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Evaluation of eyespot incidence and structure of *Oculimacula* spp. population in winter rye in Lithuania

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Abstract

Eyespot of rye, caused by two fungal pathogens *Oculimacula yallundae* and *O. acuformis,* is widespread throughout many cool and wet cereal growing regions. The study was aimed to estimate eyespot incidence in winter rye crops in three agro-ecological zones of Lithuania, and to quantify eyespot disease causal agents *O. yallundae* and *O. acuformis* in the population using the real-time polymerase chain reaction (RT PCR) method. During the 2008–2012 periods, 69 winter rye fields were surveyed and eyespot-affected stems were identified in 92.7% of the crops surveyed. The incidence of eyespot in winter rye crops varied from 8.0% to 82.7% depending on the year and location. The average disease incidence in the reviewed crops was 26.0%. The highest incidence of eyespot averaging 34.0% was identified in the winter rye crops of the Middle Lowland zone. Both eyespot causal agents *O. yallundae* and *O. acuformis* were often found to co-exist on the winter rye stems. In the majority of the winter rye crops tested, *O. acuformis* was predominant in the *Oculimacula* spp. population. In most cases, *O. yallundae* was identified only at trace-level concentrations.

Key words: eyespot, *Oculimacula acuformis*, *O. yallundae*, real-time polymerase chain reaction, *Secale cereale*.

Introduction

Among cereal species grown in Lithuania, winter rye is an important plant for bread and is best suited to grow in temperate climates. It produces a high and stable, high-quality yield of grain. Rye is very resistant to frost; its root system can take up nutrients from the deepest soil layers, so it can grow well on soils that are not suitable for growing other cereals such as wheat or barley (Bolibok et al., 2005).

Eyespot is one of the most important stem base diseases of cereals caused by the two fungi *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams, anamorph *Helgardia herpotrichoides* (Fon) Crous & W.Gams, and *O. acuformis* (Boerema, R.Pieters & Hamers) Crous & W.Gams, anamorph *Helgardia acuformis* (Nirenberg) Crous & W.Gams (Crous et al., 2003). Eyespot is widespread throughout many cool and wet cereal growing regions: temperate climate regions in Europe, especially in West European countries – the United Kingdom, Germany, France, as well as in our neighbouring country Poland, and similarly in South Africa, North America, Australia (Cromey et al., 2006; Glazek, 2009). Eyespot incidence is strongly influenced by the weather conditions; spores are formed in wet conditions only and are splashed by rain (Vanova et al., 2005). The risk of incidence depends on many other factors such as sowing time, soil type, tillage methods, crop rotation (Bock et al., 2009; Jenkyn et al., 2010; Burnett et al., 2012). This disease is particularly significant in agricultural systems with reduced rotation, because both pathogens can infect perennial grasses of the *Poaceae* family and a wide range of cereals including rye (Meyer et al., 2011). Under favourable conditions, eyespot can damage spring cereals too. According to Lemanczyk (2009) data, the greatest disease incidence (DI = 53.1%) of eyespot in spring rye was established in the year when the total rainfall was highest. In Lithuania, the incidence of eyespot in cereal crops varied depending on the year and site (Gaurilčikienė, 1998; Janušauskaitė, Čiuberkis, 2010). The symptoms of eyespot are eye-shaped, elliptical lesions on the lower internodes, which disrupt the transport of water and nutrients and predispose the plant to collapse. *O. yallundae* is a more specialised causal agent of wheat eyespot and is often referred to as W (wheat) type; however, it can affect other *Poaceae* plants, while *O. acuformis* is equally pathogenic on rye, wheat and barley and is referred to as R (rye) type. The symptoms on plants caused by *O. yallundae* and *O. acuformis* visually do not differ (Ray et al., 2006); however, they differ in pathogenicity and occurrence (Fitt et al., 2006). Visual assessment of individual pathogens in mixed infection is often difficult, because other stem base and root rot diseases have similar symptoms. Polymerase chain reaction (PCR) method is often used in research for positive diagnosis and quantification of pathogens including *Oculimacula* spp. (Nicholson et al., 1997). Studies have shown that a higher incidence of eyespot is established using PCR method compared with visual assessment (Jackson et al., 2007). RT PCR assay is much faster and more accurate. This method was used to quantify *Oculimacula* spp. in the eyespot-diseased winter rye stems (Walsh et al., 2005). In Lithuania, the ratio of *O. yallundae* to *O. acuformis* in the population has not been studied before.

The research was aimed to estimate eyespot incidence in winter rye crops in different agro-ecological zones of Lithuania, and to quantify eyespot disease causal agents *Oculimacula yallundae* and *O. acuformis* in the population using the real-time polymerase chain reaction (RT PCR) method.

Materials and methods

Assessment of eyespot incidence, field samples. To estimate eyespot incidence in winter rye in Lithuania, plant samples were collected in 2008–2012 from the farms of agricultural partnerships and private farmers. The sampling was done at cereal ripening stage (BBCH 85–89) in different agro-ecological zones of the country: in the Middle Lowland from 13 administrative districts, in Western zone from 8 and in Eastern zone from 11. A total of 250 stems (50 stems from 5 places) from a field were randomly collected for eyespot incidence assessments. Eyespot diseased stems were counted and the disease incidence (percentage of diseased stems) was determined. The lower three internodes of infected stems were cut off, labelled and stored in fabric bags in a refrigerator for further analyses.

Investigation of Oculimacula spp. population structure. Deoxyribonucleic acid (DNA) extraction. Fungal DNA was extracted from rye stems. Each sample collected from commercial fields was composed of 10 randomly selected eyespot-affected stems. Stem segments approximately 2.5 cm in size (Bateman et al., 2000) were ground in a Cyclotec™ 1093 mill (FOSS, Denmark) and homogenised in liquid nitrogen. DNA was extracted from 0.1 g homogenised sample in two replications using a commercial GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, USA).

Real-time polymerase chain reaction (RT PCR). The standard curve (absolute quantitation, AQ) method was used for *O. yallundae* and *O. acuformis* DNA quantification in eyespot-affected stems of winter rye. Plant EF1α Hor1F and Hor2R primers were used to normalize reactions (Nicolaisen et al., 2009). The volume per reaction was 20 μl consisting of 10 μl Maxima™ SYBR Green qPCR Master Mix (Thermo Fisher Scientific, Lithuania), 6.9 μl water (nuclease free), 0.3 μl Ocu-R and 0.3 μl YallFHF, 0.3 μl Ocu-R and 0.3 μl AcFDF or 0.3 μl Hor1F and 0.3 μl Hor2R and 2.5 μl DNA (Table 1). For development of standard curves of *Oculimacula* spp. fungi, DNA was isolated from pure *O. yallundae* and *O. acuformis* cultures, obtained from BCCM/IHEM Biomedical Fungi and Yeasts Collection, the Mycology Laboratory at the Scientific Institute of Public Health in Brussels (Belgium). DNA extracted from wheat (*Triticum aestivum* L.) leaves was used for standard curve of plants. To normalize reactions, plant DNA was used from the same sample as the fungus analysed. DNA sample (mg ml^{-1}) concentrations were checked by a Biophotometer (Eppendorf, Germany). Individual standard curves were formed from different concentrations of DNA of the tested fungi and plants, having done 5 dilutions of the initial DNA sample. For *O. yallundae* standard curve we used 45 to 0.000045 ng μl^{-1}, for *O. acuformis* – 26 to 0.000026 ng μl^{-1} and for plants – 55 to 0.000055 ng μl^{-1} concentrations of DNA. RT PCR was performed on a 7900HT Fast, Sequence Detection System (Applied Biosystems, USA) using the following cycling regime: 95ºC – 10 min (95ºC – 15 s, 60ºC – 30 s, 72ºC – 1 min)$^{40\ \text{cycles}}$. RT PCR of one sample was performed in three replicates.

Table 1. Specific primers and their sequences used for real-time polymerase chain reaction (RT PCR) analyses

Target	Primer	Sequence (5’-3’)
Oculimacula acuformis	AcFDF	GCCACCCTACTTCGGTAA
O. yallundae	YallFHF	GGGGGCTACCCTACTTGGCAG
Oculimacula spp.	Ocu-R	ATTCAAGGGTGGAGGTCTGRA
Triticum aestivum L.	WpalF	CGTTCTTGGTCGCGTTGTG
	WpalR	ACTCTTGACAGCATTCTTGACATTCT
Plant EF1α	Hor1F	TCTCTGGGTTTGAGGGTGAC
	Hor2R	GGCCCTTGTACCAGTCAAGGT

For the results of eyespot incidence and amounts of fungal DNA, a standard deviation was calculated using the statistical data processing software *Stat Eng*. Tables present standard deviation (SD), mean, the least (Min) and the highest (Max) disease incidence.

Results

The incidence of eyespot in winter rye. During the period 2008–2012, in Western zone of Lithuania 17 fields, in the Middle Lowland 23 fields and in Eastern zone 29 fields of winter rye were inspected for eyespot incidence (Table 2). Eyespot was identified in 92.7% of the surveyed winter rye crops. The incidence of eyespot in assessed winter rye crops markedly varied between locations and years. In 69 rye fields assessed during 2008–2012 the eyespot incidence averaged 26.0%. Depending on the year, the disease incidence in different locations varied from 8.0% to 82.7%. The highest incidence was identified in the Middle Lowland zone in 2009 (82.7%) and the lowest in the Eastern zone in 2011 (8.0%). According to the data averaged over five years, the highest eyespot incidence was in Middle Lowland (34.0%) and the lowest in Eastern zone (18.6%), while in Western zone eyespot affected 25.3% of winter rye stems.

Oculimacula spp. population structure in winter rye crops. Both eyespot causal agents *O. yallundae* and *O. acuformis* were often found to co-exist on the winter rye stems. The concentrations of *O. yallundae* and *O. acuformis* DNA in winter rye stems varied between experimental years and sites. Of all the samples of winter rye collected during 2010–2012, the highest DNA concentrations of the pathogens were detected in those collected in 2012, while the lowest concentrations were identified in the ones collected in 2010 (Table 3). In almost all winter rye crops tested, *O. acuformis* predominated in the *Oculimacula* spp. population, except only in one crop in 2012 in Jonava where the amount

Table 2. The incidence of eyespot (%) in winter rye in three agro-ecological zones of Lithuania during in 2008–2012

District	Experimental year					
	2008	2009	2010	2011	2012	Average 2008–2012
Western zone						
Kelmė	*	–	26.0	32.0	–	29.0
Klaipėda	4.0	16.3	–	–	28.0	16.1
Raseiniai	–	–	28.0	32.0	–	30.0
Plungė	–	–	–	–	44.0	44.0
Šilalė	–	–	–	23.0	–	23.0
Šilutė	–	–	–	–	4.0	4.0
Tauragė	24.0	–	–	–	20.0	22.0
Telšiai	20.0	65.3	–	0	52.0	34.3
Average	**16.0 (n** = 3)**	**40.8 (n = 2)**	**27.0 (n = 3)**	**21.8 (n = 4)**	**29.6 (n = 5)**	**25.3 (n = 17)**
SD	10.58	27.58	1.41	2.31	19.10	14.95
Middle Lowland						
Biržai	–	–	20.0	–	–	20.0
Jonava	–	–	–	–	68.0	68.0
Jurbarkas	–	–	–	0	20.0	10.0
Kaišiadorys	–	–	4.0	12.0	20.0	12.0
Kaunas	8.0	–	–	–	–	8.0
Kėdainiai	76.0	88.0	36.0	8.0	48.0	51.2
Marijampolė	55.0	84.0	–	–	–	69.5
Panevėžys	23.0	–	–	–	–	23.0
Pasvalys	–	76.0	–	–	–	76.0
Prienai	–	–	8.0	–	36.0	22.0
Radviliškis	–	–	–	28.0	–	28.0
Šakiai	–	–	–	12.0	–	12.0
Šiauliai	40.0	–	–	–	44.0	42.0
Average	**40.4 (n = 5)**	**82.7(n = 3)**	**17.0 (n = 4)**	**12.0 (n = 5)**	**39.3 (n = 6)**	**34.0 (n = 23)**
SD	26.61	6.21	14.38	8.87	18.32	31.60
Eastern zone						
Alytus	28.0	–	–	–	–	28.0
Anykščiai	18.0	28.0	16.0	0	20.0	16.4
Ignalina	33.0	–	–	–	–	33.0
Molėtai	–	–	–	20.0	20.0	20.0
Rokiškis	–	–	–	0	–	0
Šalčininkai	–	–	16.0	–	–	16.0
Trakai	–	–	–	4.0	–	4.0
Ukmergė	–	–	0	16.0	32.0	16.0
Utena	–	4.0	–	–	–	4.0
Varėna	33.4	–	30.0	–	50.0	37.8
Vilnius	16.0	–	36.0	–	36.0	29.3
Average	**25.7 (n = 10)**	**16.0 (n = 2)**	**19.6 (n = 6)**	**8.0 (n = 5)**	**31.6 (n = 6)**	**18.6 (n = 29)**
SD	8.23	16.97	14.03	8.33	12.52	9.79
Average in Lithuania	**29.1 (n = 18)**	**53.2 (n = 7)**	**20.0 (n = 13)**	**13.7 (n = 14)**	**33.5(n = 17)**	**26.0 (n = 69)**
SD	19.45	33.3	12.43	10.29	16.45	14.95
Min	4.0	4.0	0	0	4.0	0
Max	76.0	88.0	36.0	32.0	68.0	88.0

* – not assessed, ** – number of crops assessed; SD – standard deviation, Min – the least disease incidence, Max – the highest disease incidence

of both *O. acuformis* and *O. yallundae* was comparable, and in 2011 in one crop in Anykščiai where *O. yallundae* outnumbered *O. acuformis*. In most cases, *O. yallundae* was identified at trace-level concentration. The highest amount of *O. acuformis* DNA was established in the sample collected in Kaišiadorys in 2012, it is almost twice as much as in Telšiai, Ukmergė, Vilnius and Molėtai. A similar amount of both pathogens was established in 2010 in Raseiniai, Kelmė and Šalčininkai, in 2011 in Telšiai, Radviliškis, Kaišiadorys and Anykščiai, and in 2012 in Šilutė and Jonava. In all tested samples from Western zone and from Middle Lowland (Kėdainiai, Šiauliai and Prienai districts) the amount of *O. yallundae* DNA was identified at trace-level concentrations.

The averaged data of *O. yallundae* and *O. acuformis* DNA concentration in eyespot-infected winter rye stems differed between agro-ecological zones annually (Fig.). In 2010, the amount of DNA of *O. yallundae* in Middle Lowland and Eastern zone was identified at very low concentration, whereas *O. acuformis* was determined in all samples from all agro-ecological zones, and in Middle Lowland was five times as high as in Western and Eastern zones. In 2011, the highest concentrations of the *O. acuformis* DNA were detected in the samples collected in Eastern zone, which were three times higher than in those from the Middle Lowland and twice as high as in the ones from the Western zone. The amount of DNA of *O. yallundae* in winter rye samples from all locations was very low, and in Western and Middle Lowland zones only trace-level concentrations were identified. In 2012, the largest amount of DNA of both fungus species was determined. That year, in Eastern zone the amount of *O. acuformis* DNA was 18 times higher than that of *O. yallundae*, in Middle Lowland 31 times and in Western zone as many as 112 times. In 2011 and 2012, the greatest amount of *O. acuformis* DNA was detected in the samples from Eastern zone, which was probably caused by rye domination in the crop rotation

Table 3. The amount of deoxyribonucleic acid (DNA) of *Oculimacula yallundae* and *O. acuformis* in winter rye samples collected in Lithuanian fields in 2010–2012

District	*Oculimacula* spp. DNA (fungal DNA ng / plant DNA ng)					
	2010		2011		2012	
	O. yallundae	*O. acuformis*	*O. yallundae*	*O. acuformis*	*O. yallundae*	*O. acuformis*
Western zone						
Kelmė	0.036	0.050	0.236	7.371	–	–
Klaipėda	–	–	–	–	0.154	31.346
Raseiniai	1.714	3.079	0.086	1.300		
Plungė	–	–	–	–	0.049	27.205
Šilalė	–	–	0.005	4.878	–	–
Šilutė	–	–	–	–	0.439	3.794
Telšiai	–	–	0.093	0.190	0.795	117.315
Middle Lowland						
Biržai	0.134	12.350	–	–	–	–
Jonava	–	–	–	–	4.723	4.701
Kaišiadorys	0.003	0.044	0.011	0.057	3.171	211.030
Kėdainiai	0.246	2.436	0.038	7.269	0.034	5.879
Prienai	0.301	17.387	–	–	0.061	34.737
Radviliškis	–	–	0.052	0.299	–	–
Šiauliai	–	–	–	–	0.581	10.524
Eastern zone						
Anykščiai	0.019	2.999	0.332	0.238	–	–
Molėtai	–	–	0.475	9.684	1.661	65.836
Šalčininkai	0.205	0.594	–	–	–	–
Trakai	–	–	4.475	10.962	–	–
Ukmergė	–	–	–	–	4.107	94.718
Vilnius	0.006	1.076	–	–	9.678	118.435
Standard deviation	0.350	6.150	1.380	4.390	2.910	93.870
The least amount	0.0	0.04	0.01	0.06	0.03	3.79
The highest amount	1.11	17.39	4.48	10.96	9.68	211.03

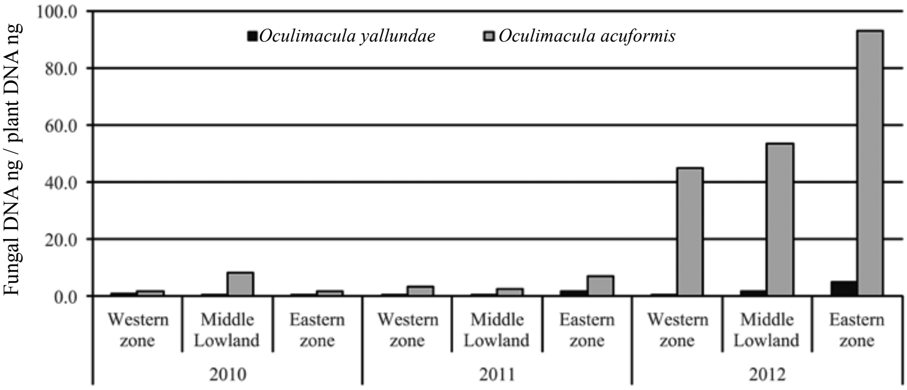

Figure. *Oculimacula yallundae* and *O. acuformis* deoxyribonucleic acid (DNA) in the winter rye stem-base from three agro-ecological zones of Lithuania (real-time polymerase chain reaction (RT PCR) absolute quantification (AQ) method)

in this agro-ecological zone. Low productivity soils in Eastern zone are more suitable for rye than for wheat in comparison with Middle Lowland and Western zone.

Discussion

The findings of the 2008–2012 field surveys indicated that the incidence of eyespot in winter rye differed between years, locations and fields. Research done in Lithuania in previous years also showed differences in eyespot incidence between years and locations. In 1994–1996, in Dotnuva, Kėdainiai distr., in tetraploid rye crops eyespot affected only single plants (Gaurilčikienė, 2000); however, in Vėžaičiai, Klaipėda distr., eyespot-infected rye stems accounted for 3.0–42.8%, and Perloja, Varėna distr. – for 0.9–19.2%. The most severe eyespot incidence was in early sown winter rye crops; however, the disease incidence varied depending on year conditions in particular agro-ecological zone (Gaurilčikienė, 1998). Crop rotation, soil type, sowing date, rainfall during spring period and cultivation method are crucial to the intensity of the disease (Burnett, 2005). In the current study, in rye crops inspected for eyespot presence all of these factors were different, which resulted in great dispersal of disease incidence data in different regions and fields.

Both *O. yallundae* and *O. acuformis* are widespread in many cereal production regions of the world, except for South Africa where only *O. yallundae* has been detected. Literature sources indicate that *Oculimacula* pathogens infect different plant species at different levels, are not specific to one host-plant species and are characterised by a wide specialization (Kayser, Heitefuss, 1992). *O. acuformis* has been reported to be equally identified in wheat and rye, while *O. yallundae* is more severe in wheat than in rye (Lucas et al., 2000). Both pathogens can co-exist on one stem and can cause symptoms of eyespot that visually hardly differ on various *Poaceae* family plants (Fitt et al., 1987). Previous investigations performed in Lithuania showed that both *O. yallundae* and *O. acuformis* were identified on eyespot diseased cereal stems according to fungi morphological characteristics as well as PCR test. Both eyespot fungi occurred at quite similar incidence on rye, wheat, triticale and barley stems and often co-existed on the same stem (Gaurilčikienė et al., 2010; Ramanauskienė et al., 2014). Pooling the averaged data on the quantity of *O. yallundae* and *O. acuformis* DNA concentrations in eyespot-infected winter rye stems, it was estimated that the quantities differed between years and agro-ecological zones annually (Fig.). Many researchers have reported that eyespot pathogen *O. acuformis* is dominant of *Oculimacula* spp. in cereals (Ray et al., 2004). According to our results, using RT PCR analysis, *O. acuformis* was clearly prevalent in the *Oculimacula* spp. population in the majority of rye crops tested; only in one field in Anykščiai (2011) *O. yallundae* DNA slightly exceeded *O. acuformis*, and in one field in Jonava (2012) DNA amount of both pathogens was equal (Table 3). *O. yallundae* usually developed earlier than *O. acuformis*. Nicholson et al. (2002) suggest that at stem elongation stage (BBCH 31–34) the amount of *O. yallundae* DNA was significantly higher than that of *O. acuformis*. This can be explained by the fact that at early plant growth stages *O. yallundae* infects plants and penetrates leaf sheaths more quickly than *O. acuformis* (Ray et al., 2006).

Both the eyespot incidence and concentrations of *O. yallundae* and *O. acuformis* DNA in winter rye stems varied between experimental years and sites. In the present study, from 2010–2012 the highest incidence of eyespot in winter rye crops was in 2012 and the lowest in 2011. In 2012, the highest concentration of *O. acuformis* DNA in eyespot infected rye stems was found too, while in 2010 and 2011 the amounts of pathogen DNA detected were considerably lower and comparable. However, eyespot incidence in separate fields most often was not corresponded with fungal DNA amounts from diseased stems (Tables 2–3). We did not find any literature to support the relationship between eyespot incidence and amounts of *Oculimacula* spp. DNA in rye crop. As has been reported by Turner et al. (2001), visual and PCR analyses on wheat stems generally supported each other, but there were often variances in relating disease incidence or the severity of symptoms to the amount of pathogen DNA by regression analyses; however, the relationships between symptoms and eyespot pathogen DNA were less clear on some cultivars. Ray at al. (2004) similarly indicated that the relationship between *O. acuformis* DNA and eyespot incidence or severity in wheat was weak.

In summary, the results presented in this study indicate that eyespot incidence in rye in some fields could be economically important, and that the prevalence of *O. acuformis* in the *Oculimacula* population is an important factor for eyespot control in rye crops.

Conclusions

1. Eyespot was identified in 92.7% of the winter rye crops inspected. The disease incidence averaged 26.0%. It occurred at higher incidence rates in the winter rye crops in the Middle Lowland zone. Depending on the year and site, eyespot incidence varied from 8.0% to 82.7%. The highest incidence was identified in the Middle Lowland zone in 2009, while the lowest incidence was recorded in the Eastern zone in 2011.

2. The concentrations of *Oculimacula yallundae* and *O. acuformis* deoxyribonucleic acid (DNA) in infected winter rye stems varied between experimental years and sites. Of all the samples of winter rye collected during 2010–2012, the highest DNA concentrations of the tested pathogens were detected in the samples collected in 2012, while the lowest concentrations were identified in those collected in 2010. In the majority of winter rye crops tested, *O. acuformis* predominated in the *Oculimacula* spp. population, *O. yallundae* was identified at trace-level concentration.

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Stiebalūžės išplitimas žieminiuose rugiuose ir jos sukėlėjų *Oculimacula* spp. populiacijos įvairovė Lietuvoje

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Santrauka

Rugių stiebalūžė, kurią sukelia patogenai *Oculimacula yallundae* ir *O. acuformis*, yra išplitusi daugelyje vėsaus klimato šalių. Tyrimų tikslas – įvertinti stiebalūžės išplitimą žieminių rugių pasėliuose įvairiose Lietuvos agroekologinėse zonose ir nustatyti patogenų *O. yallundae* bei *O. acuformis* kiekį populiacijoje, taikant tikrojo laiko polimerazės grandininės reakcijos metodą. 2008–2012 m. buvo įvertinti 69 žieminių rugių pasėliai, iš kurių 92,7 % buvo rasta stiebalūžės pažeistų rugių stiebų. Ligos išplitimas žieminių rugių pasėliuose įvairavo tarp įvairių vietovių ir tyrimų metų (nuo 8,0 iki 82,7 %). Vidutinis stiebalūžės išplitimas tirtuose pasėliuose buvo 26,0 %. Didžiausias stiebalūžės išplitimas nustatytas Vidurio žemumos zonoje (vidutiniškai 34,0 %). Abu – ir *O. yallundae*, ir *O. acuformis* – patogenai koegzistavo ant stiebalūžės pažeistų žieminių rugių stiebų. *Oculimacula* spp. kiekybinė tikrojo laiko polimerazės grandininės reakcijos analizė atlikta tiriant žieminių rugių stiebalūžės pažeistus stiebus, 2010–2012 m. surinktus iš įvairių Lietuvos agroekologinių zonų. Beveik visuose tirtuose rugių pasėliuose *Oculimacula* spp. populiacijoje vyravo *O. acuformis*. Daugeliu atvejų ant stiebalūžės pažeistų rugių stiebų buvo nustatyti labai maži kiekiai *O. yallundae* DNR.

Reikšminiai žodžiai: *Oculimacula acuformis*, *O. yallundae*, stiebalūžė, tikrojo laiko polimerazės grandininė reakcija, žieminiai rugiai.