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EFFECT OF STEM RUST AND CHEMICAL CONTROL ON PERENNIAL RYEGRASS SEED PRODUCTION

ABSTRACT

Effect of *Puccinia spp*. and application of chemical control on seed yield components and seeds germination capacity of perennial ryegrass was investigated. Field trials were carried out over two seasons evaluating triazole fungicides applied one or three time. The weather condition were favoured for rust development in both years of the experiment. The rust disease symptoms were observed only during the first year. It was possible to observe a big effect of rust disease and application of chemical control on seed yield components and seeds germination capacity.

Key words: fungicides, germination capacity, *Lolium perenne* L., *Puccinia spp*, seed yield components, stem rust

INTRODUCTION

The importance of turf and forage grasses seed production significantly increased during last years. Perennial ryegrass (*Lolium perenne* L.), also called English ryegrass, is one of the most important cool-season perennial bunchgrass species in many countries. It is widely distributed throughout the world, including Europe, North and South America, New Zealand, and Australia (Jung *et al.* 1966, Walton 1983, Balasko *et al.* 1995). *Lolium* species occurs in many different ecological stands and it is the main component of grass communities on pasturelands, meadows and recreational grounds (Cullen 1964). Perennial ryegrass is valued for high yield potential, fast establishment, reduced tillage renovation applications, and use on heavy and waterlogged soils. This species is very valued for use in dairy and sheep forage systems. However, it express low tolerance to biotic and abiotic stresses. Plant pathogens are the one of the most important and may cause yield reductions. Losses may be more severe when highly susceptible varieties are widely grown. The most obvious and harmful of perennial ryegrass diseases are rusts (Jung *et al.* 1996). Stem rust caused by *P. graminis* f. sp. *graminicola* often is a problem in late spring and early summer. Crown rust caused by *Puccinia coronata* Corda and stem rust occur in late summer and early fall. Both of them occurs all over the Europe

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(Smiley *et al.* 1992, Cagas 1996, 1997, Lewis 1996, Reheul *et al.* 2000). Development of these fungi is favored by humid weather with 7 h dark period at 21 – 24°C followed by a light period with at 29 – 35°C, during which leaves dry gradually (Lamey *et al.* 1996, Pflender 2003, 2004). They can reduce the green mass yield of almost 20% and quality (Lancashire and Latch 1966, Potter 1987, Thomas 1994). Seed crop losses due to stem rust, which occur most year during reproductive development, in many times are more than 30% and sometimes 90%. A lot of varieties formerly recommended in many countries is actually beginning abandoned due to the ever increasing susceptibility to infection by *Puccinia* spp. Differences in susceptibility of these varieties over and within countries indicating the presence of different strains of rusts in different countries and / or an important interaction with climatic conditions (Reheul *et al.* 2000, Wolters 2000). The incidence of rust appears to be extremely variable between years, locations and between spontaneous and artificial infections and this constrains selection response (Reheul and Ghesquiere 1996). Fall planting date also affect stem rust level in first-year perennial ryegrass (Pfender 2003, 2004). Late autumn planting might reduce disease in spring but a late-planted crop may produce lower yield than an early-planted crop in the absence of disease. Also, if late planting results in later maturity, the crop may be exposed to a more prolonged rust epidemic during summer before harvest. Breeding for resistance also appears to be negatively correlated with yield (Hides and Wilkins 1978, Meyer 1982, Reheul and Chesquiere 1996, Humphreys *at al.* 2000). Studies on the genetic nature of resistance to crown rust in ryegrass have indicated both qualitative and quantitative control (Hayward 1977; Wilkins 1975; Posselt 1994; Roderick *et al.* 2000).

Fungicides are commonly used in perennial ryegrass seed production (Gingrich, Mellbye 1997; Rolston *at al*. 2002). The two main fungicide groups are used on ryegrass seed crops are the DeMethylation Inhibitor -triazole (DMI-triazole) group (fenarimol, propiconazole and triadimefon) and the newly developed strobilurin group. Those chemicals do not kill the fungus but morely inhibit its growth by preventing germination of spores or by stoping growth of the hyphae or mycelium after germination. Even though they are fungistats, when applied as eradicants (i.e., after the disease is present), they will stop the spread of a fungus by preventing it from entering new plant tissue (Vargas 1994). Results obtained by Cagas (1992), Lewis (1992), Welty (1991), Welty and Azevedo (1994), Welty and Barker 1994 and Rijckaert (1995) showed that propiconazole applied as a foliar spray to swards of grasses reduced incidence of foliar diseases, increased the number of leaves per tiller and reduced the proportion of dead leaves. However, further work is still necessary to determine whether consistent seed yield increases can be obtained by fungicide application, and to investigate the effect of fungicides on leaf micoflora. Therefore the purpose of this study is to report the response of ryegrass seed production on rust and fungicide application. Rust severity was compared also with yield components and germination capacity.

MATERIALS AND METHODS

Plant material

The experiment was carried out using 6 cultivars of perennial ryegrass: Barball, Barclay, Nira, Stadion, Stoper and Taya with different resistance levels to rust disease.

Field experiment and disease assessment

The effect of fungicide and rust disease was studied in field trials conducted at Radzików, near Warsaw, Poland during 2001 – 2003.

To obtain seedlings for the experiments cultivars were seeded in 14 June 2000. Seedlings were fertilized with 30 kg N, 60 kg P_20_5 and 60 kg K_20 per hectare in August 2000 and 30 kg N and 60 K_20 in April 2001. Than, plants were planted to 10 litre pots with a mixture of peat (44%), soil (44%) and sand (12%). Five plants of each cultivar were grown per pot.

Experiment was conducted in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar.

In the treatment B ioculation was conducted twice: during summer of 2001 and 2002 using population of *Puccinia* spp. obtained from infected plants of *Lolium perenne* L.

In treatments with chemical control fungicide Tilt 250 EC or Amistar were used. Active ingredient in fungicide Tilt 250 EC is propiconazole 250g × l^{-1} and in fungicide Amistar is azoksystrobin 250g × l^{-1}.

In the treatment C fungicide Tilt 250 EC was applied 1 time in the beginning of June in 2001 and 2002 in the rate 0.5 l × ha^{-1} (mid head emergence stage). In the treatment D fungicides were applied 3 times from May to July in 2001 and 2002, respectively (from mid head emergence stage to flowering stage).

All treatments were replicated four times in a randomised block design. Pots were fertilized with 60 kg N, 60 kg P_20_5 and 60 kg K_20 per hectare in August 2001 and 60 kg N and 60 K_20 per hectare in April of 2002.

At each assessment the infection types were scored on 1 - 9 scale, according to the percentage of leaf area infected (adapted from Birckenstaedt *et al.*, 1994).

Score:

1 — More than 75% of foliage area infected
3 — 60% of foliage covered with rust
5 — 25% of foliage covered with rust
7 — 5% of foliage covered with rust
9 — No rust

Seed yield and its components

Weight of head (forty heads were taken from each genotype: 10 heads x 4 replication) was determined for all genotypes in 2001 and 2002. Based on the results obtained by Czembor (2003), where correlation between weight of head and weight of seed per head was founded, weight of seed per head was determined only in 2001, when the rust symptoms on the tested plants were observed. Thousand seed weight [g] was determined in 2002. After seed harvest, excess stems and leaves were removed from the plots.

Germination capacity

Seed germination was measured for seed samples in 2000 and 2001. It was done on the moistened paper at 10°C in darkness during the first 3 days and at 18°C during the next 28 days. For each seed-lot three replicates of fifty seed were tested. Germination was assessed as the percentage of seeds producing normal seedlings as defined by ISTA (International Seed Testing Association) Rules (ISTA, 1993). This assessment was done after 28 days.

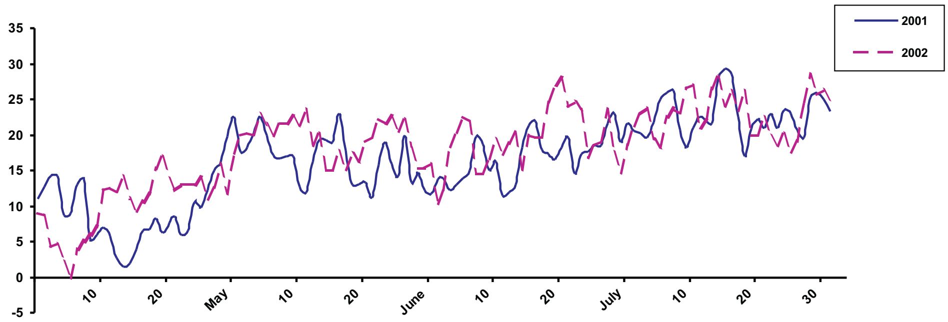

Fig. 1. The weather conditions during experiment at Radzikow during 2001 – 2002: mean of the air temperature

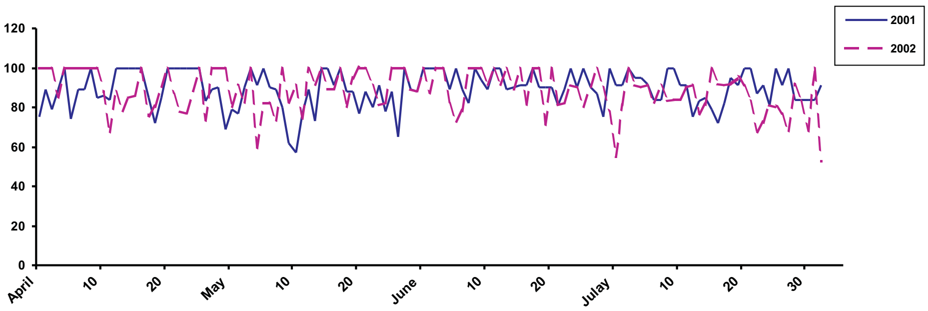

Fig. 2. The weather conditions during experiment at Radzikow during 2001 – 2002: humidity

The weather conditions

Data were provided by the Plant Breeding and Acclimatization Institute, Radzikow. Observations were made at the Field Laboratory for 24-hr periods. The weather station was located about 100 m from test plots. The data are presented as daily total precipitation recorded between 1 April and 31 June for each of the 3 yr (Fig. 1, Fig. 2) of the studies. Temperature data presented are combined average daily maximum and minimum temperatures recorded 1 m above the ground.

Data analysis

Data analysis was conducted using program Statistics for Windows StatSoft Inc. (1997). Disease scores and seed yields were subjected to analysis of variance, and treatment means were compared using the Tukey separation test at P=0.01, 0.05 or 0.1.

RESULTS

However the weather condition were favoured for rust development in both years 2001 and 2002, the rust disease symptoms were observed only in the 2001 (Table 1, Fig. 1, Fig. 2). Assessment of rust disease done in 2001 and 2002 showed high effect of inoculation by population of *Puccinia* spp. (treatment B).

Table 1

Comparison of rust resistance of tested varieties in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar during 2001 and 2002.

No	Variety	Treatment A		Treatment B		Treatment C		Treatment D	
		2001	2002	2001	2002	2001	2002	2001	2002
1	Barball	4.3	9.0	1.0	9.0	5.0	9.0	7.0	9.0
2	Barclay	4.5	9.0	1.0	9.0	4.5	9.0	7.0	9.0
3	Nira	4.0	9.0	1.0	9.0	4.8	9.0	6.8	9.0
4	Stadion	4.5	9.0	1.0	9.0	4.8	9.0	6.8	9.0
5	Stoper	5.0	9.0	1.0	9.0	4.8	9.0	7.5	9.0
6	Taya	4.3	9.0	1.0	9.0	5.0	9.0	6.3	9.0
	Mean	4.3	9.0	1.0	9.0	4.8	9.0	6.9	9.0
	Cv [%]	11.4	0.0	0.0	0.0	15.0	0.0	8.9	0.0
	LSD (Tukey)	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

In 2001, varieties used in the experiment were scored as a susceptible. In the treatment with natural infection the level of the rust resistance on them was scored on 4.3 (more than 25% of foliage area infected) and was on the same level of resistance as the score of plants growing in the treatment with one application of the fungicide Tilt 250 EC (4,8). After inoculation in 2001, more than 70% of foliage area of tested plants were infected by rust disease and resistance of all tested varieties was scored for 1.0. Rust disease on the plants growth in the treatment with three application of chemical control (every 3 weeks) was observed much more sporadically and was scored for 6.9 (5% of leaf area infected).

In 2001 significant differences in weight of head, weight of seed per head and germination capacity between treatments were observed (Tables 2, 3, 4, 6). Mean weight of head was from 0.18 g (treatment with inoculation) to 0.28 g (treatment with three application of fungicides). The lowest number of the seeds per head were obtained in the treatment with inoculation (0.03 g). In the treatments with natural infection and with one application of the fungicide Tilt 250 EC weight of seeds per head was 0.07 g and 0.05 g, respectively. After three application of the fungicide it

Table 2

Comparison of weight of head of tested varieties in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar during 2001 and 2002

No	Variety	Treatment A		Treatment B		Treatment C		Treatment D	
		2001	2002	2001	2002	2001	2002	2001	2002
1	Barball	0.30	0.11	0.15	0.18	0.20	0.12	0.29	0.13
2	Barclay	0.18	0.13	0.17	0.15	0.23	0.15	0.27	0.11
3	Nira	0.25	0.14	0.20	0.21	0.21	0.15	0.26	0.15
4	Stadion	0.25	0.14	0.16	0.19	0.23	0.15	0.29	0.15
5	Stoper	0.20	0.10	0.20	0.20	0.19	0.11	0.24	0.12
6	Taya	0.21	0.16	0.20	0.18	0.20	0.15	0.31	0.14
	Mean	0.23	0.14	0.18	0.19	0.21	0.14	0.28	0.13
	CV[%]	23.9	27.75	19.1	19.39	0.17	24.04	17.7	26.60
	LSD (Tukey)	0.05	Ns	Ns	Ns	Ns	Ns	Ns	Ns

Table 3

Comparison of weight of seed per head of tested varieties in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar in 2001

No.	Variety	Treatment A	Treatment B	Treatment C	Treatment D
1	Barball	0.075	0.034	0.030	0.17
2	Barclay	0.053	0.020	0.027	0.15
3	Nira	0.080	0.039	0.035	0.14
4	Stadion	0.070	0.023	0.080	0.15
5	Stoper	0.054	0.039	0.056	0.17
6	Taya	0.072	0.029	0.084	0.16
	Mean	0.07	0.03	0.05	0.16
	CV (%)	28.6	29.5	50.0	16.6
	LSD (Tukey)	Ns	0.008	Ns	Ns

Table 4

Germination capacity (%) of tested varieties in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar in 2001 and 2002

No	Variety	Treatment A		Treatment B		Treatment C		Treatment D	
		2001	2002	2001	2002	2001	2002	2001	2002
1	Barball	58.2	96.5	64.6	94.5	65.5	94.0	95.5	94.0
2	Barclay	61.7	96.5	25.4	95.5	50.6	98.5	94.5	93.5
3	Nira	60.0	95.6	29.3	93.0	59.5	98.0	92.5	94.5
4	Stadion	63.0	94.5	30.7	98.5	55.6	97.5	95.0	96.0
5	Stoper	69.5	95.5	54.2	99.5	69.4	96.5	94.5	95.5
6	Taya	64.5	97.0	50.3	99.0	44.2	97.0	94.5	92.5
	Mean	67.8	95.9	42.5	96.7	57.5	96.9	94.4	94.3
	Cv (%)	75.9	4.1	20.1	4.4	55.6	3.2	3.9	5.5
	LSD (Tukey)	15.6	Ns	14.3	Ns	12.7	Ns	Ns	Ns

was possible to obtain 0.16 g seeds per head. In 2001 the lowest germination capacity on the moistened paper was observed after inoculation by *Puccinia* spp. (42.5%). Also in the treatments with natural infection and with one application of the fungicide Tilt 250 EC germination capacity was low: 67,8% and 57.5%, respectively. Only in the treatment with three application of chemical control germination capacity was 94.4%.

In 2002 significant differences between seed germination capacity on moistened paper and thousand seed weight were not observed (Tables 4, 5).

Table 5

Thousand seed weight (g) of tested varieties in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar in 2002

No.	Variety	Treatment A	Treatment B	Treatment C	Treatment D
1	Barball	0.14	0.15	0.14	0.14
2	Barclay	0.14	0.16	0.15	0.13
3	Nira	0.15	0.16	0.15	0.14
4	Stadion	0.13	0.13	0.14	0.13
5	Stoper	0.14	0.14	0.13	0.13
6	Taya	0.16	0.16	0.15	0.13
	Mean	0.18	0.15	0.14	0.13
	Cv (%)	9.67	10.84	10.46	10.09
	LSD (Tukey)	0.014	0.013	Ns	Ns

Table 6

Comparison of mean value of rust resistance, weight of head, weight of seed per head, thousand seed weight (g) and germination capacity (%) of tested varieties in four treatments: A – control (with natural infection by *Puccinia* spp.), B - inoculation by population of *Puccinia* spp., C – one application of fungicide Tilt 250 EC and D – three application of fungicide Tilt 250 EC or Amistar in 2001 and 2002

Parameter/Year	Means				LSD (Tukey)
	Treatment A	Treatment B	Treatment C	Treatment D	
Rust disease					
2001	4.3	1.0	4.8	6.9	0.3
2002	9.0	9.0	9.0	9.0	Ns
Weight of head [g]					
2001	0.23	0.18	0.21	0.28	0.02
2002	0.14	0.19	0.14	0.13	0.015
Weight of seed per head [g]					
2001	0.07	0.03	0.05	0.16	0.009
Thousand seed weight [g]					
2002	0.18	0.15	0.14	0.13	0.006
Germination capacity [%]					
2001	67.8	42.5	57.5	94.4	10.95
2002	95.9	96.7	96.9	94.3	Ns

DISCUSSION

Efforts to reduce yield losses caused by fungal diseases are mainly based on the use of host resistance and of fungicides. Recently improved environmental quality, energy and resource conservation, and increased economic sustainability through better fungicide use is the long-term goal of many project and the knowledge about this is still re-evaluated. Investigations conducted by Hampton and Hebblethwaite (1984, 1985), Welling and Nordestgaard (1988, 1991), Cagas (1992, 1997), Welty (1991), Welty and Azevedo (1994), Rijckaert (1995) and Czembor (2003) showed that diseases or fungicide application have any yield response and in another time fungicide application produced some unexpected increases in seed yield.

The fungicides evaluated in these trials represent the range of fungicides commonly used on ryegrass seed crops at the time the trials were carried out. Our study indicate a big effect of *Puccinia spp*. and application of chemical control on seed yield components and seeds germination capacity of the perennial ryegrass during the first year of the experiment. The very early date of planting help for disease development during first year of the experiment (Pflender 2003, 2004). In the second year of the experiment the rust disease was not observed in all treatments, however the weather conditions were favourable for the infection. Pflender (2004) also observed that severity of stem rust epidemic differs from year to year and the most severely diseased fields in any given year are usually first – year stands.

Therefore, the effect of the rust disease on the yield components and on the seed germination capacity was possible to observe only in 2000 year. The lowest weight of head and weight of seeds per head was in the treatment with inoculation by population of *Puccinia spp.,* when more than 70% of leaf area and stems of tested plants were infected. One application of the fungicide had no effect on the level of the infection by *Puccinia spp.* In the treatment with natural infection and in the treatment with one application Tilt 250 EC severity of stem rust was high and significantly greater than in treatment with three application of fungicides Tilt 250 EC or Amistar. In those treatments more than 24% of leaf area of tested plants were infected. In those cases the weight of head, weight of seed per head and seed germination capacity on the moistened paper was very low in comparison to the treatment with three application of fungicides Tilt 250 EC or Amistar. In the treatment used as a control (natural infection) and after one application of the chemical control weight of head was 0.07 g – 0,05g and germination capacity 67.8% - 57.5%. After three application of the fungicide it was possible to obtain 0,16 g seeds per head and germination capacity was 94.4%.

Pflender (2003) showed also, that seed yield potential of first-year perennial ryegrass was affected by fall planting date.

During the second year of the experiment, in the absence of the infection by *Puccinia spp.* any relationship between fungicide application and weight of head, thousand seed weight and germination capacity was founded.

Horeman (1987, 1989) found no relationship between Kentucky bluegrass seed yield and severity of rust attack, but did report increased ryegrass seed yields following fungicide application, even in the absence of fungal pathogens. Seed yield increases in the absence of the disease have also been reported by Cagas (1992) and

Czembor (2003) in Kentucky bluegrass, by Rolston *et al.* (1985) in praire grass (*Bromus willdenowi* Kunth) and in cocksfoot (*Dactylis glomerata* L.).

Hampton and Hebblewaite (1984, 1985) and Hampton *et al.* (1985) suggested that improved seed yield accorded by fungicides may be attributed to increase in leaf area duration brought about by delays in sentence of phothsynthetic tissue, rather than to pathogenic effect.

Further observation are still necessary to predict the relationship of yield on fungicide dose and timing of application. A lot of models have been developed by several workers for cereals or other crops. Effect of variation in fungicide, pathogen, host and environment will be assessed for grasses using this hypothetical framework.

CONCLUSIONS

Seed production plots of perennial ryegrass is affected by stem rust caused by caused by *P. graminis* f. sp. *graminicola.* Initial experiments were carried out to investigate fungicide effectiveness and application options. Fungicide application were made to small, replicated plots. Visual evaluations to determine rust infection levels were made to compare effectiveness of fungicide application one or three time (fungicides Tilt 250 EC or Amistar). A big effect of rust disease and application of chemical control on seed yield components and seeds germination capacity of the perennial ryegrass was observed only during the first year of the experiment. In this time it was no differences between treatment used as a control and treatment with one application of fungicide Tilt 250 EC and in both of them the level of the disease was very high. A good results were obtained in the treatment with three application of chemical control where the level of the disease was very low. During the second year of the experiment rust disease was not observed in any treatment.

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