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Article

## Temperature and humidity effects on physogastric development and reproduction of the mushroom mite *Dolichocybe pernicios* (Acari: Dolichocybidae)

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### Abstract

The effects of temperature and humidity on physogastric development and reproduction of the mushroom mite *Dolichocybe pernicios* (Acari: Dolichocybidae) were observed at five temperatures (8, 13, 18, 25 and 28°C), five relative humidities (22, 55, 65, 81 and 92% RH) and dark condition. Temperatures significantly affected the settling time, pre-physogastry time, physogastry rate, generation time and the number of progenies in physogastry, while humidity had little effect on these factors. Settling rate of wondering adult females increased and pre-physogastry time shortened with the increase of temperature. In the range of 13°C to 28°C more than two thirds of wondering females settled within 72 hours which was significantly higher than that at 8°C. About 60 to 64 progenies were produced when temperature was above 18°C. *Dolichocybe pernicios* had the ability to survive at temperatures as high as 38°C and as low as 0°C after 24 hours but failed to settle and feed at –20°C or 42°C. It was capable to recover after being treated at 0°C for 72 hours.

**Key words:** Prostigmata, pest mites, biology, edible fungus, *Flammulina velutipes*

### Introduction

The genus *Dolichocybe* (Acari: Dolichocybidae) contains about eleven species (Rahiminejad *et al.* 2011; Khaustov 2017). Most species are considered to be fungivorous, living in subcortical habitats and also active on edible fungi (Walter *et al.* 2009). Among them two were found to be serious pests of mushroom. *Dolichocybe pernicios* Zou & Gao, 1996 is known to be the most destructive mite pest on the golden needle mushroom, *Flammulina velutipes* (Curtis) and the silver ear fungus, *Tremella fuciformis* Berk (Zou *et al.* 1992; Lan *et al.* 2016). It is also a pest on other mushroom, such as *Auricularia auricular-judae* (Bull.), *A. polytricha* (Mont.), *Ganoderma lucidum* (Curtis), *Lentinula edodes* (Berk.) and *Panus giganteus* (Berk.) (Zou *et al.* 1992; Wu & Li 2009; Lan *et al.* 2016). This tiny mite species is able to infest the mycelium and fruiting body of mushrooms and also spread mold, *Fusarium* and *Trichoderma*, causing reduction of spawning and casing, even turning the culture substrate to black (Zou & Gao 1996). It occurs commonly in huge numbers in a short period of time under ideal ambient condition and often causes devastating damages to the spawn production and cultivation of mushrooms (Zou *et al.* 1992). Lan *et al.* (2016) described the damage characteristics of *Dolichocybe pernicios* on stock culture, mother spawn, spawn on artificial bed-log of *Flammulina velutipes* and revealed that this mite could even destroy the mushroom throughout the cultivation period.

Despite its economic importance the biology of *D. perniciosus* is little known. Zou *et al.* (1992) briefly described its life cycle. Pregnant female develops a cylindrical, oval or spherical physogastry which is similar to other species in the superfamilies Dolichocyboidea, Pyemotoidea and Pygmephoridea (Walter *et al.* 2009). Eggs develop directly to adults inside the physogastry of the mother. A newly emerged female settles down and becomes immobile after locating at a proper feeding site (Zou & Gao 1996; Lan *et al.* 2016). There is no study on the effect of temperature and humidity on *D. perniciosus*. The purpose of the current study was to investigate the effects of temperature and humidity on the physogastric development and reproduction of *D. perniciosus* so as to provide a theoretical basis for the prevention and control of this pest mite in the cultivation and management of edible fungi.

## Materials and methods

### *Stock culture of mites*

*Dolichocybe perniciosus* was originally collected from a bag of *Flammulina velutipes* in Luoyuan County, Fujian province, China. The mites were purified on glass Petri dishes (30 mm diameter) and tubes (length 180 mm, diameter 18 mm) with mycelia of *Flammulina velutipes* (strain F46 from Institute of Edible Fungi, Fujian Academy of Agricultural Sciences) on a thin layer (1 mm thick) of Potato Dextrose Agar (PDA) at 25°C, 75±5% RH in a dark culture incubator (SPX-250B-Z).

### *Temperature study*

*Effects of temperature on the development and reproductivity.* The mycelia of *Flammulina velutipes* on PDA (5 mm thick) in Petri dishes were used as food source for mites. A circular area (10 mm diameter) of PDA was removed from the centre of each Petri dish with an angle of 45 degrees edge as the experimental arena. A total of ninety female mites emerged within 24 hours were transferred onto the arenas at each temperatures (8, 13, 18, 25 and 28°C) in incubators with 75±5% relative humidity and no light. The mites were observed daily and developmental status, settling and feeding status, number of adult mites within physogastric mother and emerging time of progenies were recorded.

*Effects of extreme temperature.* A total of sixty female mites emerged within 24 hours were transferred onto the arenas at each temperatures (−20, 0, 38 and 42°C), respectively. The temperatures of −20 and 0°C were achieved in a freezer and a refrigerator, respectively, and the temperatures 38 and 42°C were obtained in incubators. The mites were observed daily and the survival and physogastric development were recorded. Mites without physogastric development after 72 hours were kept at 23°C and observed daily for survival and physogastric development.

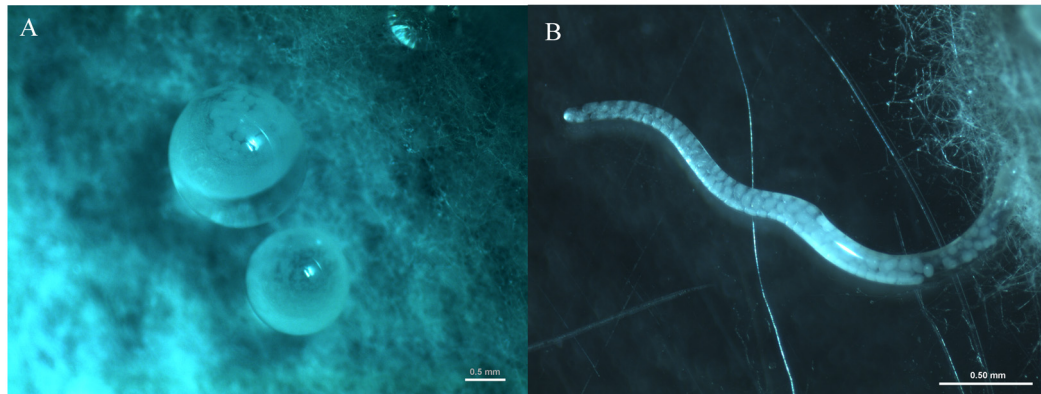
### *Humidity study*

Saturated solutions of reagent chemicals, CaCl<sub>2</sub> 260g/100mL, Mg(NO<sub>3</sub>)<sub>2</sub> 200g/100mL, NH<sub>4</sub>NO<sub>3</sub> 100g/100mL, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 80g/100mL and Na<sub>2</sub>CO<sub>3</sub> 15g/100mL were applied to acquire the different relative humidities, 22, 55, 65, 81 and 92%, respectively, at 25°C (Wang 1993). The relative humidities were checked daily. Saturated reagent chemical solutions were placed in the bottom of plastic containers (28 by 21 by 19 mm) in an incubator set at 25°C, 75±5% RH without light.

A total of ninety female mites emerged within 24 hours were transferred onto the arenas at each humidity. Observations were made daily and developmental status, settling and feeding status, number of mites within physogastric mother and emerging time of progenies were recorded.

### Data analysis

Differences in the data were analyzed using an ANOVA and Tukey's method for multiple comparisons (DPS7.05) to determine whether any significant differences occurred among different temperatures and humidities.



**FIGURE 1.** *Dolichocybe pernicioso*, physogastry. A. spherical; B. cylindrical.

## Results

### Temperature study

#### *Effects of temperature on the development and reproductivity* (Table 1)

In the temperature range from 8°C to 28°C more than 50% of newly emerged *D. pernicioso* settled and started to feed within 72 hours and the higher the temperature the more mites settled. The settling rates were significantly ( $p < 0.01$ ) higher for the mites at temperatures from 13°C to 28°C compared to those at 8°C. Physogastry (Fig.1) presented much earlier as temperature increased. Mites under 18°C to 28°C became physogastric significantly ( $p < 0.01$ ) faster than those at 13°C which were also significantly ( $p < 0.01$ ) faster than those at 8°C. None had developed to physogastric status at the temperatures below 18°C within 72 hours. The optimal temperature for physogastric development was 25°C at which the physogastric rate was significantly ( $p < 0.01$ ) higher than that at 28°C within 72 hours. Progeny developed more rapidly at higher temperatures above 13°C but did not emerge at 8°C within 72 hours. The significant shorter average time for progeny developing and emerging was observed for the temperatures of 28°C and 25°C while the emerging of progeny was much delayed at 18°C and 13°C. The number of progenies emerged was also closely related to temperature when it was below 18°C. No progeny was observed at 8°C though the physogastry developed in about 38 days. The numbers of progenies recorded were similar at 18°C, 25°C and 28°C, and about 60 to 64 progenies per mother female.

#### *Effects of extreme temperature* (Table 2)

The extreme low and high temperatures greatly affected the survival rate of *D. pernicioso*. All mites kept at -20°C died after 24 hours and 15% of mites kept at 0°C for 24 hours survived. The survived mites kept at 0°C for 24, 48 and 72 hours and later transferred to 23°C completed their physogastric status and reproduced normally. Mites kept at 38°C after 24 hour developed well and 45% of them became physogastric but, their settling rate and physogastric rate were much lower than those at 25°C, and in the meantime the hyphae of *Flammulina velutipes* became very susceptible to bacterial infection. Only 3% of mites kept at 42°C for 24 hours survived and *F. velutipes* started to decay and became smelly.

**TABLE 1.** Development of *Dolichocybe pernicioso* at 75±5% RH and different temperatures.

Temperature (°C)	Settling rate (%) in 72 h	Pre-physogastry period (d) (mean±SD)	Physogastry rate (%) in 72 h	Progeny emerging time (d) (mean±SD)	Number of progeny (mean±SD)
8	51.11 <sup>Bb</sup>	36.67±2.08 <sup>Aa</sup>	0.00	NA	0.00
13	76.67 <sup>ABa</sup>	11.33±1.52 <sup>Bb</sup>	0.00	40.67±1.15 <sup>Aa</sup>	21.00±8.54 <sup>Bb</sup>
18	82.22 <sup>ABa</sup>	6.67±1.15 <sup>Cc</sup>	0.00	15.33±1.15 <sup>Bb</sup>	62.33±4.04 <sup>Aa</sup>
25	86.67 <sup>Aa</sup>	3.67±0.58 <sup>Ccd</sup>	21.11 <sup>Aa</sup>	7.33±1.15 <sup>Cc</sup>	59.67±20.50 <sup>Aa</sup>
28	93.33 <sup>Aa</sup>	2.33±0.58 <sup>Cd</sup>	12.22 <sup>Bb</sup>	6.33±0.58 <sup>Cc</sup>	63.67±8.39 <sup>Aa</sup>

Means values followed by the same upper case letter or lower case letter in a column are not significantly different at  $p<0.01$  or  $p<0.05$ , respectively (Tukey test).

Note: Pre-physogastry period is the time before the posterior part of idiosoma starting expanding. Physogastry rate is the number of physogastric females at a specific time (here is 72 h) divided by the total number of mites observed.

**TABLE 2.** Development of *Dolichocybe pernicioso* at extreme temperatures.

Temperature (°C)	Survival rate (%) within 24 h	Settling rate (%) in 24 h
-20	0.00	0.00
0	15.56	7.78
38	85.00	50.00
42	3.33	0.00

### Humidity study (Table 3)

Compared to the temperature, the effects of humidity on the development and reproductivity of *D. pernicioso* were not significant. Mites completed their physogastric development and reproduction processes at all humidities. More than 90% of newly emerged *D. pernicioso* settled and started to feed within 72 hours at all humidities. The settling rate at 22% RH was slightly lower than those at other humidities. Physogastry presented at about the same time in the range from 22% to 92% RH. Within 72 hours, 14% to 30% mites had developed to physogastric status in the humidity range from 22% to 92% RH. No significant differences were recorded in the emerging time of progenies at all humidities. It took about six to seven days for the progenies to develop and emerge from the physogastric mother. The largest number of progenies was recorded at 55% RH and followed by 92% RH.

**TABLE 3.** Development of *Dolichocybe pernicioso* at 25°C and different relative humidities.

Relative humidity (%)	Settling rate (%) in 72 h	Pre-physogastry period (d) (mean±SD)	Physogastric rate (%) in 72 h	Progeny emerging time (d)	Number of progeny (mean±SD)
22	91.11 <sup>b</sup>	2.67±1.15 <sup>a</sup>	14.44 <sup>a</sup>	6.00±0.00 <sup>a</sup>	34.33±9.02 <sup>b</sup>
55	95.56 <sup>ab</sup>	2.00±0.00 <sup>a</sup>	30.00 <sup>a</sup>	6.33±0.58 <sup>a</sup>	58.67±8.51 <sup>a</sup>
65	98.89 <sup>a</sup>	1.33±0.58 <sup>a</sup>	25.56 <sup>a</sup>	6.67±0.58 <sup>a</sup>	40.67±5.51 <sup>ab</sup>
81	94.44 <sup>ab</sup>	1.33±0.58 <sup>a</sup>	22.23 <sup>a</sup>	6.67±0.58 <sup>a</sup>	39.33±7.09 <sup>b</sup>
92	97.78 <sup>a</sup>	1.33±0.58 <sup>a</sup>	28.89 <sup>a</sup>	7.00±0.00 <sup>a</sup>	52.67±4.51 <sup>ab</sup>

Means values followed by the same letter in a column are not significantly different at  $p<0.05$  (Tukey test).

## Discussions

There was no previous study about the effects of temperature and humidity on the development and reproduction of *D. perniciosus*. As in mite species in the relative lineage, superfamily Pygmephoroidae (Gao & Zou 2001; Bussaman *et al.* 2017), the development and reproduction of *D. perniciosus* were also temperature dependent. All newly emerged adults successfully developed their physogastrics and as the temperature increased, the development time decreased under the five temperatures tested. The optimum temperatures for settling, physogastry forming and reproducing of *D. perniciosus* were between 25–28°C.

*Dolichocybe perniciosus* showed high tolerance to temperature extremes. This was observed that 15% and 85% of *D. perniciosus* exposed to 0°C and 38°C, respectively, for 24 hours survived and continued their life cycles after being transferred to an ideal temperature environment. This finding explains why *D. perniciosus* could successfully overwinter in the abandoned edible fungus wastes. It also indicates that using thermal treatment of *D. perniciosus* in edible fungus wastes or facilities requires additional detailed consideration.

Ambient humidity is critical to the development of most mite species. *Dolichocybe perniciosus* developed considerably well at low relative humidity. This is probably because the humidity was compensated by the PDA media which maintained a considerably high humidity within the limited time. Similar results were also acquired in a pyemotid mite, *Pyemotes tritici*, by Bruce (1984) who found that the relative humidity was not as critical as temperature in the production of mite progeny once the mother mite settled down on her host.

The factors determining the shape of the physogastry of *D. perniciosus* is unclear. Zou *et al.* (1992) noted that the physogastric shape was mainly determined by the living space of the mites. The majority of mites became spherical when the living space was larger or became cylindrical when the living space was cramped (Zou *et al.* 1992). We observed that the shape of the physogastry was quite variable. It seemed that other factors were also involved in the determination of the physogastric shape as mites with spherical, cylindrical or semi-oblong physogastrics presented in the unconstrained space. Temperature and humidity are probably the main factors affecting the physogastric shape. Further studies are needed to find out the main determination factors.

The current study provides insight into the development and reproduction of *D. perniciosus* under different temperatures and humidities. The results revealed the favourable temperature range and humidity range for settling, physogastric forming and reproducing of *D. perniciosus*. The results of our experiments also indicate the possible use of extreme high or low temperature in the control of *D. perniciosus* in the mushroom houses as a hygienic measure.

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