北海道で収集されたメドウフェスクのエコタイプにおけるエンドファイトAcremonium uncinatumの検出

誌名	草地試験場研究報告 : s ō chi shikenj ō kenky ū h ō koku = Bulletin of the National Grassland Research Institute
ISSN	03850196
著者名	古賀,博則 月星,隆雄 植松,勉
発行元	農林省草地試験場
巻/号	49号
巻号補足	
掲載ページ	p. 35-41
発行年月	1994年2月

Incidence of the Endophytic Fungus, *Acremonium uncinatum*, in Meadow Fescue (*Festuca pratensis*) Ecotypes in Hokkaido

Hironori KOGA, Takao TSUKIBOSHI and Tsutomu UEMATSU

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

Received September 27, 1993

ABSTRACT

Koga, H., T. Tsukiboshi and T. Uematsu (1994) : Incidence of the Endophytic Fungus, *Acremonium uncinatum*, in Meadow Fescue (*Festuca pratensis*) Ecotypes in Hokkaido. Bull. Natl. Grassl. Res. Inst. 49 : 35~41

Intercellular hyphae were observed in the leaf sheaths of meadow fescue plants from 7 of the 8 ecotype collections from Hokkaido. The hyphae were convoluted, hyaline, with infrequent branches, and ran parallel to the vascular bundles. It was confirmed using scanning electron microscopy that the hyphae in the leaf sheaths were confined to the intercellular spaces. Colonies of the endophytic fungus grew very slowly on PDA, 0.2-0.5 mm/day at 25℃ on PDA, with the optimum temperature for growth being 25℃. The colonies were whitish to rosy buff, felted, forming aerial hyphae from which numerous phialides arose. The phialides were straight or slightly flexuous, 4-17μm long, and 0.8-1.6μm wide gradually tapering to 0.6-0.9μm towards the tips. Each phialide produced a single uncinate conidium 5-12μm long and 1.0-1.6μm wide. The morphology of the phialides and conidia, and the appearance of the colonies are identical to those described for *Acremonium uncinatum* in meadow fescue in Europe and New Zealand. Accordingly the endophytic fungus in meadow fescue in Hokkaido has been identified as *Acremonium uncinatum* Gams, Petrini et Schmidt.

Key words : endophyte, *Acremonium uncinatum*, meadow fescue (*Festuca pratensis*), scanning electron microscopy (SEM)

INTRODUCTION

Meadow fescue (*Festuca pratensis* Huds.) has been reported as being the host of two endophytic fungi (Gams *et al.* 1990), *Acremonium uncinatum* Gams, Petrini et Schmidt (Gams *et al.*, 1990) and a *Phialophora*-like species (Latch *et al.*, 1984), although the latter was mistakenly listed as *A. typhinum* (Leuchtmann 1993). Meadow fescue infected with *A. uncinatum* is known to contain loline alkaloids (Christensen *et al.*, 1993) which are toxic to several insect pests, particularly some species of aphids such as *Rhopalosiphum padi* L. and *Schizaphis graminum* Rondai (Siegel *et al.*, 1990). *In vitro*, the growth of *Drechslera sorokiniana* was inhibited by the endophytes of meadow fescue (Schmidt 1990). The beneficial effects conferred by *A. uncinatum* to meadow fescue plants make determination of its presence essential when plants are being selected for use as forage grasses.

Nothing is known about the incidence of endophytes in meadow fescue in Japan, although meadow fescue is an important pasture grass especially in Hokkaido. This paper describes the incidence, isolation and identification of endophytic fungi in some ecotype collections of meadow fescue in Hokkaido. The

relationship between the hyphae of endophytic fungi and vegetative tissue of meadow fescue plants was also confirmed using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Eight ecotypes of meadow fescue (*Festuca pratensis*) collected in Hokkaido by Dr. Ikegaya *et al.* (1988) were used in this study. The collection sites are shown in Fig. 1. Seven of the ecotypes were derived from seeds harvested at the collection sites in 1984-85 which were stored at 2-5℃ and low humidity. The ecotype collected at Niikappu was derived from seedlings collected in 1984 from which seeds were harvested in 1986.

Seedlings from each of the eight ecotype seed collections were grown in a green house maintained at 20-25℃. Twenty plants from each ecotype collection were examined for the presence of intercellular mycelium. Strips of epidermis pulled from the adaxial surface of the distal end of the leaf sheaths of the tillers were mounted in lactophenol aniline blue on glass slides and examined using a light microscope.

The endophytic fungi were isolated from the leaf sheaths of the infected plants. Leaf sheaths were immersed in 70% ethanol for 30 sec, soaked in chlorine bleach solution (1% available chroline) for 2-5 min, and then washed in sterile water. After drying on sterile filter paper, the samples were cut into small pieces and incubated on potato dextrose agar (PDA) containing 5μg/ml oxytetracycline in the dark at 25℃. Selected isolates were grown at 5, 10, 15, 20, 25 and 30 ℃ to determine the optimum temperature for growth of this endophytic fungus.

The grass/fungus associations were examined by the method using SEM as reported by Koga *et al.* (1993). Leaf sheaths of selected plants were fixed in 2.5% glutaraldehyde in 0.05 M cacodilate buffer pH 7.2 for 12 hr, and then post fixed by 1% OsO_4 for 2 hr. Fixed specimens were dehydrated using a graded ethanol series, and the specimens in 100% ethanol were transferred to gelatin capsules and sealed. Capsules were frozen in liquid nitrogen, and then split using a razor blade and hammer. The split pieces were thawed in 100% ethanol. They were then transferred to isoamyl acetate. After critical point drying, the specimens were coated with platinum and observed under the field emission scanning electron

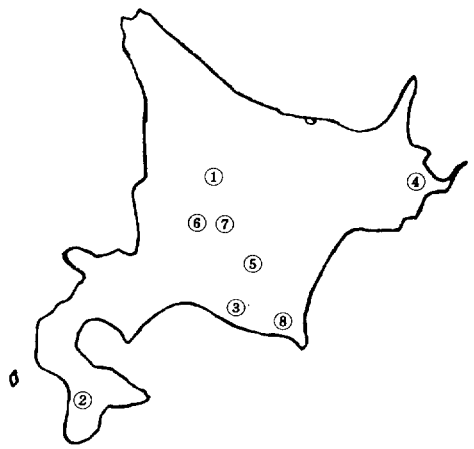


Fig. 1. Collection sites of meadow fescue ecotypes in Hokkaido. 1. Asahikawa, 2. Kaminokuni, 3. Niikappu, 4. Nemuro, 5. Hidaka, 6. Takikawa I, 7. Takikawa II, 8. Urakawa

microscope.

Conidia and phialides formed on *in vitro* were fixed with vapour of OsO_4 and were transferred into a sealed copper container. The container was frozen in liquid nitrogen and then dried for three days under vacuum (Nemoto and Miyazawa 1992). The specimens were coated with platinum in vacuum and observed as previously described (Koga *et al.* 1993).

RESULTS AND DISCUSSION

Meadow fescue plants containing intercellular hyphae in their leaf sheaths were observed in seven of the eight ecotype collections. The percentage of infected plants ranged from 80 to 100% (Table 1). The hyphae, 0.9–1.7 μm in diameter, were hyaline, convoluted and infrequently branched, and ran parallel to the vascular bundles (Fig. 2). SEM examination confirmed that all hyphae were contained in the intercellular spaces (Fig. 3) as previously reported with the *Acremonium* endophytes in perennial ryegrass (Koga *et al.* 1993). No remarkable changes were observed in appearance of the host cells adjacent to the hyphae.

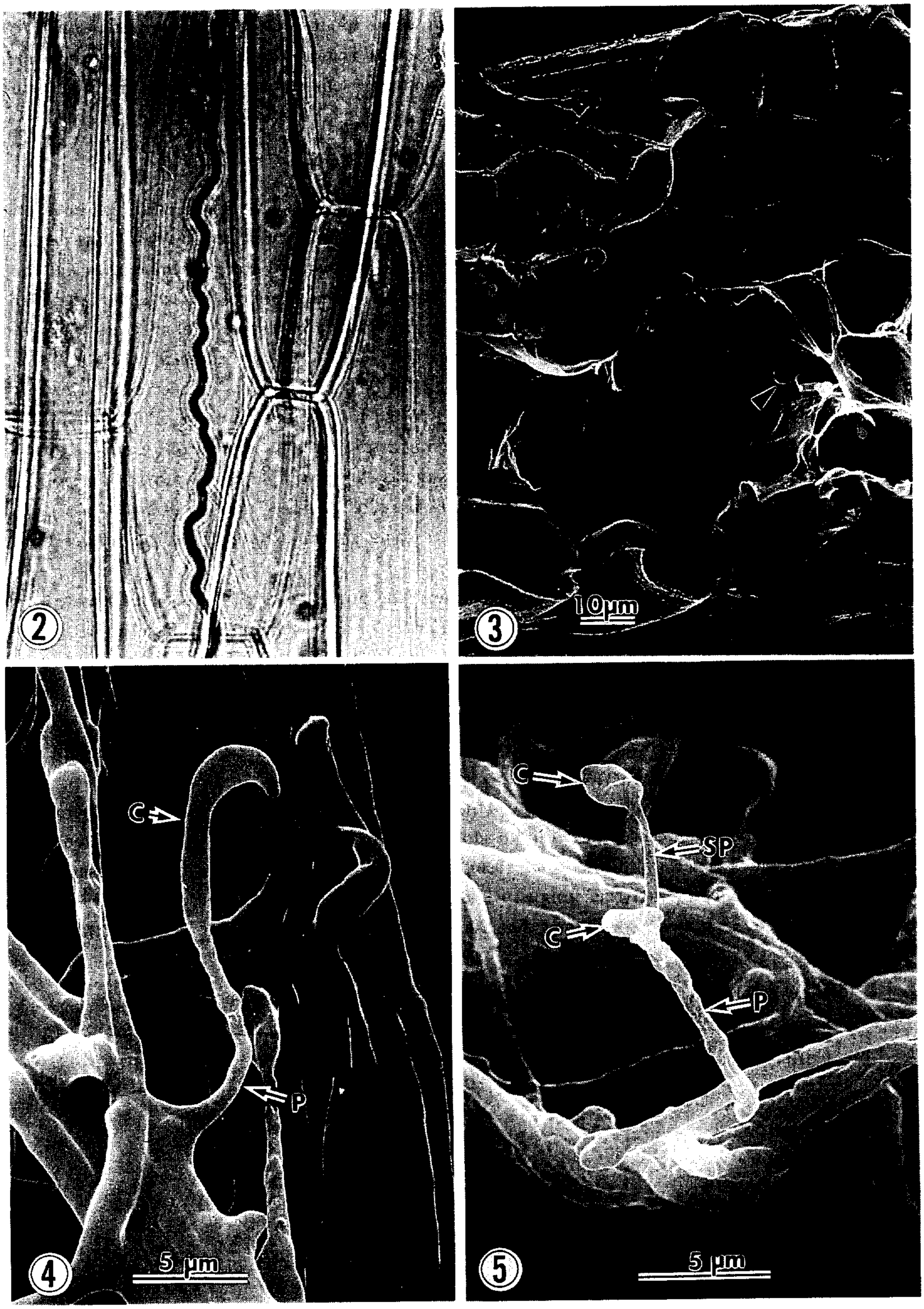
Ten or more days were required for the development of colonies from the pieces of leaf sheaths incubated on PDA. The colonies were cottony and whitish to rosy buff in color. The colonies grew slowly (0.2–0.5 mm/day), with maximum growth occurring at 25℃ (Fig. 6). Numerous phialides arose on the aerial mycelium. The phialides were straight or slightly flexuous, 4–17 μm long, and 0.8–1.6 μm wide gradually tapering to 0.6–0.9 μm towards the tips. Each phialide produced a single uncinate non-septate conidium 5–12 μm in length and 1.0–1.6 μm wide (Fig. 4). Some conidia were observed to have germinated *in situ* to produce a secondary phialide (Fig. 5).

The morphology of the phialides and conidia, and the appearance of the colonies isolated from meadow fescue in Hokkaido are identical to those described for *A. uncinatum* from meadow fescue in Europe and New Zealand (Gams *et al.*, 1990 ; Christensen *et al.*, 1993). Accordingly the endophytic fungus in meadow fescue in Hokkaido has been identified as *Acremonium uncinatum* Gams, Petrini et Schmidt. Christensen *et al.* (1993) reported that there are two strains of *A. uncinatum*, distinguished by having conidia that were typically either strongly curved or straight. However, all isolates in the present study had conidia that were strongly curved.

Meadow fescue was first introduced into Japan from Europe about 100 years ago (Ehara, 1977). *Acremonium uncinatum* has been reported in meadow fescue in Austria, France, the Federal Republic of Germany, Italy and Switzerland (Gams *et al.*, 1990), and thus it is likely that the meadow fescue plants introduced to Japan contained this fungus. The reason why no endophytes were observed in the ecotype collected at Urakawa is presumed that the endophytes in the seeds had died after the harvest.

Table 1. Incidence of endophytes in meadow fescue ecotypes collected in Hokkaido

Collection site	Number of observed plants	Infection percentage
Asahikawa	20	95
Kaminokuni	20	80
Niikappu	20	80
Nemuro	20	95
Hidaka	20	85
Takikawa I	20	95
Takikawa II	20	100
Urakawa	20	0


2
3
10μm
4
C
P
5 μm
5
C
SP
C
P
5 μm

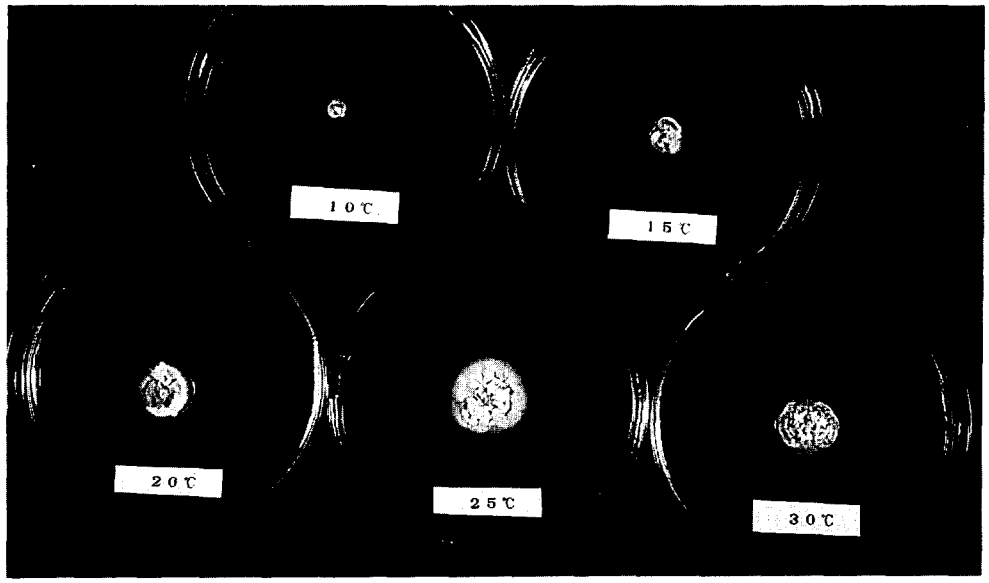


Fig. 6. Colonies of the endophyte isolated from a meadow fescue ecotype in Hokkaido, after 64 days incubation on PDA at 10, 15, 20, 25, 30℃ in the dark.

The *Phialophora*-like endophyte, readily identified in leaf sheaths as the hyphae are much branched and ramify throughout the tissue, was not detected during the present study. This is in contrast to Europe where the *Phialophora*-like endophyte is reported to be commonly found in meadow fescue (Gams *et al.* 1990).

The endophyte infected meadow fescue plants from Hokkaido were not colonized by *R. padi* aphids (Kanda *et al.* 1993). This indicates that they contain loline alkaloids as reported in meadow fescue in Europe and New Zealand (Schmidt *et al.* 1991 ; Christensen *et al.*, 1993). Many grasses infected with *Acremonium* endophytes contain a number of alkaloids which are toxic to mammals, notably lolitrem B and ergovaline (Siegel *et al.*, 1990). However, these toxic alkaloids have not been detected in meadow fescue plants containing *A. uncinatum* and there have been no reports of mammalian toxicity associated with this pasture species (Christensen *et al.*, 1993). The protection against insect pests provided by *A. uncinatum*, and the absence of mammalian toxins, suggest that the meadow fescue plants containing this endophytic fungus are beneficial to agronomic purposes.

ACKNOWLEDGEMENTS

We thank Dr. H. Daido, Hokkaido National Agricultural Experiment Station for kindly making available their ecotype collections. We are greatly indebted to Mr. M.J. Christensen, New Zealand Pastoral Agriculture Research Institute, and Dr. A. Tajimi, National Grassland Research Institute, for

Fig. 2. Light microscopy of the endophyte hypha in the leaf sheath of the meadow fescue ecotype.

Fig. 3. Scanning electron microscopy of the endophyte hypha (arrow) in the intercellular space of the leaf sheath of the meadow fescue ecotype.

Fig. 4. A phialide (P) with attached conidium (C) on the colony isolated from the leaf sheaths of the meadow fescue ecotype.

Fig. 5. A phialide with attached conidium producing a secondary phialide (SP) and conidium.

critical reading of the manuscript.

REFERENCES

Christensen, M.J., D. Hopcroft, D. D. Rowan and B. A. Tapper (1993) : Conidial morphology, alkaloid production, and host specificity of *Acremonium uncinatum*, an endophyte of *Festuca pratensis*. *In* Proc. 2nd. Int. Symp. *Acremonium*/Grass Interactions. (Hume, D. E., G. C. M. Latch and H. S. Easton eds.), Palmerston North, pp. 39–42.

Ehara, K. (1977) : Pasture grasses. Yokendo Press. Tokyo. pp.327.

Gams, W., O. Petrini and D. Schmidt (1990) : *Acremonium uncinatum*, a new endophyte in *Festuca pratensis*. Mycotaxon 37 : 67–71.

Ikegaya, F. *et al.* (1988) : A list of ecotype collections of meadow fescue. *In* A list of ecotype collections of grasses. National Grassland Research Institute. 80–91.

Kanda, K., H. Koga and Y. Hirai (1992) : Resistance of *Acremonium* endophyte-infected grasses to oat birdcherry aphid, *Rhopalosiphum padi* Linne. Proceedings of the Kanto-Tosan Plant Protection Society **39** : 191–192.

Koga, H., T. Kimigafukuro, T. Tsukiboshi, and T. Uematsu (1993) : Incidence of endophytic fungi in perennial ryegrass in Japan. Ann. Phytopathol. Soc. Japan. **59** : 180–184.

Latch,G. C. M., M. J. Christensen and G. J. Samuels (1984) : Five endophytes of *Lolium* and *Festuca* in New Zealand. Mycotaxon **20** : 535–550.

Leuchtmann, A. (1991) : Systematics, distribution and host specificity of grass endophytes, Natural Toxins **1** : 150–162.

Nemoto, N., S. Miyazawa, H. Kume, and M. Abe (1992) : Observation method of freezing dry for fungi. J. Technic. Res. Conf. Medi. and Biol. Electron Microsco. **6** : 78–79.

Schmidt, D. (1990) : Antagonistic effect of *Acremonium* spp. against potential pathogens. *In* Proceedings of the International Symposium on *Acremonium*/grass Interactions, New Orleans, (S.S.Quisenberry and R. E. Joost, eds) Louisiana Agricultural Experimental Station, Baton Rouge. 52–57.

Schmidt, D. (1991) : Les endophytes de la fetuque des pres. Revue suisse Agric. **23** : 369–375.

Siegel, M.R., G.C.M. Latch, L.P. Bush, F.F. Fannin, D.D. Rowan, B.A. Tapper, C.W. Bacon and M.C. Johnson (1990) : Fungal endophyte-infected grass : Alkaloid accumulation and aphid response. Journal of Chemical Ecology, **16** : 3301–3315.

北海道で収集されたメドウフェスクのエコタイプにおけるエンドファイト *Acremonium uncinatum* の検出

古賀博則・月星隆雄・植松　勉

環境部　作物病害研究室

（平成5年9月27日受理）

要　旨

古賀博則・月星隆雄・植松　勉 (1994)：北海道で収集されたメドウフェスクのエコタイプにおけるエンドファイト *Acremonium uncinatum* の検出．草地試研報 49：35～41

北海道に自生するメドウフェスクのエコタイプについてエンドファイトの有無を検討した。メドウフェスクの葉鞘組織を光学顕微鏡で観察した結果，上ノ国，滝川Ⅰ，滝川Ⅱ，根室，日高，新冠，および旭川で採集されたエコタイプに *Acremonium* エンドファイトが80-100％の高率で観察された。滅菌した葉鞘組織をPDA培地上に静置し，25℃に保って，エンドファイトの分離を行った。分離されたエンドファイトの菌叢は白色～薄いピンク色で，生育の最適温度は25℃で，その条件下でPDA培地上での菌叢の伸展は0.2～0.5 mm/日であった。フィアライドは菌糸の側面から直立して生じ，その先端には5-12×1.0-1.6μmの鈎形の分生子が1個形成されていた。

走査電子顕微鏡観察の結果，エンドファイトの菌糸は，植物組織の細胞内に侵入することはなく細胞間隙を伸展していることが明かとなった。

以上のことから，このエンドファイトを，*Acremonium uncinatum* Gams, Petrini et Schmidt と同定した。

草地試研報 49：35～41（1994）