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Article

Biological parameters and host preference of *Tyrophagus putrescentiae* (Schrank) on different *Pleurotus ostreatus* cultivars

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Abstract

Tyrophagus putrescentiae (Acari: Acaridae) is a destructive pest of edible fungi. Different species of edible fungi have variable effects on the growth, development and fecundity of *T. putrescentiae*, but it is unclear whether these effects exist in the same species. We used nine cultivars of the oyster mushroom (*Pleurotus ostreatus*) to evaluate the development and reproduction parameters of *T. putrescentiae* at 26 ± 1°C and 80 ± 5% humidity. Different *P. ostreatus* cultivars had significant effects on the immature stages, female fertility, and reproductive lifespan. Total egg-to-adult development time ranged from 10.0 ± 1.2 d (on Po389 cultivar) to 12.5 ± 1.0 d (on Po62 cultivar). Mite mortality ranged from 54.3 ± 4.2% (on Po86 cultivar) to 100.0 ± 0.0% (on Po62 cultivar). The number of eggs laid per female ranged from 3.2 ± 0.4 (on Po45 cultivar) to 9.1 ± 1.1 (on Po86 cultivar). The average lifespan of females ranged from 3.0 ± 0.5 d (on Po45 cultivar) to 16.3 ± 1.7 d (on Po86 cultivar). Host preference of the mite was significantly different among the nine cultivars with a preference for cultivars Po65, Po80, Po389, and Po86. These results indicate that *P. ostreatus* cultivars significantly affect the biology of *T. putrescentiae* and the relationship between mite damage and mushroom yield.

Keywords: mushroom pest, resistant cultivar, developmental parameters, acarid mite

Introduction

Tyrophagus putrescentiae (Schrank) is an important pest in the edible fungi industry, which destroys more than one million tons of fungi annually (Hubert *et al*. 2004). There are more than thirty mushroom hosts of *T. putrescentiae* including *Pleurotus ostreatus* (Jacq. Ex. Fr.), *Lentinula edodes* (Beck), *Agaricus bisporus* (J. Lange), *Ganoderma lucidum* (Curtis), *Flammulina velutipes* (Curtis), and *Auricularia auricula* (Bull) (Qu *et al*. 2015). *T. putrescentiae* can adapt to various food resources under a wide range of temperatures (10–34°C) and relative humidities (30–100%) (Sánchez-Ramos & Castañera 2005; Sánchez- Ramos *et al*. 2007; Abou & Osman 2016). Mites can move from the openings of mushroom cultivation bags or bottles into the substrate, infesting the mycelium and fruiting body of mushrooms and spreading pathogens (Qu *et al*. 2015).

Pleurotus ostreatus is commonly known as the oyster mushroom, and has an annual output of 4,000 tons in China. The oyster mushroom is considered a delicious species and is popular with consumers. It is nutritious and has anti-virus and anti-tumor activity (Lee *et al*. 2021). *P. ostreatus* is mainly produced in China by smallholder farmers under conditions of warm temperature, high humidity and poor ventilation (Zhang *et al.* 2007). The intensive cultivation of *P. ostreatus* is often

affected by bacteria, insects, and mites that can cause substantial production losses (Bellettini *et al*. 2018). The negative effects of relying on pesticide control include toxicity to non-targets and beneficial organisms, chemical residues on mushrooms, and increased pest resistance to pesticides (Simon 2011). Resistant cultivars are a simple and effective option to reduce the economic losses caused by mites. This requires minimal grower knowledge and skills to implement (Keskin & Kumral 2015).

In light of this, evaluation of the mite-resistant properties of *P. ostreatus* should include fecundity, survival rate, duration of mite development, and the mite growth index. These are the most reliable indicators of mite resistance (Qu *et al*. 2018; Gomes *et al*. 2017; Doumma *et al*. 2010; Sewsaran *et al*. 2019). This study evaluated the resistance of different *P. ostreatus* cultivars to *T. putrescentiae*. The above indicators were used to evaluate the effects of *P. ostreatus* cultivars on the biology, growth, development, fecundity and longevity and host preferences of the *T. putrescentiae*. The data can be used to improve pest management strategies.

Materials and methods

Mite and Mushroom

Tyrophagus putrescentiae used in this study originated from fungi (*Auricularia polytricha* (Mont.) Sacc) collected in Feng County, Jiangsu in 2012. Mites were reared on *Lentinula edodes* at 26 ± 1°C and 80 ± 5% relative humidity (RH).

The nine oyster mushroom cultivars were obtained from the Chinese Academy of Agricultural Sciences. They were Po45, Po60, Po62, Po63, Po65, Po80, Po86, Po88 and Po389. The mycelium was freshly inoculated onto potato dextrose agar plates in 90 mm petri dishes. The dishes were covered with lids and maintained in a growth chamber at 25°C and 80% RH in darkness (Qu *et al*. 2015). Two weeks before the start of the experiment, the cultivars were inoculated onto wheat grains in a 25 mL conical flask and cultured, at 25°C and 80% RH in darkness. The wheat grains were soaked overnight and sterilized 30 min before the inoculation.

To determine the effects of different oyster mushroom cultivars on the biological parameters of *T. putrescentiae*, mites were transferred to different mushroom hosts for five generations.

Host selection of T. putrescentiae

We used a four-point chemotaxis experiment to evaluate the host preferences of the mite to different mushroom cultivars. A petri dish (d=15 cm) was divided into four quadrants. One was empty as the blank control (marked IV), and the others (marked I, II, and III) each contained one of three 0.2 g different mushroom cultivars (Fig. 1A). Nine cultivars of the oyster mushroom were randomly divided into different groups, and each group used three replicates to determine mite preference. A total of thirty adult mites were placed in the middle of the petri dish and allowed to crawl freely. After twenty minutes, the number of mites in each quadrant or feeding on the mushroom grains were treated as valid data (Hsueh *et al*. 2017). The chemotaxis index (*CI*) was calculated by the following formula:

$$(CI)=[(X) - \#(IV)] / (I + II + III + IV) \quad (1)$$

X is the number of mites in quadrants *I*, *II*, *III*, and *IV*. The chemotaxis rate is obtained by subtracting the mites in the blank quadrant from the mites in quadrant *X* and dividing them by the total number of mites. Chemotaxis rate indicates the preference of mites for different *P. ostreatus* cultivars.

We also used a six-well selection experiment to determine the preference of mites to the nine oyster mushroom cultivars. A 0.5 g amount of each cultivar was put into separate orifice plates.

Then, about 1500 adult mites were placed into the holes and covered them with a lid for 24 h. We then counted the number of mites on each plate (Fig. 1B). The trapping rate of each cultivar was recorded as the percentage of mites' occurrence. The experiments were conducted at 26±1°C and 80±5% RH.

Development duration and survival of T. putrescentiae

A 0.25 g amount of the test oyster mushroom cultivar was placed into 25 mL culture flasks. Thirty newly laid (1 d old) eggs were placed, with a fine brush, onto the corresponding mushroom mycelia using an S8APO dissection microscope (Leica, Wetzlar, Germany). The flasks were tightly covered with lids and placed in an SPX-2501C type growth chamber (Suzhou Jiangdong Precision Instrument Co., Ltd., China) at 26±1℃ and 80±5% RH. The developmental stage of each individual was recorded every 12 h until all mites had either died or became adults. Mortality was calculated using death records of all immature stages for 15 d. The experiments were repeated five times.

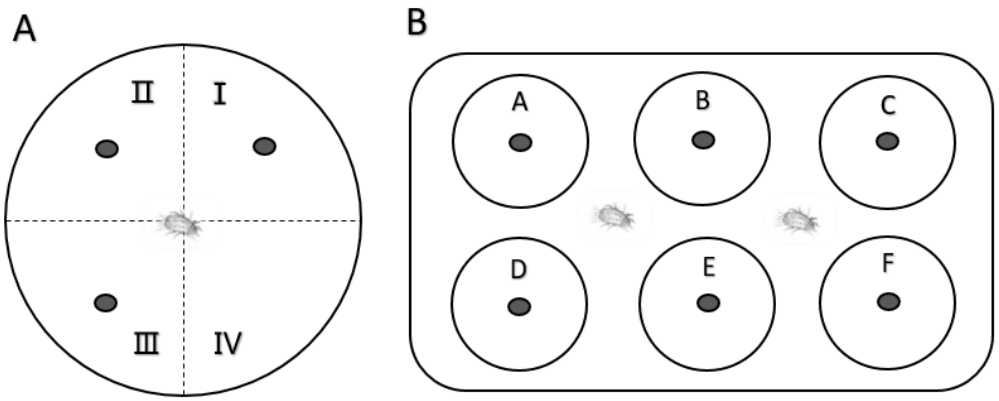

FIGURE 1. Two different methods used to evaluate the host selection of *T. putrescentiae* to different mushroom cultivars. A. Schematic diagram of four–point chemotaxis experiment method. I, II, and III are different oyster mushroom strains, and IV is a blank control. B. Schematic diagram of six–well plate experiment. A, B, C, D, E, F are different strains of oyster mushroom, and mites are placed in the middle of the grid.

Fecundity and demographic parameters of T. putrescentiae

Newly molted female and male adults were transferred into a new 25 mL culture flask containing the corresponding mushroom cultivar (0.25 g) for mating. Ten pairs of mites were tested for each cultivar. The adults were removed and transferred to a new clean petri dish after 24 hr. The number of eggs were recorded for each adult pair for five times according to the spawning period of the cultivars. The entire experiment was replicated ten times. Each of the nine cultivars was examined at 9:00 am and 3:00 pm every day to determine the number of eggs laid, number of eggs hatched, sex ratio, and longevity parameters (Qu *et al.* 2015). The survival rate was calculated for all immature stages. All tests were performed in the laboratory at 26 ± 1°C and 80 ± 5% RH.

Statistical analysis of data

The statistical analysis of study data was performed using IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA). Before data analysis, the data were tested for homogeneity of variance. One-way analysis of variance (ANOVA) was used to evaluate the effects of different *P. ostreatus* cultivars on biological parameters and host preference of the mite, followed by an LSD test ($P = 0.05$).

Results

Host selection of T. putrescentiae

Preferences of *T. putrescentiae* for nine oyster mushroom cultivars are shown in Fig. 2. Po65, Po80, Po86, and Po389, were more favored by *T. putrescentiae* with CI values above 0.3 obtained in a four–point chemotaxis experiment. However, the CI values of Po45, Po63, and Po62 were less than 0.2 (Fig. 2A). We also verified the results of the tropism experiment through the six-well selection experiment. In this test, different *P. ostreatus* cultivars were put into the six wells of a plate, and then 1500 adult mites were placed in the middle of the plates. Under the competition conditions of the six hosts, cultivars Po86, Po80, Po389, and Po65 were consistently attractive to adult mites with trapping rates above 20%. The trapping rates of cultivars Po60, Po62, Po63, and Po45 were less than 8% (Fig. 2B).

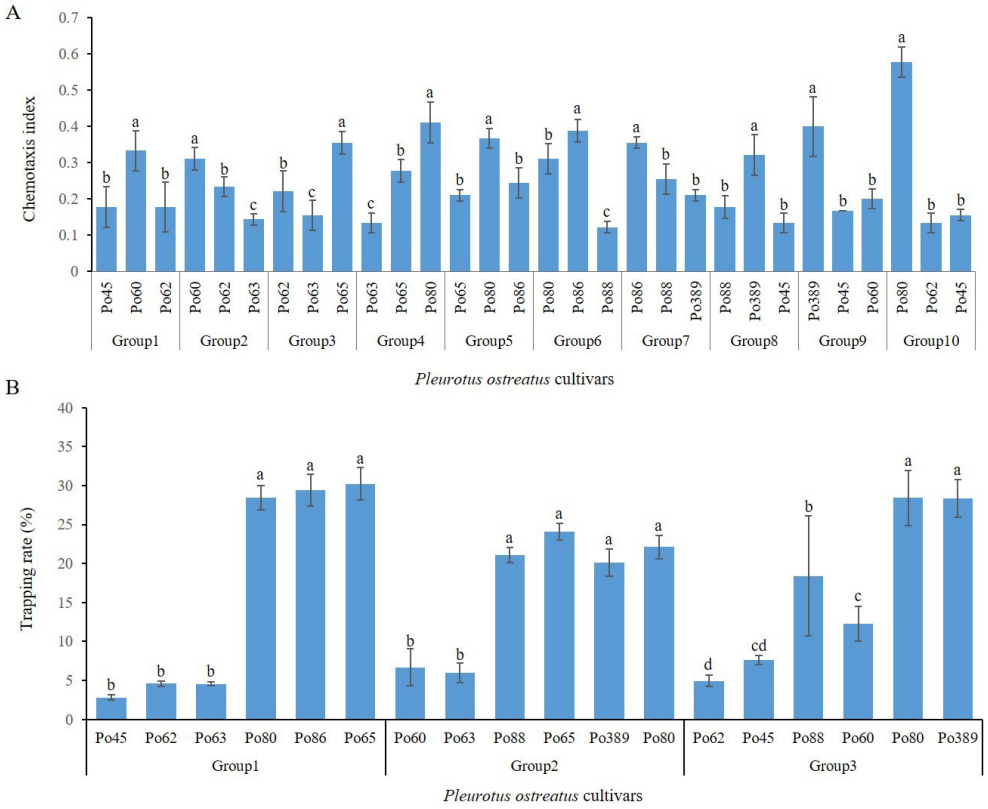

FIGURE 2. The host preference of *T. putrescentiae* to different mushroom cultivars. A. The chemotaxis rate of *T. putrescentiae* among different combinations analyzed by four-point chemotaxis experiment method at 26℃ and 80% RH. B. The trapping rate of each oyster mushroom strain to *T. putrescentiae* analyzed by six–well plate experiment at 26℃ and 80% RH. Means within a column followed by the same letter are not significantly different (LSD test：$p<0.05$).

Development duration and survival of T. putrescentiae

Tyrophagus putrescentiae successfully developed from egg to adult when fed on different mushroom cultivars at 26°C and 80% RH (Table 1). The total developmental time, egg hatchability, and the mortality of the mites were influenced by specific the mushroom cultivars. There were significant differences ($P < 0.001$) in the egg stage, the tritonymph stage, egg hatchability and mortality (Table 1). However, no significant difference was found in the protonymphal stage. The

total developmental time, from egg to adult, ranged from 10.0 ± 1.2 d (fed on Po389) to 12.5 ± 1.0 d (fed on Po62). No significant difference was observed in the total developmental time when the mite fed on Po60, Po80, Po86, Po80, Po45 and Po389 (Table 1). The shortest egg stage was 1.3 ± 0.1 d when fed on Po63, while the longest egg stage was 3.0 ± 0.3 d when fed on Po80. The tritonymphal stage lasted 5.6 ± 1.9 d and 5.8 ± 2.1d when fed on Po62 and Po63, respectively, but the stage was more than two times longer when fed on Po80, Po389, and Po65. The 15 d mortality is one of the most important evaluation criteria. The highest mortality was observed when the mites were reared on Po62 (100.0 ± 0.0%), Po63 (96.9 ± 3.2%), and Po45 (96.7 ± 3.2%). These mortality values were followed by Po88, Po60, Po389, Po65, Po80, and Po86, in descending order. Among them, the lowest mortality rates were in Po86 (54.3 ± 4.2%) and Po80 (59.2 ± 7.1%) (Table 1). Egg hatchability varied greatly from 72.7 ± 1.8% (fed on Po45) to 94.7 ± 3.8% (fed on Po62).

The results of the growth and development traits indicated that Po62, Po63, and Po45 were the poorest hosts for *T. putrescentiae*, compared to Po80 and Po86.

TABLE 1. The effect of different *P. ostreatus* hosts on the growth and development of *T. putrescentiae* at 26℃ and 80% RH.

Strain	Stage					Egg hatchability (%)**	Mortality in 15 d (%)**
	Egg**	Larvae*	Protonymph	Tritonymph**	Total*		
Po86	2.6±0.2ab	2.1±0.4ab	2.5±0.6	3.0±0.5b	10.5±0.7b	86.0±4.3ab	54.3±4.2d
Po63	1.3±0.1d	2.3±0.4ab	2.5±1.4	5.8±2.1a	11.9±0.8a	86.0±1.5ab	96.9±3.2a
Po60	2.3±0.2bc	1.84±0.7b	2.3±0.7	3.3±1.5b	10.0±1.5b	76.7±9.7bc	83.5±19.0b
Po80	3.0±0.3a	2.3±0.6ab	2.6±0.2	2.4±0.7b	10.6±0.5b	80.0±6.2bc	59.2±7.1d
Po45	1.6±0.5cd	2.3±0.6ab	3.6±0.7	2.8±0.3b	10.5±1.0b	72.7±1.8c	96.7±3.2a
Po88	1.9±0.4cd	2.6±0.2a	2.5±0.4	3.1±0.3b	10.4±0.8b	89.3±4.3ab	84.1±11.0b
Po389	2.3±0.3bc	1.5±0.7b	3.3±0.5	2.4±0.8b	10.0±1.2b	91.3±4.5ab	78.9±4.4bc
Po62	1.3±0.3d	2.7±0.4a	3.0±0.8	5.6±1.9a	12.5±1.0a	94.7±3.8a	100.0±0.0a
Po65	2.5±0.4b	2.1±0.5ab	3.3±0.8	2.7±0.9b	10.8±0.6ab	86.7±5.3ab	71.4±6.6c

Values are presented as mean ± SE.

Means within a column followed by the same letter are not significantly different (LSD test：$P < 0.05$).

Asterisk indicates that there are statistically significant differences between 9 *Pleurotus ostreatus* hosts at each parameter level (* $P < 0.05$ and ** $P < 0.01$).

TABLE 2 Egg laid, egg hatchability, survival rate and lifespan of *T. putrescentiae* reared on different *P. ostreatus* strains at 26℃ and 80% RH.

Strain	Number of eggs laid per female**	Egg hatchability (%)**	Survival rate (%)**	Average lifespan of female mites (d)**	Sex ratio (female/ male) **
Po62	3.3±0.6e	89.5±4.6b	72.9±8.8b	4.3±1.2d	1.4±1.0a
Po63	3.6±0.4e	91.8±5.9ab	78.7±7.7b	4.1±0.8d	0.9±0.2b
Po80	9.1±1.1a	97.1±4.2a	96.1±3.5a	15.5±2.4ab	1.6±0.4a
Po86	8.9±1.3a	98.7±1.5a	99.3±1.6a	16.3±1.7a	1.5±0.2a
Po389	6.0±0.8c	93.0±4.7ab	92.4±7.1a	12.1±2.2b	1.9±1.0a
Po65	7.5±1.0b	98.6±1.9a	95.5±3.2a	15.0±1.5ab	1.3±0.2a
Po45	3.2±0.4e	85.0±3.3b	77.3±5.8b	3.0±0.5d	0.9±0.3b
Po60	5.4±0.4cd	96.8±2.9a	92.4±2.1a	11.0±0.8b	1.2±0.2a
Po88	5.1±0.9d	96.2±4.2ab	96.6±3.7a	8.0±1.1c	1.2±0.4a

Values are presented as mean ± SE.

Means within a column followed by the same letter are not significantly different (LSD test：$P<0.05$).

Asterisk indicates that there are statistically significant differences between different *Pleurotus ostreatus* hosts at each parameter level (* $P<0.05$ and ** $P<0.01$).

Fecundity and demographic parameters of T. putrescentiae

Pleurotus ostreatus cultivars had a significant effect on the population growth of *T. putrescentiae* ($P < 0.001$) (Table 2). Po80 and Po86 had more eggs than the other cultivars and had almost triple the number of eggs in Po45, Po62, and Po63. The survival rates on Po80 and Po86 were 96.1± 3.5% and 99.3 ± 1.6%, respectively, and these were 20% higher than survival on Po45, Po62 and Po63. The lifespan of females was an important indicator of population growth. Females had a significantly longer lifetime when fed on Po86 (16.3 ± 1.7 d), Po80 (15.5 ± 2.4 d) and Po65 (15.0 ± 1.5 d), which were almost triple the lifespan of females fed on Po45 (3.0 ± 0.5 d), Po62 (4.3 ± 1.2 d) and Po63 (4.1 ± 0.8 days) (Table 2). The survival rate and sex ratio were also significantly higher than those on Po45, Po62, and Po63.

Discussion

Evaluating the effects of mushroom cultivars on the development and reproductive traits of *T. putrescentiae* is important for the use of resistant hosts and understanding the interactions between mushroom hosts and pest mites.

The mushroom host can affect the feeding preference, reproduction, longevity and life table parameters of pests (Kheradmand *et al.* 2007; Qu *et al.* 2015). Compared with other fungi, such as *Fusarium* spp. (Nesvorná *et al.* 2012) and *Saccharomyces cerevisiae* (Hansen) (Silva *et al.* 2018), *T. putrescentiae* had a higher intrinsic rate of increase ($r_m > 0.20$) when reared on major edible mushroom species, *F. velutipes*, *A. bisporus*, and *A. auricula* (Qu *et al.* 2015). *T. putrescentiae* had a statistically different performance on nine *P. ostreatus* cultivars. The mite preferred cultivars Po80, Po86, and Po65 (CI >0.3) (trapping rate > 20%), which resulted in the highest egg hatchability, eggs laid, survival rate, and the longest female lifetime. Population growth, reproduction, and longevity were negatively affected by cultivars Po62, Po63 and Po45. We previously found many secondary metabolites in mushroom hosts with potent and unique properties. Some volatile compounds from *F. velutipes*, such as (−)–alloaromadendrene, 2–methylnaphthalene, and cyclopentadecane, were involved in the host recognition. These have a potential higher binding capacity with the chemosensory protein gene TputCSP1 of *T. putrescentiae* (Qu *et al*. 2016). Furthermore, β–caryophyllene, benzaldehyde, and pentadecane from mushrooms play an important role in the host selection. Some terpenes induced by *T. putrescentiae*, such as caryophyllene oxide, bicyclogermacrene, and (−)–spathulenol, strongly repelled the mite and have potential value in pest control (Li *et al*. 2018).

Awmack and Leather (2002) and Wetzel *et al* (2016) noted the importance of plant nutritive and defensive traits for herbivore performance and population dynamics. Variability in plant nutrients can reduce insect herbivore performance. In the present study, the fecundity and demographic parameters of *T. putrescentiae* were adversely affected by cultivars Po45, Po62, and Po63. Compared to cultivars Po80, Po86, and Po65, the mites that fed on Po45, Po62 and Po63 laid fewer eggs, had less than a 20% survival rate, and had a female lifespan of fewer than ten days. This is consistent with the indicators considered by Dehghan *et al* (2009) to screen for resistant varieties. Through advanced breeding of different *P. ostreatus* cultivars, the influence of host resistance on the growth and development of mites would increase. Our results confirmed that the resistant cultivars affect the mite population by affecting mite reproduction. Cultivar resistance to the spider mite *Tetranychus evansi* was also observed in some plant hosts, such as *Solanum sarrachoides* (Sendtn.) (Murungi *et al*. 2010) and soybean cultivars LWK and Gorgan 3 (Dehghan *et al*. 2009). This host-plant resistance model has become a paradigm for plant breeding or genetic engineering related to resistance.

Biological parameters and host preference of *T. putrescentiae* reared on nine *P. ostreatus* cultivars help evaluate their resistance. The life parameters of *T. putrescentiae* are affected by the different components of mushroom hosts and this is an important factor when mite management procedures. In this study, the cultivars Po45, Po62 and Po63 were found to have possible value as sources of genes for resistance to *T. putrescentiae* and for breeding new resistant cultivars.

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