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Evaluation of red clover (*Trifolium pratense* L.) resistance to Sclerotinia crown and root rot (*Sclerotinia trifoliorum*) in the laboratory and field conditions

Jovita MIKALIŪNIENĖ, Nijolė LEMEŽIENĖ, Vida DANYTĖ, Skaidrė SUPRONIENĖ

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: jovita.bukauskaite@lzi.lt

Abstract

Red clover (*Trifolium pratense* L.) is very productive in the first two years of harvest but afterwards it tends to thin out dramatically and finally die. In Lithuania, the main disease causing this problem is Sclerotinia crown and root rot (*Sclerotinia trifoliorum* Eriks.). The present study was aimed to determine red clover resistance to Sclerotinia crown and root rot in the laboratory and field conditions. Investigations were carried out at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Resistance of red clover varieties and breeding lines to Sclerotinia crown and root rot was investigated in 2012–2014 in the field under natural and artificial infection conditions. Diploid (43: 20 varieties, 20 breeding lines, 2 wild ecotypes and 1 semiwild ecotype) and tetraploid (41: 24 varieties and 17 breeding lines) red clover was investigated. In the laboratory experiment, red clover seeds were treated with oxalic acid which is the main toxin of *Sclerotinia* spp. This experiment enabled us to evaluate seedling resistance to Sclerotinia crown and root rot. The same varieties and breeding lines as in the field experiment were investigated. The seedlings were treated with 0, 5, 7.5, 10, 15, 20 and 30 mM oxalic acid concentrations. The most resistant varieties were found to be 'Kaive', 'Kvarta', 'Arimaičiai', 'Skriveru tetra', 'Van', 'Ostro', 'Radviliai' and 'Jogeva 433', and breeding lines Nos. 2102, 1582, 2190, 2087 and 2739. The most sensitive varieties were 'Sara', 'Sabtoron', 'Britta', 'Triton' and 'Astra', and breeding lines Nos. 2297, 2268, 2093, 2296, 2300 and 2298.

Key words: oxalic acid, resistance, *Sclerotinia trifoliorum*, *Trifolium pratense*.

Introduction

Red clover (*Trifolium pratense* L.) is an important forage legume that is widely cultivated in Europe because it is a valuable nitrogen-fixing component of leys, has beneficial effects on soil structure, and is an appreciated component of livestock diets (Öhberg et al., 2008). Red clover is a perennial forage legume of limited persistence, mainly used for cutting in grass-clover leys 2–4 years of duration, but also occurs naturally in permanent grassland.

Among the forage legumes, in terms of seed produced and marketed worldwide, and in numbers of cultivars available, red clover ranks second after alfalfa (*Medicago sativa* L.) but still higher than white clover (*Trifolium repens* L.), although the latter has been gaining importance in the last decades (Boller et al., 2010).

However, red clover is infected by several fungal pathogens affecting its growth, persistence and overwintering capacity (Wallenhammar et al., 2006). One of the most destructive diseases is Sclerotinia crown and root rot, which is caused by the fungal pathogen *Sclerotinia trifoliorum* Erikson. The pathogen infects plants in the autumn and develops in the plant under snow cover during the winter. The disease symptoms rapidly appear on the plant once temperatures rise and the snow melts (Klimenko et al., 2010). In late summer and autumn apothecia develop from soil-borne sclerotia. Ascospores are released and cause necrotic spots on stems and leaves. These local infections do not develop further until the plants are subjected to weather conditions favourable for the fungus, between late autumn and early spring. Then the fungus starts to grow systemically through the petioles and stems into the plants' crowns, and eventually into their taproots (Öhberg et al., 2008). Furthermore, infection may also spread to other plants by means of mycelial growth, thus creating patches of dead plants in the field. In spring, infected plants are covered by slightly grey mycelia, with black sclerotia forming in dead plant tissues at soil level, or in soil close to dead plants. Sclerotia are well adapted for survival and can retain viability in soil for several years (Williams, Western, 1965; Öhberg et al., 2008).

Sclerotinia crown and root rot has been investigated for a long time and the investigations were comprehensive. Various infection backgrounds, methods of inoculation and various stages of development were explored (Svirskis, 1972; Dixon, Doodson, 1974; Marum

et al., 1994; Delclos et al., 1997; Pokorny et al., 2003; Dabkevičienė, Dabkevičius, 2005; Öhberg et al., 2008). Svirskis (1972) investigated red clover and alfalfa varieties and breeding lines for resistance and survival under natural conditions and artificial inoculation, selected varieties, breeding lines and separated individuals for further breeding, defined some biological characteristics of Sclerotinia crown and root rot and fungus of *Fusarium* genus. Dixon and Doodson (1974), Marum et al. (1994), Delclos et al. (1997) and Pokorny et al. (2003) investigated various inoculation methods in the laboratory and fields conditions. Dabkevičienė and Dabkevičius (2005) investigated resistance of artificially inoculated wild red clover populations. Öhberg et al. (2008) carried out the investigation in North and South Sweden, where 15 red clover varieties were tested in the field conditions.

In Lithuania, Sclerotinia crown and root rot causes big losses of red clover foliage and seeds (Vilčinskas, Dabkevičienė, 2009). In Lithuania, the first investigations on red clover resistance to Sclerotinia crown and root rot were performed in 1958 (Žemaitienė, 1962). Žemaitienė (1962) inoculated various red clover varieties by pure Sclerotinia crown and root rot culture in a greenhouse. Бразаускене (1985) collected Sclerotinia crown and root rot material from red and white clover, separated 30 strains from it, evaluated their aggressiveness and possibilities to mix them for inoculum preparation. She compared different methods of evaluation of white clover breeding material resistance to Sclerotinia crown and root rot and found that for comparison of resistance and selection of resistant individuals direct and stepped-up methods are the most suitable.

Most investigations were oriented towards varietal resistance estimation, but few of them were aimed to create a method for selection of resistant individuals during short time. In literature, there is little information about investigations with oxalic acid, which is the main toxin of Sclerotinia crown and root rot. Jančys and Vyšniauskienė (2002) carried out investigations with red clover and Liatukienė et al. (2012), to a greater extent, with alfalfa. The advantage of this method is the possibility of selection of resistant individuals during germination which lasts only a few days. It is easy to select a required percent of resistant plants from whole material by choosing the most suitable oxalic acid concentration. The investigations done with red clover and alfalfa according to this method showed good differentiation. Sensitive to Sclerotinia crown and root rot red clover varieties more distinctly responded to oxalic acid (Jančys, Vyšniauskienė, 2002; Liatukienė et al., 2012).

It is necessary to use fungicides to prevent Sclerotinia crown and root rot damage in red clover seed crops. Another, more effective way is choice of the right variety. The currently available varieties are productive and of good nutritional quality, but they produce insufficient seed yield, are moderately resistant to frost and insufficiently resistant to diseases (Gaurilčikienė, Staniulis, 2006).

Our study was designed to determine red clover resistance to Sclerotinia crown and root rot in the laboratory and field conditions.

Material and methods

Field experiment. Investigations were carried out at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Resistance of red clover varieties and breeding lines to Sclerotinia crown and root rot was investigated in 2012–2014 in the natural field conditions and using inoculation by sclerotiums of *Sclerotinia trifoliorum* Eriks. Diploid (43: 20 varieties, 20 breeding lines, 2 wild ecotypes and 1 semiwild ecotype) and tetraploid (41: 24 varieties and 17 breeding lines) red clover was investigated. The red clover genetic collection was established on a calcaric shallow gleyic *Endocalcari-Epihypogleyic Cambisol* (*CMg-p-w-can*) light loam. The collection was sown on the 8th of June in 2012. Each red clover variety and breeding line was sown in two 5 m long rows in three replications. The distance between the rows of accessions was 0.5 m; the distance between accessions was 1.0 m. Interrows were applied with glyphosate using a hand sprayer. Sclerotinia crown and root rot damage in red clover crop was evaluated in spring in 2013 and 2014 after resumption of vegetation using a 1–9 score scale and was calculated to percent: 1 score – 0%, 2 scores – 0.1%, 3 scores – 1%, 4 scores – 5%, 5 scores – 10%, 6 scores – 20%, 7 scores – 40%, 8 scores – 60%, 9 scores – 80% (Hartung, Piepho, 2007; Poland, Nelson, 2011).

Evaluation of resistance to Sclerotinia trifoliorum. Sclerotinia crown and root rot sclerotia were collected in the spring of 2012 from the crowns of dead red clover plants. They were sterilized in a solution of 5% sodium hypochlorite and after that in 70% alcohol and washed with sterile water. After sterilization, sclerotia were placed on 2% agar medium and kept till the begging of mycelium growth. Actively growing isolates without other fungus and bacteria infection were selected. The mycelium was transferred on to the potato dextrose agar medium (40 g l^{-1}). The mycelium was grown and the cultures were kept in the dark at 20°C temperature. Fresh mycelium was used for investigation. When the mycelium was grown up, its surface was shaved off, put to water, shook up, filtrated and diluted till the required concentration (10000 spores ml^{-1}). This solution was used for artificial inoculation of red clover. Spray application was done in October 2012 (Marum et al., 1994; Kanbe et al., 2002; Yli-Mattila et al., 2009).

Evaluation of resistance to oxalic acid. Red clover resistance to oxalic acid was evaluated as percent of germinated seeds using adapted method used in the researches of Rowe (1993), Jančys and Vyšniauskienė (2002) and Liatukienė et al. (2012). Seeds were scarified and surface sterilized in solution of 5% sodium hypochlorite for 15 min and rinsed three times in distilled water. Fifty sterilized seeds were placed in one plastic Petri plate (diameter 9 cm) on filter paper moistened with 5 ml of water solution of oxalic acid. Three replications

were used for each clover entry per one concentration of oxalic acid. The seven concentrations of oxalic acid used were: 0, 5, 7.5, 10, 15, 20 and 30 mM. Petri plates with seeds were maintained in the dark at 20°C for seven days. Germinated seeds with actively growing roots were counted as resistant. Relative percent of germination was calculated for detailed analysis of clover accessions' reaction to oxalic acid. Percent of germinated seeds in 0 mM solution of oxalic acid was equated to 100%. Relative percent of germinated seeds in other concentrations was calculated as:

$$RG = (GS_{OAx}/GS_{OA0}) \times 100,$$

where RG is the relative percent of germinated seeds, GS_{OAx} – the germinated seeds % in corresponding oxalic acid concentration, GS_{OA0} – the germinated seeds % in oxalic acid concentration 0 mM (Liatukienė et al., 2012).

Meteorological conditions. The summer of 2012 was warm, rainy and windy. There was a sufficient amount of moisture in the soil for red clover germination and further development. The autumn was warm, rainy and windy. The average air temperature was 1.4°C higher compared with the multiannual average, and precipitation was 132% of the multiannual average. The winter of 2012–2013 was early and long with a permanent snow cover and long soil freeze. December was cold, windy and with a lot of snow. The spring was late and short. In May, the weather was hot, similar to summer's weather. It was favourable for perennial grass growth. The summer was warm and with a fluctuating amount of precipitation. The autumn was warm, dry and long.

In the winter 2013–2014 the snow cover appeared only on the 13th of January and melted on the 8th of February. The weather in January was unusually warm, rainy and cloudy. The temperature in February was 4.7°C higher compared with the multiannual average. Foggy and windy weather prevailed. Such weather conditions favoured Sclerotinia crown and root rot development.

Statistical analysis. The research data were processed by the analysis of variance (Tarakanovas, Raudonius, 2003).

Result and discussion

Disease resistance is one of the most important traits of a good red clover variety. In our investigation, 84 genotypes of various origin were investigated for resistance to Sclerotinia crown and root rot (*Sclerotinia trifoliorum* Eriks.), which is the main disease of red clover. It occurs in the regions with cold winters, such as Lithuania. Resistance of red clover to *S. trifoliorum* depends on pathogen aggressiveness, plant genotype, phenotype (late or medium-late flowering type) and other local environmental abiotic and biotic factors (Öhberg et al., 2005; 2008). The development of clover crown and root rot depends heavily on the weather conditions. Favourable conditions – humid autumn and a warm, humid winter with short periods of frost – can lead to complete destruction of crops (Marum et al., 1994). In our investigation, the average of damaged red clover plants was more than three times lower in 2013 compared with 2014. In 2013 it was 7.8% and in 2014 it was as high as 24.9% (Table 1). More favourable weather conditions for development of rot infection in 2014 probably influenced the differences between years. As expected, higher damage was in the artificially infected field. In 2013, it was 11.0% compared with 4.7% in the uninfected field and in 2014 it was 30.7% and 19.1%, respectively.

Table 1. Percent of damaged red clover plants

Field type	2013				2014				2013–2014			
	2n	4n	average	LSD_{05}	2n	4n	average	LSD_{05}	2n	4n	average	LSD_{05}
Uninfected field	5.3	4.0	4.7	1.25	18.5	19.6	19.1	2.72	11.9	11.8	11.9	1.96
Infected field	14.7	7.1	11.0	3.03	30.2	31.2	30.7	3.14	22.5	19.2	20.9	2.80
LSD_{05}	2.66	1.85	1.70	–	2.61	3.26	2.07	–	2.24	2.59	1.71	–

There was a significant difference in the percentage of damaged plants between diploid and tetraploid red clover in 2013, when the infection was not high. In 2014, the infection was 4–5 times higher and there was no significant difference between the percentage of damaged diploid and tetraploid plants. Some authors maintain that tetraploid red clover varieties are more resistant to Sclerotinia crown and root rot compared with diploid varieties (Dijkstra, 1964; Raynal, 1985), others maintain that ploidy does not have any influence on the resistance to this disease (Ortega et al., 1997; Öhberg et al., 2005).

Weather conditions and infection level by Sclerotinia crown and root rot have strong influence on red clover wintering (Sprainaitis, 2000). The weather conditions were favourable for the spread of Sclerotinia crown and root rot in the autumn of 2012, which was long, warm and with a lot of rain. The soil was frozen and snow cover lasted all winter. In the spring of 2013, the breeding lines and varieties of red clover in artificially uninfected field were damaged by Sclerotinia crown and root rot from 0% to 15.8% (Table 2). Varieties 'Vesna', 'Kvarta', 'Divaja', 'Skriveru tetra' and 'Jogeva 205', and breeding lines Nos. 2096, 2300, 2301, 2190 and 1589 were not damaged (0%) by Sclerotinia crown and root rot. Low resistance to the disease was shown for the varieties 'Amos', 'Vyčiai', 'Sprint', 'Spurt' and 'Astra'. They were damaged by 10.8–12.5%. The least resistant breeding lines Nos. 2108, 2297, 2331, 2281 and 2105 were damaged from 8.3% to 15.8%.

Table 2. Resistance of red clover breeding lines and varieties to Sclerotinia crown and root rot in uninfected field, 2013–2014

Breeding line/variety	Disease severity %		Breeding line/variety	Disease severity %	
4n	2013	2014	2n	2013	2014
1582	0.0	21.7	2086	7.5	9.2
2095	5.8	18.3	2087	1.7	20.0
2096	0.0	15.0	2088	3.3	15.8
2098	3.3	21.7	2091	5.8	17.5
2114	5.0	15.0	2093	7.5	29.2
2190	0.0	17.5	2094	8.3	29.2
2268	7.5	27.5	2101	5.8	8.3
2274	2.5	18.3	2102	1.7	15.0
2281	10.0	28.3	2103	2.5	11.7
2282	1.7	14.2	2104	7.5	17.5
2286	2.5	31.7	2105	8.3	16.7
2293	5.0	27.5	2106	1.7	13.3
2296	0.8	35.8	2107	3.3	18.3
2298	5.8	26.7	2108	15.8	15.8
2300	0.0	25.8	2177	5.8	30.0
2301	0.0	28.3	2188	6.7	12.5
2302	7.5	21.7	2270	1.7	18.3
Amos	12.5	4.2	2295	3.3	15.0
Beskyd	10.0	4.2	2297	13.3	23.3
Blizard	0.8	21.7	2299	2.5	25.0
Cyklon	9.2	15.0	2331	11.7	25.0
Divaja	0.0	25.0	2433	7.5	20.0
Dolina	5.0	4.2	2739	0.8	18.3
Dolly	7.5	34.2	Arija	1.7	12.5
Ilte	1.7	22.5	Arimaičiai	5.0	15.0
Kaive	2.5	12.5	Astra	10.8	18.3
Kiršinai	3.3	25.0	Britta	2.5	20.0
Kvarta	0.0	2.5	Jancis	2.5	20.0
Nodula	10.0	5.0	Jogeva 433	5.8	12.5
Ostro	5.8	14.2	Jogeva 205	0.0	12.5
Rejista	2.5	5.8	Kamaniai	1.7	25.8
Sadūnai	7.5	25.8	Liepsna	9.2	18.3
Sara	0.8	30.8	Marita	1.7	14.2
Skriveru tetra	0.0	11.7	Nemaro	3.3	10.8
Sprint	11.7	19.2	Palma	1.7	23.3
Tempus	3.3	7.5	Raba	5.0	16.7
Triton	0.8	37.5	Radviliai	0.8	19.2
Varte	0.8	34.2	Sabtoron	7.5	24.2
Vesna	0.0	6.7	Sandis	0.8	28.3
Vyliai	1.7	20.8	Spurt	11.7	27.5
Vulkan	7.5	19.2	Start	8.3	15.8
	–	–	Van	4.2	18.3
	–	–	Vyčiai	11.7	19.2
Min.	0.0	2.5	Min.	0.0	8.3
Max.	12.5	37.5	Max.	15.8	30.0
Average	4.0	19.6	Average	5.3	18.5
$\overline{x} \pm S\overline{x}$	0.4	1.0	$\overline{x} \pm S\overline{x}$	0.4	0.8

The winter of 2013–2014 was also favourable for Sclerotinia crown and root rot spread. It was warm and rainy. A thin snow cover appeared in the middle of January and remained till the 8th of February. In 2014, red clover in artificially uninfected field was much more damaged by Sclerotinia crown and root rot compared with 2013. All red clover varieties and breeding lines were damaged by Sclerotinia crown and root rot. The plants were damaged by disease from 2.5% to 37.5%. The most resistant varieties were 'Kvarta', 'Beskyd', 'Dolina', 'Amos' and 'Nodula' (damaged from 2.5% to 5.0%), and breeding lines Nos. 2101, 2086, 2103, 2188 and 2106 (damaged from 8.3% to 13.3%). The least resistant varieties were 'Triton', 'Varte', 'Dolly', 'Sara' and 'Sandis' (damaged from 28.3% to 37.5%), and breeding lines Nos. 2296, 2286, 2177, 2094 and 2093 (damaged from 29.1% to 35.8%).

Methods of evaluation of disease resistance in field are effective if inoculation is used at the weather conditions favourable for disease development (Jančys, Vyšniauskienė, 2002). Inoculation in the genetic red clover collection was made in the end of October in 2012, when the weather conditions were favourable for pathogen spread – it was warm and rainy.

In the artificially infected field Sclerotinia crown and root rot severity in red clover was 0–41.7% (Table 3). Varieties 'Divaja', 'Ilte', 'Varte', 'Arimaičiai' and 'Sara', and breeding lines Nos. 2106, 2282, 2190, 2096 and 2301 remained undamaged even after inoculation. But there were some sensitive varieties 'Beskyd' (27.1%), 'Start' (27.1%) and 'Nodula' (20.8%), and breeding lines Nos.

Table 3. Resistance of red clover breeding lines and varieties to Sclerotinia crown and root rot in the infected field, 2013–2014

Breeding line/variety	Disease severity %		Breeding line/variety	Disease severity %	
4n	2013	2014	2n	2013	2014
1582	2.1	18.8	2086	29.2	31.3
2095	2.1	33.3	2087	6.3	29.2
2096	0.0	20.8	2088	25.0	22.9
2098	2.1	33.3	2091	4.2	29.2
2114	4.2	31.3	2093	16.7	33.3
2190	0.0	33.3	2094	41.7	35.4
2268	10.4	41.7	2101	29.2	27.1
2274	6.3	22.9	2102	14.6	16.7
2281	4.2	35.4	2103	12.5	22.9
2282	0.0	31.3	2104	22.9	37.5
2286	2.1	33.3	2105	22.9	20.8
2293	10.4	35.4	2106	0.0	27.1
2296	8.3	41.7	2107	16.7	29.2
2298	8.3	43.8	2108	25.0	25.0
2300	12.5	47.9	2177	25.0	45.8
2301	0.0	31.3	2188	10.4	25.0
2302	4.2	31.3	2270	16.7	35.4
Amos	16.7	47.9	2295	22.9	25.0
Beskyd	27.1	20.8	2297	39.6	27.1
Blizard	10.4	20.8	2299	29.2	33.3
Cyklon	10.4	25.0	2331	16.7	29.2
Divaja	0.0	33.3	2433	10.4	41.7
Dolina	4.2	20.8	2739	8.3	31.3
Dolly	4.2	20.8	Arija	16.7	29.2
Ilte	0.0	41.7	Arimaičiai	0.0	22.9
Kaive	6.3	27.1	Astra	4.2	39.6
Kiršinai	8.3	27.1	Britta	4.2	39.6
Kvarta	12.5	31.3	Jancis	12.5	41.7
Nodula	20.8	33.3	Jogeva 433	12.5	27.1
Ostro	10.4	27.1	Jogeva 205	2.1	29.2
Rejista	12.5	43.8	Kamaniai	4.2	25.0
Sadūnai	8.3	31.3	Liepsna	4.2	31.3
Sara	0.0	39.6	Marita	14.6	27.1
Skriveru tetra	8.3	27.1	Nemaro	6.3	22.9
Sprint	10.4	22.9	Palma	6.3	29.2
Tempus	8.3	12.5	Raba	6.3	31.3
Triton	14.6	31.3	Radviliai	10.4	25.0
Varte	0.0	50.0	Sabtoron	12.5	29.2
Vesna	2.1	29.2	Sandis	12.5	52.1
Vyliai	2.1	27.1	Spurt	6.3	33.3
Vulkan	16.7	20.8	Start	27.1	22.9
	–	–	Van	8.3	16.7
	–	–	Vyčiai	16.7	43.8
Min.	0.0	0.0	Min.	0.0	16.7
Max.	27.1	50.0	Max.	41.7	52.1
Average	7.1	31.2	Average	14.7	30.2
$\bar{x} \pm S\bar{x}$	0.8	1.2	$\bar{x} \pm S\bar{x}$	1.2	1.1

2101, 2086, 2299, 2297 and 2094. Severity of sensitive breeding lines was from 29.1% to 41.7%. In the second experimental year the severity of Sclerotinia crown and root rot was much higher compared with the first year and there were no undamaged varieties and breeding lines. The disease damaged from 12.5% to 52.1% of plants. The most resistant varieties were 'Tempus' (12.5%) and 'Van' (16.7%), and breeding lines Nos. 2102, 1582, 2105 and 2096. Breeding lines were damaged from 16.7% to 20.8%. The most sensitive varieties were 'Rejista', 'Amos', 'Varte' and 'Sandis'. They were damaged from 43.7% to 52.1%. The most sensitive breeding lines Nos. 2298, 2177 and 2300 were damages from 41.7% to 45.8%.

As expected, red clover plants were much more damaged in infected field compared with uninfected. In uninfected field in 2013 disease severity was from 0% to 15.8%, and in infected field from 0% to 41.7%. In 2014, in uninfected field disease severity was from 2.5% to 37.5%, and in infected field from 12.5% to 52.1%. The resistance of varieties and breeding lines to Sclerotinia crown and root rot and disease pressure differed between experimental years. However, there were some varieties and breeding lines which showed similar resistance to Sclerotinia crown and root rot in both years in both fields. The most resistant varieties were 'Tempus', 'Dolina', 'Vesna', 'Nemaro' and 'Arimaičiai', and breeding lines Nos. 2096, 2106, 1582 and 2282. The most susceptible varieties were 'Vyliai', 'Sandis' and 'Varte', and breeding lines Nos. 2094, 2177, 2297 and 2299.

Treating plant seedlings with high oxalic acid concentrations is a fast, straightforward and reliable method for the selection of plants most resistant to Sclerotinia crown and root rot. This method allows executing 2–3 sampling cycles during one year by using laboratory and greenhouse methods. In the field conditions we can execute one sampling cycle during two years, if we use inoculation or during three years without inoculation if weather conditions are favourable for disease spread (Kanbe et al., 1997).

As distinct from field investigation data, in the laboratory experiment with oxalic acid tetraploid red clover plants were more sensitive compared with diploid plants (Table 4). At 5, 7.5 and 10 mM concentrations the germination of tetraploid plants was significantly lower compared with diploid red clover plants. At 15, 20 and 30 mM concentrations the germination of tetraploids was also lower; however, the differences were not significant.

Table 4. Percent of germinated red clover plants at different oxalic acid concentrations

Ploidy	n	Oxalic acid concentrations mM					
		5	7.5	10	15	20	30
		Germination %					
2n	43	87.8	80.0	66.4	52.1	40.3	21.9
4n	41	80.2	68.5	59.4	49.4	37.8	21.3
Average		84.1	74.0	63.0	50.8	39.1	21.6
LSD_{05}		4.34	5.28	5.70	6.21	5.73	3.61

For the evaluation of red clover resistance to different oxalic acid concentrations we used the same varieties and breeding lines as in the field investigation. The breeding line 2433 showed the highest resistance in the laboratory conditions, where its germination at 5, 7.5, 10 and 15 mM oxalic acid concentrations was 99.1–92.3% and at 30 mM concentration it was 71.1%. However, in the field experiment this breeding line did not show good results of resistance to Sclerotinia crown and root rot.

Germination of varieties and breeding lines at 5 mM concentration was 99.1–50.0%, at 7.5 mM concentration – 95.6–0%, at 10 mM – 93.1–0%, at 15 mM – 92.6–0%, at 20 mM – 79.6–0% and at 30 mM – 71.1–0%. Red clover varieties were more resistant to Sclerotinia crown and root rot than breeding lines. The percent of germination of the most resistant varieties were rather stable at 5, 7.5, 10 and 15 mM and sharp decrease occurred at 20 and 30 mM oxalic acid concentrations (Table 5). A sharp decrease in the germination of susceptible varieties and breeding lines occurred already at 5 mM concentration.

The most resistant varieties to oxalic acid were 'Sadūnai', 'Jancis', 'Van' and 'Radviliai'. Their germination at 5 and 7.5 mM concentrations was 92.4–78.7%, and at 20 and 30 mM concentartion it was 71.3–37.5%. Variety 'Sara' and breeding line No. 2096 can be defined as sensitive to oxalic acid, as germination of variety 'Sara' at 7.5 mM concentration and germination of breeding line 2106 at 10 mM oxalic acid concentration was 0%. Low germination at 30 mM oxalic acid concentration was of varieties 'Sara' (0%), 'Sabtoron' (5.6%), 'Raba' (12.6%) and 'Britta' (12.7%). These varieties also showed low resistance to Sclerotinia crown and root rot in the field conditions.

Dabkevičienė and Dabkevičius (2005) suggest that resistance of *T. pratense* wild ecotypes to Sclerotinia crown and root rot can be varying. Most of them are usually sensitive or moderately resistant to Sclerotinia crown and root rot and just a few are resistant. According to the data from their investigation, 38.5% of wild red clover ecotypes were moderately resistant. In our investigations three wild breeding lines were

Table 5. Germination of red clover genotypes at different oxalic acid concentrations

Breeding line/ variety	Oxalic acid concentration mM						Breeding line/ variety	Oxalic acid concentration mM					
4n	5	7.5	10	15	20	30	2n	5	7.5	10	15	20	30
1582	93.4	86.7	58.2	57.4	48.4	22.7	2086	84.7	72.9	67.5	0.0	0.0	0.0
2095	90.4	77.8	60.1	48.0	38.9	27.3	2087	76.6	61.9	58.7	54.8	37.6	30.2
2096	34.6	0.0	0.0	0.0	0.0	0.0	2088	81.1	82.6	68.7	60.7	28.2	16.6
2098	20.2	22.7	16.9	0.0	0.0	0.0	2091	84.1	77.1	68.8	0.0	0.0	0.0
2114	58.6	24.1	20.7	17.2	13.8	0.0	2093	80.6	77.4	49.3	44.8	42.2	8.2
2190	98.0	94.0	89.1	82.1	71.9	25.0	2094	89.0	58.6	41.2	36.1	26.8	26.8
2268	82.2	81.2	58.9	51.9	36.9	19.4	2101	78.5	60.9	56.9	48.5	39.9	7.2
2274	44.8	28.8	14.9	7.0	0.0	0.0	2102	94.4	87.3	70.6	48.1	29.0	26.9
2281	79.7	67.7	46.8	25.8	12.6	6.4	2103	96.1	86.6	51.7	57.3	39.7	11.3
2282	62.9	57.3	40.3	20.8	13.0	10.4	2104	95.6	86.5	79.2	57.2	49.2	22.9
2286	65.2	55.4	55.7	43.1	23.1	9.5	2105	67.6	64.8	31.1	31.1	20.7	0.0
2293	77.9	70.6	68.1	60.8	42.2	28.5	2106	50.0	45.8	0.0	0.0	0.0	0.0
2296	57.1	49.0	26.1	12.3	7.2	0.0	2107	79.8	78.0	32.4	36.2	28.0	8.0
2298	71.2	78.6	65.7	52.2	31.2	16.3	2108	77.3	85.3	73.5	45.7	36.2	27.3
2300	79.5	69.0	77.5	52.6	39.9	16.7	2177	94.3	75.9	60.4	49.4	38.8	28.2
2301	60.3	7.3	58.7	44.5	7.3	3.7	2188	93.6	80.9	76.6	42.6	29.8	17.0
2302	81.2	69.4	63.2	50.0	41.8	40.0	2270	80.3	62.2	28.7	31.4	15.0	5.5
Amos	92.0	81.1	67.7	65.5	58.6	25.1	2295	69.4	61.1	52.4	0.0	3.4	0.0
Beskyd	98.7	87.6	77.6	70.7	64.4	19.1	2297	86.9	72.8	56.1	34.9	18.5	9.1
Blizard	90.0	75.1	58.9	49.8	34.4	22.0	2299	90.0	80.6	62.5	53.5	40.6	15.9
Cyklon	85.9	77.5	63.4	52.2	40.2	29.0	2331	93.4	70.9	59.4	50.0	36.1	24.6
Divaja	55.3	52.6	37.0	21.1	10.5	5.3	2433	99.1	94.0	93.1	92.3	79.6	71.1
Dolina	80.0	73.2	61.6	59.3	45.1	22.5	2739	88.7	78.5	64.0	55.3	49.5	37.8
Dolly	95.1	86.0	74.9	62.1	50.2	37.4	Arija	92.6	93.0	88.7	81.4	71.0	20.7
Ilte	89.0	81.9	68.1	52.0	40.2	26.4	Arimaičiai	97.4	94.2	90.1	87.1	69.8	36.2
Kaive	96.7	92.7	78.2	62.2	46.5	32.4	Astra	92.9	92.4	86.4	77.2	28.1	18.3
Kiršinai	96.3	75.2	57.4	45.9	29.4	15.6	Britta	91.9	85.9	69.3	52.8	25.9	12.7
Kvarta	92.7	85.4	74.5	66.4	55.8	34.9	Jancis	89.1	85.5	73.4	64.5	55.6	37.8
Nodula	91.1	87.5	79.9	74.5	64.8	36.7	Jogeva 433	95.3	87.7	81.8	74.7	58.9	34.7
Ostro	90.2	81.1	68.3	56.2	45.7	34.3	Jogeva 205	89.7	84.3	76.9	71.9	60.6	23.0
Rejista	96.6	90.2	83.5	77.5	68.3	36.4	Kamaniai	87.8	75.0	70.0	67.1	61.3	20.7
Sadūnai	92.4	86.5	89.8	87.5	71.3	46.4	Liepsna	94.3	87.3	79.7	66.9	55.8	14.2
Sara	71.4	0.0	0.0	0.0	0.0	0.0	Marita	91.2	84.7	74.9	63.3	52.5	32.6
Skriveru tetra	91.2	80.6	66.3	55.3	45.1	33.7	Nemaro	95.8	88.2	77.5	66.6	49.3	25.4
Sprint	87.1	78.1	64.9	54.5	44.8	33.7	Palma	96.7	89.1	92.0	83.4	67.4	29.0
Tempus	84.2	67.8	56.8	45.1	38.1	27.8	Raba	73.1	69.0	49.4	33.6	16.8	12.6
Triton	92.7	84.5	82.5	82.5	58.8	16.7	Radviliai	87.7	86.5	82.6	79.2	70.3	41.8
Varte	84.0	75.8	63.1	51.2	40.6	28.7	Sabtoron	89.6	85.6	75.9	0.0	6.5	5.6
Vesna	93.9	91.1	75.0	64.3	50.5	21.7	Sandis	95.1	87.3	77.1	66.9	56.3	31.4
Vyliai	98.2	95.6	90.6	81.7	73.1	25.1	Spurt	89.3	75.8	59.8	50.0	42.6	29.5
Vulkan	86.7	76.3	64.0	53.2	41.4	32.0	Start	85.9	69.2	59.1	51.4	42.4	31.2
–	–	–	–	–	–	–	Van	90.9	78.7	65.2	59.7	47.8	37.5
–	–	–	–	–	–	–	Vyčiai	98.2	82.4	81.3	68.6	60.7	35.7
Min.	20.2	0.0	0.0	0.0	0.0	0.0	Min.	50.0	45.8	0.0	0.0	0.0	0.0
Max.	98.7	95.6	90.6	87.5	71.3	46.4	Max.	99.1	94.2	93.1	92.3	79.6	71.1
Average	80.2	68.5	59.4	49.4	37.8	21.3	Average	87.8	80.0	66.4	52.1	40.3	21.9
$\bar{X} \pm S\bar{X}$	1.9	2.4	2.1	2.1	2.0	1.2	$\bar{X} \pm S\bar{X}$	1.1	1.2	1.9	2.2	2.0	1.3

investigated. Two of them – Nos. 2177 and 2331 did not show good resistance to oxalic acid in the laboratory and to Sclerotinia crown and root rot in the field conditions. But breeding line No. 2739 can be defined as resistant, because germination of this breeding line at 5, 7.5 and 10 mM oxalic acid concentration was 88.7–64.0%, and at 30 mM concentration it was 37.8%.

Germination of Lithuanian varieties 'Sadūnai', 'Radviliai', 'Vyčiai' and 'Arimaičiai' was the highest compared with other Lithuanian varieties. It was 98.2–81.3% at 5, 7.5 and 10 mM oxalic acid concentration and 46.4–35.7% at 30 mM concentration. The lowest germination percentage among the Lithuanian varieties at 30 mM oxalic acid concentration was of 'Kiršinai'

(15.6%) and 'Kamaniai' (20.7%). In the field experiments, the best resistance to Sclerotinia crown and root rot was shown by the variety 'Arimaičiai'. The Lithuanian varieties 'Arimaičiai' and 'Liepsna' were previously investigated by Jančys and Vyšniauskienė (2002). He has referred to 'Arimaičiai' as resistant and 'Liepsna' as sensitive variety. In the experiment of Dabkevičienė and Dabkevičius (2005) 'Liepsna' and 'Vyliai' did not show high Sclerotinia crown and root rot resistance either.

Low oxalic acid concentrations can be insufficiently effective for the selection of varieties and breeding lines resistant to Sclerotinia crown and root rot (Rowe, Welty, 1984). Germination of varieties and breeding lines decreases with increasing concentration of oxalic acid. The higher (20 and 30 mM) oxalic acid concentrations enable a more precise evaluation of resistant varieties and breeding lines. In our investigations, there was no correlation between the data from the field method and laboratory method. However, there were some varieties and breeding lines which showed the same resistance in different years and at different investigation methods. Resistant varieties were 'Kaive', 'Kvarta', 'Arimaičiai', 'Skriveru tetra', 'Van', 'Ostro', 'Radviliai' and 'Jogeva 433', resistant breeding lines were Nos. 2102, 1582, 2190, 2087 and 2739.

Conclusions

1. No correlation was found between the field and laboratory susceptibility of investigated red clover genotypes to Scerotinia crown and root rot. However, there were some varieties and breeding lines which showed good resistance using both methods.

2. Tetraploid red clover plants are more resistant in the first growth year compared with diploids, but there is no difference in the second year.

3. The most resistant varieties to oxalic acid were 'Sadūnai', 'Jancis', 'Van' and 'Radviliai'.

4. The most resistant varieties to Sclerotinia crown and root rot in the field conditions were 'Tempus', 'Dolina', 'Vesna', 'Nemaro' and 'Arimaičiai' and breeding lines Nos. 2096, 2106, 1582 and 2282, and the most sensitive varieties were 'Vyliai', 'Sandis' and 'Varte' and breeding lines Nos. 2094, 2177, 2297 and 2299.

5. The results of our investigations showed that the most resistant varieties to both Sclerotinia crown and root rot in the field conditions and to oxalic acid in the laboratory conditions were 'Kaive', 'Kvarta', 'Arimaičiai', 'Skriveru tetra', 'Van', 'Ostro', 'Radviliai' and 'Jogeva 433' and breeding lines Nos. 2102, 1582, 2190, 2087 and 2739. The most sensitive varieties were 'Sara', 'Sabtoron', 'Britta', 'Triton' and 'Astra' and breeding lines Nos. 2297, 2268, 2093, 2296, 2300 and 2298.

6. Only one wild population (No. 2739) from all investigated showed good resistance to Sclerotinia crown and root rot.

7. Among the Lithuanian varieties the best resistance to Sclerotinia crown and root rot in the field experiments was exhibited by the variety 'Arimaičiai'. Germination of this variety in oxalic acid also was very good.

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Raudonojo dobilo (*Trifolium pratense* L.) atsparumo vėžiui (*Sclerotinia trifoliorum*) įvertinimas laboratorinėmis ir lauko sąlygomis

J. Mikaliūnienė, N. Lemežienė, V. Danytė, S. Supronienė

Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas

Santrauka

Raudonieji dobilai yra labai produktyvūs pirmais ir antrais naudojimo metais, tačiau vėliau jų pasėliai smarkiai išretėja arba visai žūva. Daugelyje Lietuvos vietovių pagrindinė liga, išretinanti raudonųjų dobilų pasėlį, yra vėžys (sukėlėjas *Sclerotinia trifoliorum* Eriks.). Tyrimo tikslas – nustatyti raudonojo dobilo veislių ir selekcinių linijų atsparumą vėžiui laboratorinėmis ir lauko sąlygomis. Raudonojo dobilo tyrimai atlikti 2012–2014 m. Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės instituto Žolių selekcijos skyriaus sėjomainos laukuose. Tyrimai atlikti lauko sąlygomis esant natūraliai ir dirbtinei *Sclerotinia trifoliorum* Eriks. infekcijai. Raudonųjų dobilų genetinėje kolekcijoje buvo tirta 43 diploidai (iš jų 20 veislių, 20 selekcinių linijų, 2 laukiniai ir 1 pusiau laukinis ekotipai) ir 41 tetraploidas (iš jų 24 veislės ir 17 selekcinių linijų). Laboratorinėmis sąlygomis panaudota oksalo rūgštis, kuri yra pagrindinis *Sclerotinia* spp. toksinas. Oksalo rūgšties panaudojimas sėklų dygimo metu per keletą dienų sudarė galimybę atrinkti vėžiui atsparius individus. Siekiant nustatyti atsparumą oksalo rūgščiai, tirtos 84 raudonojo dobilo veislės ir selekcinės linijos. Panaudotos įvairios oksalo rūgšties koncentracijos: 0, 5, 7,5, 10, 15, 20 ir 30 mM. Taikant lauko ir laboratorinį metodus, galima greičiau atrinkti atsparias veisles ir selekcines linijas. Tyrimų metu nustatytos atspariausios veislės buvo ‘Kaive’, ‘Kvarta’, ‘Arimaičiai’, ‘Skriveru tetra’, ‘Van’, ‘Ostro’, ‘Radviliai’ bei ‘Jogeva 433’ ir selekcinės linijos Nr. 2102, 1582, 2190, 2087 bei 2739. Jautriausios veislės buvo ‘Sara’, ‘Sabtoron’, ‘Britta’, ‘Triton’ bei ‘Astra’ ir selekcinės linijos Nr. 2297, 2268, 2093, 2296, 2300 bei 2298.

Reikšminiai žodžiai: atsparumas, oksalo rūgštis, *Sclerotinia trifoliorum*, *Trifolium pratense*.