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Isolation of an entomogenous fungus, *Erynia delphacis* (Entomophthorales: Entomophthoraceae), from migratory planthoppers collected over the Pacific Ocean

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Abstract
Four species of planthoppers were collected using net traps and an aspirator on a weather ship above the Pacific Ocean. An entomogenous fungus, *Erynia delphacis* (Entomophthorales), was isolated from a specimen of the whitebacked rice planthopper, *Sogatella furcifera*. The fungal isolate was infectious to 3 species of planthoppers, *Sogatella furcifera, Nilaparvata lugens, Laodelphax striatellus*, and the green rice leafhopper, *Nephotettix cincticeps*, under laboratory conditions. The results suggest that *E. delphacis* migrated into Japan from overseas through migratory planthoppers.

Key words: Entomogenous fungus, *Erynia delphacis*, migratory planthoppers, *Sogatella furcifera*

INTRODUCTION

Planthoppers and leafhoppers are major insect pests of rice fields. Two species of planthoppers, *Sogatella furcifera* and *Nilaparvata lugens*, cause serious damage to rice leaves by direct feeding. A planthopper species, *Laodelphax striatellus*, and a leafhopper species, *Nephotettix cincticeps*, are also well known as vectors of viral diseases. Among these insects, the former two species are known to have immigrated into Japan from the southern part of China on a low level jetstream during the rainy period in early summer (Asahina and Turuoka, 1970; Kisimoto, 1971, 1975; Hirao and Ito, 1980; Watanabe and Seino, 1991). This migratory phenomenon has been studied on weather ships of the Meteorological Agency of Japan.

Natural enemies of planthoppers, such as leafbugs of egg predators and dryinid parasites, were found above both the Pacific Ocean and the East China Sea (Asahina and Turuoka, 1970; Hirao and Ito, 1980). No entomogenous fungus, however, has been detected in planthoppers collected above the ocean. The possibility of the immigration of entomogenous fungi into Japan from overseas by infected planthoppers was researched by isolating the fungi from planthoppers collected on a weather ship above the Pacific Ocean.

MATERIALS AND METHODS

Collection of planthoppers. The study was conducted on a weather ship named "Keifu-maru" in an area from latitude 31° 30′ N and longitude 134° E to 29° N and 135° E on the Pacific Ocean during the period from June 27 to July 6 in 1992. Planthoppers were collected both on a moving and an anchored ship every 3 h from 6 : 00 a.m. to 0 : 00 a.m. using 3 net traps (1 m in diam.) which were tied to the mast. Insects gathered on the lights of the anchored ship at night were also collected using an aspirator.

Isolation of entomogenous fungi from planthoppers. The body surfaces of all collected insects were sterilized by immersing them into 70% ethanol for 2 s, a sodium hypochlorite solution (1% available chloride) for 1 min, and then thoroughly rinsing in sterilized water to remove the residual chloride. Some planthoppers caught by net traps were placed on 1.5% water agar plates (9 cm in diam.). All other planthoppers were dried with silica gel, and placed on water agar plates after they were brought back to the laboratory. These plates

were kept in an incubator at 25°C for 14 d. After incubation, fungi on cadavers were transferred onto fresh plates of Sabouraud's dextrose agar with yeast extract (abbreviation SDY; peptone 10 g; yeast extract 10 g; dextrose 20 g; agar 15 g per liter of water), and then all colonies that formed on the medium were isolated. In contrast, a cadaver producing spores of Entomophthorales was placed, with SDY, into a Petri dish (9 cm in diam.) so the spores could adhere, and later the Petri dish was kept in an incubator. Colonies from discharged spores on SDY medium were transferred to fresh plates of Nemoto's medium (peptone 10 g; dextrose 20 g; dried egg yolk 43 g; agar 15 g per liter of water) for subculturing. All isolates were maintained on SDY or Nemoto's medium. The primary spores, secondary spores and cystidia were observed with a light microscope, and the genus of the fungi were identified from morphological structures.

Pathogenicity of the fungal isolate to planthoppers and leafhoppers. An entomophthoralean isolate, obtained from a planthopper collected above the ocean, infected three species of planthoppers (*S. furcifera*, *N. lugens*, *L. striatellus*) and one species of leafhopper (*N. cincticeps*), in the pathogenicity test. These insects were bred in growth chambers at 25°C under a photoregime of 16 h light and 8 h dark. Sporulating colonies of the fungal isolate were adhered to the inner surface of the lids of Petri dishes. These lids were placed on the top of glass cylinders (20 cm length × 9 cm in diam.) containing 8–18 healthy planthopper or leafhopper adults and rice seedlings. These lids were kept for 24 h at room temperature to inoculate discharged spores. After inoculation, lids with the fungus were replaced by sterilized lids, and the glass cylinders were kept in a growth chamber at 25°C under a photoregime of 16 h light and 8 h dark for 14 d. The glass cylinders were checked for mortalities for 3 to 14 d. The number of spores inoculating to the insects was estimated by the average number of spores dropped in Tween 80 solution (1,000 ppm), 2 h before and after the inoculation. The spores in the suspension were counted by a hemocytometer.

RESULTS

Planthoppers collected on the ocean

Four species of planthoppers; *S. furcifera, N. lugens, L. striatellus* and *Nilaparvata muiri*, were obtained during the period from July 1 to July 6, 1992. The numbers of insects collected through out the period are shown in Table 1. A total of 1,150 planthoppers, 1,101 *S. furcifera*, 44 *N. lugens,* 4 *L. striatellus* and 1 *N. muiri* were collected. No insects were collected during the period from June 27 to June 30 (Table 1).

Fungi isolated from planthoppers

A total of 15 isolates were obtained from 15 individuals among 1,145 planthoppers (1,101 *S. furcifera* and 44 *N. lugens*) caught by net traps and by an aspirator. Most of the fungal isolates were identified as *Penicillium* spp. Only one isolate which was obtained from a female of *S. furcifera* collected by using the net trap on July 5 was identified as a species of Entomophthorales. The characteristics of the entomophthoralean fungus collected were as follows. Conidiophores were branched. Primary spores (water mounted), $25.8 \pm 0.40 \times 15.9 \pm 0.21$ μm (mean ± SE, $n = 50$), were uninucleate, long ovoid with a papilla at the basal part. The morphological characteristics of the fungal conidia closely resembled those of *Entomophthora delphacis* Hori (Table 2). Secondary spores were directly formed from

Table 1. Numbers of planthoppers collected in 3 net traps and an aspirator over the Pacific Ocean

Date	*Sogatella furcifera*	*Nilaparvata lugens*	*Nilaparvata muiri*	*Laodelphax striatellus*
27 June	0	0	0	0
28 June	0	0	0	0
29 June	0	0	0	0
30 June	0	0	0	0
1 July	2	0	1	0
2 July	1	0	0	0
3 July	16	0	0	0
4 July	587	17	0	0
5 July	289[a]	16	0	0
6 July	206	11	0	4
Total	1,101	44	1	4

[a] *Erynia delphacis* was isolated from one specimen.

Table 2. Conidial measurement

Species	Size of conidia (μm)
Entomophthora delphacis S.Hori sp.nov[a]	22–28 × 10–14
Entomophthora delphacis Hori[b]	22.9–36.8 × 12.4–20.3
Erynia delphacis[c]	17.4–31.2 × 12.8–19.3

[a]Hori (1906), [b]Shimazu (1976), [c]the authors.

primary spores, and were almost the same size as primary spores, with a papilla at the basal part (Fig. 1). Cystidia distinctly extended from the sporulating layer, were 390 μm to over 800 μm in length, and 10.0 to 22.5 μm at the basal width. Resting spores were not observed on Nemoto's medium. Although we could not observe resting spores, other morphological characteristics coincided with the description for *Erynia delphacis* (Hori, 1906; Shimazu, 1976). Therefore, the authors identified the fungus as *Erynia delphacis* (Hori).

Pathogenicity of the causal fungus

The isolate of *E. delphacis* collected over the ocean infected all 4 insects (Table 3). Infectivity of *E. delphacis*, however, depended on the number of spores inoculated. A significant number of insects died of infections with a large number of *E. delphacis* spores.

DISCUSSION

Significant numbers of planthoppers were caught over the Pacific Ocean. There is evidence to show that planthoppers have been immigrating into Japan from the southern part of China on the low level jet streams during the rainy season in early summer (Asahina and Turuoka, 1970; Kisimoto, 1971; Hirao and Ito, 1980; Watanabe and Seino, 1991). It is known that a large number of planthoppers such as, *S. furcifera* and *N. lugens*, fly to the southern part of Japan every year (Seino et al., 1987; Watanabe et al., 1994).

Several kinds of entomogenous fungi, including *E. delphacis*, were isolated from planthoppers in paddy fields (Okada, 1971; Shimazu, 1976, 1979; Rombach et al., 1986, 1987; Cai, 1987; Holdom et al., 1988, 1989; Rombach and Roberts, 1989; Li et al., 1992). However, no entomogenous fungus had been isolated from migratory planthoppers.

Fifteen isolates of fungi were obtained from insects collected over the Pacific Ocean. Most isolates belonged to the *Penicillium* genus. The *Penicillium* genus is known to be a saprophytic group of fungi, and not to be entomopathogenous. In contrast, fungi belonging to En-

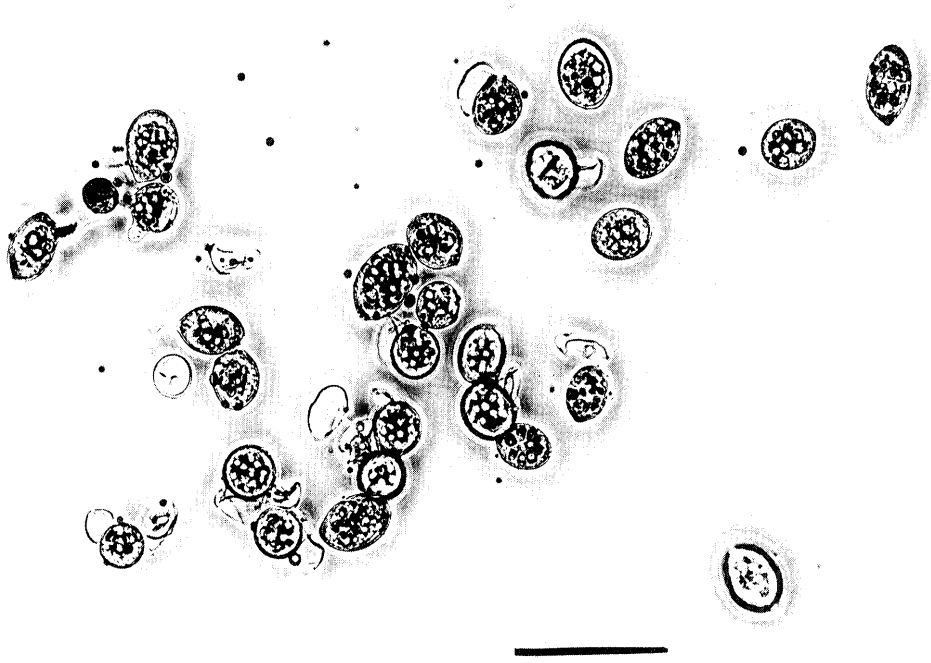

Fig. 1. Spores of *Erynia delphacis* in pure culture on SDY medium. Bar: 50 μm.

Table 3. Infectivity of *Erynia delphacis* isolated above ocean against both planthopper and leafhopper species

Insect species	Trial	No. of spores inoculated[a] ($\times 10^5$)	No. of insects tested	No. of insects dead	No. of insects infected
Sogatella furcifera	1	0	9	3	0
		22.9	10	9	5
	2	0	10	5	0
		1.9	10	9	1
	3	0	10	5	0
		3.1	12	12	9
Nilaparvata lugens	1	0	9	4	0
		27.2	10	3	2
	2	0	10	3	0
		1.4	10	2	0
	3	0	11	7	0
		15.4	11	9	0
Laodelphax striatellus	1	0	8	0	0
		19.4	9	1	0
	2	0	9	0	0
		1.2	14	12	7
	3	0	15	3	0
		56.4	15	15	8
Nephotettix cincticeps	1	0	10	1	0
		30.9	8	7	6
	2	0	12	7	0
		1.3	9	5	0
	3	0	18	4	0
		3.3	11	6	3

[a] Estimated numbers of discharged spores from an inoculum in 24 h.

tomophthorales have been classified as pathogens of insects causing epizootics. In this study, one species of Entomophthorales, *E. delphacis*, was isolated from an immigrating planthopper caught in a net trap. It could be speculated that planthoppers served as carriers of this fungus. After arriving in Japan, the infected insects would serve as vectors causing infection in populations of Japanese planthoppers.

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