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First Report of *Fusarium poae* Associated with and/or Causing Silvertop on Loloid-type *Festulolium* in the Czech Republic

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Abstract

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Silvertop is a serious disease of grasses, and severe infestations cause a great deal of damage every year (particularly to seed producers). Inflorescences of infected plants dry prematurely and are sterile. The genera *Festuca, Poa, Agrostis, Trisetum,* and *Cynosurus* are the most frequent hosts of this disease, which has newly been reported in a loloid-type intergeneric *Festulolium* hybrid. The disease was also observed in the genus *Lolium*, with only rare previous description. The suspicion that *Fusarium poae* is the causal agent of severe silvertop was confirmed, and the pathogen was morphologically described and confirmed by molecular analysis. The meadow plant bug (*Leptopterna dolabrata*) may be a vector transferring the pathogen to plants, although a direct pathogen transfer was not demonstrated. Grass colonisation by the pathogen therefore apparently occurs after plant tissues injury by sucking of the meadow plant bug.

Keywords: disease; fertile stems; causal agents; grass hybrids; *Leptopterna dolabrata*

Silvertop, a disease affecting fertile stems and inflorescences of certain cultured and wild grass species (also known as white head or white ear), results in the loss of the ability to create developed seeds. This important disease may cause even total losses in seed production. Although it infects most grasses, its typical symptoms associated with almost complete coverage can be observed in only a few species from the genera *Festuca* (red fescue, meadow fescue, sheep's fescue), *Poa* (smooth meadow grass, swamp meadow grass, wood meadow grass, flattened meadow grass, and rough meadow grass are severely infected), *Agrostis* (common bent, creeping bent, and black bent are highly sensitive), *Trisetum* (golden oat grass), and *Cynosurus* (crested dog's-tail). Severe silvertop, whereby the entire inflorescence is destroyed, is typical for most species susceptible to this disease, while less severe silvertop is characteristic of such other species as common bent (Wetzel 1968; Cagaš 2004). The main symptom of the disease is complete drying and necrosis of the fertile plant stem at an early development stage. After grasses head, inflorescences cease to develop and do not blossom. The fertile stems remain short, dry, and become yellowish or silvery in colour, markedly differing from the previously green stems and later straw-coloured mature stems. The overall appearance of the infected stems is described similarly to a shepherd's crook due to its characteristic curvature (Cagaš 2004). Silvertop may be brought on by both abiotic and biotic factors. Frequent abiotic causes of silvertop include environmental conditions of the stand, late spring frost, and insufficient plant nutrients (Smith *et al.* 1989).

As the most frequent biotic causal agents of the so-called parasitic silvertop, the literature mentions various insect species as well as mites as vectors (Mühle *et al.* 1971) acting together with the fungus

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Table 1. Degree of severity of silver top on different grass species

Latin name	Common name	Degree of severity
Festuca	fescue	3
Poa	meadow	3
Trisetum	golden oat-grass	3
Agrostis	bentgrass	3
Cynosurum	crested dogs tail	1
Lolium	ryegrass	0
Festulolium	festulolium	0

Degree: 3 – very significant, 2 – significant, 1 – negligible, occasional occurrence, 0 – unimportant, possible occurrence

Fusarium poae. This type of silvertop is characterised by shrinking, darkening, and subsequent necrosis of the infected stem in the basal section of the last internode. The disease primarily affects older stands and is certainly one of the most serious diseases for grasses grown for seed (Cagaš 2004). The importance of silvertop for different grass species is reported by Kůdela *et al*. (2012) (Table 1).

Schurr *et al*. (1963) and Arnott *et al*. (1967) supported the insect vector theory and demostrated that symptoms of silvertop were eliminated by insecticide, while elimination by acaricide was not proved. Cagaš and Rotrekl (2014, personal communication) provided similar results. Silvertop symptoms were eliminated by insecticide application.

The objective of the present study was to test and possibly confirm the relatively old hypothesis that the fungus *F. poae* is a causal agent of parasitic silvertop (Steward & Hodgkiss 1908). Another hypothesis previously confirmed in field experiments states that the main vector of *F. poae* transfer to plants is the meadow plant bug (*Leptopterna dolabrata* L.), an insect from the order Hemiptera, family Miridae. A further objective was to determine how *L. dolabrata* contributes to the transfer of *F. poae* to plants.

MATERIAL AND METHODS

Stands established at Troubsko and Zubří, Czech Republic were monitored during 2012–2014. These areas included plots with various grasses and clover grass mixtures as well as surrounding areas which could act as reservoirs for causal agents of silvertop.

Silvertop stem collection. Silvertop stems were collected from experimental plots once per developmental cycle during the flowering stage of the stand (BBCH 65–69). The experiment was established in 2011 and randomised in two variants (monocultures of smooth meadow grass and golden oat grass) and three repetitions. Experimental plot size was 10 m^2.

The number of collected infected plants differed in the course of the project. This was due to small to non-existent disease incidence in newly established stands and stands in their first year of use, when at most two silvertop stems per experimental plot were recorded. In certain experimental plots, silvertop was not recorded at all during the first year of use. Only in the second and third years of use it was possible to secure a sufficient amount of material for subsequent laboratory analysis. At that time, 15 plants infected with silvertop were collected from each plot. Entire plants were collected including plant bases and root system sections. Numbers of infected plants are given in Table 2.

At the same time, various grass species (swamp meadow grass, wood meadow grass, Kentucky bluegrass, golden oat grass, festulolium) infected with silvertop were collected as part of screening in the surroundings of Zubří from the so-called open areas (grass stands for seed production, meadows, ditches).

Table 2. The silvertop occurence (%) per plot in the years (2011–2014)

	Smooth meadow-grass (*Poa pratensis* L.)						Golden oat grass (*Trisetum flavescens* (L.) P.B.)					
	1st replication		2nd replication		3th replication		1st replication		2nd replication		3th replication	
	Troubsko	Zubří	Troubsko	Zubří	Troubsko	Zubří	Troubsko	Zubří	Troubsko	Zubří	Troubsko	Zubří
2011	–	–	–	–	–	–	–	–	–	–	–	–
2012	0	+	0	0	0	0	0	+	0	+	0	0
2013	0.2	0.5	0.1	0.3	0.2	0.1	0.3	0.1	0.1	0.7	0.2	0.4
2014	3	2	2.5	1	2.5	1	3	2	0.5	3	2.5	1.5

+ sporadic occurrence of the silvertop on the trial plot

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Again, where possible, entire plants with bases and root system sections were collected. The collected samples were placed in cold storage and transferred for laboratory analysis within 24 hours. Fertile stem ends were pulled out of the flag leaf sheathes. Basal stems, characteristically shrunken and dried, were cut into 1–2 mm segments. These segments were placed into Petri dishes with a growth medium (potato dextrose agar) and cultivated in an incubator at 24°C. Dishes were evaluated after five days.

Meadow plant bug capture. Meadow plant bugs, currently considered to be one of the main causal agents (vectors) of parasitic silvertop in grasses, were captured at two locations: Zubří (Vsetín District) and Skalička (Přerov District). At Zubří, the capture at three-year monoculture stands of smooth meadow grass and golden oat grass was established as part of the project. At Skalička, meadow plant bugs were collected from a five-year-old seed-production stand of smooth meadow grass. During the capture of meadow plant bug imagines, stand growth phase was between the end of tillering and the end of heading (BBCH 29–59). Capture itself began on April 14, 2014 and continued at ca. 14-day intervals. An entomological sweep net was used with a frequency of 10 sweeps per 20 m of stand. In addition, yellow entomological trap bowls filled with detergent were placed in stands at the Zubří location, with two bowls per plot (10 m^2).

Meadow plant bug imagines were not captured successfully until the end of May. Earlier attempts to obtain meadow plant bugs were not successful, apparently due to unfavourable weather.

At the Troubsko location, no meadow plant bugs were observed or captured in the given year, even though silvertop did occur at that location during the same year. Captured meadow plant bugs were killed in ethyl acetate ($CH_3COOCH_2CH_3$). Depending upon the subsequent laboratory processing method, they were then either immediately frozen to −18°C or kept in a refrigerator for at most 24 hours.

A total of 60 individuals were captured by sweep net and then transferred for mycological analysis. For 30 individuals, following surface sterilisation in 96% ethanol for 1 min and washing with distilled sterile water, the intestine was dissected and cultivated on potato dextrose agar in a Petri dish. The bodies of the remaining 30 individuals were crushed between two sterile glass slides and then the samples were removed using an inoculation loop to a dish with growth medium. Bacteria from the symbiotic microflora of the gastrointestinal tract were abundant in these samples. To limit the growth of unwanted microflora, the antibiotic neomycin at 1 g/l concentration was used during the further meadow plant bug preparation.

Further 140 individuals of *L. dolabrata* were captured from experimental areas at the Zubří location on two dates (June 20 and July 4, 2014), and 40 individuals were captured from the Skalička location. Half of these were always surface-sterilised, while the other half were kept without surface sterilisation. The method of crushing the body and subsequently removing the sample to a Petri dish was used for all individuals.

RESULTS AND DISCUSSION

In 2013, silvertop was observed at the Zubří location for the first time on a loloid-type intergeneric hybrid of Italian ryegrass (*Lolium multiflorum*) and tall fescue (*Festuca arundinacea*) (cv. Bečva). Microscopic analysis confirmed the presence of *F. poae*. In 2014, this disease was again observed on grasses from the genus *Lolium* – *L. multiflorum* and perennial ryegrass (*Lolium perenne*) (Gerlach & Nirenberg 1982). Mycological analyses at the Research Institute for Fodder Crops Troubsko laboratories again confirmed the presence of *F. poae*. The results of mycological determinations were confirmed by molecular analysis. The pathogen was re-isolated from infected basal stem. Species-specific primers Fp82 F and Fp82 R (Parry & Nicholson, 1996) were used to confirm the identification of *F. poae*. Reaction products obtained with this primers set were 220 bp long. Complete silvertop were not previously recorded in intergeneric hybrids of ryegrass and fescue, and this is the first report of the disease occurring in these host species. In all Petri dishes with infected plant segments massive growths of *F. poae* mycelia have developed. *Fusarium poae* is an occasionally pathogenic saprophyte which is widespread across the temperate zone. It colonises plant tissues, cereals, and dead plant matter. Its fast-growing and floccose mycelia are initially white and later beige to pink. The reverse side of the Petri dish is usually red to burgundy in colour. Its conidiophores are branched, while its monophialides are short and wide. Its single-cell microconidia are shaped like teardrops and are 6–10 × 5.5–7.5 µm in size. Identical *F. poae* morphology has been described by Gerlach and Nierenberg (1982), Kubátová (2006), and Leslie and Summerell (2006).

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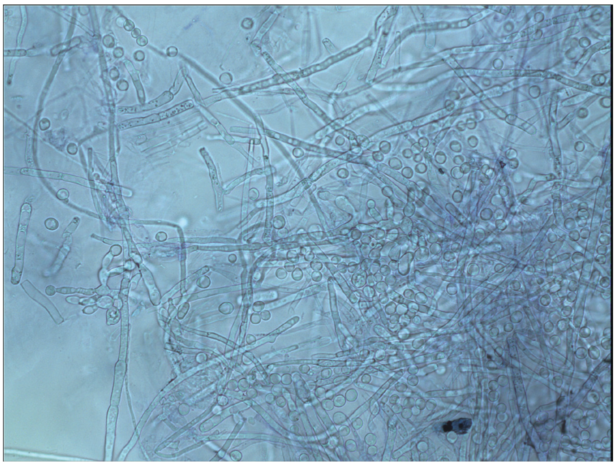

Figure 1. Mycelium and microconidia of *Fusarium poae*

Figure 2. Imago of the meadow plant bug

At the Troubsko location, silvertop was observed over the course of three years, and *F. poae* was identified on the grass species smooth meadow grass (*Poa pratensis*) and golden oat grass (*Trisetum flavescens*), both on experimental plots and in the surroundings. Similarly at the Zubří location, infected plants were collected from the species *P. pratensis*, wood meadow grass (*Poa nemoralis*), golden oat grass (*T. flavescens*), and cocksfoot (*Dactylis glomerata).* At the same time, increasing occurrence of parasitic silvertop with stand age was confirmed at both locations.

Microscopic analysis of meadow plant bugs confirmed the occurrence of fungi from the genus *Arthrinium* in 60% of individuals as well as the sporadic occurrence of sterile mycelia from the genera *Cladosporium* and *Penicillium*. None of these fungi are entomophagous; they are saprophytic fungi. There was one instance (on an imago from experimental areas) of *Fusarium oxysporum*. *Fusarium poae* was not detected in any bug sample.

Although the experimental analyses did not therefore confirm the hypothesis that *L. dolabrata* is the main vector transferring the pathogen, it can be expected that by feeding on plants it creates conditions suitable for predisposing the plant to the pathogen entry.

Based on the finding of mycelia on the damaged parts of silvertop stems, Steward and Hodgkiss (1908) had already designated *F. poae* as one possible causal agent of silvertop. Sprague (1950) judged this fungus to be a weak parasite or saprophyte. According to certain authors, its mycelium is transferred by mites (Hodgkiss 1908). Cherewick and Robinson (1958) demonstrated not only that *Siteroptes graminum* mites transfer *F. poae* spores, but also that by sucking on stem plant tissue they cause injury and thereby enable subsequent infection and mycosis entry. The mites may also use the fungus mycelium as a food source (in addition to stem dermal tissue) (Reuter 1909). In contrast, Hardison (1959) and Wetzel (1968) stated that colonisation of silvertop stem sections infected by this fungus ranged around 15–20%, so it cannot in any way be considered the immediate causal agent of this disease, and Hardison (1959) proposed that insects instead of mites are the

Figure 3. Silver top

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main causal agent. Berkenkamp and Meeres (1975) reported similar results from their study.

Schurr and Bean (1963) and Arnott and Bergis (1967) supported the insect vector theory and emphasised that silvertop was effectively eliminated in stands through the use of insecticides in contrast with the results from applying acaricides.

According to Cagaš (2004), the dominant causal agents of parasitic silvertop in grasses within the Czech Republic are the meadow plant bug (*L. dolabrata*) as vector and *F. poae*. This type of disease is characterised by a correlation between stand age and intensity of silvertop stem occurrence. In seed-production cultures, infection rates are – with some exceptions – very low in the first year of use and then reach their peak in the last year of use for seed regardless of the monitored species, as a grass sward provides the insect vector with sufficient options for hiding both in winter and during plant development. Mites, and particularly the species of the genera *Steneotarsonemus* and *Siteroptes*, have not been demonstrated as causal agents of this disease in the Czech Republic.

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