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Successive Transmission of Resistance to Bluegrass Webworm to Perennial Ryegrass and Tall Fescue Plants by Artificial Inoculation with *Acremonium* Endophytes

Hironori KOGA*[1], Yoshio HIRAI*[2], Ken-ichi KANDA, Takao TSUKIBOSHI and Tsutomu UEMATSU*[3]

Department of Environment, National Grassland Research Institute
(Nishinasuno, Tochigi, 329-27 Japan)

Abstract

Perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) plants were imparted the resistance to the bluegrass webworm (*Parapediasia teterrella Zincken*) by artificial infection with *Acremonium* sp. and *A. coenophialum*, respectively. The *Acemonium* endophytes were isolated from perennial ryegrass and tall fescue plants which were resistant to the larvae of the bluegrass webworm. The endophytes were inoculated by inserting the mycelia from the pure culture into the meristematic region. The presence of hyphae of the *Acremonium* endophytes in the inoculated plants was monitored by light microscopy 1-2 months after inoculation. The percentages of successful infection of perennial ryegrass with *Acremonium* sp. were 9-33%, and that of tall fescue with *A. coenophialum* was 7%. There were no differences in the appearance between the endophyte-infected plants and uninfected ones. The plants infected with the endophytes had acquired a feeding deterrent to the bluegrass webworm and the survival rate of the larvae on the infected leaf blades was definitely lower than that of the larvae on the uninfected leaf blades. The endophytes were transmitted to all seeds which were produced by the artificially infected perennial ryegrass and tall fescue plants. The plants grown from the seeds had also acquired the feeding deterrent to bluegrass webworm.

Discipline: Biological control
Additional key words: feeding deterrent

Introduction

The term endophyte has been used to define an organism contained or growing entirely within a plant, practically or symbiotically[18]. Endophytic fungi have been reported in seeds and tillers of perennial ryegrass[8], tall fescue[9] in Japan. Symptomless endophytic fungi belonging to the genus *Acremonium* sect. *Albo-lanosa* are known to produce a number of alkaloids which may affect grazing animals and insects[16,18]. The endophytes in perennial ryegrass and tall fescue were identified as *Acremonium* sp. and *A. coenophialum*, respectively[8,9].

The larvae of the bluegrass webworm (*Parapediasia teterrella* Zincken) feed on the sprouts of turf grasses and induce severe damage to them all over Japan except northern part of the country[19]. Mathias et al.[15] reported that perennial ryegrass (*Lolium perenne*) infected with *Acremonium* endophytes was resistant to the bluegrass webworm. Kanda et al.[5] also confirmed the resistance of endophyte-infected perennial ryegrass to the bluegrass webworm in Japan. They showed that not only perennial ryegrass but also tall fescue (*Festuca arundinacea*) infected with *Acremonium* endophytes had acquired a feeding deterrent to bluegrass webworm.

Present address:
*[1] Research Institute of Agricultural Resources, Ishikawa Agricultural College (Suematsu, Nonoichi, Ishikawa, 921 Japan)
*[2] Department of Insect Physiology and Behavior, National Institute of Sericultural and Entomological Science (Tsukuba, Ibaraki, 305 Japan)
*[3] Department of Research Planning and Coordination, National Agriculture Research Center (Tsukuba, Ibaraki, 305 Japan)

The artificial inoculation method was developed by Latch and Christensen[10]. Several researchers[11,14,17] have reported that plants were successfully infected by artificial inoculation. The present study was carried out to breed perennial ryegrass and tall fescue plants resistant to the bluegrass webworm by artificial inoculation of the *Acremonium* endophytes.

Materials and methods

1) Plants

Two pasture-type cultivars, "Kiyosato" and "Friend", 1 turf-type cultivar, "Manhattan II" of perennial ryegrass (PR) were used for the artificial inoculation in addition to 1 pasture-type cultivar of tall fescue (TF), "Kentucky 31" (Takii Co. Ltd.). To avoid the use of endophyte-infected seeds for the artificial inoculation, the seeds of these cultivars were preliminarily confirmed to be free from infection with fungal endophytes by light microscopy according to the method reported previously[9].

To sterilize the surface of the seeds, they were soaked in 50% H_2SO_4 for about 30 min then washed with water. They were soaked in 1% NaClO for about 30 min then washed in sterilized water. The sterilized seeds were placed onto 4% water agar and incubated at 25°C for 4–6 days. The germinated seeds were used for the artificial inoculation, described as follows.

2) Endophytes

Two isolates (Isolates no. 92-002 and 92-008) of *Acremonium* sp. obtained from perennial ryegrass, cultivar Omega II and 1 isolate (Isolation no. 91-018) of *A. coenophialum* obtained from tall fescue, cultivar Titan were used for the artificial inoculation. These isolates obtained from the plants which had acquired a feeding deterrent to the bluegrass webworm[5] were incubated on PDA medium.

3) Artificial inoculation

Acremonium sp. and *A. coenophialum* isolates were inoculated to PR and TF, respectively. The agar pieces (approximately 0.5 × 0.5 mm) including mycelia of the endophytes were cut from the pure culture on PDA and inserted into the meristematic region at the junction of the mesocotyl and coleoptile of 4–6 day-old seedlings from PR and TF. The inoculated plants were maintained at 25°C in the dark for 7–10 days, and grown in a greenhouse conditioned at 25°C after transfer into pots[11].

4) Examination of endophyte infection

In order to determine whether the inoculated plants were infected with the endophytes or not, strips of epidermis were removed from the leaf sheaths of vegetative tillers, stained with lactophenol aniline blue, and examined using a light microscope[12]. The artificially infected plants were also observed 2 years after the inoculation to determine whether the infected endophytes were still present.

To examine seed transmission of the endophytes from artificially infected plants (parent), the seeds were harvested from the infected plants. Then the seedlings developed from the seeds (progeny) were monitored for endophytic infection by the same method as that described above.

Bioassay of resistance to bluegrass webworm

Adults of the bluegrass webworm moths, *Parapediasia teterrella* (Zincken), were collected in the field at Nasu in Tochigi Prefecture and caged in plastic cups from which eggs were collected. Eggs hatched on moist filter paper and the first instar larvae were used immediately for bioassay studies. Three to 5 leaf blades were placed at the bottom of a plastic petri dish (3.5 cm in diameter) on the water-moistured filter paper. Leaves approximately 3 cm long from the PR and TF plants artificially infected with endophytes and uninfected plants were placed in each test plate. Ten larvae were stuck individually at the tip of a moistened paint brush and released onto each test plate. The test plates were sealed with thin vinyl tape to prevent the larvae from escaping. The plates were kept in a growth chamber conditioned at 20°C, under a 16 h light regime. Counts of living larvae were made at 2-day intervals. Mortality was evaluated based on the number of the living larvae after 8 days. The experiments were replicated 10 times in the artificially inoculated parent plants and uninfected ones, and 5 times in their progenies.

Results

In the preliminary test no endophytes were detected in more than 100 seeds of the cultivar used for the artificial inoculation. After inoculation, hyaline, convoluted, infrequently branched hyphae of the *Acremonium* endophytes were observed under light microscopy in the leaf sheaths of the plants successfully infected (Plate 1). No differences in

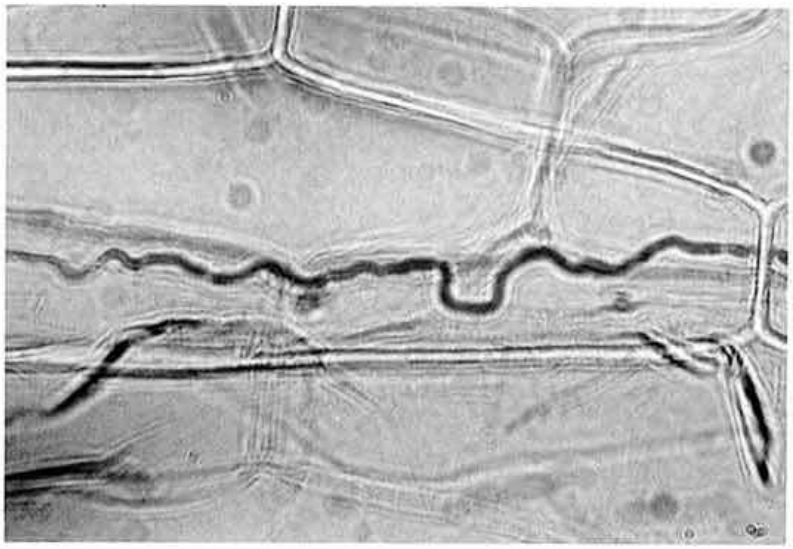

Plate 1. Light microscopy photograph of a hypha of the *Acremonium* endophyte in the leaf sheath of perennial ryegrass (cultivar Kiyosato) about 2 months after inoculation

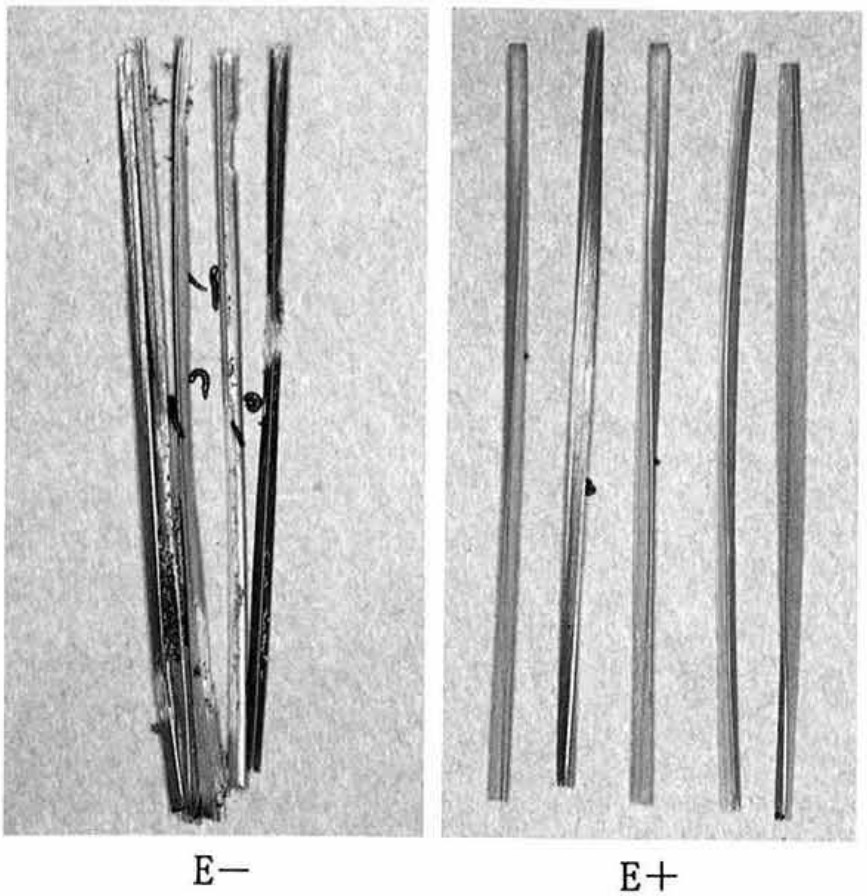

Plate 2. Feeding trials of the first instar larvae of bluegrass webworms on the leaf blades of perennial ryegrass artificially infected (E +) with *Acremonium* endophyte and uninfected (E −)
The photograph was taken 8 days after feeding trials on these leaf blades.

Table 1. Percentages of perennial ryegrass and tall fescue seedlings successfully infected with *Acremonium* endophytes by artificial inoculation[a)]

Cultivar	Endophytes (isolate)	Number of seedlings examined (A)	Number of infected seedlings (B)	B/A (%)
Perennial ryegrass				
Kiyosato	*Acremonium* sp.			
	(92-002)	10	2	20
	(92-008)	17	3	18
Friend	*Acremonium* sp.			
	(92-002)	12	4	33
	(92-008)	19	3	16
Manhattan II	*Acremonium* sp.			
	(92-002)	11	1	9
	(92-008)	20	6	30
Tall fescue				
Kentucky 31	*A. coenophialum*			
	(91-018)	30	2	7

a): The inoculated seedlings were examined 1-2 months after inoculation.

the appearance between the endophyte-infected and uninfected plants were observed. The percentages of the PR and TF seedlings successfully infected were 9-33% and 7%, respectively (Table 1). About one-third to half of the inoculated seedlings died due to the damage associated with the inoculation as reported by Latch and Christensen[10]. Therefore, the actual percentages of successful infection of the inoculated seedlings were lower than the data recorded. The percentages of successful infection varied with the combination of host cultivars and endophyte isolates. All the endophytes in the PR and TF plants artificially infected survived for about 2 years after inoculation.

In feeding trials, the larvae of the bluegrass webworm on the endophyte-infected and uninfected leaf blades ate small amounts of the infected leaf blades and eventually died. On the other hand, they fed on the uninfected ones well and most of them were alive even 8 days after incubation (Plate 2). Survival rates of the larvae on the infected leaf blades of PR, cultivar Kiyosato decreased to 22% after 4 days of incubation and less than 10% after 8 days of incubation (Fig. 1). However, the survival rate on the uninfected leaf blades which decreased slowly was 68% after 8 days of incubation. The effects on bluegrass webworm were almost the same in both the PR plants infected with *Acremonium* sp. and TF plants infected with *A. coenophialum*. The survival rates on the infected leaf blades of 3 PR cultivars and 1 TF cultivar remarkably decreased to 0-4.1% after 8 days of incubation, while those on the uninfected leaves remained at 75.1-91.6% (Table 2).

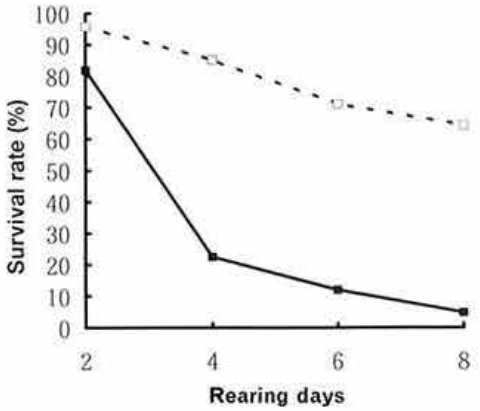

Fig. 1. Survival rate of bluegrass webworm feeding on endophyte-infected (■) and uninfected (□) leaf blades of perennial ryegrass, cultivar Kiyosato

All the seedlings of the progenies grown from the seeds produced on the parent plants artificially infected with the endophytes were also infected with the endophytes in 4 cultivars of PR and TF (Table 3). The seedlings of the progenies had also acquired the feeding deterrent to the bluegrass webworm (Table 2). In the combinations of PR cultivars, Kiyosato and Friend infected with the isolate 92-008

Table 2. Survival rate of bluegrass webworm larvae on artificially infected plants with the *Acremonium* endophyte and on their progenies

Cultivar/Inoculated endophyte isolate number[a]		Survival rate (%)[b]
Perennial ryegrass		
Kiyosato (uninfected)		75.1
Kiyosato/*Acremonium* sp. 92-008	Parent	0
Kiyosato/*Acremonium* sp. 92-008	F1	0
Friend (uninfected)		80.5
Friend/*Acremonium* sp. 92-008	Parent	4.1
Friend/*Acremonium* sp. 92-008	F1	0
Manhattan II (uninfected)		91.6
Manhattan II/*Acremonium* sp. 92-002	Parent	3.4
Manhattan II/*Acremonium* sp. 92-002	F1	24.2
Tall fescue		
Kentucky 31 (uninfected)		85.1
Kentucky 31/*A. coenophialum* 91-018	Parent	1.0
Kentucky 31/*A. coenophialum* 91-018	F1	34.4

a): Parent; artificially inoculated plants with the endophyte, Progeny (F1); seedlings grown from seeds from the parent plants infected with the endophyte.
b): The survival rate was estimated after 8 days of incubation.

Table 3. Percentages of transmission of *Acremonium* endophyte through seeds on artificially infected perennial ryegrass and tall fescue plants

Inoculated parent plant cultivar/ Inoculated endophyte isolate	Number of F1 seedlings examined[a]	Percentage of F1 seedlings infected with endophyte
Perennial ryegrass		
Kiyosato/*Acremonium* sp. 92-008	18	100
Friend/*Acremonium* sp. 92-008	31	100
Manhattan II/*Acremonium* sp. 92-008	35	100
Tall fescue		
Kentucky 31/*A. coenophialum* 91-018	63	100

a): The F1 seedlings were grown from the seeds obtained from artificially inoculated parent plants. The seeds were sowed and the developed seedlings were examined 1-2 months after sowing.

of *Acremonium* sp., the survival rates of the bluegrass webworm were 0-4.1% in both the artificially inoculated plants and their progenies. However, in the combinations of Manhattan II (PR) infected with the isolate 92-008 of *Acremonium* sp. and Kentucky 31 (TF) infected with the isolate 91-018 of *A. coenophialum*, the survival rates in the progenies were fairly high compared with those of the parents.

Discussion

It had already been reported that *Acremonium* endophytes isolated from PR and TF could infect their natural hosts by artificial inoculation but that the percentages of infection ranged from 2.0 to 53.8%[3]. The present results also showed that the percentages of infection of PR with *Acremonium* sp. and TF with *A. coenophialum* were in the 7-33% range. In these experiments, the mycelia of the endophytic fungi were inoculated by insertion into the meristematic regions of the seedlings. Another method using calli of PR and TF has been developed[4,6]. In that case, the inoculation was carried out by inserting the mycelia into the calli.

The plants artificially infected with the endophytes had acquired the feeding deterrent to the bluegrass webworm. Therefore, it is concluded that the feeding deterrent to the bluegrass webworm could be imparted by artificial inoculation of the endophytes.

In the present study the survival rate of the

bluegrass webworm feeding on the endophyte-infected grasses was high until 3 days, but rapidly decreased from 4 days onward and most of the larvae were dead within 8 days. Kanda et al.[5] demonstrated experimentally that the pattern of the survival rates on the endophyte-infected grasses was almost the same as that of the survival rates on water. Therefore, it was concluded that the larvae starved to death by avoiding endophyte-infected grasses. However, it remains to be determined what kind of compounds are related to the feeding deterrent to the bluegrass webworm although several alkaloids were reported as feeding deterrents for insects[10,16,18]. Further studies should be carried out to clarify the mechanisms of feeding deterrents in the endophyte-infected plants.

The percentages of seed transmission of the endophyte from PR and TF plants artificially infected were 100% in the present study. These results were consistent with those reported in PR artificially infected with *A. loliae*[10]. Since the endophytes used in the present study were transmitted by seed, infected seeds could be obtained by multiplying the infected plants.

The progenies of artificially inoculated PR infected with *Acremonium* sp. 92-008 had acquired a distinct feeding deterrent as well as their parents, while in those infected with *Acremonium* sp. 92-002 and *A. coenophialum* 91-018 the feeding deterrent to bluegrass webworm was not as strong as that of their parents. Since the characteristics of the isolates of *Acremonium* endophytes were variable during incubation[1,2], it is possible that *Acremonium* sp. 92-008 was more stable than other isolates. It is important for the breeding of bluegrass webworm-resistant plants that the feeding deterrent to bluegrass webworm remained stable in the successive generations.

In several useful grasses such as bentgrass and Kentucky bluegrass, natural infection with *Acremonium* endophytes has not been detected. Therefore attempts were made to infect these grasses with *Acremonium* endophytes artificially. When the endophytes were artificially inoculated into the non-host plants, some of them were effective while the others were not effective[13]. Whether the infection was successful or not depends on the specificity between plant and endophyte associations[3,13,14]. It was already reported that the intercellular matrix degenerates between the hyphae of the endophyte and host cell wall in incompatible association of *A. coenophialum* and PR[7]. Further studies on the mechanism of specificity will be important in order to inoculate the endophytes to the grasses.

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