rate of AMF under different treatments.

F: fungicide, NF: no fungicide. Letters indicate significant difference at $P < 0.05$ in descending order.

3.2. PT and GR of Caterpillars

Warming significantly affected the PT of both female and male pupal weight ($F_{1,14}$ = 22.188, $P_{1,14}$ < 0.001, $F_{1,16}$ = 69.597, $P_{1,16}$ < 0.001). The advancing of pupation time of female (2%) and male (4%) in the W treatment was stronger than that in NW treatment (Figure **2**). The expanding of difference between the pupation of female and male caterpillars in the W treatment was larger by 25% than that in the NW treatment (Figure **2**). The effects of warming (F = 56.109, $P < 0.001$), fungicide (F = 9.292, P = 0.003) and their interaction (F = 4.345, P = 0.040) on GR of caterpillars were significant. GRs in the both WNF and WF treatments were higher by 34% and 16% than that tin NWNF treatment (Figure **3**). The GR under WF was lower by 13% than that under WNF (Figure **3**).

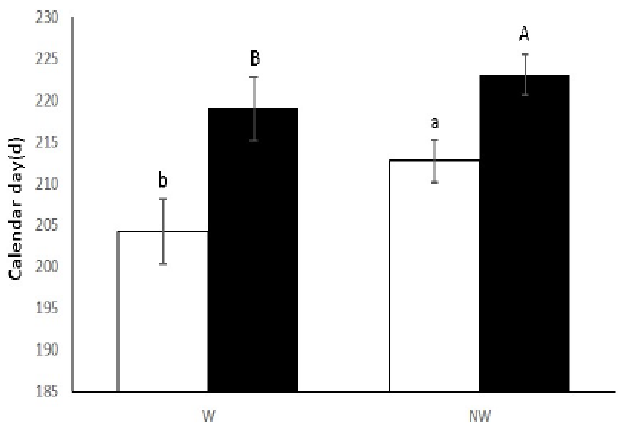

Figure 2: Pupation time of male (open bars) and female (filled bars) under different treatments.

W: warming, NW: no warming. Letters indicate significant difference at $P < 0.05$ in descending order.

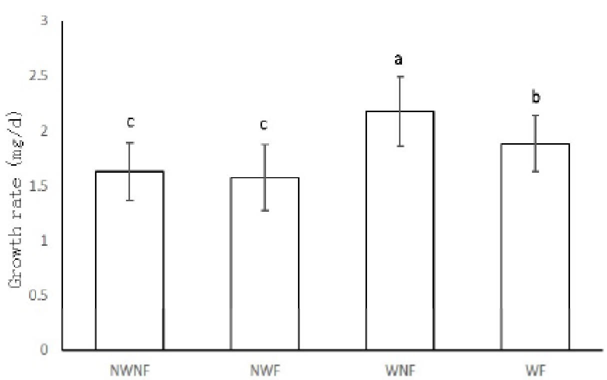

Figure 3: Growth rate of caterpillars under different treatments.

NWNF: no warming with no fungicide, NWF: no warming with fungicide, WNF: warming with no fungicide, WF: warming with fungicide. Letters indicate significant difference at $P < 0.05$ in descending order.

DISCUSSIONS

The life cycle of *G. menyuanensis* consist of larva, pupa, adult and egg [29]. As a holometabola species, they acquire enough energy for their subsequent development and reproduction through their larvae. However, during larva stage, there are some factors threatening their survivability such as extreme environment, prey of enemy and amensalism [27, 30]. Our results showed that warming advanced the pupation time of *G. menyuanensis*, suggesting warming can help *G. menyuanensis* avoid the some threats of extreme environment, and then increase their generation number and expand their population over the long term [27]. In addition, protandry, as an evolution phenomena under natural selection, is helpful to mate successfully for insects [31]. So, protandry can effectively increased mating success. Although there was no direct protandry evidence of *G. menyuanensis*, the advancing of pupation can indirectly imply its ahead protandry in our study, suggesting that warming could enlarge mating success of *G. Menyuanensis* .

GR represent the energy converting ability of insects. The higher GR means that insects are likely to prevail in competition [33]. There is an optimum temperature range for each insect species, and either the high or low temperature is adverse to their development and reproduction [34]. Considering the cold environment, warming properly is always favorable for the improvement of net primary productivity and the surviving of organism on the QTP [35]. The beneficial impacts of warming on GR of *G. menyuanensis* in this study was in accordance with Yu *et al*. [23].

Previous studies showed also that insects were more sensitive to elevated temperatures in comparison with plant. So, the impacts of warming on herbivores are mainly depended on its direct effects of AMF rather than its indirect effects of through their host plants [2]. The higher nitrogen content of food is, the more rapidly *G. menyuanensis* development [28], but the reduced nitrogen content of *E. nutans* induced by fungicide had no influence on the GR of *G. menyuanensis* (data not shown). However, the combined treatments of both warming and fungicide significantly suppressed the GR of *G. menyuanensis*. It was likely that warming accelerated the growth of *G. menyuanensis*, and then increased their demand for the nutrition of changing food [36], and enlarge the impacts of fungicide on *G. menyuanensis*. In other words, AMF strengthen the beneficial effects of warming on *G. menyuanensis* by providing them with much nitrogen nutrient. It indicated that elevated fitness of *G. menyuanensis* induced by AMF would exacerbate the grassland degeneration under the condition of warming [28]. However , AMF can increased plant nutrition and the adaption of the majority of grasses in the long run,, meaning the much quantity and higher quality of food for insect, which in part alleviate the disequilibrium of relation between plants and herbivores [37, 38]. Our findings suggested that AMF might buffer the adverse impacts of warming on growth of *G. menyuanensis*.

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