ALEKSANDRAS STULGINSKIS UNIVERSITY
LITHUANIAN RESEARCH CENTRE FOR AGRICULTURE AND FORESTRY

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POPULATIONS STRUCTURE COMPOSITION AND DYNAMICS OF THE BLACKLEG CAUSAL FUNGI *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA*

Summary of doctoral dissertation
Agricultural sciences, agronomy (01 A)

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The doctoral dissertation and summary is available at the Libraries of Lithuanian Research Centre of Agriculture and Forestry and Aleksandras Stulginskis University.

ALEKSANDRO STULGINSKIO UNIVERSITETAS
LIETUVOS AGRARINIŲ IR MIŠKŲ MOKSLŲ CENTRAS

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RAPSŲ FOMOZĘ SUKELIANČIŲ GRYBŲ *LEPTOSPHAERIA MACULANS* IR *L. BIGLOBOSA* RŪŠIŲ SANTYKIS BEI DINAMIKA POPULIACIJŲ STRUKTŪROJE

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INTRODUCTION

Brassica is a genus of plants in the *Brassicaceae* family. The genus is known for containing many important agricultural and horticultural crops. Common types of *Brassica* are used as oilseed crops, vegetables, forage crops, dietary fibers and spices (Sirvastava et al., 2010). As of 2012–2013, oilseed rape (*Brassica napus* L.) was the second largest source of oilseed after soybean and third largest source of vegetable oil worldwide (USDA, 2013).

Fungi are the most important pathogens of cultivated plants, causing approximately 20% yield losses worldwide (Evans, 2010). Their phenotypic diversity and genotypic plasticity enable fungi to adapt to new host species and farming systems and to overcome new resistance genes deployed in attempts to limit losses in crop yields (Jestin et al., 2011; Bousset, Chevre, 2013). Blackleg (phoma blackleg, phoma stem canker) is one of the most devastating fungal diseases for *Brassica* species. The disease is caused by *Leptosphaeria maculans*/*L. biglobosa* complex (Mendes-Pereira et al., 2003). *L. maculans* is generally more damaging than *L. biglobosa*, with *L. maculans* more frequently associated with cortical infection near the base of the stem and *L. biglobosa* with upper stem lesions in oilseed rape (Fitt et al., 2006). The pathogens are a serious concern around the world, where oilseed rape is extensively grown. Although not common, seasonal losses of up to 90% have been recorded (Raman et al., 2013)

Growing area of oilseed rape in Lithuania had been constantly increasing from 60.000 ha to 263.000 ha over a period of ten years from 2002 to 2012. Recent studies show a quick spread of the blackleg disease in Lithuania. *Leptosphaeria* spp. ascospore is a primary inoculum causing blackleg leaf spotting, later turning into stem canker disease. The identification of species composition of the airborne spore samples is of prime importance, as *L. maculans* and *L. biglobosa* differ in aggressiveness and sensitivity to fungicides (Kaczmarek et al., 2009). The ascospores of *L. maculans* and *L. biglobosa* are indiscernible phenotypically, therefore molecular tools have been developed and applied (Shoemaker, Brun 2001). Information on the dynamics and species composition of these fungi ascospores in spore samples help to predict disease severity and optimize fungicide timing. Peak ascospore numbers vary between years and regions (Jedryczka et al., 2008), and therefore the information about timing and intensity of ascospore release is necessary to control blackleg epidemics.

A number of *L. maculans* populations originating from oilseed rape have been analysed to date, however there is lack of information on how isolates from other *Brassicas* may be different in terms of race or population specificity (Dilmaghani et al., 2012; Dilmaghani et al., 2013). Furthermore, other *Brassicas* may serve as source of *L. maculans* on oilseed rape (Hall, 1992).

Sexually reproducing species are usually characterized as population of higher genetic diversity in comparison to asexually reproducing species.

Leptosphaeria spp. sexual stage is extremely important in the disease cycle by generating genetic variation and as the source of ascospores, which are the primary inoculum for disease spread (Cozijnsen et al., 2000). One of the main differences between *L. maculans* and *L. biglobosa* species is that *L. maculans* develops gene for gene interaction with *Brassica* host while this interaction was not observed for *L. biglobosa* (Vincenot et al., 2008). Due to the mixed reproductive strategies displayed by *L. maculans*, this phytopathogen has the ability to adapt and overcome novel host resistance (Rouxel, Balesdent, 2005).

Hypothesis

Composition of *L. maculans* and *L. biglobosa* during oilseed rape growing season varies from year to year. Isolates of *L. maculans* and *L. biglobosa* isolated from different species of plants of the genus *Brassica* genetically are closely related. Oilseed rape plants after inoculation with *L. maculans* or *L. biglobosa* express specific genes associated with the pathogenesis.

Research objective

To assess species ratio of *L. maculans* and *L. biglobosa* during oilseed rape growing season and determine the response of oilseed rape plants to *L. maculans* and *L. biglobosa* infection.

Statements to be defended:

1. Depending on meteorological conditions, quantity and species composition of *Leptosphaeria* spp. ascospores in the air varies among different years.
2. *Leptosphaeria maculans* and *L. biglobosa* species are common in Lithuania on winter oilseed rape (*B. napus var. oleifera*), cabbage (*B. oleracea var. capitata*) and broccoli (*B. oleracea var. italica*).
3. Isolates of *L. biglobosa* from cabbage (*B. oleracea var. capitata*) are genetically closer to the *L. biglobosa* isolates from broccoli (*B. oleracea var. italic*) than to the *L. biglobosa* isolates from winter oilseed rape (*B. napus var. oleifera*).
4. Oilseed rape plants express stress-related genes during infection of *L. maculans* and *L. biglobosa*.

Experimental objectives:

1. To investigate seasonal dynamics of *Leptosphaeria* spp. ascospore release and investigate species composition of *L. maculans* and *L. biglobosa* ascospores in spore samples using real time PCR.
2. To assess the occurrence of *L. maculans* and *L. biglobosa* on different *Brassicaceae* plants (*B. napus var. oleifera*, *B. oleracea var. capitata* and *B. oleracea var. italic*).
3. To investigate species diversity of *L. maculans* and *L. biglobosa* in the population using molecular markers.
4. To assess dynamics of blackleg sympthoms after inoculation with *L. maculans* and *L. biglobosa* and identify differentially expressed genes in oilseed rape.

Novelty of the research

The prevalence of *Leptosphaeria* species on *Brassica* plants has been investigated only on oilseed rape in Lithuania. This work showed that *L. maculans* and *L. biglobosa* can cause blackleg not only on winter oilseed rape (*B. napus var. oleifera*), but also on cabbage (*B. oleracea var. capitata*) and broccoli (*B. oleracea var. italica*). Composition of *L. maculans* and *L. biglobosa* during oilseed rape growing season varies from year to year.

Practical application

Information on the dynamics of ascospore release and species composition of *Leptosphaeria* spp. ascospores in spore samples could be used to predict disease severity, to control blackleg epidemics, optimizing the time of spraying fungicides. The knowledge on the occurrence of *Leptosphaeria* spp. on various *Brassica* plants could be used to prevent blackleg in these crops. Differentially expressed genes identified in this study are putative targets for further plant-pathogen interaction studies and molecular breeding.

Approval of the experimental results

Resreach results had been published in 3 scientific articles refereed in ISI WOS and presented at 6 conferences.

Volume and structure of the dissertation

The dissertation is written in Lithuanian. It is composed of an introduction, literature overview, description of materials and methods, experimental results and discussion, conclusions, list of references and list of publications published by the autor in cooperation with co-authors. The dissertation comprises 86 pages, research results are presented in 20 tables and 23 figures, a total of 204 literature references had been cited.

MATERIAL AND METHODS

Investigation of seasonal dynamics of *Leptosphaeria* spp. ascospore release was carried out at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry and at the Institute of Plant Genetics, Polish Academy of Sciences. Genetic diversity of *L. maculans* and *L. biglobosa* isolates and investigation of dynamics of blackleg sympthoms in the greenhouse was carried out at the Laboratory of Genetics and Physiology, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry and at the Institute of Plant Genetics

Spore trapping. Volumetric samplers (Burkard Manufacturing Company Ltd., UK, and Lazoni Ltd., Italy) were used for *Leptosphaeria* spp. ascospore monitoring; both samplers were based on identical mode of action, differing only in design and producer. Spore collection was done in autumn from 1st September until 31st October (2009–2012) in Poland and in autumn from 1st September until 31st October (2010–2011) and in spring-summer from 1st April until 31st August (2011–2012) in Lithuania. The sampling in Poland was done in Krasne, Carpathian Foothills region (50º03′06.5″ N, 22º05′06.5″ E) and

sampling in Lithuania was done in Akademija, Kėdainiai district (55º24′25.32″ N, 23º52′04.06″ E). The spore trap was placed in the centre of a circular area of winter oilseed rape stem debris infected by blackleg (about 800 stems) from the previous growing season. In Lithuania, the spore trap was placed in close proximity (60 m) to the oilseed rape field in the autumn of 2010 and in the centre of a circular area of winter oilseed rape stem debris in the autumn of 2011. Volumetric spore samplers used for monitoring had a power suction of 10 L min^{-1}. Spores were collected using a Vaseline-coated cellophane tape (Burkard Manufacturing Company Ltd.), which was placed on a rotating drum. The tape was collected weekly and cut into seven 48 mm pieces (one piece representing 1 day). Each piece of the tape from Poland was cut in half lengthwise and one half was mounted onto a microscope slide for counting ascospores. Spore counts were done using the whole area of the slide (half-tape), with the use of microscope Axiostar ("Zeiss", Germany) under 200× magnification. The second half of the tape was stored at -20ºC until DNA extraction. Tape from Lithuania was used for counting *Leptosphaeria* spp. ascospores under a microscope Eclipse E600 ("Nikon", Japan) under a 200× magnification. The spores were counted and recorded as daily ascospore number per m^3 of air.

DNA extraction from spore samples. DNA was extracted from the tape fragments using a CTAB protocol, as described by Kaczmarek et al. (2009).

Real-time PCR conditions. For real-time PCR, a 10 µl reaction contained 2.5 µl (1:4 aqueous dilution) of DNA template, 5 µM of each *L. maculans* or *L. biglobosa* species-specific primers (Mahuku et al., 1996), 5 µl of SYBR Green JumpStart Taq ReadyMix, 1.9 µl of nuclease-free sterile water ("Sigma", UK). Cycling parameters were: 95ºC for 2 min, 95ºC for 15 s, 60ºC for 30 s, 72ºC for 45 s, 38 cycles in total. Nuclease-free water was used as no-template control.

Weather conditions. The weather parameters included mean daily temperature, rainfall and air humidity. Meteorological station in Lithuania is located in Akademija, Kėdainiai district approximately 1.5 km distance from the spore trap. In Poland, the weather station is located in Rzeszów-Jesionka airport (50º01′46.5″ N, 22º01′05.5″ E) in the close proximity to Krasne (distance of 7 km). The weather data of the experimental years were compared to the multiannual average. The average was calculated for the period of 14 years (1999–2012).

Fungal isolates. Leaves of blackleg-affected broccoli (*Brassica oleracea var. italica*), cabbage (*Brassica oleracea var. capitata*) and oilseed rape (*Brassica napus var. oleifera*) plants (11 to 14 plants per location) were collected at three sites: Babtai (broccoli) and 2 sites in Akademija (cabbage and oilseed rape) in Lithuania in autumn 2012, the distance between Babtai and Akademija fields was approximately 35 km and between oilseed rape and cabbage collection sites in Akademija it was about 1.5 km. One lesion per leaf per plant was taken for pycnidium isolation with two pycnidia from one lesion

retained for further molecular analysis. Single pycnidiospore isolates were derived using Sosnowski et al. (2001) method.

DNA extraction and specific PCRs. Genomic DNA was extracted from fungal mycelia grown at 20ºC in V8 liquid medium for 4 days as described earlier (Brazauskiene et al., 2011). Species-specific primers were used for *L. maculans* and *L. biglobosa* identification (Liu et al., 2006). Mating types for *L. maculans* were determined by PCR with mating type-specific primers (Cozijnsen, Howlett, 2003).

AFLP analysis. AFLP analysis was performed as in Bachem et al. (1998) with minor modifications. Selective amplification reaction was carried out in a total volume of 15 µl, containing 7.5 µl of 20× diluted pre-amplification product, 0.5 µM of *AseI* primer, 0.03 µM *TaqI* primer, 1× DreamTaq buffer (with 20 mM $MgCl_2$), 0.2 mM dNTPs, 0.2 U DreamTaq polymerase. PCR reactions were performed with the following profile of 12 cycles of 30 s at 94ºC, 30 s at 65ºC (with a 0.7ºC reduction per cycle), 60 s at 72ºC and 24 cycles of 30 s at 94ºC, 30 s at 56ºC, 60 s at 72ºC.

For fragment detection 0.2 µl of amplification product was mixed with 0.8 µl Blue Stop Solution (Li-Cor Biosciences, USA), denatured at 95ºC for 5 min and then electrophoresed on a 6.5% denaturing polyacrylamide gel using Li-Cor 4300 DNA Analyzer (Li-Cor Biosciences, USA). Amplified bands were analyzed with the software Saga Generation 2 (Li-Cor Biosciences, USA) by comparing with the size standart IR Dye ® 800 (Li-Cor Biosciences, USA). AFLP procedure was applied at least twice for all samples and only reproducible bands were scored.

Plant growth. For analysis of blackleg symptoms after inoculation was investigated in two commercial cultivars of winter oilseed rape 'SunDay' (Lantmännen SW Seed, Sweeden) and 'DK Secure' (Monsanto, France) were used. Plants were grown in a glasshouse (+18ºC, 16 h photoperiod) and were inoculated with *L. maculans* or *L. biglobosa* pycnidiospore when they reached growth stage 1.2 (Sylvester-Bradley, Makapeace, 1985).

For identification of differentially expressed genes in oilseed rape after inoculation with *L. maculans* or *L. biglobosa* two genotypes of oilseed rape were used '02-22-2-1' (lacking Rlm5) and 'Westar' (no known resistance genes) (INRA, France). Seeds were sown in pots containing peat substrate. Plants were grown in a greenhouse (+18ºC, 16 h photoperiod) and were inoculated when they reached growth stage 1.2 (Sylvester-Bradley, Makapeace, 1985).

Preparation of pycnidiospore suspension. A pycnidiospore suspensions of *L. maculans* and *L. biglobosa* were prepared from a 21-day-old culture of isolates KED09–23 (*L. maculans*) and VA09–3 (*L. biglobosa*), obtained from single ascocpores from naturally infected winter oilseed rape stem base debris collected in 2010. Isolates were transfered on 1.5% V8 agar and incubated at room temperature for 7 days. Then incubated at 20ºC for 14 days, 12 h light/12

h dark. Distilled water was added to suspend the pycnidiospores. The concentration of the pycnidiospores suspension was adjusted to 10^6 pycnidiospore per ml.

Plant inoculation. For investigation of blackleg symptom dynamics after inoculation, two methods of inoculation were used: (1) spraying of the whole plant with pycnidiospore suspension; (2) point inoculation with a 10 µl drop of pycnidiospore suspension on a site that had been wounded using a sterile pin. Six plants of each cultivar were inoculated with *L. maculans* pycnidiospore suspension by both methods (three plants inoculated using the first (1) method and three plants inoculated using the second (2) method) and six plants of each genotype were inoculated with *L. biglobosa*. Six plants of each cultivar were inoculated with sterile water representing a control group. For point inoculation only the first and the second leaves of each plant were inoculated; 12 wounded sites on each plant were inoculated. After inoculation, plants were sprayed with water and covered with polyethylene bags to maintain wetness of the leaf surface for 72 h. The incubation period (from inoculation to the appearance of first blackleg leaf spots) for each treatment was recorded. Four blackleg leaf spots from each plant inoculated with point inoculation were measured after 15, 20, 25 and 30 days post inoculation.

For identification of differentially expressed genes in oilseed rape under infection, point inoculation was used. Nine plants of each genotype were inoculated with *L. maculans* pycnidiospore suspension, nine plants were inoculated with *L. biglobosa* pycnidiospore suspension, and nine plants were treated with sterile water (control). After inoculation, plants were covered with polyethylene to maintain leaf surface wetness for 72 h. Leaf samples were taken at 3, 5, and 7 days after inoculation. Leaves from three plants of each group (3 biological replicates) inoculated with different species of fungus were harvested at each time point. Samples were immediately frozen in liquid nitrogen and stored at -80ºC until RNA isolation.

RNA isolation and cDNA synthesis. Total RNA was isolated with the Rneasy® Plant Mini Kit (QIAGEN, USA). Poly(A)$^+$RNA from total RNA was further purified with the Dynabeads® mRNA Purification Kit (Invitrogen, Netherlands). Poly(A)$^+$RNA was reverse transcribed with an anchored oligo dT18 primer using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher, Lithuania). 20 µl of reverse transcription reaction product were then immediately used in a second strand cDNA synthesis reaction. The double-stranded cDNA was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and quantified by running 6 µl of cDNA on the agarose gel.

cDNA-AFLP analysis. The cDNA-AFLP procedure was conducted as described above. The selective PCR products and size standard GeneRuler Low Range DNA Ladder (Thermo Fisher, Lithuania) were mixed with 2X RNA Loading Dye (Thermo Fisher, Lithuania), denatured at 95ºC for 5 min, then

electrophoresed on a 15% denaturing polyacrylamide gel and stained by silver nitrate.

Identification and cloning of DEF. The reproducibility of differentially expressed fragments (DEF) was tested by repeating the PCR amplification 2 times. Only repeatable bands were included it this study. DEFs were excised from polyacrylamide gel with the sterile needle, purified from polyacrylamide using elution buffer and incubated at 37ºC overnight. The gel pieces were pelleted by centrifugation. The supernantant was placed in a sterile 2 ml tube containing 5 M NaCl and 1 ml 96% ethanol, then centrifuged and supenatant was poured away. DNA pellets were washed with 70% ethanol, dried, and dissolved in TE buffer. DNA fragments were ligated into pJET1.2/blunt vector using the CloneJET™ PCR Cloning Kit (Thermo Fisher, Lithuania) according to the manufacturer instructions. Plasmid was cloned to *E. coli* strain XL1–Blue. Plasmid DNA was isolated using GeneJET Plasmid Miniprep Kit (Thermo Fisher, Lithuania) and sequenced at GATC Biotech (European Genome and Diagnostic Centre, Germany). DNA sequences were revised using ChromasPro 1.5 software (Technelysium Pty Ltd, Australia). The sequences were blasted against the non redundant protein database of NCBI with BLASTX.

Data analysis. The correlation between the number of ascospores on tape and the amount of DNA was calculated using *STATISTICA* version 7.0 for *Windows* (StatSoft Inc, Tulsa, USA). As ascospores of *L. maculans* and *L. biglobosa* are indiscernible under microscope, a total amount of *Leptosphaeria* spp. DNA detected was used to calculate the coefficients of correlation.

The observed mating type ratio was tested with a χ^2 significance test, which tests the probability that the deviation from the expected ratio is due to chance alone.

AFLP profiles were scored for the presence (1) or absence (0) of bands in the range of 50-450 bp. A cluster analysis was carried out using a distance matrix based on the Jaccard's genetic distance (Jaccard, 1908) and the unweighted pair group method with arithmetic average (UPGMA). The distance between populations was calculated using Cavalli-Sforza & Edwards chord distance (1967). The analysis of molecular variance (AMOVA) was applied to partition the total genetic variance within and among the populations. All these calculations were performed using Fingerprint Analysis with Missing Data (FAMD) software version 1.3 (Schluter and Harris, 2006).

RESULTS AND DISCUSSION

***Leptosphaeria* spp. ascospore release in autumn.** Seasonal dispersal of ascospores in Lithuania in 2010 and 2011 differed greatly. In 2010, ascospores were released during the first days of September, preceded by the heavy rain on last days of August. In the second half of August there were 7 rainy days (cumulative rainfall of 32.6 mm). The highest peak of spore release was recorded on 9th September (13 ascospores m^{-3} of air). In October just a few days

of ascospore release were detected. First half of October in 2010 was dry and no ascospores were detected on microscopic slides. In 2011 the first discharge of ascospores was detected on the 12th of September. The maximum number of ascospores (34 ascospores m^{-3} of air) was trapped on the 24th of October. In 2011, more ascospores were trapped in days with high air humidity (>90%), however, in September 2010 ascospores were found on nine consecutive days and during these days air humidity was not very high (70–79%), but air temperature was below 15ºC. Lower autumn temperatures in 2010 reduced ascospore release from pseudothecia. Pseudothecia development and maturation on stem residues require higher rainfall and higher air temperatures. Drier and cooler conditions retard pseudothecia maturation process (Kaczmarek et al., 2010). It is worth mentioning that both in Poland and Lithuania the pattern of rainfall events was similar, and fluctuations in ascospore release were alike. Much higher rainfall in Lithuania in the autumn of 2011 resulted in much higher ascospore release as compared to the same period in Poland (Fig. 1b and 2c).

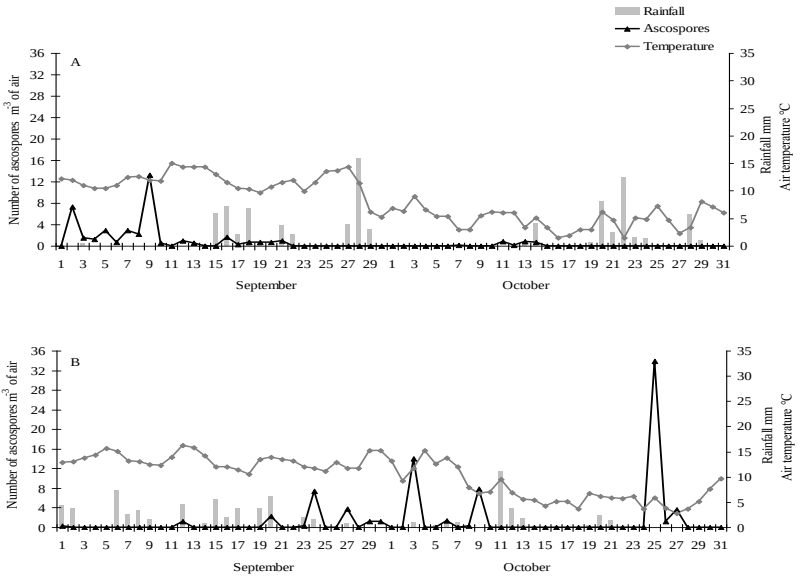

Figure 1. Seasonal dispersal of *Leptosphaeria* spp. ascospores and main meteorological data over September–October in 2010 (A) and 2011 (B) in Akademija, Lithuania.

In Krasne (Poland) the numbers of spores and their fluctuations were different in each autumn season. The majority of spores were released in October. The highest peak of ascospores was detected in 2009 on the 28th of October (41 ascospore m^{-3} of air) and in 2012 on the 5th of October (35 ascospores m^{-3} of air) (Fig. 2).

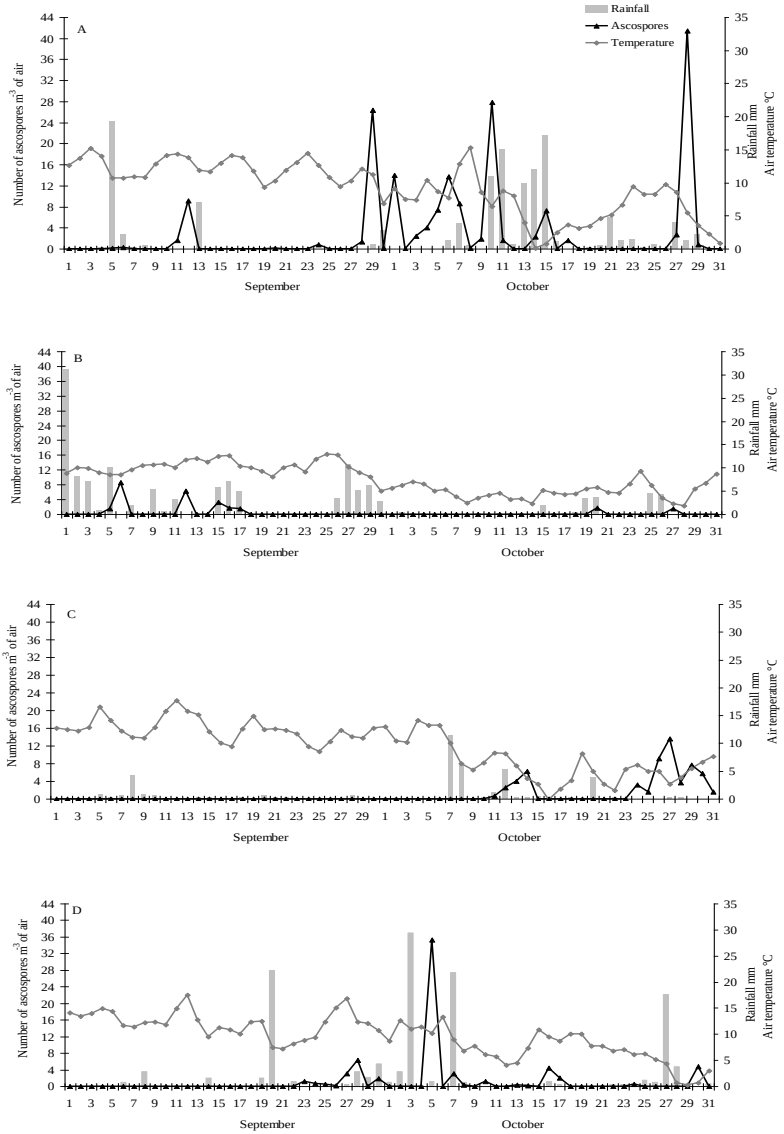

Figure 2. Seasonal dispersal of *Leptosphaeria* spp. ascospores and main meteorological data over September–October in 2009 (A), 2010 (B), 2011 (C) and 2012 (D) in Krasne, Poland.

In 2009, the first ascospores in the spore samples were detected on the 12th of September. In this year four peaks with 14 ascospores m^{-3} of air were detected, most of the ascospores were released at the end of September and

during first half of October. Lower temperatures and rainy days could influence ascospore release from pseudothecia during this period.

In mid-September ascospores were not observed and this coincided with no rainfall during this period. In 2010, the first *Leptosphaeria* spp. ascospores were detected on the 5th of September. During this season the lowest number of ascospores was detected (maximum 9 ascospores m^{-3} of air), as compared to the other three seasons. Unlike 2010, September of 2011 was warmer (15.8ºC) and much drier (9.4 mm of rainfall) month and no ascospores were detected in the samples. This period was most likely un-favourable for pseudothecia maturation on oilseed rape stem debris from the previous year. In 2011, first ascospores were detected only in mid-October after rainfall (Fig. 2). Air temperature, soil moisture and air humidity all contribute to the maturation of pseudothecia of both *L. maculans* and *L. biglobosa* (West et al., 2002 a). Wetness provided by the rainfall is essential for ascospore release during this period (Huang et al., 2005). In 2012 the first half of September was warm and relatively dry, consequently the first *Leptosphaeria* spp. ascospores were detected at the end of September. In Krasne (Poland) September is usually characterized by a relatively high air temperature, probably too high for pseudothecia maturation. Dawidziuk et al. (2010) noted that the amounts of ascospores in the eastern part of Poland are low compared to regions located in the western part of the country.

Our results show that time of ascospore release differed between studied seasons. In 2009 and 2010 in Krasne, the first ascospores were found on the first days of September like in Akademija in 2010 and 2011. In 2011 and 2012, the first ascospores were detected later, i.e. at the end of September (2012) or in mid-October (2011). In Krasne, September months were drier than Octobers (except 2010) during experimental period and in Lithuania, on the contrary, October months were drier than September months. Autumn season's mean air temperature in Akademija was from 0.6ºC to 2.2ºC lower than in Krasne.

***Leptosphaeria* spp. ascospore release during seasons of spring-summer in Akademija.** In 2011 in Akademija first ascospores were detected at the end of April. In May and June the number of ascospores wasn't very high. The first higher peak of ascospores amount was detected on the 16th of July (32 ascospores m^{-3} of air). The maximum number of ascospores (42 ascospores m^{-3} of air) was trapped on the 1st of August. In 2011 six peaks with 15 ascospores m^{-3} of air were detected, largest amount of ascospores was released in August (Fig. 3).

In 2012 first *Leptosphaeria* spp. ascospores were detected at the end of April like in 2011. The first higher peak of the number of ascospores was detected on the 14th of May (76 ascospores m^{-3} of air). During June there were 18 days when ascospores were observed. The maximum number of ascospores (100 ascospores m^{-3} of air) was trapped on the 8th of July, also on that day heavy rain (41.3 mm) was recorded. During August, the ascospores were found in the spore

samples on the first half of the month. In 2011 and in 2012 the second half of April was favourable for the ascospore dispersal, when the air temperature was higher than 5ºC. The ascospore dispersal was more intense and abundant in spring-summer season in 2012 than in 2011. It seem that lower air temperature and higher rainfall were favourable for *Leptosphaeria* spp. pseudothecia maturation in 2012.

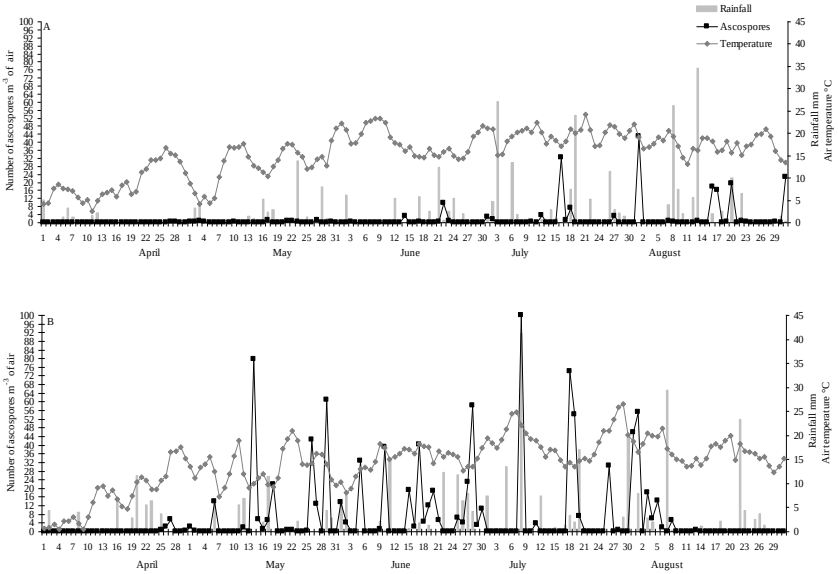

Figure 3. Seasonal dispersal of *Leptosphaeria* spp. ascospores and main meteorological data over April – August in 2011 (A), 2012 (B) in Akademija, Lithuania.

Species composition in spore samples from the air in Krasne. The detection and quantification of *L. maculans* and *L. biglobosa* species from cellophane tape (samples from Poland) was performed using a quantitative real-time PCR method. There was significant positive correlation detected between ascospore counts and quantities of DNA of *Leptosphaeria* spp. (Table 1). The coefficient of correlation shows the relation between the number of ascospores on the tape and the amount of DNA of *Leptosphaeria* spp. The strongest correlation was in 2009, when ascospores were detected in samples of more than 20 days by both methods. In the samples originating from 2009 both species were detected. The first ascospores detected in 2009 belonged to the species of *L. biglobosa*. There were more days with *L. biglobosa* DNA (22 days) than with *L. maculans* (9 days). The detection of *L. maculans* DNA always coincided with the detection of *L. biglobosa*. The amounts of *L. biglobosa* DNA ranged from 0.05 to 5.5 pg (picograms) and *L. maculans* – from 0.4 to 41.0 pg per sample (day). In spore samples, *L. biglobosa* was more frequently detected but lower

DNA amounts were found than in the case of *L. maculans*. In total, the amount of *L. maculans* DNA was 7 times higher than that of *L. biglobosa*. It means that release of *L. biglobosa* ascospores was more evenly distributed over the whole period of monitoring and the release of *L. maculans* ascospores was more weather-driven and sudden, resulting in higher numbers of spores at a given time. During other three seasons of 2010–2012 only *L. biglobosa* was detected in the spore samples from the air, with no traces of *L. maculans* (Table 1).

Table 1. Seasonal differences of *Leptosphaeria* spp. ascospore release in the autumn detected by microscope and real-time polymerase chain reaction (PCR) in Krasne, Poland.

Year	2009	2010	2011	2012
Date of the first ascospore detection by microscope	5 September	5 September	11 October	23 September
Date of the first *L. maculans* DNA detection by real-time PCR	12 September	n.d.	n.d.	n.d.
Date of the first *L. biglobosa* DNA detection by real-time PCR	5 September	5 September	15 October	9 September
Number of days of ascospore detection by microscope	25	8	12	16
Number of days of *Leptosphaeria* spp. DNA detection by real-time PCR	22	9	8	6
Date of the detection of the highest number of ascospores by microscope	28 October	6 Septmber	27 October	5 October
Date of the highest number of *Leptosphaeria* spp. DNA detected by real-time PCR	28 October	9 September	26 October	16 October
Correlation coefficients between the number of ascospores and the amount of DNA of *Leptosphaeria* spp.	0.828*	0.632*	0.483*	0.550*

n.d. – not detected, * $p < 0.05$

During this study, the dominating species in the spore samples was *L. biglobosa*. In 2010–2012, *L. biglobosa* was the only species detected in the spore samples. The finding is in agreement with the study of Jedryczka et al. (2008), who found more isolates of *L. maculans* in the western part of Poland and more isolates of *L. biglobosa* in the eastern part of Poland, where Krasne is located.

Species composition on Brassica plants. For species identification 105 isolates were used. The ratio of *Leptosphaeria* species between isolates from cabbage (*Brassica oleracea var. capitata*), broccoli (*Brassica oleracea var. italica*) and oilseed rape (*Brassica napus var. oleifera*) was variable. All isolates from *Brassica oleracea var. capitata* (40 isolates) were identified as *L. biglobosa*, while both species were detected on *Brassica oleracea var. italica* and *Brassica napus var. oleifera*. In total, 40 isolates of 46 were identified as *L.*

maculans on *Brassica oleracea var. italica* and 7 isolates of 19 were identified as *L. biglobosa* on *Brassica napus var. oleifera*. *L. maculans* was dominant species in both populations. There is evidence that both species coexist on broccoli (Moreno Rico et al., 2001) and on cabbage in Mexico (Dilmaghani et al., 2010).

Mating type distribution. Mating types were identified for *L. maculans* populations as PCR products corresponding to one of the two mating type idiomorphs, MAT1-1 or MAT1-2. In the *L. maculans* population from *Brassica oleracea var. italica* the ratio between mating types was close to 1 as well as in the *L. maculans* population from *Brassica napus var. oleifera*. Mating type ratio was tested with χ^2 significance test, and the data showed no significant ($p < 0.05$) deviation between the observed and expected 1:1 mating type ratio. In some cases both mating types were identified in isolates from the same leaf lesion. Three isolates from *Brassica oleracea var. italica* (Br-59, Br-93, Br-95) failed to amplify any PCR fragment with mating-type-specific primers, despite the fact that species-specific primers produced a clear 331 bp fragment specific for *L. maculans* isolates. Dilmaghani et al. (2013) indicate that sexual stage is seldom exploited by the pathogen and is believed to play little or no part in the blackleg disease cycle on *B. oleracea*. *L. maculans* isolated from cabbage in Mexico had disproportionate distribution of mating types within population. Lack of sexual reproduction leads to a very high clonal fraction and low gene diversity in fungal populations (Dilmaghani et al., 2013).

AFLP analysis. AFLP is sensitive and useful technique for *L. maculans* and *L. biglobosa* genetic analysis (Purwantara et al., 2000; Barrins et al., 2004). Three primer combinations used in the AFLP analyses yielded 283 fragments in total. The number of amplified fragments ranged from 78 (IR800 + AG/T + AA primer combination) to 110 (IR800 + AC/T + A primer combination). The average number of fragments per isolate was 38.2 within the size range of 52 to 525 bp. 277 fragments out of 283 were polymorphic among isolates. Overall 51 (18.0%) fragment was determined as being specific to the *L. maculans* isolates from *Brassica oleracea var. italica* and 60 (21.2%) fragments specific to *L. maculans* isolates from *Brassica napus var. oleifera* population. Furthermore, 22 (7.7%) fragments were unique to the *L. biglobosa* isolates from *Brassica oleracea var. capitata*, 8 (2.8%) fragments unique to isolates from *Brassica oleracea var. italica* and 38 (13.4%) fragments specific to isolates from *Brassica napus var. oleifera* population. Two identical AFLP patterns were found among isolates from *Brassica napus var. oleifera*; Ca-36 and Ca-37. These isolates were obtained from the same leaf lesion, but from different pycnidia. All other isolates had unique AFLP patterns.

Leptosphaeria spp. isolates were clustered using the UPGMA method. There was a clear differentiation among *L. maculans* and *L. biglobosa* isolates. The dendrogram disclosed two particular groups (Fig. 4).

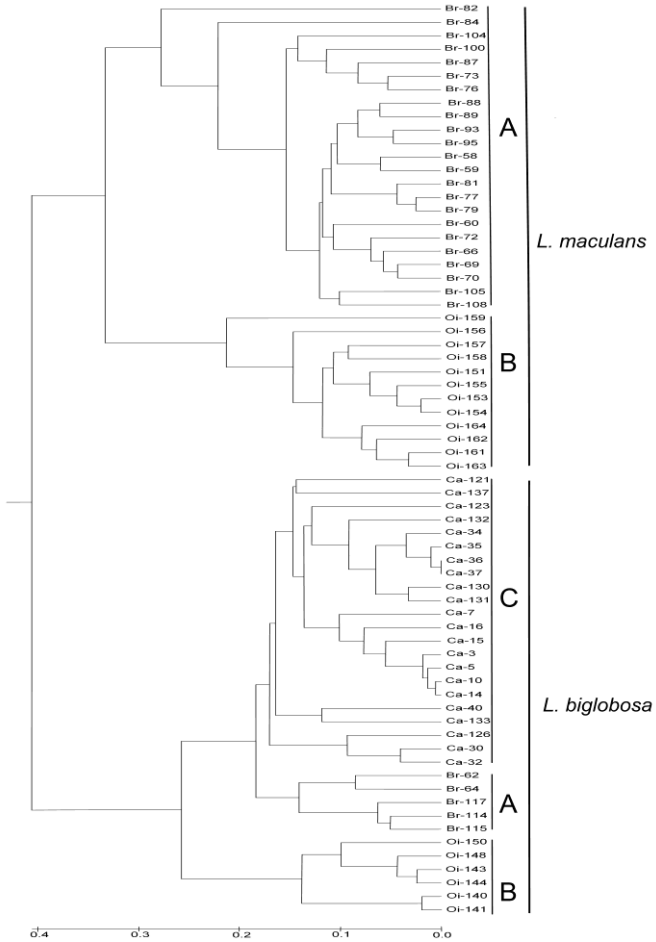

Figure 4. UPGMA dendrogram from AFLP data for 68 isolates of *Leptosphaeria spp.* amplified with 3 primer pairs. UPGMA cluster analysis was based on Jaccard genetic distance, A – isolates from broccoli, B – isolates from oilseed rape, C – isolates from cabbage.

The first group consisted of the isolates identified as *L. maculans*. All these isolates were divided into 2 subgroups. The first subgroup had isolates from *Brassica oleracea var. italica* and the second subgroup clustered isolates from *Brassica napus var. oleifera*. The second group partitioned *L. biglobosa* isolates. This group was divided into 3 subgroups. These subgroups partitioned isolates from, from *Brassica oleracea var. capitata*, *Brassica oleracea var. italica* and *Brassica napus var. oleifera* respectively. There was no obvious grouping in cluster analyses of *L. maculans* isolates according to mating types.

The analysis has also revealed that isolates of *L. biglobosa* from *Brassica oleracea var. capitata* were genetically closer to the *L. biglobosa* isolates from *Brassica oleracea var. italica* (genetic distance = 0.09) than to the *L. biglobosa* isolates from *Brassica napus var. oleifera* (genetic distance = 0.17).

Isolates of *L. biglobosa* from different populations were much closer genetically compared to the genetic distances among isolates of *L. maculans* from different populations. Genetic distance between *L. maculans* from *Brassica oleracea var. italica* and *L. maculans* from *Brassica napus var. oleifera* was 0.23. Furthermore, isolates of *L. maculans* from oilseed rape were slightly closer to *L. biglobosa* isolates from *Brassica oleracea var. italica* (genetic distance = 0.38) than to *L. biglobosa* from *Brassica napus var. oleifera* (genetic distance = 0.40). AMOVA results showed that greater amount of variance of *L. maculans* was distributed within populations, however the distance between two fields was short (about 35 km) and 42.1% of total variance was distributed among populations (Table 2).

Table 2. Analysis of molecular variance (AMOVA) for two *L. maculans* populations

Source of variation	df	SSD	Precentage of variation (%)	Φ_{ST}	p^*
Among populations	1	1.016	42.08	0.42	0.000
Within populations	66	6.903	57.91		
Total	67	7.920			

df: degree of freedom; SSD: sum of squared deviations; $^*p$: Probability of obtaining value estimated from 1000 randomisations.

Similar results were published in Canada, where just 54.5% of total variance was distributed within field populations (Mahuku et al., 1997). Other studies showed that almost all of the diversity is found within field populations in France (Gout et al., 2006) and in Australia (Barrnis et al., 2004). One of the reasons why we have found large proportion of this variance between fields in our study is that that the source of sexual spores was different for each field. What is less clear is what caused high genetic diversity of *L. biglobosa* between populations (73.3%) (Table 3).

Table 3. Analysis of molecular variance (AMOVA) for three *L. biglobosa* populations.

Source of variation	df	SSD	Precentage of variation (%)	Φ_{ST}	p^*
Among populations	2	2.575	73.3	0.73	0.000
Within populations	65	5.345	26.6		
Total	67	7.920			

df: degree of freedom; SSD: sum of squared deviations; $^*p$: Probability of obtaining value estimated from 1000 randomisations.

It might show that sexual reproduction is very rare and fungus seemingly spreads only within the field. A limited sample size of isolates used in this study, however, could also have an effect on the genetic diversity estimation between isolates.

cDNA-AFLP analysis. cDNA-AFLP analysis was performed using 176 primer pair combinations on inoculated and control plant samples and revealed 10,988 fragments with an average of 62 fragments per primer pair. The size of the fragments ranged from 15 bp to 700 bp. 11 fragments were identified as being differentially expressed (DEFs). The expression pattern included 10 up-regulated and 1 down-regulated DEFs. All 11 DEFs were extracted from polyacrylamide gels, cloned and sequenced. A total of seven DEFs revealed significant (E value < $1e^{-10}$) sequence similarities in a BLASTX search against the non-redundant (nr) protein database of GenBank (Table 4). Four sequences showed homology to *A. thaliana*, two sequences showed homology to *B. rapa* and one sequence showed homology to *B. distachyon*. Identified genes can be classified in three groups: genes involved in stress response, genes involved in photosynthesis control and genes acting in regulatory processes.

Table 4. Functional annotation of the 7 TDFs revealing significant (E < $1e^{-10}$) sequence similarities in BLASTX.

Number	Homologue description	Function	Organism
1	UMP synthase	Catalyzing the final 2 steps of *de novo* pyrimidine biosynthesis	*Arabidopsis thaliana*
2	Chlorophyll a-b binding protein 2/3 (CAB2)	Binds chlorophyll molecules	*Arabidopsis thaliana*
3	Nitrate reductase	Catalyzes NAD(P)H reduction of nitrate to nitrite	*Brassica rapa*
4	Protein N-MYC downregulated-like 2 (NDL2)	Act in a signaling pathway that modulates root auxin transport	*Arabidopsis thaliana*
5	Eukaryotic translation initiation factor 4 gamma 2-like	Involved in translation process	*Brachypodium distachyon*
6	Uncharacterized protein (CEST)	Stress-response protein for abiotic stresses	*Arabidopsis thaliana*
7	Avirulence-responsive protein-related protein	Stress-response protein for biotic stresses	*Brassica rapa*

Nitrate reductase (NR) is one of the NO-producing enzymes in plants (Desikan ir kt., 2002). NO was hypothesized to take part in the plant defensive response, but there is no direct evidence. However, Yamamoto et al. (2003) showed that expression of the *NR* gene was induced in potato tubers inoculated with an incompatible race of *P. infestans*. Subramanian et al. (2005) conducted a comparative study with two species *B. carinata* (resistant for blackleg) and *B. napus* (sensitive for blackleg). Resistant phenotype, after inoculation (48 hours) with *L. maculans*, accumulated 28 different proteins including NR.

CONCLUSIONS

1. *Leptosphaeria* spp. ascospore release from pseudothecia depend on the weather conditions, mainly rainfall, air temperature and relative air humidity. On days without rain, spores were present in spore samples

when average relative air humidity exceeded 90% and day temperature was below 15ºC.

2. In Krasne, as in other parts of Eastern Poland *L. biglobosa* is dominant species. *L. maculans* species was detected in spore samples only in 2009, *L. biglobosa* species was found throughout the whole period of 2009–2012.
3. In autumn season fungi belonging to the genus *Leptosphaeria* spp. are found in different proportions on winter oilseed rape (*Brassica napus var. oleifera*), cabbage (*Brassica oleracea var. capitata*) and broccoli (*Brassica oleracea var. italica*) in Lithuania. Both species were detected on winter oilseed rape (*B. napus var. oleifera*) and broccoli (*B. oleracea var. italica*) with *L. maculans* prevalence, while only *L. biglobosa* was identified on cabbage (*B. oleracea var. capitata*).
4. The sexual reproduction cycle of *Leptosphaeria maculans* dominates in Lithuania. Both mating types were detected and their distribution was equal.
5. Isolates of *L. maculans* and *L. biglobosa* differ from each other. Isolates of *L. biglobosa* from cabbage (*Brassica oleracea var. capitata*) are genetically closer to the *L. biglobosa* isolates from broccoli (*Brassica oleracea var. italica*) than to the *L. biglobosa* isolates from winter oilseed rape (*Brassica napus var. oleifera*).
6. Blackleg symptoms caused by *L. maculans* and *L. biglobosa* on winter oilseed rape (*B. napus var. oleifera*) leaf develop at different rate.
7. Oilseed rape plants express stress-related genes during *L. maculans* and *L. biglobosa* infection. Eleven differentially expressed fragments were identified using cDNA-AFLP method. Homologies for 7 genes were obtained with three genes products being involved in stress response.

LIST OF PUBLICATIONS

Articles in journals indexed in the ISI WOS database:

1. Brazauskienė I., Piliponytė A., Petraitienė E., Brazauskas G. 2011. Diversity of *L. maculans*/*L. biglobosa* species complex and epidemiology of phoma stem canker on oilseed rape in Lithuania. Journal of Plant Pathology, 93 (3): 577–585

2. Brazauskienė I., Petraitienė E., Piliponytė A., Brazauskas G. 2012. The peculiarities of phoma stem cancer (*Leptosphaeria maculans*/*L. biglobosa* complex) infections in winter and spring oilseed rape (*Brassica napus* L.). Zemdirbyste=Agriculture, 99 (4): 379–386.

3. Piliponytė-Dzikienė A., Kaczmarek J., Petraitienė E., Kasprzyk I., Brazauskienė I., Brazauskas G. Jędryczka M. 2014. Microscopic and molecular detection of *Leptosphaeria maculans* and *L. biglobosa*

ascospore content in air samples. Zemdirbyste=Agriculture vol 101 (3): 303–312

Abstracts in conference proceedings:

1. Piliponytė A., Brazauskienė I., Petraitienė E., Brazauskas G. Dynamics of ascospore dispersal and pathogenicity of *L. maculans* and *L. biglobosa* on winter oilseed rape. Plant biotechnology advances in agriculture: 22 psl. Kaunas, 2011 spalio 27–28, Lietuva.

2. Piliponytė A., Brazauskienė I., Petraitienė E., Brazauskas G. Genetic diversity of *Leptosphaeria maculans* and *L. biglobosa* of phoma stem canker on oilseed rape in Lithuania. 8 colloque de laSociété Française de Phytopathologie, 87 psl., Paris 5–8 June, 2012, France.

3. Piliponytė-Dzikienė A., Andriūnaitė E., Brazauskienė I., Petraitienė E., Brazauskas G. Identification of differentially expressed genes in oilseed rape – *Leptosphaeria* spp. interaction. 5th Baltic Congress of Genetics, 50–51 psl., Kaunas 2012 spalio 19–22, Lietuva.

4. Jędryczka M., Kaczmarek J., Piliponytė A., Irykowski W., Kasprzyk I. The use of molecular aerobiology methods for the detections of spores of plant pathogens and human allergens. Microorganisms – plant – environment under changing climate, 35 psl., Pulawy–Lublin, 12–15 May, 2013, Poland.

5. Piliponytė-Dzikienė A., Kaczmarek J., Petraitienė E., Brazauskienė I, Jędryczka M., Brazauskas G. *Leptosphaeria* spp. askosporų analizė oro mėginiuose. Doktorantų stažuotės užsienio mokslo centruose 2012–2013: 51–52 psl. Vilnius, 2013 spalio 11, Lietuva.

6. Piliponytė-Dzikienė A., Andriūnaitė E., Petraitienė E., Brazauskienė I, Brazauskas G. Fitopatogeninių grybų *Leptosphaeria maculans* ir *L. biglobosa* rūšių genetinė įvairovė ir paplitimas bastutiniuose augaluose Lietuvoje. Jaunieji mokslininkai - žemės ūkio pažangai: 87–88 psl. Vilnius, 2013 lapkričio 21, Lietuva.

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I am grateful to my husband Paulius, parents and friends for patience and support during my PhD studies.

Short profile of the PhD student

Agnė Piliponytė-Dzikienė was born in Kaišiadorys on the 1st of January, 1986. In 2004 she finished Kaišiadorys Vaclovas Giržadas school and the same year entered Vytautas Magnus University, Faculty of Natural Sciences. In 2008 she finished bachelor studies and received a bachelor degree in biology. In 2008 Agnė Piliponytė-Dzikienė started master studies in biology in Vytautas Magnus University. In 2010 she finished master studies and started her PhD studies at the Lithuanian Research Centre for Agriculture and Forestry.

REZIUMĖ

Disertacija rengta 2010–2014 metais Lietuvos agrarinių ir miškų mokslų centro filiale Žemdirbystės institute.

Hipotezė. Patogeninių grybų *L. maculans* ir *L. biglobosa*, sukeliančių rapsų fomozę, santykis atskirais metais bei rapsų vegetacijos metu kinta, o *L. maculans* ir *L. biglobosa* izoliatai, išskirti iš skirtingų bastutinių šeimos augalų, genetiškai yra labai artimi. Po *L. maculans* ar *L. biglobosa* infekcijos rapsų augalai ekspresuoja specifinius su patogeneze susijusius genus.

Tyrimų tikslas: nustatyti *L. maculans* ir *L. biglobosa* rūšių santykį rapsų vegetacijos metu, bei identifikuoti diferenciškai ekspresuojamus rapsų augalų genus *L. maculans* ir *L. biglobosa* infekcijos metu.

Tyrimų uždaviniai:

1. Ištirti *Leptosphaeria* spp. aukšliasporių plitimo dėsningumus bei *L. maculans* ir *L. biglobosa* rūšių santykį grybo populiacijoje rapsų vegetacijos metu iš aukšliasporių panaudojant tikrojo laiko PGR su rūšims specifiniais pradmenimis.
2. Nustatyti *L. maculans* ir *L. biglobosa* rūšių paplitimą ant įvairių bastutinių šeimos augalų rūšių (*Brassica napus var. oleifera, B. oleracea var. capitata, B. oleracea var. italica*).
3. Molekulinių žymeklių pagalba įvertinti *L. maculans* ir *L. biglobosa* rūšių skirtingumą ir izoliatų giminingumą rūšies viduje.
4. Ištirti fomozės požymių dinamiką po rapsų augalų užkrėtimo *L. maculans* ir *L. biglobosa* piknosporomis (dirbtinio užkrėtimo sąlygomis šiltnamyje), bei identifikuoti diferenciškai ekspresuojamus rapsų augalų genus *L. maculans* ir *L. biglobosa* grybų piknosporomis užkrėstuose augaluose.

Disertacijos ginamieji teiginiai:

1. *Leptosphaeria* spp. aukšliasporių kiekis bei rūšių santykis atskirais metais kinta priklausomai nuo meteorologinių sąlygų.
2. Lietuvoje fomozę sukeliančios *L. maculans* ir *L. biglobosa* rūšys paplitusios ant žieminio rapso (*B. napus var. oleifera*), baltagūžio kopūsto (*B. oleracea var. capitata*), brokolio (*B. oleracea var. italica*).
3. Fitopatogeno *L. biglobosa* izoliatai išskirti nuo brokolio (*B. oleracea var. italica*) yra genetiškai artimesni izoliatams išskirtiems nuo baltagūžio kopūsto (*B. oleracea var. capitata*) nei izoliatams išskirtiems nuo žieminio rapso (*B. napus var. oleifera*).
4. Po *L. maculans* ar *L. biglobosa* infekcijos rapsų augalai ekspresuoja su specifinius genus.

Mokslinio darbo naujumas

Lietuvoje *Leptosphaeria* spp. paplitimas bastutinių (*Brassicacea*) šeimos augaluose buvo tyrinėtas tik rapsų pasėliuose, nebuvo žinomas šių rūšių santykis kituose bastutinių šeimos augaluose. Šiame darbe nustatyta, kad Lietuvoje fitopatogeniniai grybai *L. maculans* ir *L. biglobosa* gali sukelti fomozę ne tik ant žieminio rapso (*B. napus var. oleifera*), bet ir ant baltagūžio kopūsto (*B. oleracea var. capitata*) bei brokolio (*B. oleracea var. italica*). Užkrėstuose *L. maculans* ir *L. biglobosa* rapsų augaluose identifikuoti trys nauji genai, kurie dalyvauja streso atsako procesuose. Nustatyta, kad *L. maculans* ir *L. biglobosa* rūšių santykis atskirais metais bei rapsų vegetacijos metu kinta.

Praktinis pritaikymas

Leptosphaeria spp. aukšliasporių monitoringo metu surinkta informacija gali būti panaudota fomozės epidemijos prognozei ir kontrolei, optimizuojant fungicidų purškimo laiką. *Leptosphaeria* spp. rūšių paplitimo įvairiuose bastutiniuose augaluose gauti rezultatai gali būti naudojami fomozės prevencijai šių augalų pasėliuose. Gauti rezultatai, gali būti pritaikomi tolimesniuose augalo ir patogeno sąveikos tyrimuose bei nustatant naujus atsparumo fomozei genus.

Išvados

1. *Leptosphaeria* spp. aukšliasporių išbarstymą iš pseudotecių įtakoja meteorologinės sąlygos – kritulių kiekis, oro temperatūra bei santykinė oro drėgmė. *Leptosphaeria* spp. aukšliasporės mėginiuose buvo aptiktos ir dienomis be lietaus, kuomet santykinė oro drėgmė siekė daugiau nei 90 %, o dienos oro temperatūra buvo žemesnė nei 15 ºC.
2. Krasne vietovėje, kaip ir kitose Rytinės Lenkijos dalyse, dominuoja *L. biglobosa* rūšis. Surinktuose sporų mėginuose (2009–2012 m.) agresyvesnė *L. maculans* rūšis buvo nustatyta tik 2009 metais, *L. biglobosa* rūšis aptinkama visais tyrimų metais (2009–2012).
3. Lietuvoje rudens laikotarpiu *Leptosphaeria* genčiai priklausančios grybų rūšys ant žieminio rapso (*B. napus var. oleifera*), baltagūžio kopūsto (*B. oleracea var. capitata*) bei brokolio (*B. oleracea var. italica*) aptinkamos skirtingomis proporcijomis. Ant žieminio rapso (*B. napus var. oleifera*) bei brokolio (*B. oleracea var. italica*) identifikuotos abi rūšys su *L.*

maculans dominavimu. Ant baltagūžio kopūsto (*B. oleracea var. capitata*) nustatyta tik *L. biglobosa* rūšis.

4. Lietuvoje vyrauja lytinis *L. maculans* dauginimosi ciklas. Izoliatai išskirti nuo brokolio (*B. oleracea var. italica*) bei žieminio rapso (*B. napus var. oleifera*) augalų priklausė abiem lytinio dauginimosi tipams, o jų pasiskirstymas buvo tolygus.
5. Izoliatai priklausantys *L. maculans* ir *L. biglobosa* rūšims tarpusavyje skiriasi. *L. biglobosa* izoliatai surinkti nuo brokolio (*B. oleracea var. italica*) bei baltagūžio kopūsto (*B. oleracea var. capitata*) augalų yra genetiškai artimesni tarpusavyje nei lyginant juos su izoliatais surinktais nuo žieminio rapso (*B. napus var. oleifera*). Nustatyta, kad tarp *L. maculans* izoliatų vyrauja didesnė genetinė įvairovė populiacijų viduje nei tarp populiacijų.
6. *L. maculans* ir *L. biglobosa* sukelti fomozės požymiai ant žieminio rapso (*B. napus var. oleifera*) augalų lapų vystosi skirtingu greičiu. Fomozės požymiai greičiau išryškėjo *L. biglobosa* užkrėstuose augaluose, tačiau fomozės dėmės ant lapo sparčiau vystėsi *L. maculans* užkrėstuose augaluose.
7. Rapsų augalai, *L. maculans* ir *L. biglobosa* sukeltos infekcijos metu, ekspresuoja su stresu susijusius genus. kDNR-AFLP analizės metu rasta 11 diferenciškai ekspresuojamų fragmentų. Identifikuoti 7 genai, kurių 3 genų produktai dalyvauja su stresu susijusiuose procesuose: nitrato reduktazė, chloroplasto baltymas didinantis pakantumą stresui, su virulentiškumo atsako baltymu susijęs baltymas.

Trumpos žinios apie disertantę

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