INCIDENCE OF FUSARIUM SPECIES ON RED CLOVER SEED

Vesna Krmjača, Jelena Lević, M. Ivanović, Zorica Tomić, Slavica Mrfat-Vukelić

Abstract: Phytopathological investigation of seed of three commercial red clover cultivars – K9, K17 and NS Kolubara, and two pre-treatments of seeds – rinsing and surface disinfection of seeds (T1) and surface disinfection of seeds (T2) have determined that frequency of species within the genus Fusarium varied from 0 to 13%. By use of T1 pre-treatment the presence of Fusarium species on the seed was observed in neither of examined cultivars. In this pre-treatment the presence of 1% Penicillium spp. was observed on seed of cultivar K17 only, while the seeds K9 and NS Kolubara were non-infected. By use of T2 pre-treatment, the frequency of Fusarium species varied from 1 to 13%, depending on cultivar. From the seeds of all three examined cultivars, Fusarium verticillioides (2-13%) and F. proliferatum (2-6%) were isolated and from the seed of cultivar K9, F. oxysporum (4%). F. solani (2%) and undetermined Fusarium species (3%) were isolated, while F. subglutinans (1%) was isolated only from the seed of NS Kolubara. Besides Fusarium species, by application of pre-treatment T1, in all examined cultivars the species from genera Cladosporium (6-14%), Alternaria (6-7%) and Acremonium (1-5%) were isolated, and Penicillium spp. (1%) from the seed of cultivar K17. The results obtained indicate that frequency of mycobiota on the seed of red clover depended more on the seed pre-treatment than on the examined cultivars.

Considering the significant differences gained in health state of seed by application of T1 and T2 pre-treatments, further investigations should be directed towards the development of a method which would show real health state of seed, that is, natural contamination of seed. In this case it is the use of pre-treatment T2.

Key words: Fusarium spp., incidence, red clover (Trifolium pratense L.), seed infection.

Introduction

Seed of grown plants represents favourable substrate for development of numerous phytopathogene and saprobe fungus. Degree of change, accordingly, depends on type of seed, pathogens and environmental factors (Mišošević et al., 1995). Symptoms expressed on red clover seed and seed of other legumes, caused by pathogen organisms, are manifested as seed rot, reduction of seed germination, damping-off, and post emergence seedling root-rot (Tyler et al., 1956; Schmitthenner, 1964; Hancock, 1983). Seed coat, cotyledons, young shoots and leaf primordia of red clover and alfalfa could be invaded by hyphae pathogen fungus from the

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genus *Fusarium* (Chi et al., 1964). Red clover, alfalfa, sweetclover, and Ladino white clover were extremely susceptible to some species of *Pythium* when inoculated at the time of seeding or within 1 day thereafter (Halpin and Hanson, 1958).

According to the method Kilpatrick et al. (1954a), *Fusarium* spp. have been isolated from diseased red clover roots more frequently than any other fungi (Kilpatrick et al., 1954b; Fulton and Hanson, 1960). The most frequent being *Fusarium moniliforme* Sheldon, *F. oxysporum* Schlecht., *F. solani* (Mart.) App. & Wr. and *F. roseum* Lk. From other species of fungi usually the species from the genera *Gliocladium*, *Rhizoctonia*, *Pythium*, *Phoma*, *Pencillium*, *Trichoderma*, *Alternaria*, *Aspergillus*, *Mucor*, *Chaetomium* and other *Pythium* spp. were most commonly isolated from very young plants of red clover (Kilpatrick et al., 1954b; Fulton and Hanson, 1960).

During 1997, in the area of Kruševo town severe wilting of red clover on the surfaces meant for the production of fodder and seeds was observed. Disease was caused by the infection of root and tracheomycosis by *Fusarium* species (Urošević et al., 1999). Obtained isolates of this species have expressed pathogenicity to red clover (60%), alfalfa (65%), white clover (67%), soybean (40%), beans (23%), peas (17%), lupin (13%) and fodder peas (7%). According to authors’ description regarding morphological properties of fungi it could be concluded that it was *Fusarium oxysporum*.

In Serbia there are little data on agents of red clover, especially of certain species of the genus *Fusarium*. Also, as there is little routine experience on their identification, and in seed health testing the of commercial red clover cultivars only the genus *Fusarium*, but not the species, is being determined. Considering the importance of use of healthy seeding material, and in order to determine the significance of certain species of the *Fusarium* genus within etiology of red clover seed diseases, in this paper the study on the nature of infection of the seed of commercial red clover cultivars most often grown in Serbia was carried out.

**Material and Methods**

Investigations were carried out in Laboratory for Phytopathological Research in the Maize Research Institute in Zeman Polje. As initial material in this research seed of three red clover cultivars (K9, K17 and NS Kolubara), of the 2001 harvest was used.

For isolation of fungi from red clover seed the method of nutrient media was used with seed pre-treatment. In first pre-treatment (T1) seed was rinsed for 4 hours in running tap water, surface disinfected for 5 min. in 1% sodium hypochlorite solution (NaOCl), rinsed three times in sterile water and dried between two filter papers. In second pre-treatment (T2) surface disinfec tions with of seed was carried out. Of each cultivar 100 seeds were examined being distributed in 10 Petri dishes, 10 seeds per dish, were seeded to water agar and incubated on room temperature.

After 10 days colonies developed around seed were analysed microscopically and for further identification sample of colony was transferred on two media: synthetic low nutrient media (SNA) with 2 × 4 cm sterile filter paper (Nirenberg and O'Donnell, 1998) in Petri dishes of diameter of 9 cm, and on media containing of 2% agar and 5 sterile carnation leaves (CLA) in Petri dish of 6 cm diameter (Fisher et al., 1982).

Determination of *Fusarium* species was carried out by colony inspection in *situ* and native preparations prepared from fungus cultures grown on SNA and CLA nutrient media (Nelson et al., 1983; Burgess et al., 1994).
Results

Microscopic observation of fungus colonies isolated from red clover seed has determined smaller differences in regard to infection of seed between investigated cultivars than in different pre-treatments (Table 1). In pre-treatment T₁ no infection of seed by the species from *Fusarium* genus was observed except for the presence of 1% *Penicillium* spp. on seed of cultivar K17. By application of pre-treatment T₂, *Fusarium* species were isolated from 1 to 13%, depending on tested cultivar. *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *Fusarium moniliforme*) was determined in high percentage (13%) on seed of cultivar K17, relatively high percentage on seed of cultivar NS Kolubara (5%) and in low percentage on seed of cultivar K9 (2%). Presence of *F. proliferatum* (Matsumoto) Nirenberg was determined in relatively high percentage on seed of cultivar K9 (6%) and in low percentage on seed of cultivar NS Kolubara (3%) and K17 (2%). From seed of K9 cultivar some other species of *Fusarium* genus were isolated and they are *F. oxysporum* (4%), *F. solani* (2%) and *Fusarium* spp. (3%), and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas (1%) from the seed of cultivar NS Kolubara.

In seed pre-treatment T₂, besides *Fusarium* species, the species from genera *Acremonium, Alternaria, Cladosporium* and *Penicillium* were isolated as well. By application of this pre-treatment the most often isolated species were those from genus *Cladosporium*, and that in high percentage on seed of cultivar NS Kolubara (14%) and K9 (12%) and in relatively high percentage on seed of cultivar K17 (6%). Relatively high percentage of presence of *Alternaria* spp. was determined on seed of all investigated cultivars (6-7%). Presence of *Acremonium* spp. was established in relatively high percentage on seed of cultivar NS Kolubara (5%) and in low percentage on seed of cultivars K9 and K17 (1%), while species from *Penicillium* genus (1%) was established only on seed of cultivar K17.

By application of pre-treatment T₁ the interior (natural) seed contamination was established and with the application of pre-treatment T₂ the surface infection of seed of red clover was established (*Lević, personal communication*).
Table 1. Frequency (%) of Fusarium, Acremonium, Alternaria, Cladosporium and Penicillium species on seed of various red clover cultivars (Trifolium pratense L.)

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<tr>
<th>Cultivar/ Sorta</th>
<th>Intensity of seed infection/ Intenzitet infekcije semena (%)</th>
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- * pathogens were not found/ nisu pronađeni patogeni

Discussion

Many diseases of red clover (Trifolium pratense L.) are limiting the yield, quality and longevity of crops of this important fodder plant for animal feeds. One of the major problems in the production of red clover is the establishment and maintenance of stands. Lack of persistence is a major problem. Several factors are involved, one of which is the root and crown disease complex. Stand losses are most conspicuous in the second year, but also occur commonly in the first. In the humid areas of the United States, few red clover fields remain productive beyond the second year after seeding (Kilpatrick et al., 1954a; Moody et al., 1967).

Studies on mycosphaera of seed of red clover cultivars most often grown in Serbia have shown that, in natural conditions, they are, depending on cultivar, infected by the species
from *Fusarium* genus up to 13%, the frequency of *F. verticillioides* being very high therein. According to literature references *F. moniliforme* (syn. *F. verticillioides*), *F. oxysporum* and *F. solani* could be considered as potential inducers of disease in red clover. Mentioned species have proved their pathogenicity in regard to root of red clover and other hosts when inoculated at the tip of root (Leath and Kendall, 1978).

In laboratory tests on red clover seedlings isolates *F. oxysporum*, *F. solani* and *F. roseum* Link have caused a disease whose intensity ranged from 23 to 95%. Isolates of *Chaetomium, Mucor, Aspergillus, Alternaria, Penicillium*, and *Trichoderma* were in general either non-pathogenic or only weakly pathogenic of red clover seedlings (Kilpatrick et al., 1954b). Results of the tests made in greenhouse show that all the isolates of *Pythium debaryanum* Hesse tested and some of the isolates of *F. oxysporum*, *F. roseum*, and *F. solani* were very pathogenic on red clover seedlings while some isolates *Rhizoctonia*, *Phoma* and *Gliocladium* were moderately pathogenic (Kilpatrick et al., 1954b).

Symptoms incited by pathogenic isolates included pre-emergence killing, post-emergence damping-off, stunting, and rotting of the roots and hypocotyls (Kilpatrick et al., 1954b). Many pathogenic isolates of *F. oxysporum* and *Phoma* spp. caused post-emergence damping-off. The most pathogenic isolates of *F. oxysporum* caused a severe necrosis of the upper part of the primary root and lower part of the hypocotyl. Also, in these parts of root dark brown to black necrosis was caused by pathogenic isolates from *Phoma* and *Rhizoctonia* species. When the rotting was severe the seedlings would fall to the ground and die. *F. solani* caused a severe dark brown to black decay of the entire root system. The pathogenic isolates of *F. roseum* caused a brown rot of the roots and lower part of the hypocotyl. *Gliocladium roseum* Banier caused a dark brown rot of the roots and frequently of the lower part of hypocotyl. The most severe type of pre-emergence killing was caused by *P. debaryanum*.

In our country Todorov et al. (1995) have established that wilting of red clover is caused by *F. oxysporum* Schl. f. sp. *medicaginis* (Weimer) Snyder & Hansen, while Radović et al. (1999) observed that genotypes of red clover which have proved resistant to fusaric acid - toxin, have also proved resistant to *F. oxysporum* f. sp. *medicaginis*.

Obtained results indicate the significance of rinsing of seed (T₁) in elimination of presence of fungi on seed. This measure contributes to improvement of health condition of red clover seed. In practice, however, there is no example confirming the actual application of this or similar method of preventive protection of seed from infection. One of the reasons is insufficient knowledge of the role of seed as carrier of some pathogen fungi that are important from the economical aspect, including species of *Fusarium* genus, as well as economical and practical importance.

**Conclusions**

Results obtained on the basis of conducted research indicate that:

Two different seed pre-treatments, which mutually differed in 4h rinsing of seed (T₁) and non-rinsing of seed (T₂) before surface-sterilisation have brought about. By application of pre-treatment T₁ in all tested cultivars the presence of *Fusarium* species from 1 to 13% was observed. *Fusarium verticillioides* and *F. proliferatum* were isolated from seed of all tested cultivars, while *F. oxysporum*, *F. solani*, *F. subglutinans* and *Fusarium* spp. were isolated from the seed of one cultivar only. Also, the fungi from genera *Acremonium*, *Alternaria*,
**Cladosporium and Penicillium** were isolated. Considering the significant differences gained in health state of seed by application of T₁ and T₂ pre-treatments, further investigations should be directed towards the development of method which would show real health state of seed, that is, natural contamination of seed. In this case it is the use of pre-treatment T₂.

**UČESTALOST FUSARIUM VRSTA NA SEMENU CRVENE DETELINE**

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**Rezime**

FITOPATOLOŠKIM ISPITIVANJIMA SEMENA TRI KOMERCIJALNA KULTIVARA CRVENE DETELINE - K9, K17 i NS Kolubara, i dva pretretmana semena - ispiranje i površinska dezinfekcija semena (T₁) i površinska dezinfekcija semena (T₂), utvrđeno je da je učestalost vrsta iz roda *Fusarium* varirala od 0 do 13%. Primjenom T₁ pretretmana nije utvrđeno prisustvo *Fusarium* vrsta na semenu ni u jednom ispitivanoj kultivari. U ovom pretretmanu je samo na semenu kultivara K17 ustanovljeno prisustvo 1% *Penicillium* spp., dok je semen K9 i NS Kolubara bilo bez zaraze. Primjenom T₂ pretretmana, učestalost *Fusarium* vrsta je varirala od 1 do 13%, zavisno od kultivara. Iz semena sva tri ispitivana kultivara izolovane su *Fusarium verticillioides* (2-13%) i *F. proliferatum* (2-6%) dok su iz semena kultivara K9 izolovane *F. oxysporum* (4%), *F. solani* (2%) i nedeterminisane *Fusarium* vrste (3%), a *F. subglutinans* (1%) jedino iz semena NS Kolubara. Pored *Fusarium* vrsta, primjenom pretretmana kod svih ispitivanih kultivara, izolovane su vrste iz roda *Cladosporium* (6-14%), *Alternaria* (6-7%) i *Acremonium* (1-5%), a *Penicillium* spp. (1%) iz semena kultivara K17. Dobijeni rezultati ukazuju da je učestalost mikrobiota na semenu crvene deteline više zavisila od pretretmana semena nego od ispitivane sorte.

S obzirom da su konstatovane značajne razlike u zdravstvenom stanju semena primjenom T₁ i T₂ pretretmana, buduća istraživanja trebalo bi usmeriti u pravcu razvoja metode koja pokazuje realno zdravstveno stanje semena, odnosno prirodnu zarazu semena. U ovom slučaju to je primena pretretmana T₁.

**Ključne reči:** crvena detelina (*Trifolium pratense* L.), *Fusarium* spp., infekcija semena, učestalost.

**Literature**

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