Population Growth and Dispersal Behavior of *Histiogaster* sp. (Acari: Acaridae) on Several Economically Important Fungi

Kimiko OKABE

*Kyushu Research Center, Forestry and Forest Products Research Institute,
4-11-16 Kurokami, Kumamoto 860, Japan*

(Received 4 December, 1991; Accepted 3 September, 1992)

Population growth of *Histiogaster* sp. was observed on three well-known Imperfect fungi (*Aspergillus oryzae*, *A. niger* and *Fusarium oxysporum*), five popular Basidiomycetes (commercial mushrooms of *Lentinus edodes*, *Pholiota nameko*, *Pleurotus ostreatus*, *Flammulina velutipes* and *Grifola frondosa*) and an important mushroom pest fungus (*Hypocrea nigricans*). The mites showed steady population growth on most of the Imperfect fungi and on *H. nigricans*. *Hypocrea nigricans* and *A. niger*, among those, provided a high growth rate of the mite population. In Basidiomycetes, the mites showed a high growth rate on *F. velutipes*, but no sign of growth was observed on *L. edodes*. The mites grew well at 25°C and 30°C but did not grow at all at 15°C. When mite density reached a high level they started to crawl off the culture and escape. Escaping mites most frequently reached a fresh nearby plate of *H. nigricans*, where they established well. Some mites, mostly in hypopi stage, even moved to a PDA plate without any fungus. This suggests that this mite species may become a serious pest for culturing fungi.

Key words: *Histiogaster* sp., mycophagous mite, Imperfect fungi, Basidiomycetes, mushroom pest

INTRODUCTION

Many species of acaridina mites were shown to feed and increase on fungi (SINHA, 1964; THOMAS and DICKE, 1970; NOBLE and POE, 1972; RHOADES et al., 1989). For instance, the Robine bulb mite (*Rhizoglyphus robini* CLAPARÈDE), a soil-inhabiting mite, preferred *Fusarium*-infected rakkyo bulbs to healthy ones and grew better on the former (SHINKAJI et al., 1986; OKABE and AMANO, 1991). YAMADA (1974) observed conidia of *Fusarium* fungi on the traces of these mites, and this suggests that the bulb mites possibly carry pathogenic fungi while they move from infected plants to healthy ones. However, few studies have been conducted on interactions between mites and microorganisms, and many remain unknown.

The *Histiogaster* sp. used in this study was collected from *Trichoderma harzianum* culture in July 1990 in Oita Prefecture. This was the first description of this genus in Japan. *Trichoderma*, an anamorph of *Hypocrea*, is one of the most serious pathogen for cultivated mushrooms and causes economic damage (STEANE, 1978). It was recently reported in Japan that several mite species infested and caused damage to cultivated mushrooms and mushroom medium. However, little is known on fauna about mushroom pests in Japan, and relationship between mites and plant disease is not well understood. Mites are recognized as pests of stock cultures of fungi, but no effective and consistent control measure has been established.

I compared population growth of mites at various temperatures to determine whether these mites are pests of culturing fungi or of stock culture. Usually, fungi are cultured at 20°C to 25°C and stocked below 10°C. I investigated whether *Histiogaster* sp. could feed on mycelia of various fungi including three Imperfect fungi, five Basidiomycetes, all of which are well-known cultivated mushrooms in Japan, and on *H. nigricans*, a world-wide notorious pest fungus of cultivated mushrooms. I also observed escaping behavior of mites from populated cultures to new ones, and examined the habitat preference of mites for different fungi as recolonizing sites.

MATERIALS AND METHODS

Histiogaster sp. used in this study has been maintained on a *Hypocrea nigricans* culture (potato dextrose agar medium—PDA) in our laboratory.

The three Imperfect fungi used were *Aspergillus oryzae*, *A. niger* and *Fusarium oxysporum* f. sp. *allii* (SUF 1251). Five Basidiomycetes also used were *Lentinus edodes* (R6-1), *Pholiota nameko* (nameko No. 2), *Pleurotus ostreatus* (hiratake No. 8), *Flammulina velutipes* (enokitake No. 7) and *Grifola frondosa* (maitake No. 3). The mushroom-pest fungus used was *H. nigricans*, which is the perfect stage of *Trichoderma harzianum*. Two *Aspergillus* species and *F. oxysporum* were donated, respectively by the Laboratory of Environmental Biology and by the Laboratory of Plant Pathology, both from the Faculty of Horticulture in Chiba University. *Hypocrea nigricans* and five Basidiomycetes were donated by the Forest By-products Laboratory, Kyushu Research Center, Forestry and Forest Products Research Institute. All fungi were cultured on PDA (Nissui Inc.) plate medium (9 cm dia.) and they were used for experiments after the colonies were established.

Population growth. To compare population growth at various temperatures, *H. nigricans* was used as the food of *Histiogaster* sp. Five adults of each sex were applied to the *H. nigricans* culture. The Petri dishes were sealed with Parafilm® to prevent mites from escaping. Three dishes were placed in incubators adjusted variously to 10, 15, 20, 25 or 30°C under a photoperiod of 16L-8D. The number of mites at each stage (adults, immatures and hypopi) was counted using a stereo-microscope after 28 d.

Adult mites collected from the culture were sterilized by dipping them for a few minutes into 70% ethanol, and then rinsed with sterilized water. Even with this procedure, some contamination occurred due to the engulfment and eventual excretion of bacteria by the mites. Sterilized adult mites (five of each sex) were applied to each culture of fungi grown on PDA plate medium in Petri dishes. The Petri dishes were sealed with Parafilm® and placed in an incubator at 25°C and 16L-8D. The number of mites was counted in the same manner as before after 28 d. Three replicates were made for each observation. The feeding activity of mites on each fungus was also observed. Movement of mite chelicera on hyphae served as cue for their feeding behavior, and vacant spots in fungus colony and disappearance (retardation) of mycelium were also treated as feeding marks by mites. Coincidence of hyphal color with color of gut contents also provided evidence of successful feeding. Gut contents of mites was easily observed since their bodies are almost transparent and the hypha of each non-Basidiomycetes kept its original color. Hypha colors were very similar to that on the media in most Basidiomycetes, and therefore only mycelia were used for mites' food. Mycelia of Basidiomycetes were cultured in potato sucrose (PS) liquid medium, and

Table 1. Population growth of *Histiogaster* sp. on fungal culture of *H. nigricans* at different temperatures

Temperature	No. of mites after 28 d ^a				
	Female	Male	Larva & Nymph	Hypopus	Total
10°C	2.7±2.3	2.0±1.7	0.3±0.6	0	5.0±4.4
15°C	1.3±2.3	0.7±0.6	16.7±17.6	0.3±0.6	18.3±17.6
20°C	74.7±44.2	10.7±4.6	888.0±665.3	1.3±2.3	1008.0±742.4
25°C	217.3±59.5	96.3±51.8	3148.0±1178.9	140.0±46.1	3601.7±1109.3
30°C	280.0±62.9	164.0±48.7	4784.0±2661.2	197.3±65.2	5425.3±2758.7

^a $\bar{x} \pm \text{SE}$ ($n=3$).

were placed in vacant Petri dishes with mites. The mite population was estimated after 28 d.

Dispersal behavior. Two experiments were conducted to investigate dispersal behavior of mites. First, ten mites (five of each sex) were reared at 25°C and 16L–8D in each Petri dish without sealing with Parafilm®. Change in population density and escaping rate from the population were determined as follows. The Petri dish was placed in a water-filled dish (15 cm dia.), and the number of mites staying in the Petri dishes, and the number crawling off and falling on the water were counted every 7 d.

To observe which fungus the mites prefer, five adults of each sex were reared for 28 d on *H. nigricans* grown in a Petri dish in the following experiment. The Petri dish where mite population had established was placed in the center of a desiccator (plastic box type, 22×32×24 cm). Other Petri dishes of fresh fungal cultures or fresh medium were arranged around the Petri dish with mites at intervals of 3 cm. Due to the limited space in the desiccator, the experiment was divided into two halves. In the first (test A), Basidiomycetes of *F. velutipes*, *P. ostreatus* and *L. edodes* were used as target fungi. In the other (test B), preferential aggregation of the mite was compared among *H. nigricans*, *P. ostreatus* and the PDA medium with no fungus. The desiccator was placed in an incubator with the temperature maintained at 25°C and 16L–8D. Relative humidity in the desiccator was maintained at about 65% throughout the experiments. The tests were repeated three times and the arrangement of dishes was changed each time. After 7 d, the number of mites in each surrounding dish was counted under a stereomicroscope.

RESULTS

Population growth

The total number of mites was highest at 30°C and lowest at 10°C (Table 1). The same trend appeared in every stage. Hypopi increased dramatically at 25°C and the ratio of hypopus in the total population was also very high at this temperature. A few mites were found at 10°C and 15°C. At 10°C, even larvae and nymphs were scarce and hypopus was observed.

Among four non-Basidiomycetes fungi (Table 2), high population density was observed on *H. nigricans*. As a result of mite feeding, most mycelium disappeared after 28 d from cultures of *H. nigricans*, *A. niger* and *F. oxysporum*, and only film-like fragments were observed on the surface of the PDA medium. On the other hand, the colony of

Table 2. Population growth of *Histiogaster* sp. on various fungi (non-Basidiomycetes)

Fungus	No. of mites after 28 d ^a				
	Female	Male	Larva & Nymph	Hypopus	Total ^b
<i>Aspergillus oryzae</i>	11.3±11.0	3.0±0	85.0±73.9	0	99.7±84.5 c
<i>Fusarium oxysporum</i>	58.3±57.9	16.3±18.8	706.0±779.7	2.0±3.5	782.7±857.7 c
<i>Aspergillus niger</i>	269.3±160.3	185.3±121.4	1857.3±1481.0	45.3±78.5	2357.3±1651.0 cd
<i>Hypocrea nigricans</i>	217.3±59.5	96.3±51.8	3148.0±1178.9	140.0±46.1	3601.7±1109.3 d

^a $\bar{x} \pm SE$ ($n=3$).^b Means followed by the same letter are not significantly different ($p<0.05$) in DUNCAN's new multiple range test.Table 3. Population growth of *Histiogaster* sp. on various fungi (Basidiomycetes)

Fungus	No. of mites after 28 d ^a				
	Female	Male	Larva & Nymph	Hypopus	Total ^c
<i>Lentinus edodes</i>	0	1.0±1.73	0	0	1.0±1.73 d
<i>Pholiota nameko</i> ^b	26.3±40.5	8.0±13.0	34.0±40.0	0	68.3±93.3 d
<i>Pleurotus ostreatus</i>	24.7±17.6	18.7±16.3	201.0±220.2	4.7±4.0	249.0±249.1 d
<i>Flammulina velutipes</i>	161.3±100.4	65.3±18.9	1844.0±368.3	126.7±46.4	2197.3±342.6 de
<i>Grifola frondosa</i> ^b	68.0±39.6	28.0±21.2	2913.3±2921.3	53.3±65.2	3061.3±3013.6 e

^a $\bar{x} \pm SE$ ($n=3$). ^b Contamination with fungus was observed by the 28th day.^c Means followed by the same letter are not significantly different ($p<0.05$) in DUNCAN's new multiple range test.Table 4. Feeding activity of *Histiogaster* sp. on various fungi

Fungus	Feeding behavior	Mark spot	Gut contents	Growth on mycelium
<i>A. oryzae</i>	○	○	○	
<i>F. oxysporum</i>	○	○	○	
<i>A. niger</i>	○	○	○	
<i>H. nigricans</i>	○	○	○	
<i>L. edodes</i>	—	—	—	—
<i>P. nameko</i>	—	—	—	—
<i>P. ostreatus</i>	○	○	—	○
<i>F. velutipes</i>	○	○	○	○
<i>G. frondosa</i>	—	—	—	—

○: Found, —: Not found.

A. oryzae remained almost undisturbed and maintained its original shape. Mites also grew well on two Basidiomycetes, *F. velutipes* and *G. frondosa* (Table 3), and their population reached the same level as for most of the non-Basidiomycetes fungi. Fungal culture of *G. frondosa* and *P. nameko* were contaminated with non-Basidiomycetes fungi. The latter received additional contamination with some bacteria. In spite of the contamination, *G. frondosa* supported a large number of mites, while *P. nameko* kept only a few. No motile mites were found on *L. edodes* after 28 d. Regardless of the appearance

Fig. 1. Population growth of *Histiogaster* sp. (A), and number of mites escaping (B) from *H. nigricans*. ●: all motile stages with SE, ○: hypopus with SE. ▨: adult, □: larva and nymph, ▤: hypopus.

of the remaining hyphae, the feeding of mites, though with various degrees, was ascertained on *H. nigricans* and on all Imperfect fungi tested (Table 4). Colors of gut contents of mites were similar to those of hyphae, on which mites were reared, in four Basidiomycetes fungi. Feeding behavior and mark spots (vacant spot as feeding mark) on mycelium were observed only on *P. ostreatus* and *F. velutipes* among Basidiomycetes. When Basidiomycetes were cultured in PS medium without contamination, no increase in mite number was observed on *G. frondosa*.

Dispersal behavior

Figure 1 shows the population increase (A) and subsequent escaping activity of mites (B) on *H. nigricans*. The number of motile stages (Fig. 1A) represents a total of mites escaping from and staying in a Petri dish. The number of mites increased exponentially until 28th day, and then declined. No hypopi were found for the first 14 d, but between the 21st and 28th days, they increased exponentially. The number of mites escaping from cultures also increased sharply after the 21st day (Fig. 1B), of which most were immatures and hypopi.

Among three mushroom fungi of *L. edodes*, *P. ostreatus* and *F. velutipes*, the highest number of mites aggregated to the *F. velutipes* dish, and hypopi occupied a higher proportion (Fig. 2A) on the fungus. In test B, the greater number of mites escaped to the *H. nigricans* culture in preference to *P. ostreatus* culture or PDA medium with no fungus (Fig. 2B).

Fig. 2. Number of mites successfully migrating from the *H. nigricans* culture to new cultures with fungi, shown in tests A and B respectively. □: all motile stages excluding hypopus, ▨: hypopus.

DISCUSSION

There was marked population growth of *Histiogaster* sp. at 25°C to 30°C. The number of motile mites at 15°C was low, and hypopi began to appear, indicating that 15°C is unfavorable for population growth of mites. At 10°C, the number of adults decreased and neither larvae nor nymphs were detected. These results suggest that the developmental zero of this mite is between 10°C and 15°C.

Among non-Basidiomycetes fungi used, *A. niger* and *H. nigricans* colonies, as well as that of *F. oxysporum*, have a similar mat-like shape, and mites grew well on these fungi. This suggests that the colony shape might have influenced the feeding of mites and their population growth. On these fungal cultures, mycelium was not observed on the 28th day, probably due to intense feeding by mites. Among three non-Basidiomycetes fungi, mycelium of *F. oxysporum* disappeared most rapidly. This indicates that the lower population growth is attributed to the earliest lack of food (hyphae) on this culture.

NOBLE and POE (1972) investigated population growth of two acarid species, *Anoetus feroniarum* and *Rhizoglyphus robini*, on various fungi and bacteria. They found that the growth rate varies considerably depending on the cultures of fungi and bacteria on which they were isolated. *Siteroptes mesembrinae* and *Brennandania lambi*, though both of them belong to the same family Pygmephoridae, showed completely different growth patterns: the former species increased well on the Imperfect fungi but poorly on *Agaricus* of Basidiomycetes, while the latter species of mite had a reverse food preference (CLIFT and TOFFOLON, 1981). It seems that each mite species has a preferred microorganism

for its best growth. Interaction between mites and fungi seems to be an important factor for the success of mite reproduction and this interaction may also be influenced by habitat condition such as humidity and temperature. Little is known about the natural habitat of the *Histiogaster* species, but the present study revealed that this species may become a harmful pest for non-Basidiomycetes fungi. To prevent an increase of mite population, stock culture of such fungi should be maintained at 10°C or lower.

Among the five Basidiomycetes examined, rapid mite growth was seen on *G. frondosa* and *F. velutipes*. However, *G. frondosa* and *P. nameko* did not seem to be suitable food for mites, since mites failed to build up their population on non-contaminated cultures of these fungi. On the other hand, extensive feeding was observed on *F. velutipes*, and mycelium disappeared within 28 d after mites were introduced. Their feeding on *F. velutipes* was verified by observing the population increase on mycelium placed in a Petri dish without any medium. *Lentinus edodes* grew so fast that mycelium lifted the cover off the Petri dish within 35 d after an inoculation of fungus. This rapid growth of hyphae might have caused some deaths on mites, particularly on eggs and larvae in chrysalis, by covering and wrapping with fungi. On another fungus species of *P. ostreatus*, however, mites showed a steady population growth, even though this fungus grows as fast as *L. edodes*. This brings us to another assumption: *L. edodes* might contain some inhibitor(s) for mite population growth.

In the production of commercial mushrooms, the culture medium usually consists of a mixture of sawdust and rice bran. Culturing conditions such as nutrient may therefore be quite different from those in the present experiment. Nevertheless, the present study indicates the possibility of *Histiogaster* sp. becoming a serious pest for most commercial mushrooms, especially those cultured at 20°C or higher.

Many acarid species show a high rate of population growth and increase exponentially when maintained under favorable conditions. For example, *Lardoglyphus konoi* increased exponentially until the third week, reaching a level of over 3,000 mites when reared with dry yeast and powder of dry small sardines (MATSUMOTO, 1970). *Rhizoglyphus robini* also increased exponentially when reared on rakkyo bulbs infected with fungi (OKABE and AMANO, 1991). Since *Histiogaster* sp. also increased exponentially on *H. nigricans*, the fungus was judged to be a favorable food for mites. In the *Histiogaster* sp. population, hypopi began to appear on the 21st day and became abundant on the 28th day after introduction. The number of hypopi escaping from the culture increased with an increase in total population. A similar result was reported also for *L. konoi* (MATSUMOTO, 1970).

Many hypopi escaped from the original Petri dish cultures of test fungi. Though hypopi were known to have no mouth parts and be incapable of feeding, they may have some sense organs which are responsible for food and habitat selection. With these organs, hypopi might prefer *H. nigricans* and be rather sensitive to habitat conditions such as humidity and temperature. In the surrounding Petri dishes, eggs, larvae and protonymphs were rarely observed in the new culture; also, the duration of 7 d was too short for newly hatched larvae to reach adulthood. Therefore, mites found on the fungal cultures were considered which had immigrated by themselves.

Fungi preferred by *Histiogaster* sp. supported appropriate population growth: a few mites immigrated into cultures of *L. edodes* and many into the culture of *H. nigricans*. Other stage of mites also preferred *H. nigricans* culture. With further evidence of its weaker preference for PDA medium itself, *H. nigricans* seemed to be the most suitable

food for *Histiogaster* sp. among the fungi I studied.

I concluded that *Histiogaster* sp. would potentially be pests for culturing fungi, particularly for cultivating mushroom except *L. edodes*. Further study will be done on factor(s) responsible for poor growth of mites on certain fungi, particularly on *L. edodes* because it may be possible to use the interaction between fungi and mites for mushroom pest control in future.

ACKNOWLEDGEMENTS

The author thanks Mr. S. ARIMA (Oita Mushroom Research Institute), the Laboratory of Environmental Biology, the Laboratory of Plant Pathology (Faculty of Horticulture, Chiba University) and the Forest By-products Laboratory (Kyushu Research Center, Forestry and Forest Products Research Institute) for providing *Histiogaster* sp., *Aspergillus* culture, *Fusarium* culture, and *Hypocrea* and all Basidiomycetes cultures, respectively. Thanks are also extended to Dr. K. KUROSA for identification of *Histiogaster* sp., and to Dr. H. AMANO, Chiba University, for valuable comments and critical reading of the manuscript.

REFERENCES

- CLIFT, A. D. and R. B. TOFFOLON (1981) Biology, fungal host preferences and economic significance of two Pygmephorid mites (Acarina: Pygmephoridae) in cultivated mushrooms, N.S.W., Australia. *Mushroom Sci.* **11**: 245–253.
- MATSUMOTO, K. (1970) Studies on the environmental factors for the breeding of gain mites Part X. Effect of the temperature and relative humidity on the breeding and hypopus formation in *Lardoglyphus konoi*. *Jpn. J. Sanit. Zool.* **21**: 213–219 (in Japanese with English summary).
- NOBLE, W. E. and S. L. POE (1973) Attractancy of several fungi and bacteria for bulb and soil mites frequenting diseased gladiolus corms. *Proc. Fla. State Hortic. Soc.* **85**: 401–404.
- OKABE, K. and H. AMANO (1991) Penetration and population growth of the Robine bulb mite, *Rhizoglyphus robini* CLAPARÈDE (Acari: Acaridae), on healthy and *Fusarium*-infected rakkyo bulbs. *Appl. Entomol. Zool.* **26**: 129–136.
- RHOADES, M. H., S. L. POE and R. J. STIPES (1989) Transformation of hypopodes of *Rhizoglyphus robini* (Acari: Astigmata: Acaridae) on cultures of three soil fungi. *Exp. Appl. Acarol.* **6**: 91–94.
- SHINKAJI, N., K. OKABE and H. AMANO (1986) Rhizoglyphine mites (Acari: Acaridae) infesting Rakkyo (*Allium chinense* G. DON) and Chinese Chive (*A. tuberosum* ROTTLER), and their susceptibility to dimethoate and disulfoton. *Jpn. J. Appl. Ent. Zool.* **30**: 285–289.
- SINHA, R. N. (1964) Ecological relationships of stored-products mites and seed-born fungi. *Acarologia* **6**: 372–389.
- STEANE, R. G. (1978) Monitoring of “disease” and “pest” levels in the mushroom crop as a guide to the application of control measures. *Mushroom Sci.* **10**: 281–302.
- THOMAS, C. M. and R. J. DICKE (1971) Response of the grain mite, *Acarus siro* (Acarina: Acaridae), to fungi associated with stored-food commodities. *Ann. Entomol. Soc. Amer.* **64**: 63–68.
- YAMADA, K. (1974) Role of the bulb mite on disease development of bulb rot of tulip. *Kinki Chugoku Agric. Res.* **48**: 77–81 (in Japanese).