JOURNAL OF PLANT PROTECTION RESEARCH Vol. 51, No. 4 (2011)
DOI: 10.2478/v10045-011-0059-z

# EFFECT OF *NEOTYPHODIUM UNCINATUM* ENDOPHYTE ON MEADOW FESCUE YIELDING, HEALTH STATUS AND ERGOVALINE PRODUCTION IN HOST-PLANTS

*Dariusz Pańka[1]*, Małgorzata Jeske[1], Mikołaj Troczyński[2]*

University of Technology and Life Sciences in Bydgoszcz
Kordeckiego 20, 85-225 Bydgoszcz, Poland
[1] Department of Phytopathology and Molecular Mycology
[2] Department of Environmental Chemistry

*Received: April 5, 2011*
*Accepted: June 24, 2011*

**Abstract:** The objective of our research was to assess the beneficial impact of the *Neotyphodium uncinatum* (Gams, Petrini and Schmidt) Glenn, Bacon and Hanlin endophyte on its natural host – meadow fescue. Assessment was made by measuring the green mass yield, the susceptibility of the host plants to infection by pathogens, and the content of the toxic alkaloid ergovaline, in field conditions. The research involved Justa meadow fescue. The studied factors were as follows: endophyte infection (E+ and E-) and system of use (for pasture and for cut). The effect of *N. uncinatum* on Justa meadow fescue yielding in all the combinations was observed. The presence of endophyte significantly enhanced higher yields of dry matter compared to the non-infested plants. The infestation of Justa meadow fescue by the endophyte, *N. uncinatum,* significantly protected the plants from infection of the fungi which causes leaf spot. The endophyte, however, did not affect the development of powdery mildew and rust fungi. Justa meadow fescue showed a relatively high content of ergovaline when grown in the field. The level of the toxin in the season varies a lot, which suggests a high effect of external factors on its production. Due to the production of the toxin, the animal feed made from infested plants can pose a threat to animals when administered over a long period. *N. uncinatum* isolates from Justa meadow fescue cannot be used as biological control agents to improve the growth and resistance of other cultivars, due to the production of ergovaline.

**Key words:** endophyte, *Neotyphodium uncinatum* (Gams, Petrini and Schmidt) Glenn, Bacon and Hanlin, *Festuca pratensis* Huds., pathogens, ergovaline, yield

## INTRODUCTION

Meadow fescue (*Festuca pratensis* Huds.) is one of the most important pasture species of grasses in Europe. It has evolved symbiotic associations with fungi, including ecto- and endomycorrhizal fungi of the roots and fungi that systemically infect grass tillers. Among the latter, fungi which live their entire life within the host grass, and form nonpathogenic, symptomless, intercellular associations are commonly defined as grass endophytes (Bacon and De Battista 1991; Smith and Read 1997). The main two groups of these endophytes are called: e-endophytes and p-endophytes. The fungus belonging to the first group and classified in the tribe Balansiae of the family Clavicipitaceae (Ascomycetes), is known as *Neotyphodium uncinatum* (Gams, Petrini and Schmidt) Glenn, Bacon and Hanlin (Glenn *et al*. 1996). *Phialophora*-like endophytes belong to the second group of meadow fescue endophytes and they are ordered to Eurotiales (Ascomycetes) (An *et al*. 1993; Siegel *et al*. 1995). The e-endophyte – *N. uncinatum* has the greatest significance which is related to the agronomic impact, mainly to the livestock performance and persistence of the sward. This impact is highly correlated with biologically active alkaloids which are produced in the plants infected with the endophyte. The main alkaloid is ergovaline which is assumed to be responsible for fescue toxicosis in livestock. The symptoms associated with this syndrome are lower feed intake, loss of live weight and rough hair coat (Porter and Thompson 1992; Thompson and Stuedemann 1993; Oliver 2005). Long exposure on high ergovaline concentrations can even lead to an animal's death. *Neotyphodium* endophytes can also beneficially affect the host plant. They stimulate growth and development of the host plant, tillering, and enhance drought stress resistance, persistence and competitiveness compared to uncolonized grasses. Moreover, they often ensure higher resistance of the host plant to infection by pathogens and insect feeding (Johnson *et al*. 1985; Clarke *et al*. 2006; Lehtonen *et al*. 2006). These facts indicate the possibility of utilizing the endophyte isolates for improving meadow fescue growth under biotic and abiotic stress (Bouton and Easton 2005). Such a use could reduce the costs of plant cultivation and protection. However, because of the toxins produced, application is hindered.

---

*Corresponding address:
panka@utp.edu.pl

The objective of the conducted research was to assess the level of the beneficial impact of the *N. uncinatum* endophyte on its natural host – meadow fescue. Assessment was made by measuring the green mass yield, the susceptibility of the host plants to infection by pathogens, and the content of the toxic alkaloid – ergovaline in field conditions.

## MATERIALS AND METHODS

### Field experiment

The research involved Justa meadow fescue (*F. pratensis*). The plot experiment was set up in 2006, at the Experiment Station of the University of Technology and Life Sciences at Mochełek (53°13′ N 17°51′ E), on IIIB class soil of very good rye complex, as a two-factor experiment in randomized block design, in 4 reps. The seeds at the rate of 10.0 kg/ha were sown as a companion crop into spring barley (100 kg/ha). Fertilisation and cultivation were made following the instructions for the cultivar and the guidelines of the breeder. In the years that nitrogen fertilisation was used, it was applied at the dose of 60 kg/ha in spring, and under each successive regrowth. Phosphorus was applied once, in spring, at a dose of 100 kg $P_2O_5$/ha, while potassium – at the split dose of 60 kg $K_2O$/ha. Each were applied under first and third regrowth. The plot area was 14 $m^2$ (2x7 m). The first factor involved the infestation with *N. uncinatum* (combinations E+ and E-). The presence of the endophyte in plants, was confirmed with the staining method according to Saha *et al*. (1988). The level of infestation accounted for 89%. The plants without endophyte (E-) were obtained from seeds in which symbiont was removed with the thermal method, prior to the establishment of the experiment (Latch and Christensen 1982). The second factor covered the system of fescue use: pasture (P) and for cut (K). In the pasture system the plants were cut at the end of tillering. For K, the plants were cut at the beginning of the shooting stage. During plant growth, plant protection chemicals were not applied. 'Justa' meadow fescue yielding was analysed for successive cuts collected over 2007 and 2008. In the year of the establishment (2006) of the field experiment, no green forage cuts were collected due to very unfavourable weather conditions in the growing season. Long semi-drought periods resulted in the inhibition of the development of young plants and their thinning on the plots. Plant regeneration in the experiment occurred only in the autumn and spring of the following season. In 2007, four cuts were collected, while in 2008 – three. For each cut, the yield of dry matter was determined. The values obtained were verified with a two-factor analysis of variance. The significance of differences was defined with the Tukey test.

### Meadow fescue infection by pathogens

Over the research years, the infection of plants with powdery mildew [*Blumeria graminis* (DC) Speer], rusts (*Puccinia* spp.) and leaf spot complex [*Bipolaris sorokiniana* (Sacc.) Shoem., *Drechslera* spp.] was analysed. The observations were made in spring, prior to the first harvest. The observations were made again in autumn, at the end of the vegetation period. For the analysis, 50 leaves each were randomly sampled from each replicate. The degree of plant infection was evaluated using a modified Birckenstaedt *et al*. (1994) scale, where 0 was applied for healthy leaves and 8 – for the leaves on which disease symptoms were visible on over 69.3% of the leaf area for rusts and powdery mildew, and 64.7% of the leaf area for spots. The degrees of plant infection were transformed into disease index (DI%) values following the Townsend and Heuberger (Wenzel 1948) formula, and then exposed to the analysis of variance for two-factor experiments. The significance of differences was defined based on the Tukey half-intervals of confidence.

### Ergovaline analyses

Ergovaline content was determined in each cut. Samples of the tillers were randomly harvested before cuts, lyophilized, powdered in a laboratory mill, and then taken for HPLC analyses. Chemical analyses were carried out according to the modified method of Rottinghaus *et al*. (1991). A weighed portion of dry plant material (0.2 g) was transferred to sealed glass vials. Five $cm^3$ 20% acetic acid and 1 μg ergotamine as an internal standard, were added and stirred in a vortex. Then, the samples were placed for 12 h at ca. 4°C and vortexed every hour. Next, the samples were centrifuged, and 3 $cm^3$ of the extract was applied to a solid phase extraction column containing 300 mg of C18 packing material. Pre-condition of the column was performed by washing with 3 $cm^3$ of methanol followed by 4 $cm^3$ of deionized water. The column was rinsed with 2 $cm^3$ of acetic acid used for extraction, and then with 4 $cm^3$ of deionized water after passing through of the extract. The adsorbed toxins were eluted three times with an ammonia solution (0.04%) in methanol (1 $cm^3$ total volume), stirred and immediately analyzed using a Perkin-Elmer Series 200 high-performance liquid chromatography system, equipped with a fluorescence detector. The wavelength of the excitation was set at $\lambda_{ex}$ = 235 nm, and of the emission was set at $\lambda_{em}$ = 415 nm. Compounds were separated on an Alltech Komasil C18 column (150 mm). Elution was performed isocratically using methanol and 0.1 M/$dm^3$ ammonium acetate (3:1). The ergovaline concentration was calculated based on the peak area, taking into account recovery, which was determined for each sample separately on the basis of the ergotamine concentration (internal standard). For the preparation of the standard curve for quantitative calculation, ergotamine was used because of pure ergovaline unavailability. Analyses were carried out in 4 replications. Results were statistically analyzed using two-way ANOVA. Means were separated based on Tukey's test.

## RESULTS

### Endophyte effect on meadow fescue yielding

The reported Justa meadow fescue yields varied considerably in the research years (Table 1). The first year of harvest was relatively favourable to the development of grasses (Table 4). Moderate temperatures and quite a good rainfall distribution were recorded in the first year

Table 1. Dry matter yield of Justa meadow fescue (*F. pratensis*) [t DM/ha]. Mochełek 2007–2008

| Endophyte status | First cut | | | Sum of cuts | | |
|---|---|---|---|---|---|---|
| | P[2] | K | mean | P | K | mean |
| 2007 | | | | | | |
| E+[1] | 3.16 | 4.04 | 3.60 | 5.63 | 7.03 | 6.33 |
| E- | 2.32 | 2.49 | 2.40 | 4.50 | 4.73 | 4.61 |
| Mean | 2.74 | 3.26 | – | 5.06 | 5.88 | – |
| NIR $\alpha = 0.05$ | I = 0.412; II = 0.412; II/I = 0.583; I/II = 0.541 | | | I = 0.392; II = 0.392; II/I = 0.554; I/II = 0.514 | | |
| 2008 | | | | | | |
| E+ | 1.23 | 1.80 | 1.51 | 2.91 | 3.55 | 3.23 |
| E- | 1.08 | 1.40 | 1.24 | 2.26 | 2.41 | 2.34 |
| Mean | 1.16 | 1.60 | – | 2.58 | 3.00 | – |
| NIR $\alpha = 0.05$ | I = 0.149; II = 0.149; II/I = 0.211; I/II = 0.196 | | | I = 0.267; II = 0.267; II/I = 0.378; I/II = 0.351 | | |

[1] first factor (I) – endophyte infected (E+) and uninfected (E-) plants

[2] second factor (II) – type of utilization: for pasture (P) and for cut (K)

Table 2. Level of infection [DI %] of Justa meadow fescue (*F. pratensis*) by pathogenic fungi depending on utilization type. Mochełek, 2007–2008

| Endophyte status | 2007 | | | 2008 | | | 2007–2008 | | |
|---|---|---|---|---|---|---|---|---|---|
| | P[2] | K | mean | P | K | mean | P | K | mean |
| Spring observation | | | | | | | | | |
| Rusts | | | | | | | | | |
| E+[1] | 3.20 | 3.83 | 3.51 | 1.61 | 1.90 | 1.75 | 2.41 | 2.86 | 2.63 |
| E- | 4.00 | 4.22 | 4.11 | 2.22 | 2.17 | 2.19 | 3.11 | 3.20 | 3.15 |
| Mean | 3.60 | 4.02 | – | 1.91 | 2.03 | – | 2.76 | 3.03 | – |
| NIR $\alpha = 0.05$ | I = n.s.[3]; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | |
| Leaf spots | | | | | | | | | |
| E+ | 9.25 | 9.89 | 9.57 | 3.55 | 3.23 | 3.39 | 6.40 | 6.56 | 6.48 |
| E- | 10.90 | 11.31 | 11.10 | 4.44 | 4.79 | 4.62 | 7.67 | 8.05 | 7.86 |
| Mean | 10.07 | 10.60 | – | 3.99 | 4.01 | – | 7.03 | 7.30 | – |
| NIR $\alpha = 0.05$ | I = 0.65; II = n.s.; II/I = n.s.; I/II = 0.85 | | | I = 0.69; II = n.s.; II/I = n.s.; I/II = 0.91 | | | I = 0.39; II = n.s.; II/I = n.s.; I/II = 0.55 | | |
| Powdery mildew | | | | | | | | | |
| E+ | 1.67 | 1.90 | 1.78 | 0.83 | 1.03 | 0.93 | 1.25 | 1.46 | 1.35 |
| E- | 1.73 | 2.07 | 1.90 | 1.06 | 1.15 | 1.10 | 1.39 | 1.61 | 1.50 |
| Mean | 1.70 | 1.98 | – | 0.94 | 1.09 | – | 1.32 | 1.53 | – |
| NIR $\alpha = 0.05$ | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | |
| Autumn observation | | | | | | | | | |
| Rusts | | | | | | | | | |
| E+[1] | 6.92 | 7.15 | 7.03 | 7.51 | 7.99 | 7.75 | 7.21 | 7.57 | 7.39 |
| E- | 7.39 | 7.65 | 7.52 | 7.90 | 8.02 | 7.96 | 7.64 | 7.83 | 7.74 |
| Mean | 7.15 | 7.40 | – | 7.70 | 8.00 | – | 7.43 | 7.70 | – |
| NIR $\alpha = 0.05$ | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | |
| Leaf spots | | | | | | | | | |
| E+ | 12.14 | 12.50 | 12.32 | 15.21 | 14.60 | 14.90 | 13.67 | 13.55 | 13.61 |
| E- | 15.75 | 15.31 | 15.53 | 17.07 | 17.24 | 17.16 | 16.41 | 16.28 | 16.34 |
| Mean | 13.94 | 13.90 | – | 16.14 | 15.92 | – | 15.04 | 14.91 | – |
| NIR $\alpha = 0.05$ | I = 1.52; II = n.s.; II/I = n.s.; I/II = 1.99 | | | I = 1.13; II = n.s.; II/I = n.s.; I/II = 1.48 | | | I = 0.64; II = n.s.; II/I = n.s.; I/II = 0.90 | | |
| Powdery mildew | | | | | | | | | |
| E+ | 2.23 | 2.45 | 2.34 | 3.84 | 3.36 | 3.60 | 3.03 | 2.91 | 2.97 |
| E- | 1.91 | 2.79 | 2.35 | 3.74 | 3.80 | 3.77 | 2.82 | 3.30 | 3.06 |
| Mean | 2.07 | 2.62 | – | 3.79 | 3.58 | – | 2.93 | 3.10 | – |
| NIR $\alpha = 0.05$ | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | | I = n.s.; II = n.s.; II/I = n.s.; I/II = n.s. | | |

[1] first factor (I) – endophyte infected (E+) and uninfected (E-) plants

[2] second factor (II) – type of utilization: for pasture (P) and for cut (K)

[3] n.s. – not-significant difference

Table 3. Ergovaline content in green forage [µg/g] of Justa meadow fescue successive cuts depending on *Neotyphodium uncinatum* infection and type of utilization. Mochełek 2007–2008

| Endophyte status | Type of utilization in successive years of research | | | | | |
|---|---|---|---|---|---|---|
| | 2007 | | | 2008 | | |
| | P[2] | K | mean | P | K | mean |
| First cut | | | | | | |
| E+[1] | 0.775 | 2.083 | 1.429 | 1.217 | 2.297 | 1.757 |
| E- | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Mean | 0.388 | 1.041 | – | 0.609 | 1.148 | – |
| NIR $\alpha = 0.05$ | I = 0.075; II = 0.075;<br>II/I = 0.106; I/II = 0.099 | | | I = 0.110; II = 0.110;<br>II/I = 0.155; I/II = 0.144 | | |
| Second cut | | | | | | |
| E+ | 1.179 | 0.707 | 0.943 | 2.066 | 1.194 | 1.630 |
| E- | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Mean | 0.589 | 0.353 | – | 1.033 | 0.597 | – |
| NIR $\alpha = 0.05$ | I = 0.072; II = 0.072;<br>II/I = 0.102; I/II = 0.095 | | | I = 0.150; II = 0.150;<br>II/I = 0.213; I/II = 0.197 | | |
| Third cut | | | | | | |
| E+ | 3.244 | 4.836 | 4.040 | 3.940 | 0.938 | 2.439 |
| E- | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Mean | 1.622 | 2.418 | – | 1.970 | 0.469 | – |
| NIR $\alpha = 0.05$ | I = 0.092; II = 0.092; II/I = 0.130; I/II = 0.120 | | | I = 0.211; II = 0.211; II/I = 0.299; I/II = 0.277 | | |
| Fourth cut | | | | | | |
| E+ | 2.341 | 3.968 | 3.154 | – | – | – |
| E- | 0.000 | 0.000 | 0.000 | – | – | – |
| Mean | 1.170 | 1.984 | – | – | – | – |
| NIR $\alpha = 0.05$ | I = 0.116; II = 0.116; II/I = 0.164; I/II = 0.152 | | | | | |
| Mean for cuts | | | | | | |
| E+ | 1.885 | 2.899 | 2.392 | 2.408 | 1.476 | 1.942 |
| E- | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Mean | 0.942 | 1.449 | – | 1.204 | 0.738 | – |
| NIR $\alpha = 0.05$ | I = 0.030; II = 0.030; II/I = 0.043; I/II = 0.043 | | | I = 0.077; II = 0.077; II/I = 0.109; I/II = 0.109 | | |

[1] first factor (I) – endophyte infected (E+) and uninfected (E-) plants

[2] second factor (II) – type of utilization: for pasture (P) and for cut (K)

Table 4. Weather conditions during the experiments. Mochełek 2006–2008

| Month | Temperature [°C] | | | | Rainfall [mm] | | | |
|---|---|---|---|---|---|---|---|---|
| | decade | | | mean | decade | | | Σ |
| | I | II | III | | I | II | III | |
| 2006 | | | | | | | | |
| January | –3.6 | –7.3 | –12.9 | –8.1 | 0 | 0 | 2.8 | 2.8 |
| February | –3.4 | –1.1 | –4.6 | –2.9 | 9.7 | 8.2 | 1.2 | 19.1 |
| March | –6.0 | –2.0 | 3.0 | –1.5 | 2.2 | 0.3 | 24.9 | 27.4 |
| April | 5.3 | 7.3 | 8.7 | 7.1 | 7.3 | 4.6 | 65.1 | 77.0 |
| May | 12.9 | 13.1 | 11.4 | 12.5 | 7.6 | 31.1 | 21.2 | 59.9 |
| June | 11.8 | 18.9 | 19.7 | 16.8 | 5.0 | 1.0 | 15.8 | 21.8 |
| July | 22.7 | 21.8 | 22.7 | 22.4 | 4.5 | 9.7 | 10.0 | 24.2 |
| August | 17.6 | 17.4 | 15.0 | 16.6 | 80.1 | 30.3 | 18.6 | 129.0 |
| September | 15.2 | 15.7 | 14.6 | 15.2 | 30.8 | 0.3 | 9.5 | 40.6 |
| October | 12.2 | 7.3 | 9.4 | 9.6 | 5.4 | 0.0 | 6.7 | 12.1 |
| November | 4.0 | 6.1 | 5.6 | 5.2 | 10.0 | 15.5 | 8.4 | 33.9 |
| December | 5.4 | 3.7 | 2.3 | 3.7 | 11.4 | 13.0 | 7.0 | 31.4 |
| 2007 | | | | | | | | |
| January | 5.4 | 4.6 | –1.4 | 2.7 | 7.9 | 47.9 | 20.1 | 75.9 |
| February | –0.4 | –0.6 | –2.5 | –1.0 | 11.5 | 9.5 | 7.0 | 28.0 |
| March | 4.0 | 4.9 | 6.0 | 5.0 | 13.1 | 18.6 | 16.2 | 47.9 |
| April | 5.9 | 9.3 | 10.2 | 8.5 | 14.2 | 2.3 | 1.1 | 17.6 |
| May | 9.3 | 12.7 | 19.0 | 13.8 | 23.7 | 36.8 | 12.6 | 73.1 |
| June | 18.8 | 19.5 | 16.2 | 18.2 | 16.2 | 34.8 | 54.5 | 105.5 |
| July | 15.7 | 21.1 | 17.3 | 18.0 | 66.1 | 3.6 | 35.0 | 104.7 |
| August | 18.6 | 18.6 | 16.4 | 17.8 | 7.6 | 2.3 | 32.2 | 42.1 |
| September | 12.6 | 11.3 | 13.2 | 12.4 | 17.0 | 4.2 | 16.4 | 37.6 |
| October | 9.3 | 6.1 | 5.5 | 6.9 | 10.5 | 9.1 | 0.3 | 19.9 |
| November | 3.7 | –0.3 | 0.4 | 1.3 | 13.9 | 1.0 | 7.4 | 22.3 |
| December | 4.3 | 0.1 | –3.1 | 0.3 | 26.9 | 5.4 | 3.7 | 36.0 |
| 2008 | | | | | | | | |
| January | –3.1 | 1.9 | 2.4 | 0.5 | 9.6 | 12.9 | 25.7 | 48.2 |
| February | 2.7 | 0.7 | 5.1 | 2.8 | 4.9 | 1.1 | 9.9 | 15.9 |
| March | 3.9 | 3.3 | 2.0 | 3.0 | 23.2 | 24.0 | 14.0 | 61.2 |
| April | 5.7 | 6.7 | 10.4 | 7.6 | 16.5 | 18.4 | 3.8 | 38.7 |
| May | 12.6 | 13.0 | 14.0 | 13.2 | 4.3 | 7.2 | 0.0 | 11.5 |
| June | 19.1 | 15.6 | 18.0 | 17.6 | 0.0 | 3.5 | 12.0 | 15.5 |
| July | 19.1 | 18.2 | 20.3 | 19.2 | 12.7 | 44.4 | 1.6 | 58.7 |
| August | 19.1 | 18.3 | 16.2 | 17.8 | 21.7 | 53.5 | 20.3 | 95.5 |
| September | 16.8 | 9.7 | 10.7 | 12.4 | 7.0 | 1.6 | 11.6 | 20.2 |
| October | 8.9 | 9.8 | 6.8 | 8.4 | 9.2 | 27.1 | 43.7 | 80.0 |
| November | 7.1 | 5.2 | 0.5 | 4.3 | 1.6 | 14.1 | 3.7 | 19.4 |
| December | 1.6 | 1.3 | –2.0 | 0.2 | 3.2 | 18.2 | 3.4 | 24.8 |

growing season, which facilitated the harvest of 4 cuts in 2007. Good growth conditions accelerated the regeneration of plants exposed to thinning due to the long-term semi-drought periods in the growing season of 2006, when the experiment was established. Only intensive rainfall, starting in August, launched the period of plant regeneration on plots. During cultivation, however, there was a slightly lower plant thinning in the combinations with the endophyte. There was relatively less weed-infestation in the combinations with the endophyte than in the combinations with no endophyte. Thanks to favourable conditions later and the following year, the plants demonstrated very good tillering, eliminating drought damage. The second year of observations (2008) was also less favourable for plant growth due to low rainfall starting from the third decade of April to the beginning of July (Table 4). In the second year it was possible to collect only 3 cuts.

An effect of *N. uncinatum* on Justa meadow fescue yielding in all the combinations, was observed (Table 1). The presence of the endophyte significantly enhanced higher yields. The highest annual yields were reported in 2007, in combination E+ cut at harvesting maturity. They exceeded 7.0 t DM/ha. Significantly lower yields were recorded in the combination which involved pasture use. In the following year yielding was about 50% lower due to the weather conditions. In both research years the first cut was used to account for over 50% of the annual yield. The lowest yields were recorded for the last cuts. The second factor, the system of use, also affected Justa yielding significantly. The effect, however, was not clear-cut. Significantly higher yields from the plots cut at harvesting maturity were only recorded in the combinations with the plants infested by the endophyte. which resulted from *e.g.* a longer growth period of plants cut at harvesting maturity. In turn, the result was an increase in biomass production. The process was additionally enhanced by the endophyte at the physiological level. However, there were no noted differences between cuts at grazing and harvesting maturity in the combinations with the plants without the endophyte. The one exception was for the first cut in 2008, when the system of use significantly affected combination E- yielding.

### Occurrence of diseases

Analysis was done on the occurrence of fungal diseases on Justa meadow fescue depending on the infestation by *N. uncinatum* and on the system of use. The analysis demonstrated a slight effect of the first factor and no effect of the second factor at all (Table 2). On the plots, the most frequent fungi were those representing the *Puccinia* genus, causing rusts, and *Drechslera* spp., *B. sorokiniana*, responsible for spots on leaves. Powdery mildew symptoms were noted less frequently on the plants. While evaluating the condition of overwintering plants in spring, in the first and second year of use, sporadic symptoms of infection with *Microdochium nivale* (Fr.) Sam.ex I.C.Hallett were found. By isolating microorganisms from decaying tillers, the presence of the pathogen and *Fusarium* genus fungi was identified.

In 2007, weather conditions at the beginning of the vegetation period were more favourable for the development of pathogens than in 2008 (Table 4). The result was a higher infection of meadow fescue (Table 2). The level of infection of plants with pathogens causing rusts did not exceed 4.22%. However in 2008, the disease index value did not exceed 2.22%. There was no significant noted variation in the occurrence of symptoms of rust on the plants infested and non-infested by the endophyte. Neither did the system of use affect the infection of plants. The presence of the endophyte, however, affected the occurrence of leaf spot. Significantly less disease symptoms were noted on plants E+, in the spring of 2007 and 2008. The disease index of leaves reached the maximum value of 11.31% in 2007. Similarly in the case of spot, there was no observed effect of the system of use, on infection. The relatively least amount of disease symptoms were noted for powdery mildew. The highest value of the index of infection with that pathogen accounted for 2.07% in the combination with plants E- in 2007. There was no observed effect of the factors analysed on the occurrence of infection symptoms caused by *B. graminis* in spring over 2007–2008.

In the autumn of 2007 and 2008, there was a greater intensity of disease occurrence identified (Table 2). The most frequently noted symptoms were those of leaf spot, which ranged from 12.1 to 15.8% in 2007 and 14.6 to 17.2% in 2008. The rust symptoms were observed to a lesser degree. Their occurrence intensity was higher in the second research year and did not exceed 8.02%. More than half as much infection was reported for plant infection with *B. graminis*. There was neither the presence of the endophyte nor the effect of the system of use on the occurrence of rust and powdery mildew on both cultivars throughout the autumn observations, in 2007 and 2008. Leaf spots were the only one for which there was recorded a significant effect of Justa infestation with the endophyte on the decrease in infection.

### Toxins production

The analysis of the content of ergovaline in green matter in Justa showed its presence in all the cuts from plots E+ in the research years (Table 3). The lowest level of the toxin was noted in the plants of the second cut collected at harvesting maturity, in 2007. The highest toxin level was in the third cut, also at harvesting maturity, in 2007. The method of use had a significant effect on the amount of the toxin found but the effect was not clear-cut. In the first, third and fourth cut in 2007 and the first cut in 2008, higher contents were reported in green forage collected at harvesting maturity; in the other cases more was noted in the cuts at grazing maturity. Analysing the mean values, there was a higher content of ergovaline in plants, in 2007 than in 2008.

## DISCUSSION

Endophytes of the *Neotyphodium* genus, as obligatory biotrophes, take up nutrients from a living host-plant. They develop only in intercellular spaces and never penetrate inside the cells. This means that their existence depends completely on the host plant. It is ‘in the interest’ of the endophyte, therefore, to enhance the plant development, and to protect it from the effect of many stress factors which could lead to its death. Indeed, the effect of endophytes on the host plant is very complex and covers many aspects of its existence. The key components include the effect on the host growth, its reproduction, resistance to stress factors as well as competitiveness towards other plants in the ecosystem. The effect on the growth is most frequently expressed as the number of blades, leaf area and dry matter of tillers and roots. A positive effect of endophytes on the host development is widely reported. As early as 1959, Bradshaw observed a significant increase in the number of blades in *Agrostis tenuis* plants infested with *Epichloë typhina*. A negative consequence of this association was, however, a total inhibition of seed production by infested plants. An increase in the number of blades of *Danthonia spicata* infested by the endophyte, *Atkinsonella hypoxylon*, as compared with plants E- was also noted under field conditions by Clay (1984). Besides, the infested plants were more competitive towards *Anthoxanthum odoratum* than the non-infested plants. If the soil is limited in nutrients, and there are unfavourable environmental conditions, the effect of the endophyte can be negative. McCormik *et al*. (2001) observed worse development of E+ plants of *D. spicata* when exposed to water and mineral deficits in soil. A positive effect of the endophyte, *N. uncinatum,* on the development of meadow fescue was observed by Malinowski *et al*. (1997). They reported plants with the endophyte showed a significantly higher content of dry matter of tillers and roots than E- plants. A significantly greater accumulation of dry matter of E+ plants of perennial ryegrass and tall fescue after 14 weeks of growth, was also noted by Clay (1987). Similarly, in the present research, the significant effect of *N. uncinatum* on an increase in the dry matter of successive cuts in Justa meadow fescue in the field were demonstrated. The effect was especially clear in 2007. It was the first year of use after the semi-drought year of 2006. Most probably the plants infested with *N. uncinatum* survived the period better due to the symbiont, and thus, there was a higher yield under the conditions enhancing growth, in 2007. The main mechanisms of such an effect of the endophyte are mainly: an increase in the volume of the plant root system, root elongation, earlier and more rapid closing of stomata, accumulation and metabolism of carbohydrates and a greater content of phenolic compounds (Malinowski *et al*. 1997; Elmi and West 1995; Richardson *et al*. 1992). Owing to good growth conditions in 2007, E- plants regenerated and the differences between combinations E+ and E– in 2008, were smaller.

The endophyte also affected the production of ergovaline in plants. In all cuts in the research ergovaline content was relatively high. The capacity for the production of ergovaline depends mostly on the host-plant genotype and on the endophyte genotype (Faeth 2002). The present results suggest a high potential for producing toxins by association. Other factors affecting the production of the compound in the plant are as follows: growth temperature, amount of rainfall, plant development stage as well as fertilisation. Żurek *et al*. (2010) also reported higher concentrations of ergovaline in grasses utilized for cutting than for grasses for grazing. As far as weather conditions are concerned, higher amounts of alkaloids are usually observed late in spring and early autumn. Higher nitrogen fertilisation also enhances an increase in the content of ergopeptine alkaloids in grasses (Lyons *et al*. 1990; Belesky *et al*. 1988). A three-fold increase in the nitrogen dose can increase the content of alkaloids by 60–80% in tall fescue, depending on the year. In the present field experiment, the amount of ergovaline increased in successive cuts. Ergovaline reached the highest value when the temperatures were the highest in the spring vegetation period. Besides, Arechavaleta *et al*. (1992) observed that not only the nitrogen dose affects the level of ergot alkaloids but also their form and noted a higher content of toxins for $NH_4^+$ than for $NO_3^-$.

In the present research, the association of Justa meadow fescue with *N. uncinatum* did not prove to be especially resistant to infection from pathogens. Endophyte only ensured a higher level of protection towards non-infested plants, in the case of fungi causing leaf spot. Literature reports state the presence of the endophyte can decrease the susceptibility of the host-plant to infection by pathogens and feeding of pests, however, there are also known cases of a completely different effect of the symbiont on the host-plant (Bacon *et al*. 1997). Schmidt (1994) observed a greater resistance of meadow fescue with the endophyte to infection with some pathogens developing on plants after emergence. At the same time, they demonstrated that plants with the endophyte were more susceptible to infection with pathogens causing pre-emergence seedling rot. Vincelli and Powell (1991) observed a significantly lower infection of perennial ryegrass with endophyte by red thread (*Laetisaria fuciformis*). Similar results were reported by Gwinn and Gavin (1992) investigating the susceptibility of seedlings of tall fescue with the endophyte, to infection with *Rhizoctonia zeae*. There was also observed lower susceptibility of the E+ plants to infection with *Sclerotinia homoeocarpa* (dollar spot disease), *Cercospora* leaf spot disease, *Typhula ishikariensis* (grass snow mold disease) and virus BYDV (Koshino *et al*. 1987; Clarke *et al*. 2006; Lehtonen *et al*. 2006; Wäli *et al*. 2006). The infected plants can also show a lower susceptibility to infection by those pathogens which cause rusts and leaf spots and viruses (Lewis 1996a, 1996b; Pańka *et al*. 2004). No effect of the endophyte on a decrease in plant infection with rust fungi was found in the present research project.

The present results show that endophytes in the association with meadow fescue plants demonstrate both a positive and a negative effect on the host. The endophytes increase plant yielding, decrease infection by some pathogens and, at the same time, they are responsible for the production of ergovaline. Animal feed made from infested plants can, consequently, pose a threat to animals when administered over a long period of time.

## CONCLUSIONS

1. The presence of the endophyte, *N. uncinatum*, in Justa meadow fescue had a significant effect on the increase in the dry matter yield, as compared with the non-infested plants.

2. The infestation of Justa meadow fescue by the endophyte, *N. uncinatum*, significantly protected the plants from infection with fungi causing leaf spot. The endophyte, however, did not affect the development of powdery mildew and rust fungi.
3. Justa meadow fescue showed a relatively high content of ergovaline when grown in the field. The level of the toxin in the season varies a lot, which suggests a high effect of external factors on its production. Due to the toxin production, the animal feed made from infested plants can pose a threat to animals when administered over a long period.
4. *N. uncinatum* isolates from Justa meadow fescue cannot be used as biological control agents to improve the growth and resistance of other cultivars due to ergovaline production.

This work was supported by the State Committee for Scientific Research, Poland, grant No. PB 853/P06/2004/27

## REFERENCES

An Zhi-Qiang, Siegel M.R., Hollin W., Tsai Huei-Fung, Schmidt D., Schardl C.L. 1993. Relationships among non-*Acremonium* sp. fungal endophytes in five grass species. Appl. Environ. Microbiol. 59 (5): 1540–1548.

Arechavaleta M., Bacon C.W., Plattner R.D., Hoveland C.S., Radcliffe D.E. 1992. Accumulation of ergopeptide alkaloids in symbiotic tall fescue grown under deficits of soil water and nitrogen fertilizer. Appl. Environ. Microbiol. 58 (3): 857–861.

Bacon C.W., Battista De J. 1991. Endophytic fungi of grasses. p. 231–256. In: "Handbook of Applied Mycology" Vol. 1. "Soil and Plants" (D.K. Arora B. Rai, K.G. Mukerji, G.R. Knudsen, eds.). Marcel Dekker, New York, 720 pp.

Bacon C.W., Richardson M.D., White J.F.Jr. 1997. Modification and uses of endophyte-enhanced turfgrasses: A role for molecular technology. Crop Sci. 37 (5): 1415–1425.

Belesky D.P., Stuedemann J.A., Plattner R.D., Wilkinson S.R. 1988. Ergopeptine alkaloids in grazed tall fescue. Agron. J. 80 (2): 209–212.

Birckenstaedt E., Eickel P., Paul V.H. 1994. Scoring of grass diseases for the evaluation of varieties. IOBC/WPRS Bull. 17 (1): 193–200.

Bouton J., Easton S. 2005. Endophytes in forage cultivars. p. 327–340. In: "*Neotyphodium* in Cool-Season Grasses" (C.A. Roberts, C.P. West, D.E. Spiers, eds.). Blackwell Publishing, Ames, Iowa, USA, 379 pp.

Bradshaw A.D. 1959. Population differentiation in *Agrostis tenuis* Sibth. II. The incidence and significance of infection by *Epichloë typhina*. New Phytol. 58 (3): 310–315.

Clarke B.B., White Jr. J.F., Hurley R.H., Torres M.S., Sun S., Huff D.R. 2006. Endophyte mediated suppresion of dollar spot disease in fine fescues. Plant Dis. 90 (8): 994–998.

Clay K. 1984. The effect of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) on the reproductive system and demography of the grass *Danthonia spicata*. New Phytol. 98 (2): 165–175.

Clay K. 1987. Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinaceae*. Oecologia 73 (3): 358–362.

Elmi A.A., West C.P. 1995. Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. New Phytol. 131 (1): 61–67.

Faeth S.H. 2002. Are endophytic fungi plant mutualists? Oikos 98 (1): 25–36.

Glenn A.E., Bacon C.W., Price R., Hanlin R.T. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia 88 (3): 369–383.

Gwinn K.D., Gavin A.M. 1992. Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. Plant Dis. 76 (9): 911–914.

Johnson M.C., Dahlmann D.L., Siegel M.R., Bush L.P., Latch G.C.M., Potter D.A., Varney D.R. 1985. Insect feeding deterrents in endophyte infected tall fescue. Appl. Environ. Microbiol. 49 (3): 568–571.

Koshino H., Togiya S., Yoshihara T., Sakamura S. 1987. Four fungitoxic C-18 hydroxy unsaturated fatty acids from stromata of *Epichloë typhina*. Tetrahedron Lett. 28 (1): 73–76.

Latch G.C.M., Christensen M.J. 1982. Ryegrass endophyte, incidence, and control. N. Zeal. J. Agric. Res. 25 (3): 443–448.

Lehtonen P.T., Helander M., Siddiqui S.A., Lehto K., Saikkonen K. 2006. Endophytic fungus decreases plant virus infections in meadow ryegrass (*Lolium pratense*). Biol. Lett. 2 (4): 620–623.

Lewis G.C. 1996a. A review of research on endophytic fungi worldwide, and its relevance to European grassland, pastures and turf. IOBC/WPRS Bull. 19 (7): 17–24.

Lewis G.C. 1996b. Effect of cutting height on perennial ryegrass with and without infection with endophyte and ryegrass mosaic virus. IOBC/WPRS Bull. 19 (7): 55–58.

Lyons P.C., Evans J.J., Bacon C.W. 1990. Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. Plant Physiol. 92 (3): 726–732.

Malinowski D., Leuchtmann A., Schmidt D., Nösberger J. 1997. Growth and water status in meadow fescue is affected by *Neotyphodium* and *Phialophora* species endophytes. Agron. J. 89 (4): 673–678.

McCormik M.K., Gross K.L., Smith R.A. 2001. *Danthonia spicata* (Poaceae) and *Atkinsonella hypoxylon* (Balansiae): environmental dependance of a symbiosis. Am. J. Bot. 88 (5): 903–909.

Oliver J.W. 2005. Pathophysiologic response to endophyte toxins. p. 291–304. In: "*Neotyphodium* in Cool-Season Grasses" (C.A. Roberts, C.P. West, D.E. Spiers, eds.). Blackwell Publishing, Ames, Iowa, USA, 379 pp.

Pańka D., Podkówka L., Lamparski R. 2004. Preliminary observations on the resistance of meadow fescue (*Festuca pratensis* Huds.) infected by *Neotyphodium uncinatum* to diseases and pests and nutritive value. No. 401. p. 88–90. In: "Proceedings of the 5th International Symposium on *Neotyphodium*/Grass Interactions" (R. Kallenbach, C. Jr. Rosenkrans, T.R. Lock, eds.). Fayetteville, AR, USA, May 23–26, 2004, 180 pp.

Porter J.K., Thompson F.N. Jr. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70 (5): 1594–1603.

Richardson M.D., Chapman Jr. G.W., Hoveland C.S., Bacon C.W. 1992. Sugar alcohol in endophyte-infected tall fescue under drought. Crop Sci. 32 (4): 1060–1061.

Rottinghaus G.E., Garner G.B., Cornell C.N., Ellis J.L. 1991. HPLC method for quantitating ergovaline in endophyte-infested tall fescue: Seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seedheads. J. Agric. Food Chem. 39 (1): 112–115.

Saha D.C., Jackson M.A., Johnson-Cicalese J.M. 1988. A rapid staining method for detection of endophytic fungi in turf and forage grasses. Phytopathology 78 (2): 237–239.

Schmidt D. 1994. Influence of endophytes of *Festuca pratensis* on damping-off diseases of seedlings. IOBC/WPRS Bull. 17 (1): 267–271.

Siegel M.R., Schardll C.L., Philips T.D. 1995. Incidence and compatibility of nonclavicipitaceous fungal endophytes in *Festuca* and *Lolium* grass species. Mycologia 87 (2): 196–202.

Smith S.E., Read D.J. 1997. Mycorrhizal Symbiosis. 2nd ed. Academic Press, San Diego, CA, 605 pp.

Thompson F.N., Stuedeman J.A. 1993. Phytophysiology of fescue toxicosis. Agric. Ecosyst. Environ. 44 (1–4): 263–281.

Vincelli P., Powell A.J. 1991. Reaction of perennial ryegrass varieties to red thread, 1990. Biol. Cultural Tests Control Plant Dis. 6, p. 102.

Wäli P.R., Helander M., Nissinen O., Saikkonen K. 2006. Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). Can. J. Bot. 84 (7): 1043–1051.

Wenzel H. 1948. Zur erfassung des schadenausmasses in pflanzenschutzversuchen. Pflanzenschuzberichte 15: 81–84.

Żurek M., Ochodzki P., Wiewióra B. 2010. Ocena zawartości ergowaliny w trawach runi wybranych trwałych użytków zielonych na terenie województwa mazowieckiego. Biul. IHAR 257/258: 39–47.