北草研報29:78－84(1995)

Incidence of Endophyte Fungi on Forage Grasses in the Grassland in Northern and Central Regions of Hokkaido and the Prevention Methods of Infected Seeds

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道北・道央草地におけるエンドファイトの検出と感染種子の防除法

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Summary

We examined the incidence of endophyte fungi in perennial ryegrass, meadow fescue and tall fescue collected from 28 sites through the northern to central parts of Hokkaido. Of 140 plants we detected 5 plants that were infected with these fungi. This suggests that grazing animals have been hardly affected until now. However, we are afraid of importing infected seeds from abroad in future, so that attempts were made to control the seed-borne fungi.

Heat treatment of the seeds in dry state at 110℃ reduced significantly the percentage of infected seedlings, but the seed viability was also decreased by the treatment at more than 90℃. So this method could not be suitable for the practical use.

The seed treatment with fungicide, "Benomyl" was so effective to control endophyte fungi that the percentage of the infected seedlings decreased from 69% in the non-treated seedlings to 12% and did not affect the seed germination rate at all. Hence, this convenient technique of fungicide treatment appears to be available to control of a fungus endophyte in forage grass.

Key words : Endophyte, Heat treatment of seed, Fungicide, Benomyl, Perennial ryegrass, Meadow Fescue, Tall Fescue.

キーワード：エンドファイト，種子乾熱処理，殺菌剤，ベノミル，ペレニアルライグラス，メドウフェスク，トールフェスク.

Introduction

In a previous paper, we reported to find a high incidence of endophyte fungi in the seeds of perennial ryegrass varieties used for turf and forage in Hokkaido[22]. There has been several reports that the presence of endophyte fungi should relate to ryegrass staggers and fescue toxicosis[3, 5, 8, 10, 12, 13, 25, 26]. This indicates that we should take precautions against spreading

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平成6年度　研究発表会において発表

of endophyte fungus in pasture, especially of ryegrass and fescue.

Since they are seed-borne fungi, we could establish new swards with so small amount of infected plants not to cause grazing animals a disorder by using fungus-free seeds. There are several control methods such as storage for a long period, hot water treatment and chemical treatments[11, 17, 23]. These methods are available to eradication of endophyte fungus but they reduce so much seed viability at the same time. Hence, it is necessary to develop convenient techniques that meet the need for the dairy farmers.

The objectives of our studies were to survey the incidence of the fungus in the grassland in Hokkaido, and to establish convenient method to eradicate fungus in seed.

Materials and Methods

Experiment 1. Incidence of endophyte fungi in forage grasses

We collected 16 and 5 populations of perennial ryegrass in the grassland of northern and central Hokkaido, respectively. Four meadow fescue and three tall fescue populations were collected in central Hokkaido (Table 1).

Five plants of each population were grown in the planter in a greenhouse. The stem tissue of all plants were tested to determine the presence of endophyte using the ELISA technique as previously described[1]. The stem

Table 1. Collection site of the plant materials.

Collection site	materials			
	Perennial ryegrass	Meadow fescue	Tall fescur	total
Northern Hokkaido				
Hamatonbetsu-cho Usotan	5			
Nakatonbetsu-cho	5			
Saruhutsu-mura Shineshinko	5			
Saruhutsu-mura Shineshinko	5			
Saruhutsu-mura Chiraibetsu	5			
Saruhutsu-mura Chiraibetsu	5			
Saruhutsu-mura Chiraibetsu	5			
Toyotomi-cho Nishitoyotomi	5			
Toyotomi-cho Nishitoyotomi	5			
Toyotomi-cho Nishitoyotomi	5			
Toyotomi-cho Hukunaga	5			
Wakkanai-shi Toyobetsu	5			
Wakkanai-shi Toyobetsu	5			
Wakkanai-shi Toyobetsu	5			
Wakkanai-shi Ariake	5			
Wakkanai-shi Ariake	5			
Central Hokkaido				
Urausu-cho		5		
Urausu-cho	5			
Eniwa-shi Mikawa			5	
Eniwa-shi Mikawa		5		
Eniwa-shi Mikawa	5			
Eniwa-shi Mikawa	5			
Eniwa-shi			5	
Eniwa-shi			5	
Hamamasu-mura Zitsuta		5		
Hamamasu-mura Zitsuta	5			
Makkari-mura	5			
Numata-cho Gokayama		5		
total 28	105	20	15	140

in which endophyte fungus had been detected were stained with 0.1% lactophenol aniline blue followed by examining again the presence under a light microscope (×400)[19].

Experiment 2. Eradication of endophyte fungi in seeds

1) Determination of infection rate of the experimental seeds

The experimental seeds of four perennial ryegrass cultivars, that is, 'Advent', 'Citation II', 'Manhattan II' and 'Parmer II / Prelude II', were provided by Snow Brand Seed Co., Ltd. and Tokyo Green Co., Ltd. We determined the percentage of the endophyte infection of the seeds by double-check test using the ELISA technique and the staining method with 0.5% rose bengal[21].

2) Heat treatment of seeds

The seeds were treated for 30 min. at 40,50 ,60,70,80,90,100,110 and 120℃ in convection ovens. The corresponding relative humidities to the heat treatment were ranged from 10.5% in the treatment of 40℃ to 2.9% at 90℃. The seeds were transferred into a refrigerator for cooling. Then 150 seeds of each cultivar were sown in the pots filled with sterilized soil. After four weeks 30 stems of each cultivar in each treatment were examined for incidence of endophyte by ELISA method.

3) Fungicide treatment of seeds

The seeds were treated with fungicide, "Benomyl" (50W 0.5% seed weight) as seeds powder dressing. The examining method was the same as the heat treatment trial.

Results

Experiment 1. Incidence of endophyte fungi in forage grasses

We regarded the plant observed dark staining mycelium of the endophyte in the intercellular space of the stem tissue as the infected plant (Fig.1).

The sites that plants collected were shown in Fig.2. We assessed five plants from each population. Of 16 populations of perennial

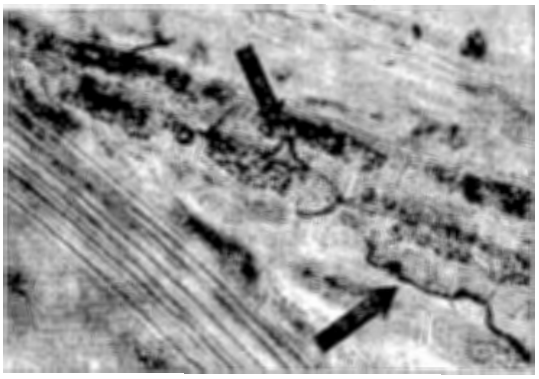

Fig. 1. Endophytic hyphae in the stem tissue of perennial ryegrass stained with 0.1% lactophenol anilin blue (×400)

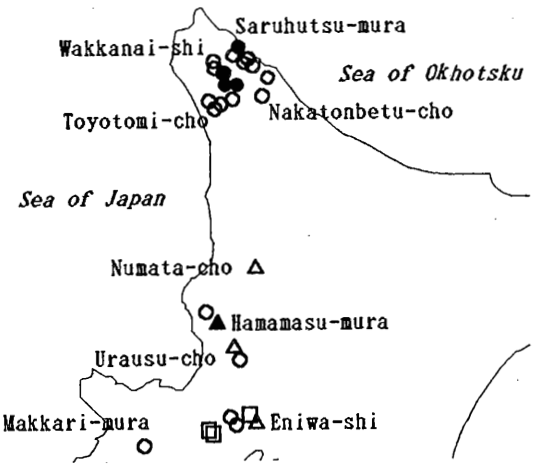

Fig. 2. Incidence of endophytic fungi in forage grass populations collected in northern and central Hokkaido.

Open circle(○), triangle(△)and square(□)shows the collecting site where no endophyte was detected in perennial ryegrass, meadow fescue and tall fescue populations, respectively. Closed circle(●)and triangle (▲)shows the site in which endophyte was detected in perennial ryegrass and meadow fescue populations, respectively.

ryegrass collected in northern Hokkaido, four populations had one infected plant, wich is 20% of the plants even in the infested population. On the other hand, no infected plant was found in perennial ryegrass and tall fescue in central Hokkaido, but one infected plant was found in only one meadow fescue population. Of 28 populations in total, five ones included infected plants, that is 18% and 4% based on poupulations and plants, respectively. This

indicated the grassland in Hokkaido did not yet attain the critical level for animal health[9, 20].

Experiment 2. Eradication of endophyte fungi in seeds

1) Determination of infection rate of the experimental seeds

We used artificially inoculated seeds with endophyte fungi for turf use. The ELISA technique revealed the difference of percentage of infected seeds among cultivars ranging from 100% in 'Advent' to 60% in 'Manhattan II' and the staining method showed that the varietal difference were 89% in 'Advent' and 68% in 'Manhattan II' (Table 2). The relative order of the cultivars thus was closely maintained in both examination methods. Therefore, the values in Fig.3 and Fig.4 showed averages of four cultivars with standard deviations.

Table 2. Incidence of seed-borne endophytic fungi in perennial ryegrass seeds of four cultivars (%)

Name of cultivar	Staining methods[1)]	ELISA technique
Advent	89	100
Parmer II/Prelude II	88	96
Citation II	76	72
Manhattan II	68	60

1) Seeds were stained with 0.5% rose begal.
2) Fifty seeds of each cultivars were examined.

2) Heat treatment of seeds

Heat treatment influenced both on occurrenced of infected seedlings and seed viability (Fig.3). The seed viability kept the original level below 80℃, above which percentage of germination decreased significantly. The heat treatment with 80℃ was ineffective for decreasing the infected seedlings. The percentage of infected seedlings was significantly decreased at 110℃ at which the percentage of germination fell drastically to below 20%.

3) Fungicide treatment of seeds

Seeds dressing with "Benomyl" powder were very effective in eradicating endophyte fungi parasitizing in the seeds (Fig.4). The average percentages of infected seedlings from the seeds decreased from 69% in the original no-treated seeds to 12%. The significant decrease in the germination rate was not observed by this treatment.

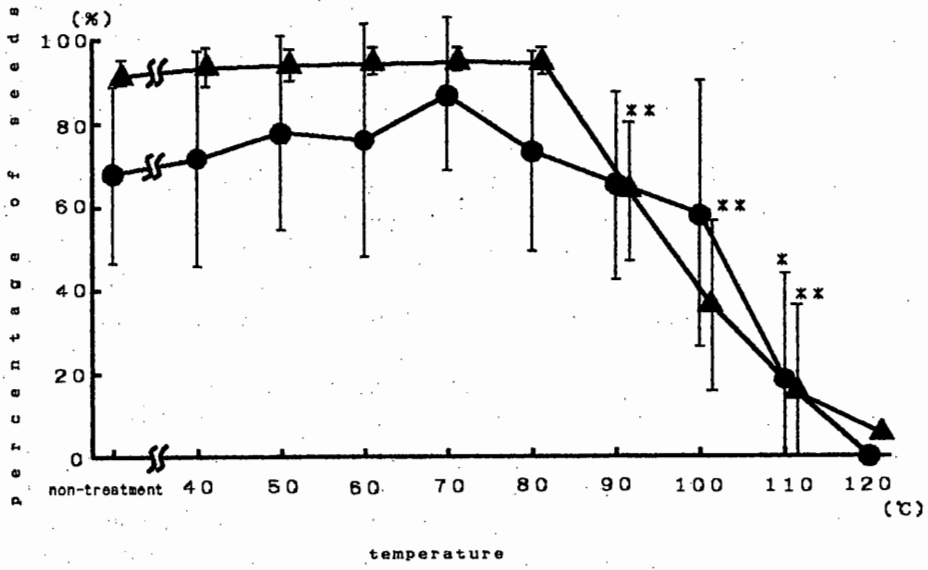

Fig. 3. Effect of heat treatment of seed on endophytic infection in seedlings and seed germination. Values are based on the average of four cultivars and the bars show standard deviation. Closed circle (●) and triangle (▲) show the percentage of infected seedlings and the percentage of seed germinition, respectively. Asterisks against symbols indicate significance difference from non-treatment at 5% level (*) or 1% level (**).

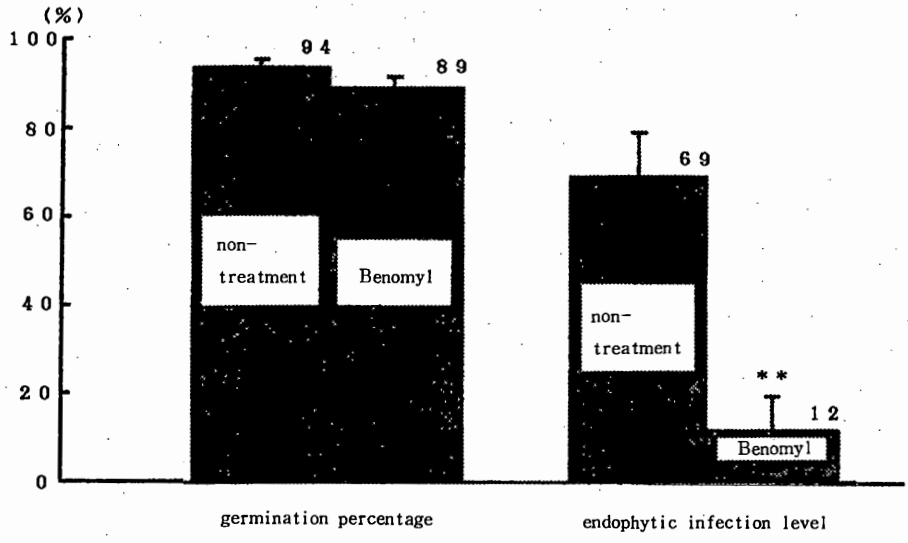

Fig.4. Effect of Benomyl (50W 0.5% seed weight) as a powder dressing on endophytic infection in seedlings and seed germination. (**) show least significant difference from non-treatment at 1% level.

Discussion

Our survery indicated that the level of endo phyte fungi infection does not attain serious conditions for animal health in the grassland investigated in Hokkaido, since grazing animals may exhibit symptoms of fescue toxicosis in the case that they graze in the swards where over 30% of plants are infected[11, 24]. We did not find infected plants in tall fescue but Koga et al. (1995) observed the infected seeds collected in Hokkaido[15, 16]. Even though there ossurs very small rate of infection at the present state, we should pay attention to the import of infested seeds from abroad.

On the other hand, the seed industry has produced the inoculated seeds for turf use as already mentioned, since a fugus endophyte imposes drought and insect resistance on the infected plants[2, 4, 18, 20, 27]. Thus we might expect the problem of endophyte infection in forage and turf grass in future, so that the detection and control methods should be estab lished.

As to the control techniques, storage for a long period, hot water treatment and several kinds of chmical treatment have been tried to make endophyte infected seeds free[11, 17, 23]. They were effective in controlling the fungus but reduced almost proportionally seed viability at the same time. The methods we tried in the experiment, heat treatments, were confirmed to be effective in fungi controlling and the former treatment also reduced seed viability.

Fungicide treatment can not eradicate completely the fungi but it may control them to the level satisfied from practical points of view. Because of nonphytoxicity to seed germination, this "Benomyl" powder dressing method is considered to be a convenient practical technique.

At last we must emphasize that it is of very importance to take precautions against endophyte infection into grassland, since the commercial inoculated seeds are practically used in turf ground that are likely to contaminate swards for animal feeding. We would like to advocate eatablishment of a new organization for test and control of endophyte infection.

Acknowledgements

We thanks Dr. H. KOGA, National Grassland Research Institute, for kindly supplying some ecotypes of endophyte fungi. We are greatly indebted to Dr. J. ABE, Hokkaido National Agricultural Experiment Station, for his critical reading of this manuscript.

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(Received on May 30,1995)

要 約

道北・道央地域の草地においてエンドファイトは140点中5点検出された。検出程度は極めて少なく、現時点では放牧家畜に害がでる可能性は低いことが示唆された。しかしながら、高い感染程度の種子を導入した際の対応のために、感染種子における乾熱処理およびベノミル（50%水和剤、0.5%種子重量）の粉衣処理による効果を検討した。

乾熱処理では110℃で発芽牧草におけるエンドファイト感染率の有為な減少が認められたが、同時に90℃以上で発芽率の有意な減少も認められ、本方法は実用面で問題があり、乾燥条件におけるエンドファイトの強い休眠性が示唆された。

ベノミルの粉衣処理では発芽牧草におけるエンドファイト感染率は69%から12%に有意に減少し、発芽率には差が認められなかった。本方法はエンドファイトの防除が完全ではないものの、操作の簡便性や発芽率に対する安全性から考え、有効な方法と考えられる。