Inhibitory Effect of an Epiphytic Fungus, *Ephelis japonica*, on the Feeding of *Mythimna* (*Pseudaletia*) *separata* (Lepidoptera : Noctuidae) and Aiolopus thalassinus tamulus (Orthoptera : Acrididae)

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Received May 12, 2000

Abstract

The effect of infection by a fungal epiphyte (*Ephelis japonica* Hennings), presents on *Digitaria eriantha* Stent and *Cynodon pletostachyrus* (K. Schm.) Pilger on the feeding of two insects, an armyworm, *Mythimna* (*Pseudaletia*) *separata* (Walker), and a grasshopper, *Aiolopus thalassinus tamulus* (Fabricius), was investigated. *M. sepatara* larvae significantly preferred *E. japonica*-free leaves compared to *E. japonica*-infected leaves of *D. eriantha*, but no significant difference on the feeding preference on *E. japonica*-infected leaves of *C. pletostachyrus*. While the adults of *A. t. tamulus* preferred *E. japonica* -free to *E. japonica*-infected leaves of *D. eriantha* and *C. pletostachyrus*. Adults of *A. t. tamulus* that fed on *E. japonica*-free *D. eriantha* leaves survived over a significantly longer period of time than those that fed on *E. japonica*-infected leaves. These sutudies indicate the presence of some factor (s) associated with leaves of the two *E. japonica* - infected grasses.

Key words: Clavicipitaceae, *Ephelis japonica*, *Mythimna* (*Pseudaletia*) *separata*, *Aiolopus thalassinus tamulus*, *Digitaria eriantha*

Introduction

Ephelis japonica Hennings (Clavicipitaceae) has been found to grow epiphytically on fifteen species of grasses on Ishigaki Island, Okinawa [3,9]. *E. japonica*-infected grasses are characterized by the presence of fungal stromata composed of dense mycelial growth that covers the inflorescences, and for most of the grasses, by the presence of white fungal growth on the leaf blades. Many fungi within the family Clavicipitaceae, in particular *Epichloë* and *Neotyphodium* spp., are known to produce alkaloids as allelochemicals, which protect host grasses from attacks by insect pests but also adversely affects the health of grazing animals[7]. Uegaki *et al.*[10] found out that *E. japonica* on grass species, *Paspalum thunbergii*, detered the feeding of two grasshoppers, *Atractomorpha lata* (Motschulsky) and *Oxya yezoensis* Shiraki. This paper reports the investigation on the inhibitory effect of the *E. japonica* infected grasses on the feeding of *Mythimna* (*Pseudaletia*) *separata* (Walker) and *Aiolopus thalassinus tamulus* (Fabricius).

Materials and Methods

1) Feeding preference

Leaves of two species of grasses, *Digitaria eriantha* Stent and *Cynodon pletostachyrus* (K. Schm.) Pilger, either infected with *E. japonica* (E+) or healthy (E-), were collected from grasslands in Ishigaki. The leaf blades (5 cm long) from E+ and E- plants of each grass species were placed side by side on a moistened filter paper in covered plastic containers (9 cm in width × 4 cm in depth). One 2nd -3rd instar larva of the armyworm, *M. separata* collected from a grassland in Ishigaki, was placed in a container replicated thirty times, and kept in the darkness at 25 ℃ . The leaf blades were examined after 24 h for feeding damage. The feeding preference of the 6th-instar *M. separata* larvae using E+ and E- *D. eriantha* leaf blades was examined after 6 h. While the feeding preference of the adults of *A. t. tamulus* grasshopper, collected from a grassland in Ishigaki, was examined after 24 h at 25 ℃ under the light. The amount of E+ and E- leaf consumed (damage ratio) by each insect was scored using 0-5 scale, namely, grade 0: 0% consumed by an individual, grade 1 : 1-20% consumed, grade 2: 21-40% consumed, grade 3: 41-60% consumed, grade 4: 61-80% consumed and grade 5: 81-100% consumed. These damage ratios were analyzed using the Wilcoxon test.

2) Insect survival

Leaf blades of E+ and E- *D. eriantha* were placed in separate covered glass containers (10 cm in width × 15 cm in depth). One adult of *A. t. tamulus* collected from the field were placed inside a container replicated 10 times for each treatment, and maintained at 22 ℃ under a 14L-10D regime. The period of survival of grasshopper in three trials was recorded, and the data were analyzed using the Mann-Whitney test.

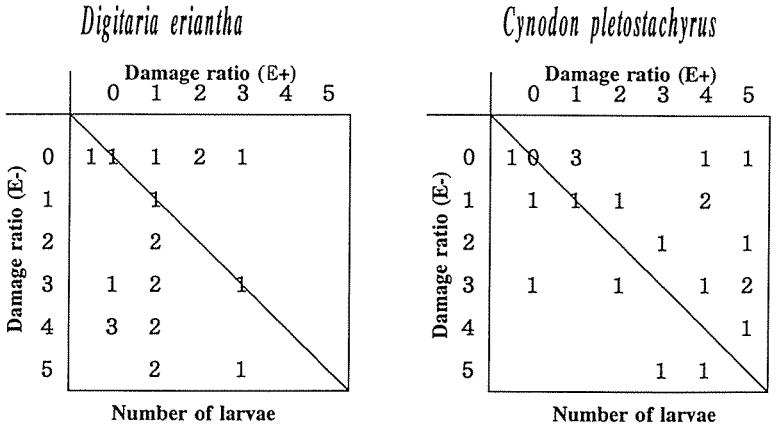

Fig.1. Comparison on the effect of two *Ephelis*-infected (E+) and *Ephelis*-free (E-) grass species on the eating preference of the 2nd-3rd instar larvae of *Mythimna* (*Pseudaletia*) *separata*.

Table1. Comparison on the effect of *Ephelis*-infected (E+) and *Ephelis*-free (E-) Digitaria eriantha on the survival of the adult *Aiolopus thalassinus tamulus*

(Starting day)		1st trial Sep. 10	2nd trial Oct. 6	3rd trial Nov. 30
Survived days	E+	13.0 ± 4.0 **(n=10)	7.5 ± 3.5 **(n=10)	8.5 ± 2.8* (n=10)
	E-	21.0 ± 7.0 (n=10)	15.8 ± 8.1 (n=10)	12.7 ± 5.0 (n=10)

** : Significantly different by Mann-Whitney test. P<0.01
* : Significantly different by Mann-Whitney test. P<0.05

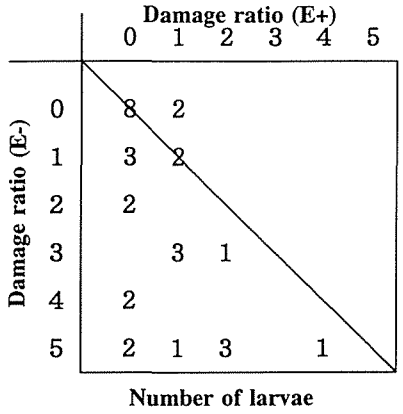

Fig. 2. The effect of *Ephelis*-infected (E+) and *Ephelis*-free (E-) Digitaria eriantha on the eating preference of the 6th instar larvae of *Mythimna* (*Pseudaletia*) *separata*.

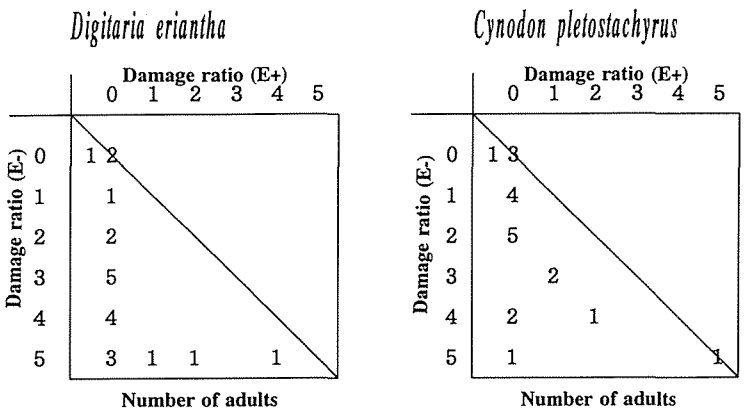

Fig. 3 .Comparison on the effect of two *Ephelis*-infected (E+) and *Ephelis*-free (E-) grass on the eating preference of the adult of *Aiolopus thalassinus tamulus*.

Results

1) Feeding preference

In all the figures, the numerals in the left part of the oblique line indicate the number of individuals that preferred E- leaves to E+ leaves. Both 2nd-3rd instar, and the 6th instar larvae of *M. separata* ate significantly ($P<0.01$)E- compared to E+ leaf of *D. eriantha* (Fig. 1, 2). However no significant differences were noted in the feeding preference of the 2nd-3rd instar larvae of *M. separata* between E+ and E- leaves on *C. pletostachyrus* (Fig. 1). Adults of *A. t. tamulus* significantly preferred E- leaves of both *D. eriantha* and *C. pletostachyrus* to E+ leaves ($P<0.01$) (Fig. 3).

E+ leaves had hyphae on the surface of the blades with the concentration being similar on each leaf. No morphological differences were observed between E+ and E- leaves.

2) Insect survival

The survival period of the adults of *A. t. tamulus* grasshoppers fed on E+ *D. eriantha* leaves was significantly shorter compared with the grasshoppers fed on E- leaves for the three trials (Table 1). The grasshoppers that fed only on E+ leaves consumed less leaf than the grasshoppers that fed only on E- leaves.

Discussion

The results of the feeding trials indicate that two species of the *E. japonica*-infected grasses found in Ishigaki contain some inhibitory factor(s) that affect insect feeding. However, the effects of the factor(s) on *D. eriantha* and *C. pletostachyrus* were different on larvae of *M. separata*. The apparent difference in the spectrum of the factor(s) may indicate genetic diversity with *E. japonica* found on grass species. Likewise, the factor(s) affect the feeding preference and survival period of *A. t. tamulus*. This result conforms with the finding of Uegaki *et al.*[10] on the two species of grasshopper. The short survival period of the grasshopper feeding on E+ *D. eriantha* leaves was attributed to a lower consumption of the leaf.

A number of alkaloids with insect deterrence or toxicity are present in grasses infected with fungi belonging to the family Clavicipitaceae. These have been extensively studied in grasses infected with *Neotyphodium* species, in particular those which form seed-borne endophytic associations with perennial ryegrass, *Lolium perenne* L. and tall fescue *Festuca arundinacea* Screb.[8]. The main alkaloids known to confer resistance are peramine, lolines and ergovaline[1]. When a grass contains peramine, *Listronotus bonariensis* (Kuschel) does not lay their eggs on the plant[6]. Lolines are toxins that occur at high concentrations, up to 10,000 ppm[2]. Ergovaline found throughout the *Balansia*[5], is mainly contributed to be a mammalian toxin but is known to enhance the protection against attacks by insect pests[4]. The identification of a specific inhibitory factor(s) of *E. japonica*-i).nfected grasses has still to be determined.

Acknowledgments

We thank Dr. Michael J. Christensen of AgResearch of New Zealand and Dr. Melanie C. Alba of Sugar Regulatory Administration of Philippines for reviewing the manuscript.

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Ephelis japonica のイネ科牧野草への感染がアワヨトウ及びマダラバッタの摂食に及ぼす阻害的な効果

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摘　要

Ephelis japonica はイネ科牧野草に感染する表生菌であるが、本菌の感染がアワヨトウ *Mythimna (Pseudaletia) separata* 幼虫及びマダラバッタ *Aiolopus thalassinus tamulus* 成虫の摂食に及ぼす影響について調査を行った。アワヨトウ幼虫は本菌の感染したパンゴラグラス *Digitaria eriantha* 葉よりも非感染葉をより好んで摂食した。マダラバッタ成虫はパンゴラグラス及びジャイアントスターグラス *Cynodon pletostachyrus* において感染葉よりも非感染葉をより好んで摂食した。またパンゴラグラスの感染葉を摂食し続けたマダラバッタ成虫の生存日数は非感染葉を摂食し続けた場合よりも 4-8 日ほど短かった。これらのことから本菌の感染した葉上には、昆虫の摂食に影響を及ぼす何らかの要因が存在している可能性が示唆された。

キーワード　；バッカクキン科 , *Ephelis japonica,* アワトヨウ , マダラバッタ , パンゴラグラス