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Resistance to anthracnose (*Colletotrichum linicola*) on different cultivars of red clover (*Trifolium pratense*) in Serbia

Original Article

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Abstract:

In addition to alfalfa, red clover (*Trifolium pratense* L.) is the most important perennial forage legume grown in Serbia. It is rich in proteins that are important for the nutrition of livestock. Red clover, like many legumes, is parasitized by fungi of the genus *Colletotrichum*, and the consequence is significant economic damage in the form of reduced yields and reduced hay quality. In this study, sixteen cultivars of red clover of different geographical origin were tested for the resistance to the isolate Coll-44 (*C. linicola*). The susceptibility of different cultivars of red clover to the tested *C. linicola* isolate was determined using the scale of 1-5. Six cultivars (Wilo, Repio, Viglana, Kolubara, K-39 and K-17) showed high levels of resistance to the tested isolate of *C. linicola*. Based on the severity index, the most resistant to the isolate of *C. linicola* was the American variety Wilo with a severity index of 1.6 (50%), while the lowest severity index of the Serbian variety K-39 was 2.6 (20%). The purpose of this research was to evaluate the susceptibility and tolerance level of 16 red clover cultivars to anthracnose causative agents, in this case, *C. linicola*.

Key words:

Colletotrichum linicola, cultivar, resistance, *Trifolium pratense*

Apstract:

Otpornost na antraknozu (*Colletotrichum linicola*) kod različitih sorti crvene deteline (*Trifolium pratense*) u Srbiji

Pored lucerke, crvena detelina (*Trifolium pratense* L.) je najvažnija višegodišnja krmna leguminoza koja se gaji u Srbiji. Bogata je proteinima koji su važni za ishranu domaćih životinja. Crvenu detelinu kao i mnoge leguminoze parazitiraju gljive iz roda *Colletotrichum*, nanoseći značajne ekonomske štete u vidi smanjenja prinosa i kvaliteta sena. U ovom radu testirano je šesnaest sorti crvene deteline različitog geografskog porekla na otpornost prema izolatu Coll-44 (*C. linicola*). Osetljivost različitih sorti crvene deteline prema ispitivanom izolatu *C. linicola* određena je prema skali od 1-5. Šest sorti Wilo, Repio, Viglana, Kolubara, K-39 and K-17 pokazalo je visok nivo otpornosti na testirani izolat *C. linicola*. Na osnovu indeksa otpornosti najotpornijom prema izolatu *C. linicola* se pokazala američka sorta Wilo sa indeksom otpornosti 1.6, dok je najniži indeks otpornosti kod srpske sorte K-39 iznosio 2.6. Cilj ovog istraživanja bio je da se oceni stepen osetljivosti i tolerantnosti kod 16 sorti crvene deteline prema prouzrokovačima antraknoze, u ovom slučaju *C. linicola*.

Ključne reči:

Colletotrichum linicola, sorta, otpornost, *Trifolium pratense*

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Introduction

Red clover (*Trifolium pratense* L.) is one of the most important forage crops in Serbia. As such, it represents an essential element in the crop rotations. In recent years, it has been observed that the life span and quality of red clover hay decreased significantly, and, as a reason for this occurrence, fungal species of the genus *Colletotrichum* (in particular, C. *trifolii* Bain et Essary, *C. destructivum* O'Gara, *C. coccodes* (Wallr.) Hughes, *C. truncatum* (Schw.) Andrus and Moore, *C. dematium* (Pers.) Grove and *C. linicol*a Pethybr. and Laff) were cited (Schubiger et al., 2004; Jacob et al., 2010; Vasić et al., 2014). Anthracnose

Fig. 1. "Shepherd's crook" symptom on red clover

decreases the vigour of the individual plants and thins out the plant population. During the summer and autumn, diseased plants start to appear in the red clover and alfalfa fields. These diseased plants have typical appearance. Plants, in the advanced stages of the disease, develop deformations of the upper part of the stem. Stem infection results in wilting and death of the upper portion of the stem and thus making the characteristic "shepherd's crook" symptom (**Fig. 1**). This is considered as the typical symptom of the disease. As the disease progresses, the infection spreads and passes from the stem to the root neck and the root area. Crown and root infection is characterized by dry rot with a change in the color of the affected tissue to blue-black (Vasić et al., 2016).

Damage caused by presence and development of pathogen agent cause the reduction of quantity and quality of fresh mass of 10% up to 30% depending on cultivar of red clover and alfalfa, species of pathogen, climatic and edaphic factors (Stuteville and Erwin, 1990; Vasić, 2010b). The diseased plants can, thus, decay during one growing season or be severely weakened, often succumbing to frost in winter conditions (Schubiger et al., 2003).

Since the growing importance of red clover crops in Serbia, the aim of this experiment was to determine the susceptibility and tolerance level of different red clovers cultivars to anthracnose agents, in this case, *C. linicola*, and to find the right sources of resistance.

Material and methods

In June-July, 2018, sixteen commercial cultivars of red clover were tested for resistance to the anthracnose causative agent *C. linicola*. The experiment was done in the greenhouse of the Institute for Forage Crops in Kruševac. Commercial red clover cultivars of different origin were used for the experiment. Cultivars were as following: K-9, K-17, K-27, K-32, K-38, K-39 (Institute for forage crops, Kruševac, Serbia), Una and Kolubara (Institute of Field and Vegetable Crops, Novi Sad, Serbia), Diplomat (Deutsche Saatveredelung AG – DSV, Lippstadt, Germany), Nigula (Estonian Crop Research Institute, Estonia), Longevo (Istituto Sperimentale per le Colture Foraggere, Lodi, Italy), Viglana, Manuela (Research Institute of Plant Production in Piestany, Slovakia) and Wilo, Repio and Diana (US Dairy Forage Research Dairy Farm, Wisconsin, USA). One isolate from Serbia, Coll-44, was used in this study and was identified as *C. linicola* originated from alfalfa from Serbia, based on morphological, pathological and molecular traits. Colonies of isolate *C. linicola* were light to dark olive green. In cultures on PDA medium, acervuli were formed. Conidia from acervuli were released in mucous masses that were orange to cream-pink in color (Vasić et al., 2014).

The sowing of the red clover seeds was done using plastic containers at a depth of 2 cm in sterile substrate in a greenhouse. The conidial suspension was prepared from cultures of the tested isolate. It was grown for ten days on PDA medium at 25 °C in the dark. The concentration of spores was determined by hemocytometer by Thoma and it was 4-6 x 10^4 spores/ml. Inoculated red clover plants were 7 weeks old and they were scarred prior to inoculation by cutting and sprayed with a spore suspension. The experiment was conducted in five repetitions, 5 plants per genotype and red clover plants inoculated with sterile water used as a negative control.

Plants were categorized in 5 score classes, based on the anthracnose reaction using the 1 to 5 scale (1 = no stem lesions or only a few small water-soaked or black spots; 2 = stems with elongated black lesions but without acervuli; 3 = stems with long, wide, but non-gridling lesions, with acervuli present; 4 = large, coalescing and sporulating lesions which kill

upper part of seedling; 5 = seedling dead) (Ivanović and Ivanović, 2005).

Based on the results, the severity index was calculated according to the following formula:

$$I=\Sigma \frac{(n \times k)}{N \times K} \times 100$$

I - severity index
N- total number of seedling
n - number of seedlings in class
K - number of categories
k - number of individual categories
Σ - sum of products

According to Ostazeski et al. (1969), where 1 and 2 are assigned to resistant plants, plants were scored individually, four weeks after inoculation. Disease intensity or severity of infected plants was calculated using the severity index. When the severity index is used as a criterion, all cultivars are classified with severity index from 2.72 to 2.25 can be classified as resistant cultivars (Ostazeski et al., 1979).

Results

The studied cultivars showed a clear difference in sensitivity to the tested isolate of *C. linicola*. The number of surviving plants varied over a wide range. Four weeks after the inoculation of the tested plants, primarily necrotic lesions occurred on the stem and there was slight twist of the top of the upper third of the stem. Necrotic lesions did spread further throughout the plant and it led to the complete drying and decay of some plants. The intensity of disease development was evaluated using the scale of 1-5. The response of the tested red clover cultivars after inoculation with the tested *C. linicola* isolate as well as the severity index score are shown in **Tab. 1**.

In the conditions of this experiment, the most resistant was the American cultivar of red clover Wilo, which had the severity index of 1.6 and 50% of resistant plants. Furthermore, Vinglana cultivar, with the severity index 2.4, and Repio, with the severity index 2.6, showed high levels of resistance. Also, Serbian varieties K-39, K-17 and Kolubara showed significant resistance, and their severity indices ranged from 2.6 to 2.8, while the percentage of resistant plants for all tested cultivars was 20. The most sensitive was cultivar Diana, with the severity index 3.6 and 10% of the healthy plants, while cultivars K-27 and Diplomat had severity indices 3.4 without healthy plants.

Discussion

According to results of Ostazeski et al. (1969), sensitive cultivars include cultivars with 10% of healthy plants, while resistant cultivars include cultivars with over 65% of healthy plants.

Vasić et al. (2015) tested thirteen (K-27, K-39,

Table 1. Evaluation of sensitivity in commercial cultivars of red clovers (*Trifolium pratense* L.) to isolate *C. linicola* (Coll-44)

Cultivars	Plants categorised by Ostazeski scale (%)					Severity index	Resistant plants (1+2%)
	1	2	3	4	5		
K-9	0	20	10	20	0	3.0	20
K-17	0	20	20	10	0	2.8	20
K-27	0	0	30	20	0	3.4	0
K-32	0	10	30	10	0	3.0	10
K-38	0	0	30	20	0	3.4	0
K-39	0	20	30	0	0	2.6	20
Diplomat	0	0	30	20	0	3.4	0
Nigula	0	10	30	20	0	3.0	10
Viglana	10	10	30	0	0	2.4	20
Manuela	0	10	20	10	20	3.4	10
Longevo	0	10	20	10	0	3.4	10
Kolubara	0	20	20	10	0	2.8	20
Una	0	10	30	10	0	3.0	10
Wilo	20	30	0	0	0	1.6	50
Repio	0	20	30	0	0	2.6	20
Diana	0	10	10	20	0	3.6	10
Control	-	-	-	-	-	-	-

K-32, K-38, Una, Kolubara, Čiroku, Lea, Virgiana, Valentine, Wilo, Repio and Diana) commercial cultivars of red clover for resistance to the anthracnose causative agent *C. destructivum*. The reactions of the tested cultivars were different. The most resistant to *C. destructivum* was the cultivar Wilo with severity index 1.9, while cultivar Valentina showed the highest sensitivity to the tested isolate. Despite the large number of studies, which indicated that resistance to anthracnose is controlled by major dominant or recessive genes and other resistance mechanisms, resistance to *C. trifolii* is not clear enough (Schubiger et al., 2004). Schubiger et al. (2003) found that the degree of resistance to *Colletotrichum trifolii* was different in different cultivars of red clover. The anthracnose resistance of the red clover plants ranged from 3% to 52% (Schubiger et al., 2004). According to Boller et al. (2004), Merula and Pavo were red clover cultivars of the Mattenklee type resistant to the causative agents of anthracnose, *C. trifolii*. According to Jacob et al. (2010), the German cultivars of red clover showed a wide range of resistance to *Colletotrichum trifolii* with plant survival from 29% to 87%. Schubiger et al. (2004) examined twelve Swiss cultivars of red clover (Leisi, Renova, Ruettinova, Milvus, Formica, Corvus, Merula, Pavo, Temara, Vanessa, Larus, Astur) considering the resistance to *C. destructivum*. In their research, they stated that none of the red clover cultivars were severely affected by *C. destructivum*. Also, Jacob et al. (2015) tested thirty-eight red clover cultivars listed in Germany, for the resistance to *C. trifolii* and found that resistance of red clover to anthracnose was highly heritable. Vasić et al. (2010b) reported that different cultivars of red clover (Manuela, Margot, K-39, K-32 and breed population L-50) show different levels of resistance depending on different *C. trifolii* isolates. The degree of resistance of the red clover cultivar depended on the virulence of the *C. trifolii* isolates. Vasić et al. (2010a) showed that alfalfa clones had different resistance to *C. trifolii*. By comparing American and Serbian cultivars, it can be noted that the American alfalfa cultivars had the highest resistance level (64.5% of plant survival). The most susceptible tested cultivar had the plant survival rate of 56.4%.

Conclusion

It can be concluded, from the presented assessment, that red clover cultivars showed variations in their resistance response to *C. linicola*. Six cultivars of Wilo, Repio, Viglana, Kolubara, K-39 and K-17 showed resistance to the tested isolate, and could be listed for cultivation under integrated production systems and used in the development of new resistant red clover cultivars.

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