

Effect of Some Factors on the Incidence of Choke (*Epichloë typhina*) in Grass Seed Stands in the Czech Republic

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Abstract

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Choke of grasses caused by *Epichloë typhina* occurred in 2008 and 2009 in more than 25% of grass seed stands of timothy (*Phleum pratense* and *P. nodosum*); a lower incidence was reported in orchard grass (*Dactylis glomerata*). A decline in disease incidence in 2010 was caused partly by a reduction in the total area of grasses grown for seed production and partly by a decrease of older grass seed stands. The incidence of choke in timothy varieties Odenwälder and Pampas was very high; they differed statistically from the other 24 grown cultivars. No difference was found among 21 varieties of orchard grass. The highest incidence of choke was observed in varieties Comer and Dolina (timothy) and Amera (orchard grass). The incidence of choke in cultivated varieties of *Phleum* spp. and *D. glomerata* was affected by stand age (the incidence of the disease in timothy was recorded in 13% of stands in the first cropping year and in 50% of stands in the fifth cropping year). Choke disease occurred in all regions where the species were grown for seed.

Keywords: *Dactylis glomerata*; *Phleum pratense*; *Phleum nodosum*; age; varieties; grass seed

Choke, caused by the ascomycete fungus *Epichloë typhina* (Pers.) Tul. & C. Tul., is a serious disease of grasses grown for seed. It has a wide host range and infects a number of cultivated and wild grasses (MÜHLE 1971). It is becoming economically important in the species grown for seed such as timothy (*Phleum pratense* L. and *P. nodosum* L.) and orchard grass (*Dactylis glomerata* L.). The causal agent of the disease is an endophytic fungus which spends its life cycle inside the host. The fungus produces mycelial stromata that develop on emerging inflorescences which are suppressed completely or seed production on the infected fertile stem is partly prevented. Long-term observations of this disease in grasses grown for seed reveal an increasing tendency (CAGAŠ 2008, 2010). A stand with more than 5 infected plants per 100 m² was rejected until recently (Regulation No. 384/2006), but at present the criteria are less strict

(Regulation No. 369/2009 of the Statute Book). The stand with infected plants is unsuitable for animal feeding because of the presence of ergot alkaloids produced by the fungus (LEUCHTMANN *et al.* 2000). For this reason ploughing under the grass stand is highly recommended. An economic impact of the disease is high – high losses due to non-marketability of seed and the necessity to re-establish the stand. The objective of the study was to assess the effects of year, variety, stand age and locality on the incidence of choke disease in main host plants (*P. pratense*, *P. nodosum*, and *D. glomerata*) in the years 2008–2010.

MATERIAL AND METHODS

Comprehensive data on the incidence of choke in grass seed stands of timothy and orchard grass

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were based on the protocols submitted by Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ). The incidence of choke of timothy was monitored in 2008 in 87 agricultural enterprises (173 seed stands) in 10 regions (Jihočeský, Jihomoravský, Karlovarský, Moravskoslezský, Olomoucký, Pardubický, Plzeňský, Středočeský, Vysočina, Zlínský). Similarly, the incidence of choke of orchard grass was monitored in that year in 41 agricultural enterprises (94 seed stands) in 11 regions (Jihočeský, Karlovarský, Královéhradecký, Moravskoslezský, Olomoucký, Pardubický, Plzeňský, Středočeský, Ústecký, Vysočina, Zlínský). In 2009 the incidence of choke of timothy was monitored in 77 agricultural enterprises (162 seed stands) in 12 regions (Jihočeský, Jihomoravský, Královéhradecký, Liberecký, Moravskoslezský, Olomoucký, Pardubický, Plzeňský, Středočeský, Vysočina, Zlínský). In that year was the incidence of choke of orchard grass monitored in 38 agricultural enterprises (81 seed stands) in 10 regions (Jihočeský, Karlovarský, Královéhradecký, Olomoucký, Pardubický, Plzeňský, Středočeský, Ústecký, Vysočina, Zlínský). In 2010 the incidence of choke of timothy was monitored in 40 agricultural enterprises (70 seed stands) in 9 regions (Jihočeský, Královéhradecký, Moravskoslezský, Olomoucký, Pardubický, Plzeňský, Vysočina, Zlínský). Similarly, the incidence of choke of orchard grass was monitored in 2010 in 18 agricultural enterprises (42 seed stands) in 7 regions (Jihočeský, Královéhradecký, Olomoucký, Pardubický, Plzeňský, Středočeský, Vysočina).

The data on the incidence of the choke disease in 2008 to 2010 were processed with regard to year, variety, stand age and growing region (locality) in the Czech Republic. ANOVA was used for the evaluation of differences in the occurrence of choke among the varieties of timothy and orchard grass.

RESULTS

Year 2008

Phleum pratense L. and *P. nodosum* L. Choke disease occurred in 26.7% of registered stands (a total of 172 stands were registered and assessed). 9.3% were rejected because of a high level of infection (more than 5 infected plants per 100 m²). A total of 25 varieties of both species of the genus *Phleum* were harvested for seed. Choke disease was

detected in the varieties Dolina, Kaba, Odenwälder, Pampas, Sobol, Timola (high incidence – more than 5 infected plants per 100 m²) and in the varieties Bobr, Classic, Comer, Erecta, Fidanza, Ragnar, Winnetou (low incidence – less than 5 infected plants per 100 m²). Choke disease was observed in stands in the second, third and fourth cropping years. The disease was detected in stands in the Moravskoslezský, Olomoucký, Pardubický, Vysočina and Zlínský regions.

Dactylis glomerata L. Choke disease occurred in 3.2% of registered stands (the total number of stands under study was 95). One stand was highly infected (the Greenly variety with more than 100 infected plants/100 m²).

A total of 18 varieties were harvested for seed in the Czech Republic. Choke disease was detected in the Greenly variety (high incidence) and in the varieties Dana and Niva (low incidence). The disease was detected in stands in the second and third cropping years in the Pardubický region.

Year 2009

Phleum pratense L. and *P. nodosum* L. Choke disease occurred in 35.4% of registered timothy stands (a total of 161 stands were registered and studied). 2.3% of stands were rejected because of a high level of infection (more than 5 infected plants per 100 m²). A total of 15 varieties of both species of the genus *Phleum* were harvested for seed. Choke disease was detected in the varieties Commer, Dolina and Kaba (high incidence), Bobr, Classic, Erecta, Latima, Levočský, Lirocco, Sobol, Summergraze, Timola and Winnetou (low incidence). The disease was observed in stands in the third, fourth, and fifth cropping years. Choke was detected in stands in the following regions: Moravskoslezský, Olomoucký, Pardubický, and Vysočina.

Dactylis glomerata L. Choke disease occurred in 9.7% of registered stands (the total number of stands was 82) while in two stands the level of infection was higher than 5 infected plants per 100 m².

A total of 18 varieties were harvested for seed in the Czech Republic. Choke disease was detected in the varieties Amera (high incidence), Niva and Zora (low incidence). The disease was observed in stands in the second, fourth and fifth cropping years in the regions Moravskoslezský, Olomoucký, and Pardubický.

Year 2010

Phleum pratense L. and *P. nodosum* L. Choke disease occurred in 2.9% of registered timothy stands (a total of 69 stands were registered and observed). One stand was rejected because of high infection. A total of 15 varieties of both species of the genus *Phleum* were harvested for seed in the Czech Republic. Choke disease was detected in the varieties Commer (high incidence) and Sobol (low

incidence). The disease was observed in stands only in the second cropping year in the Pardubický region.

Dactylis glomerata L. Choke disease occurred in 0.8% of registered stands (a total of 43 stands were registered and observed). The level of infection was lower than 5 infected plants per 100 m².

A total of 17 varieties were harvested for seed in the Czech Republic. Choke disease was detected only in the variety Otello. The occurrence of choke was recorded in three stands in the fourth crop-

Table 1. Incidence of choke (*Epichloë typhina*) in timothy (*Phleum pratense*) and small timothy (*P. nodosum*) varieties grown for seed in the Czech Republic in 2008 to 2010

No.	Variety	Incidence/number of stands			Total number of stands	Monitored
		> 5 plants/100 m ²	< 5 plants/100 m ²	no infection		
1	Bobr*	0	3	19	22	2008–2010
2	Classic	0	15	17	32	2008–2010
3	Comer	2	3	5	10	2008–2010
4	Dolina*	4	15	36	55	2008–2010
5	Erecta	0	2	4	6	2008, 2009
6	Fidanza	0	1	0	1	2008
7	Kaba	5	5	37	47	2008–2010
8	Latima ⁺	0	1	10	11	2008–2010
9	Leutimo	0	0	1	1	2008
10	Levočský	0	3	8	11	2008–2010
11	Lirocco*	0	1	17	18	2008–2010
12	Motim	0	0	2	2	2008
13	Narnia	0	0	1	1	2008
14	Obra	0	0	7	7	2008–2010
15	Odenwälder	6	0	0	6	2008
16	Pampas	1	0	0	1	2008
17	Piccolo	0	0	2	2	2008
18	Ragnar	0	1	16	17	2008–2010
19	Sirius	0	0	1	1	2008
20	Skala	0	0	4	4	2008
21	Sobol*	2	13	60	75	2008–2010
22	Summergraze	0	5	4	9	2008–2010
23	Timola	1	9	5	15	2008–2010
24	Tundra	0	0	1	1	2008
25	Winnetou	0	11	37	48	2008–2010
26	Zubr ⁺	0	0	2	2	2010
Total		21	88	296	405	

*varieties listed in the State Variety Book; ⁺*Phleum nodosum* L.

Table 2. Incidence of choke (*Epichloë typhina*) in orchard grass (*Dactylis glomerata*) varieties grown for seed in the Czech Republic in 2008 to 2010

No.	Variety	Incidence/number of stands			Total number of stands	Monitored
		> 5 plants/100 m ²	< 5 plants/100 m ²	no infection		
1	Accord	0	0	15	15	2008–2010
2	Amera	2	4	30	36	2008–2010
3	Bepro	0	0	3	3	2008–2010
4	Dana*	0	1	11	12	2008–2010
5	Dika	0	0	3	3	2008–2010
6	Greenly	1	0	21	22	2008–2010
7	Horizont*	0	0	18	18	2008–2010
8	Husar	0	0	10	10	2008–2010
9	Intensiv*	0	0	2	2	2010
10	Lidaglo	0	0	4	4	2008
11	Luron	0	0	2	2	2010
12	Niva*	0	2	7	9	2008, 2009
13	Otello	0	3	26	29	2008–2010
14	Padania	0	0	7	7	2008–2010
15	Starly	0	0	14	14	2008–2010
16	SW Luxor	0	0	5	5	2008–2010
17	Toscali*	0	0	4	4	2008–2010
18	Trerano	0	0	10	10	2008, 2009
19	Vaillant	0	0	2	2	2008, 2009
20	Vega*	0	0	8	8	2008–2010
21	Zora*	0	0	2	2	2010
Total		3	10	204	217	

*varieties listed in the State Variety Book

ping year. The disease was detected only in the Středočeský region.

DISCUSSION

Stromata of choke are regularly detected during spring inspections in some grasses grown for seed. According to long-term observations (since 1995) the incidence of choke was investigated in 0.1–3.8% of areas covered by grasses grown for seed in the Czech Republic (CAGAŠ 2010). The unusually high incidence of this disease in 2008 stimulated growers' interest in this disease, predominantly in potential disease control, and generated a lot of discussion about the meaning of both regulations governing the use of choke-infected stands. Direct economic losses resulting

from the rejection of grass seed stands in the Czech Republic were about 2.5 million CZK in 2008 (they do not include financial losses connected with killing the production stand and re-establishment of a new stand). RAYNAL (1991) estimated losses due to this disease in grass seed producing areas in France at 10–20%. PFENDER and ALDERMAN (1997) reported that this disease was unknown in Oregon until 1996 but then it became quite common. The same authors pointed out that as early as in 2000 choke disease affected 90% of orchard grass seed producing fields in the Willamette Valley, the major Oregon production area (PFENDER & ALDERMAN 2004). Choke is a “new emerging” disease of orchard grass in China (LI *et al.* 2009).

Choke disease incidence was extremely high in 2008 and 2009. In timothy (*P. pratense* and *P. nodosum*) it occurred in more than a fourth of

all grass seed producing stands. Its occurrence in orchard grass was lower and the disease was detected in 2.9 % to 9.7% of stands. A substantial choke disease decline in 2010 was attributed mainly to a marked decrease in production areas covered by the grass species (from 172 registered stands in 2008 to 69 stands in 2010 in both species of timothy and from 95 registered stands to 43 in orchard grass, respectively). Another factor involved was a reduction of old grass seed fields, which is evident in both timothy species (stands aged over three cropping years were reduced from 10.5% to 7.2%). Growing different varieties and a decline in the number of varieties in 2010 might also play an important role (a reduction from 25 varieties in 2008 to 15 varieties in both timothy species and from 18 to 17 in orchard grass). Different macroclimatic and microclimatic conditions might have affected sexual and asexual spores, infection pattern in healthy plants and stromata formation in the next year.

Varietal resistance to choke disease has not yet been discussed in plant pathological literature. In the years of observation a remarkably wide population of varieties of both timothy species (26 varieties) and orchard grass (21 varieties) was available. Objective assessment of potentially different varietal resistance was, however, complicated by the fact that only 54% of timothy (*P. pratense* and *P. nodosum*) varieties were assessed throughout the entire three-year cycle of observation. In this group of varieties no choke infection was found with the exception of the Obra variety, which was grown and assessed just on seven stands (Table 1). Similarly, only 67% of orchard grass varieties were assessed in three years, which made a comparison of their resistance quite difficult (Table 2). However, the tests of French varieties of orchard grass in Oregon did not reveal different levels of choke disease (PFENDER & ALDERMAN 1997). Our observations did not show any clear differences among varieties, even though e.g. timothy (*P. pratense* L.) varieties Comer, Dolina, and Kaba produced a higher number of choked tillers in the stand, compared with the others. The same applies to the orchard grass variety Amera. On the other hand, the comparison of triennial observations (without reference to the number of assessed years) showed the high susceptibility to choke in timothy varieties Odenwalder and Pampas, which differ statistically from the other items (Table 3). Similar results among orchard grass varieties were

Table 3. Susceptibility of timothy varieties (*Phleum pratense* and *Phleum nodosum*) grown for seed in the Czech Republic (without reference to the number of stands and cropping years) to choke of grasses (*Epichloë typhina*)

No.	Variety	Percentage of stands with > 5 attacked plants/100 m ²	Tukey 95%
1	Bobr	0.0	a
2	Classic	0.0	a
3	Comer	44.4	ab
4	Dolina	5.8	a
5	Erecta	0.0	a
6	Fidanza	0.0	a
7	Kaba	8.5	a
8	Latima	0.0	a
9	Leutimo	0.0	a
10	Levočský	0.0	a
11	Lirocco	0.0	a
12	Motim	0.0	a
13	Narnia	0.0	a
14	Obra	0.0	a
15	Odenwalder	100.0	b
16	Pampas	100.0	b
17	Piccolo	0.0	a
18	Ragnar	0.0	a
19	Sirius	0.0	a
20	Skala	0.0	a
21	Sobol	1,9	a
22	Summergraze	0.0	a
23	Timola	5.6	a
24	Tundra	0.0	a
25	Winnetou	0.0	a
26	Zubr	0.0	a

$P < 0.0001$

not investigated. The difficulties of determining differences in resistance among timothy (*P. pratense*) varieties were pointed out by SHIMANUKI and SATO (1983).

Age of grass seed stand can be one of the factors which affect the incidence of disease. Even if the disease incidence in the first cropping (seeding) year cannot be excluded (Table 4), there was a relation between this trait and the incidence of choke disease in timothy species. Whereas in

Table 4. Incidence of choke (*Epichloë typhina*) in timothy (*Phleum pratense*) and small timothy (*P. nodosum*), and orchard grass (*Dactylis glomerata*) grown for seed in different cropping years

Cropping year	Incidence/number of stands (%)			Total number of stands
	> 5 plants/100 m ²	< 5 plants/100 m ²	no infection	
Timothy and small timothy				
I.	1 (1)	14 (12)	99 (87)	114
II.	6 (5)	26 (20)	98 (75)	130
III.	9 (8)	34 (28)	77 (64)	120
IV.	5 (14)	8 (22)	23 (64)	36
V.	1(25)	1(25)	2 (50)	4
VI	0	0	1 (100)	1
Total number of stands	22	83	296	405
Orchard grass				
I.	0 (0)	0 (0)	45 (100)	45
II.	1 (2)	4 (6)	59 (92)	64
III.	1 (1,5)	1 (1,5)	63 (97)	65
IV.	0	4 (11)	34 (89)	38
V.	1 (20)	2 (40)	2 (40)	5
VI.	0 (0)	0 (0)	3 (100)	3
Total number of stands	3	11	206	220

the first cropping year the disease incidence was recorded in 13% of stands, in the second year it was in 25%, in the third and fourth years in 36% and in the fifth year in 50% of stands (in this case only 4 stands were observed; the older stands of timothy were mostly ploughed because of low seed profitability). A similar tendency can also be observed in orchard grass (Table 4). According to PFENDER and ALDERMAN (1998) the incidence of choke disease was the main reason for liquidation of older orchard grass stands in France.

The incidence of choke disease in both cultivated species of the genus *Phleum* and in orchard grass was reported in all areas of Bohemia and Moravia where grass seed fields of these species were located. No relation between disease incidence and regional conditions has been demonstrated.

The choke of grasses could become an important disease in grasses grown for seed in the near future. The only possibility how to prevent the spread of choke (the effective chemical control is not known) is the liquidation of seed stands with a high incidence of the disease. Further investigations of possibilities of the disease control are necessary.

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