Mycological investigations of seeds of cultivated grasses

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Pilzliche Krankheitserreger an Gräsersamen

1 Introduction

The grassland area of Austria amounts to two million hectares, of which 47 % is cultivated with two or more cuts per year and includes fodder-plant production. The remaining percent is extensive grassland (alpine pastures, mountainous areas). In a yearly average, 86,000 hectares are restored by sowing, oversowing and intercropping. For these purposes, 5,545 tons of seed were used annually between 2002 to 2004. It is necessary to use healthy, pathogene-free seed of excellent quality for high quality production. However, we lack information of seed-contaminating fungal pathogenes and their effect on germination. The aim was to conduct seed-pathological investigations of nine grass species important in the plant association of meadows and pastures in Austria. We also tested changes of seed germination rates in relation to the fungal infection rates of seed samples.

Simay (1989, 1990, 1991) investigated seed pathology of a number of plant species, but among them only oat and maize were of the *Poaceae* family (Simay 1992a, 1992b). He did not investigate grass seed pathology. Walcz and Horváth (1976) undertook seed pathology tests of three grass species (smooth brome, red fescue and tall fescue) during some years. They used different testing methods and found that the occurrence of *Alternaria alternata* is the most important pathogen on grass seeds. Tagenko (1974) also found *Alternaria alternata* on seeds of smooth brome. Together with *A. alternata* and *A. tenuissima,* Gannibal (2004) identified *A. infectoria* on seeds of different grass species. He also reported data on the occurrence of the *Ulocladium* and *Embellisia* genus genera near to that of

Zusammenfassung

Die Autoren haben in Österreich vergleichende mykologische Untersuchungen an Samenpartien von neun Grasarten mit zwei unterschiedlichen Methoden durchgeführt. Neben der Aufnahme samenübertragbarer Pilzgattungen wurde auch die Beeinflussung der Keimfähigkeit der verschiedenen Samenmuster in Zusammenhang mit ihrer Kontaminationsrate verfolgt. Auf Samen der untersuchten Grasarten konnten 31 Pilzgattungen nachgewiesen werden, die zu den Stämmen *Myxomycota, Ascomycota* und *Deuteromycota* gehören. Saprophyte Arten der Gattungen *Alternaria, Epicoccum, Cladosporium, Stachybotrys* erwiesen sich als dominant. Als Pflanzenpathogene konnten Arten aus den Gattungen *Bipolaris, Drechslera, Fusarium, Septoria* und *Ascochyta* nachgewiesen werden. Die Identifizierung von *Curvularia lunata, C. inaequalis, Ulocladium botrytis, U. atrum, Pithomyces chartarum, Embellisia*-Arten und *Septonema*-Arten aus Samen verschiedener Grasarten ist bis jetzt nicht beschrieben.

Schlagworte: Gräser, Keimung, pilzliche Infektion.

Summary

Using two different methods, the authors made comparative seed-pathological investigations of the seeds of nine grass species cultivated in Austria. Together with the mapping of fungal genera occurring on grass seeds, they also tested changes of germination rates related to the rate of fungal infection. 31 fungal genera were identified on the seeds of *Myxomycota, Ascomycota* and *Deuteromycota* grasses. Saprophytic *Alternaria, Epicoccum, Cladosporium, Stachybotrys* genera were dominant among them, and plant pathogenics were also detected of the *Bipolaris, Drechslera, Fusarium, Septoria* and *Ascochyta. Curvularia lunata, C. inaequalis Ulocladium botrytis, U. atrum, Pithomyces chartarum, Embellisia* sp. and *Septonema* sp. genera from grass varieties not yet described.

Key words: Grass species, germination, fungal infection.

Alternaria. VARGA et al. (2004) investigated the seeds of 30 cultivars from 11 grass species. They indicated the dominance of the *Alternaria* genus as seed pathogens. Within the genus they identified also *Alternaria alternata* and *A. tenuissima* and reported the occurrence of the *Ulocladium* genus on the seeds of perennial ryegrass. The genera of *Embellisia* and *Septonema* were also identified on seeds of perennial ryegrass (VARGA and FISCHL 2005). MAKELA (1972) also investigated seeds of more grass species and found that a number of fungal pathogen occurs on seeds of sheep fescue, while the seed samples of common bent-grass was less contaminated. PAPP et al. (1986) tested othere grass species, and the seed-borne species of the following fungal genera are considered to be important: *Drechslera, Bipolaris, Fusarium, Septoria, Colletotrichum, Mastigosporium, Scolecotrichum* (*Cercosporidium*), *Ascochyta, Stagonospora, Tilletia, Ustilago.* RADULESCU and NEGRU (1971) have given a detailed description of seed-borne diseases on 13 grass species. Together with the above mentioned, they referred to the presence of species of the *Leptosphaeria*, *Ovularia*, *Selenophoma* and *Phoma* and to the *Curvularia lunata* genera. No author mentioned seed-borne fungal pathogens of the crested dog's tail.

2 Material and methods

The investigations were undertaken at the Federal Research and Education Centre Raumberg-Gumpenstein. Analysed were the seeds of perennial ryegrass (*Lolium perenne*), hybrid ryegrass (*Lolium* × *boucheanum*), cock's-foot (*Dactylis glomerata*), creeping red fescue (*Festuca nigrescens*), common meadow grass (*Poa pratensis*), crested dog's tail (*Cynosorus cristatus*), yellow oat grass (*Trisetum flavescens*), common bent grass (*Agrostis capillaris*) and common foxtail (*Alopecurus pratensis*). The seeds originated from different harvests. Seed contamination rates were tested in thermostats and on a Jakobsen table. The seed samples were stored in paper bags in a refrigerated room at 4–6 °C. The samples were not surface sterilized and were tested according to two different methods aligned to the the prescriptions of the International Seed Testing Association (ISTA 2005). Four batches of 400 seeds were placed on filter paper in a germination cabinet, and four batches of 400 seeds of each species were also tested on a Jacobsen table. Filter papers were moistened with a 0.3 % KNO_3 solution, and prechilling for 7 days at 4–6 °C was made to break dormancy in yellow oat grass. During germination in the cabinet, 12 hours of light and 12 hours of darkness were implemented at a temperature of 20 °C. 12 hours of light was also given during germination on the Jakobsen table and 30 °C water temperature for the crested dog's-tail and yellow oat grass seeds, and 20 °C for the remaining grass species. Mycological observations were made twice simultaneously of the germination assessments. Observations were undertaken of perennial ryegrass and hybrid ryegrass on the 5th and 14th day, of common foxtail on the 7th and 14th day, of creeping red fescue, cock's foot and yellow oat grass on the 7th and 21st day, of crested dog's tail on the 10th and 21st day of common bent grass on the 7th and 28th day and of common meadow grass on the 10th and 28th day. Each seed was investigeted using a stereomicroscope and also with a light microscope at 780 times magnification of those showing symptoms of fungal contamination. For determination of the fungal genus and species, the morphology, colour and size of colonies and spores were taken into consideration according to the descriptions given by ELLIS (1971), BOOTH (1971), CHIDAMBARAM et al. (1973), SUTTON (1980) and SIVANESAN (1987), SINGH et al. (1991), MATHUR and MANANDHAR (2003). Some microphotos were also taken of the different fungal pathogens.

3 Results and conclusions

We found that despite the different age of seed samples their germination rate was excellent. It was interesting that nearly all seed samples showed a higher germination rate using the Jakobsen table compared to the thermostate method. The difference was as much as 30 % in common meadow grass and cock's-foot (Figure 1). A high rate of occurrence of saprophytic fungi was been found at the second rating the fungal contamination rate was highest on creeping red fescue, crested dog's tail and cock's-foot, while the lowest contamination rates were found on the seeds of common bent grass, hybrid ryegrass, yellow oat grass and common meadow grass. Both investigation methods showed the contamination rates, but the rate was in some cases 30–40 % higher by using the Jakobsen table (Figure 2). The number of contaminating fungal species was also found to be higher when using the Jakobsen table.

During the investigations we could identify 45 fungal species belonging to 31 fungal genera (Table 1). Species of saprotrophic genera, such as *Alternaria, Epicoccum, Cladosporium* and *Stachybotrys* were dominant. *A. alternata* and *A. tenuissima* were identified within the *Alternaria* genus.

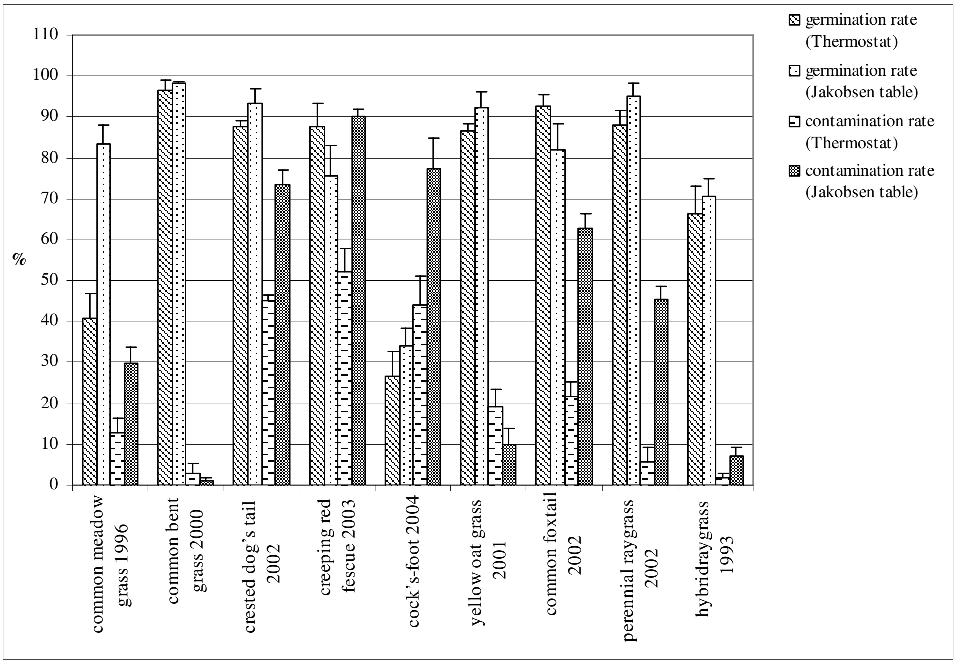

Figure 1: Germination rates and fungal contaminations at the first count

Abbildung 1: Veränderung der Keimungs- und pilzliche Infektionsraten von Samen der untersuchten Grasarten bei der Erstzählung

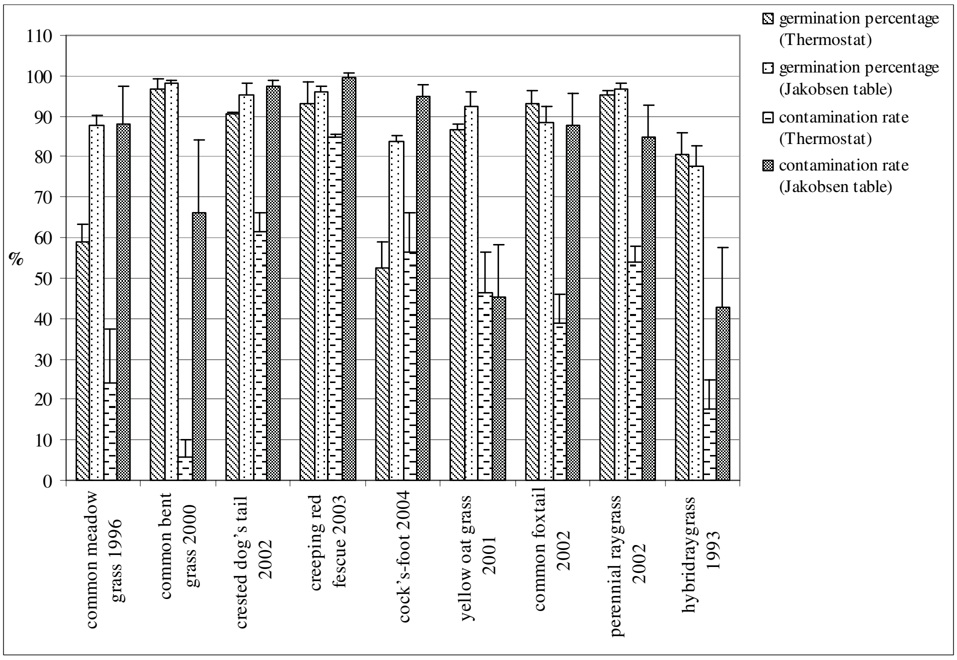

Figure 2: Germinating rates and fungal contaminations at the time of the final count

Abbildung 2: Keimungs- und pilzliche Infektionsraten von Samen der untersuchten Grasarten bei Versuchsabschluss

The contamination rate by the *Alternaria* species was the highest of all, and it even reached 75 % on the seeds of crested dog's tail, but the *Alternaria* species were also dominant on the seeds of hybrid ryegrass (71 %) and perennial ryegrass (69 %).

There was also a high occurrence of the genus *Cladosporium* species, mainly *C. cladosporioides*, but *C. herbarum* also could be identified. The rate of occurrence of this genus on the seeds of creeping red fescue was 88 % in relation to all genera. A high rate of occurrence of *Epicoccum nigrum* was observed during germination on the Jakobsen table. We found a contamination rate of 9.2 % on common foxtail with the use of a thermostat for germination, but the rate was 61.75 % when using the Jakobsen table. *Stachybotrys atra* occurred mainly with use of the the Jakobsen table: its occurrence was so high during the second observation peri-

Table 1: Fungal genera and species identified on grass seeds
Tabelle 1: Auf Grassamen identifizierte Pilzgattungen und Arten

Fungal genera	Fungal species
Alternaria	*A. alternata, A. tenuissima*
Cladosporium	*C. cladosporioides, C. herbarum*
Epicoccum	*E. nigrum*
Stachybotrys	*S. atra*
Stemphylium	*S. botryosum*
Gonatobotrys	*G. simplex*
Acremoniella	*A. atra*
Hadrotrichum	*Hadrotrichum* sp.
Oedocephalum	*O. glomerulosum*
Periconia	*P. byssoides, P. minutissima*
Torula	*T. graminicola*
Pithomyces	*P. chartarum*
Bipolaris	*B. sorokiniana, B. spicifera*
Drechslera	*D. siccans, D. dictyoides, D. dactylidis, D. biseptata, D. phlei*
Fusarium	*F. avenaceum, F. equiseti, F. graminearum, F. semitectum*
Curvularia	*C. lunata, C. inaequalis*
Embellisia	*Embellisia* sp.
Ulocladium	*U. botrytis, U. atrum*
Septonema	*Septonema* sp.
Cercosporidium	*C. graminis*
Septoria	*Septoria* spp.
Ascochyta	*Ascochyta* spp.
Phoma	*P. epicoccina, Phoma* sp.
Myrothecium	*M. graminearum*
Dinemasporium	*D. strigosum*
Dactylaria	*Dactylaria* sp.
Gibberella	*G. zeae*
Pyrenophora	*Pyrenophora* sp.
Leptosphaeria	*Leptosphaeria* sp.
Chaetomium	*Chaetomium* spp.
Physarum	*P. nutans*

od that it could hinder the assessment of other fungal species. It is interesting that during the first observation period, when using a thermostat for germination, none of the grass species showed *Stachybotrys atra*, while its occurrence in the second observation period was considerable (cock's-foot showed 31 % of all contaminations). *Pithomyces chartarum* is a saprophytic fungus, however its pathogenicity also is known. EKEN et al. (2006) reported leaf spot disease caused by this fungus on smooth brome. Occurrence of this species on various grasses is frequent and its mycotoxin, the sporidesmin, causes photosensitive skin-inflammation, mainly in sheep and occasionally in cattle. We identified this fungus from the seeds of crested dog's tail, creeping red fescue, yellow oat grass, common foxtail, common meadow grass, cock's-foot and perennial ryegrass.

Other than saprophytic species, we identified a number of plant pathogenic fungi from grass seeds. Of the genus *Bipolaris* we identified *B. sorokiniana* from the seeds of common bent grass, common foxtail, crested dog's tail, perennial ryegrass, and hybrid ryegrass, while *B. spicifera* was indentified from seeds of the common foxtail. *Bipolaris sorokiniana* is a pathogen with a broad host range, which mainly causes black seed bud on cereals, seedling rot and root rot. During the vegetation period it causes brown leaf spots with a yellow border. It also attacks a number of grass species. The *Bipolaris spicifera* can also cause root rotting. CHIDAMBARAM (1973) mentioned infected seeds of 67 plant species, including grasses, but he did not mention an occurrence on common foxtail. The species of the genus *Drechslera* cause leaf spots on grasses (MÜHLE 1971) and they play a role in damaging seed buds and death in severe cases. One of their way of distribution is via the seeds. The *Drechslera siccans* and *D. dictyoides* species were identified on perennial ryegrass and hybrid ryegrass seeds. We found *D. dactylidis* on the seeds of cock's-foot, *D. biseptata* on the seeds of creeping red fescue, and *D. phlei* on the seeds of crested dog's tail. The genera *Bipolaris* and *Drechslera* caused considerable infection of perennial ryegrass and hybrid ryegrass, their rate of occurrence was 11 % on perennial raygrass and 14.3 % on hybrid ryegrass germinated on a Jakobsen table. There is evidence that species of the genus *Curvularia* cause seed bud damage (mainly on rice plants) and they can also cause leaf spots (SPRAGUE 1950). We identified *Curvularia inaequalis* on seeds of the common foxtail and *C. lunata* on seeds of the crested dog's tail, cock's-foot, common foxtail and creeping red fescue. The *Fusarium* species damage grasses in all stages of their development. An important strategy of their distribution is via seeds. Infected seeds do not germinate or seedlings die soon after emergence. Other than on perennial ryegrass and hybrid ryegrass, we observed damage by *Fusarium* on all tested grass species. A somewhat higher infection rate was observed on the seeds of creeping red fescue and cock's-foot, but their infection rate was not higher than 2.5 % of the total. Most of them were *F. avenaceum*, but *F. equiseti, F. graminearum* and *F. semitectum* were also identified.

We were the first to isolate *Ulocladium botrytis*, *Pithomyces chartarum*, *Curvularia lunata*, *Septonema* sp. and *Embellisia* sp. from the seeds of crested dog's tail in both Austria and Hungary. We isolated *Curvularia lunata*, *C. inaequalis*, *Ulocladium botrytis* from the seeds of common foxtail and *Ulocladium botrytis* from the seeds of yellow oat grass as well as *U. atrum* from the seeds of creeping red fescue.

Through statistical analysis we were unable to prove correlation between the germination rate and fungal infection rate, neither with the use of thermostat data for germination tests (r_1 = 0,08), nor with use of the Jakobsen table (r_2 = 0,32). This means that fungal infection had no negative influence on germination. Many of the identified fungal species (*Alternaria, Fusarium, Bipolaris, Drechslera, Stemphylium*) however, may cause the death of seedlings or may hinder the vital processes of germinating seed and even saprophytic fungi on the seed surface may decrease germination rate.

In the pratice of grass production the dressing with fungicides seed treatment is not common, which is why special attention has to be paid to the health status of the seed. For maintaining seed quality appropriate storage with optimum conditions is of great importance.

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Eingelangt am 5. Jänner 2007
Angenommen am 3. Oktober 2008