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Resting Spore Formation of *Entmophthora sphaerosperma* FRESENIUS (Entomophthorales : Entmophthoraceae) in the Brown Planthopper, *Nilaparvata lugens* (STÅL) (Hemiptera : Delphacidae)

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Effects of temperature and host stages on spore form of *Entomophthora sphaerosperma* in *Nilaparvata lugens* were investigated. In each host stage, at the range from 15 to 25°C, the fungus formed more resting spores rather than conidia in proportion to the decrease of temperature. At each temperature, it formed more resting spores rather than conidia in the old nymph than the young one, in the adult than the nymph, in the macropterous than in the brachypterous adult, and in the young macropterous female than the aged macropterous female. The mortality due to this fungus was higher at lower temperature in each stage, and at each temperature it was higher in the old nymph than the young one, in the adult than the nymph, and in the macropterous than the brachypterous adult. This tendency coincided with the tendency of resting spore formation.

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (STÅL), is one of the most serious rice plant pests in the Southwest of Japan. Two major entomophthoraceous fungi, *Entomophthora delphacis* HORI (SHIMAZU, 1976) and *Entomophthora sphaerosperma* FRES., being pathogenic to *N. lugens* have been found in Chikugo, Fukuoka, Japan. Field observation showed that *E. sphaerosperma* produces two different spores, conidia and resting spores, in *N. lugens*. Life cycles of entomophthoreceous fungi are still uncertain. It is generally believed that they develop resting spores or hyphal bodies in unfavourable conditions such as winter (AOKI et al, 1976; WILDING, 1973). To study their life cycles, it is first necessary to clarify conditions which lead to formation and germination of resting spores. DUSTAN (1927) and GAUMANN and DODGE (1928) considered that climatic conditions are important for resting spore formation. WILDING and LAUCKNER (1974) and NEWMAN and CARNER (1975) considered that physiological age of the infected host is an important factor in developing resting spores. However, all these speculations are due to observations of epizootics in nature, and there is no report which confirms experimentally conditions governing resting spore formation.

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In this paper, conidia and resting spore formations of *E. sphaerosperma* at various instars, sexes, phases (wing-forms), and adult ages of inoculated *N. lugens* were studied at various temperatures.

MATERIALS AND METHODS

(1) *Effects of host instar, sex and wing-form at various temperatures.* A stock culture of *E. sphaerosperma* isolated from infected *N. lugens* in the field was subcultured on SABOURAUD's dextrose agar containing yeast extract (1%). During the subculture, it was passed through *N. lugens* to prevent reduction of its virulence. *N. lugens* were reared on rice seedlings at 25 °C in the laboratory. For inoculation tests, the insects were put into small test tubes (18×100 mm) and fed rice seedlings. Seeds and roots were covered with absorbent cotton to keep them clean and moist. Five nymphs or four adults were put in each tube. The fungus was cultured on SABOURAUD's dextrose agar containing 1% yeast extract, in petri dishes of 9 cm diameter at 15 °C. Several days later, colonies of the fungus on the plate were cut into pieces about 5 mm square and attached to cork plugs with vaseline. These cork plugs were put into each test tube containing insects, and left for one night. Thus the insects were inoculated with discharged conidia. Then the cork plugs were replaced by cotton ones, and the test tubes were kept for 14 days. All cadavers were examined under a microscope to investigate formation of spores and their form (conidia or resting spores). In this experiment, 1st, 2nd, 3rd, 4th and 5th instar nymphs, and two wing-forms (brachypterous and macropterous) of both sexes were used. Both wing-forms of both sexes of adults were put together in each test tube. The experiments were carried out at temperatures of 15, 20 and 25°C under a long day condition (16L–8D) with 2 repetitions.

(2) *Effects of host age.* Macropterous adults of *N. lugens* within 3 days and more than 10 days after emergence were inoculated and observed in the same way as mentioned earlier at 25 °C. The fungus used in this experiment was newly-isolated from the field. The experiment was carried out 2 times.

RESULTS

The cadavers killed by *E. sphaerosperma* were divided into 3 types; type C (developing conidia), type R (developing resting spores), and type H (developing only hyphae but neither conidia nor resting spores). The cadavers of type C were covered with white velvet-like conidiophores, and discharged conidia (Fig. 1A). Those of type R were covered with cotton like hyphae or none on their surface, mummified, and their bodies were filled with resting spores (Fig. 1B). Usually, conidia and resting spores did not coexist on an individual, but rarely (maximum 4.3%, average 0.3%) both spores were formed on the same individual.

(1) *Effects of host instar, sex and wing-form at various temperatures*

Table 1 shows the results of inoculation to the host at various stages (instars, sexes, and wing-forms) and temperatures. The tendency to form resting spores is indicated by the ratio of the percentage of insects of type R (r) to that of type C+type R (c+r). At 15 °C, the number of insects of type R were greater than that of type C (r/(c+r)>

Table 1. Spore Formation of *Entomophthora sphaerosperma* in Various Stages of *Nilaparvata lugens* at Various Temperatures

Stage	Repeat	15°C						20°C						25°C					
		No. of insects	c[a]	h[b]	r[c]	c+h+r	r/(c+r)	No. of insects	c[a]	h[b]	r[c]	c+h+r	r/(c+r)	No. of insects	c[a]	h[b]	r[c]	c+h+r	r/(c+r)
1st, 2nd	1	52	11.5	23.1	3.8	38.5	0.25	101	7.9	20.8	0.0	28.7	0.00	100	5.0	6.0	0.0	11.0	0.00
	2	100	15.0	41.0	3.0	59.0	0.17	100	9.0	39.0	2.0	50.0	0.18	100	6.0	13.0	0.0	19.0	0.00
	av.						0.21						0.09						0.00
3rd	1	100	9.0	48.0	22.0	79.0	0.71	100	11.0	48.0	4.0	63.0	0.27	100	5.0	11.0	0.0	16.0	0.00
	2	100	8.5	49.0	21.5	79.0	0.72	100	14.0	33.0	5.0	52.0	0.26	100	9.0	38.0	15.0	62.0	0.63
	av.						0.72						0.27						0.32
4th, 5th	1	100	10.5	42.0	17.5	70.0	0.63	100	5.0	24.0	5.0	34.0	0.50	100	2.0	20.0	0.0	22.0	0.00
	2	100	4.0	29.0	42.0	75.0	0.91	100	6.0	52.0	4.0	62.0	0.40	100	1.0	28.0	0.0	29.0	0.00
	av.						0.77						0.45						0.00
Macropterous male	1	37	2.7	45.9	32.4	81.1	0.92	59	3.4	47.5	11.9	62.7	0.78	45	2.2	48.9	0.0	51.1	0.00
	2	81	0.0	66.7	29.6	96.3	1.00	43	2.3	67.4	7.0	76.7	0.75	30	0.0	40.0	10.0	50.0	1.00
	av.						0.96						0.77						0.50
Brachypterous male	1	23	0.0	78.3	4.3	82.6	1.00	20	10.0	50.0	10.0	70.0	0.50	45	6.7	20.0	0.0	26.7	0.00
	2	20	15.0	40.0	35.0	90.0	0.70	11	9.1	36.4	9.1	54.5	0.50	18	5.6	22.2	0.0	27.7	0.00
	av.						0.85						0.50						0.00
Macropterous female	1	18	0.0	38.9	38.9	77.8	1.00	61	10.7	47.5	10.7	68.9	0.50	45	8.9	35.6	0.0	44.4	0.00
	2	50	2.0	58.0	32.0	92.0	0.94	43	2.3	65.1	7.0	74.4	0.75	29	10.3	41.4	0.0	51.7	0.00
	av.						0.97						0.63						0.00
Brachypterous female	1	42	4.8	33.3	45.2	83.3	0.90	60	10.0	28.3	13.3	51.7	0.57	45	2.2	31.1	0.0	33.3	0.00
	2	49	5.1	59.2	21.4	85.7	0.81	43	4.7	53.5	4.7	63.9	0.50	23	4.4	34.7	0.0	39.1	0.00
	av.						0.86						0.54						0.00

[a] Percentage of the cadavers of type C containing a half percentage of the cadavers on which both conidia and resting spores were formed together.

[b] Percentage of the cadavers of type H.

[c] Percentage of the cadavers of type R containing a half percentage of the cadavers on which both conidia and resting spores were formed together.

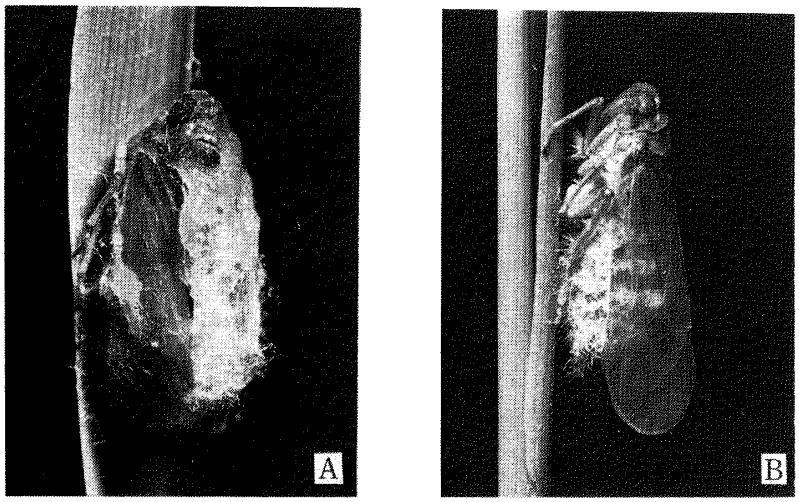

Fig. 1. *Nilaparvata lugens* killed by *Entomophora sphaerosperma* on which conidia were formed (A), and in which resting spores were formed (B).

0.5), except the plot of 1st and 2nd instar nymphs. At 20°C, the number of insects of type C and type R were nearly equal ($r/(c+r) \fallingdotseq 0.5$). At 25 °C, very few resting spores were formed ($r/(c+r) \fallingdotseq 0$). These results suggest that in this range of temperature, the fungus form more resting spores in cadavers in proportion to the decrease of temperature in each stage. At each temperature, r/(c+r) value was larger in the adult than in the nymph, in the old than in the young instar nymph, and in the macropterous than in the brachypterous adult. No significant difference in r/(c+r) value was found between sexes. The percentage of type H in all cadavers did not vary clearly with temperatures or host stages. The mortality due to this fungus (c+h+r) was larger at lower temperature in each stage. And at each temperature, mortality was larger in the adult than in the nymph, in the old than in the young instar nymph, and in the macropterous than in the brachypterous adult. This tendency coincided

Table 2. SPORE FORMATION OF *Entomophthora sphaerosperma* IN YOUNG AND AGED MACROPTEROUS ADULTS OF *Nilaparvata lugens*

Days after emergence	Sex	Repeat	No. of insects	c[a]	h[b]	r[c]	c+h+r	r/(c+r)
Within 3	Male	1	40	2.5	75.3	5.0	82.5	0.67
		2	43	2.3	34.9	20.9	58.1	0.90
		av.						0.79
	Female	1	40	2.5	75.0	15.0	92.5	0.86
		2	13	0.0	46.1	23.1	69.2	1.00
		av.						0.93
Mare than 10	Male	1	38	0.0	60.5	2.6	63.2	1.00
		2	40	7.5	22.5	15.0	45.0	0.67
		av.						0.84
	Female	1	38	2.6	65.8	2.6	71.1	0.50
		2	40	15.0	25.0	25.0	65.0	0.63
		av.						0.57

[a,b,c] See Table 1.

with the tendency of resting spore formation.

(2) *Effects of host age*

The difference in resting spore formation owing to the age of adults were not clear in male. In female, however, r/(c+r) value of young adults was larger than that of aged adults (Table 2). In this experiment, in spite of the temperature of 25°C, r/(c+r) value was very large and the mortality was very high; probably owing to the high virulence of newly-isolated fungus used.

DISCUSSION

NEWMAN and CARNER (1975) investigated the spore form of *Entomophthora gammae* in *Pseudoplusia includens*, and found that the spore form was not affected by temperature and photoperiod of incubation, and that conidia were predominant spore form in small larvae while resting spores were predominant in large larvae, and suggested that the age of the larvae bearing the infection played a very significant role in spore form determination. WILDING and LAUCKNER (1974) suggested from the observation of *Entomophthora muscae* on *Leptohylemyia coarctata* that resting spore formation is affected by the age of the fly. In the present experiment, it is suggested that not only age of the infected insects but temperature is also an important factor determining spore form. However, at each temperature, the resting spore form ratios varied with instar of nymphs, and wing-forms and age of adults. Therefore, these results indicate that spore form is not determined by direct effect of temperature on the fungus, but according to insect states affected by temperature. SAWYER (1931) suggested that the resting spore production of *E. sphaerosperma* were promoted by high temperature, and that chemical activators present in egg yolk and absent in potato were also factors to produce resting spores. If temperature acts directly on the fungus to form resting spores, his explanation is not suitable for the results of the present experiment.

The rate of resting spore in the brachypterous adult was lower than that in the macropterous adult, and were similar to those in the 4th and 5th instar nymph. Thus, it may be considered that brachypterous adult is more similar to the nymph, in its physiological response to the fungous infection, than to the macropterous adult. The wing-form of *N. lugens* is determined by the density and food of nymphal stage. KISIMOTO (1957) studied the wing-forms of plant hoppers, and considered the macropterous-brachypterous form relation as the adult-juvenile relation, on the bases that the macropterous form is a form adapted to unfavourable conditions for the species and to find new habitat, whereas brachypterous form is adapted to rapid population growth under favourable conditions. In his classification, brachypterous form resembles nymph rather than macropterous form in their physiological state. The relationship between brachy- and macropterous form in the present experiment coincided with KISIMOTO's classification.

The difference in spore formation owing to the age of adult was found only in macropterous female. This suggests that physiological reaction against the fungus in macropterous females varied with their age. According to KISIMOTO (1965), the average pre-ovipositional period of macropterous female of *N. lugens* is 7.5 days and that of brachypterous female is 3.0 days at 25 °C. In the present experiment, the females used were within 3-days old and more than 10-days old, and they might

be thought as immature and mature, respectively. The fungus formed more resting spores in immature macropterous females than in mature ones.

The mortalities due to the fungous infection were higher at low temperature in each stage. They were also higher at each temperature, in the adult than in the nymph, in the old than in the young instar nymph, and in the macropterous than in the brachypterous adult. This relationship between susceptibility and insect stages was similar to that obtained from the infection experiment of *E. delphacis* to *N. lugens* (SHIMAZU, 1977), although *E. delphacis* was found to form only conidia and no resting spore.

The mortalities caused by the infection showed a tendency similar to the resting spore formation rates. This fact suggests that the factor of host insect which stimulates this fungus to form resting spores, may be connected with the susceptibility of the insect to the fungus.

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