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EFFECT OF LITHUANIAN GENOTYPES RESISTANCE TO ANTHRACNOSE (*COLLETOTRICHUM* SPP.) IN *LUPINUS ANGUSTIFOLIUS* L.

Breeding for disease resistance is one of the most difficult tasks in breeding work. Rapid mutation of pathogen's populations, its ability to continuously form a large amount of races differing in virulence and aggressiveness are the chief obstacles in the development of disease-resistant lupine varieties. Of 2019—2021 year twelve narrow-leafed forage lupine genotypes developed by the individual selection methods and characterised by a high resistance to anthracnose (*Colletotrichtum gloesporoides* (Penz.) Penz & Sass.) resistance 7—9 points, rapid growth rate, and a high seed yield 1.3—3.8 t/ha were tested at the competitive variety testing trials. The investigated lines are a valuable material from the viewpoint of genetics, breeding and agronomic characteristics which will be used in subsequent breeding work and the most promising lines will be transferred to the official variety testing.

narrow-leafed forage lupine; individual selection; variety; resistance to anthracnose diseases

One of the key challenges that Lithuania has currently to deal with is rational use of land, since any agricultural activity either increases or declines natural soil fertility. Consequently, it is very important to select a farming method which would prevent any direct or indirect damage to the environment.

Recent research has shown that soil biological fertility increasing methods are the most promising. Out of all known microorganisms the most efficient nitrogen fixations are legume bacteria in symbiosis with legume plants [1, 2]. Depending on these bacteria and legume crops properties and growing conditions, the symbiotically fixed nitrogen can total from 45 to 460 kg/ha in year [3], therefore many countries researchers suggest rational use of mineral nitrogen and maximal use of biological nitrogen. With growing demand for ecologically clean agricultural produce increasingly more attention is being paid to ecological agriculture, which restricts the use of fertilisers, pesticides, and herbicides. Therefore, in the situation of increased environmental pollution legume crops are of special value in ecological agriculture.

One of the oldest legume crops, grown world-wide, suitable not only for forage production as protein source but also for soil culturing, increasing of its natural fertility is lupine [4].

Lupine has been grown in Lithuania since olden times; however, the area sown with this crop is not large. The chief reason why the area sown with lupine is declining is the spread of new fungal diseases. Lupine anthracnose (*Colletotrichtum gloesporoides* (Penz.) Penz & Sass.) [5, 6] is one of the most harmful lupine fungal diseases, which occurs on all lupine species.

Although in contrast to other plants, lupines do not suffer from many diseases, their cultivation remains so far not so easy issue. A range of lupine diseases, most of which are caused by fungal and viral pathogens, give rise to different types of injuries [7] leading to yield losses reaching 75—100% [8]. Based on observations carried out over the past 15 years, fungal diseases, such as lupine anthracnose and fusarium wilt of lupines, gray mold, and sclerotinia, are among the most common lupines crops [9, 10]. Anthracnose, caused by *Colletotrichum gloeosporioides* phytopathogen, is the most devastating fungal disease, rapidly spreading and highly reducing lupine seeds and green weight yields, and thus almost completely destroys the crops during epidemic [11]. Symptoms such as brown areas of dead tissue (necrotic lesions) on leaf blades, stalks and stems, flower stalks and seed pods, and slimy mass of orange-coloured spores etc., were observed to be almost the same in all lupine species [12, 14].

The objective of our work is to develop high-yielding, fungal disease-resistant, early maturing varieties of narrow-leafed forage lupine, suitable for cultivation in all climatic zones of Lithuania.

Materials and Method. Tests of the new narrow-leafed forage lupine genotypes for anthracnose resistance were carried out during the final stage of the breeding process. The trials were set up Vokė Branch of Lithuanian Research Centre for Agriculture and Forestry (Baltic Sea region, 54°33'49.8"N 25°05'12.9" E) during 2019—2021. Field experiments were carried out in 5-field selection crop rotation. The soil was sandy loam *Haplic Luvisol* according to the FAO [13]. The agrochemical characteristics of the arable layer (0—20 cm) were defined to be as follows: pH_{KCl} (4.8—5.0), mobile Al^{3+} (20—35 mg/kg), available phosphorus (52—55 mg/kg P_2O_5) and potassium (80—95 mg/kg K_2O), and organic carbon (0.89—1.01%).

Soil for the lupines was prepared according to routine technology: deep ploved in autumn, 2 times cultivated in spring. Herbicide Stomp (455 g/l pendimethalin) (2.0—2.5 l/ha) was used for weeding control.

Eleven narrow-leafed forage lupine breeding genotype were investigated. Lupine variety 'Boruta' was used as a control. Since during the last eight year period anthracnose had occurred annually, the tests were conducted under natural conditions.

The area of the record plots was 6 m^2, four replications were used. Randomised plot design was employed.

During the growing season resistance to fungal diseases was estimated at three plant growth stages: seedling, bud formation — flowering, and shiny pods. 1-to — 9-point scale was used: 1 — very low resistance, diseased plants over 50%, 3 — low resistance, diseased plants 26—50%, 5 — moderate resistance, diseased plants 11—25%, 7 — high resistance, diseased plants 2.5—10%, 9 — very high resistance, diseased plants less than 2.5% [11]. With this end in view, at complete emergence plants were counted in A and C replications, at seedling, bud formation — flowering, and shiny pods stages anthracnose-affected plants were counted and removed from the plot. At complete maturity stage healthy plants were counted and their productivity was estimated.

Percent of fungal-disease affected plants was identified according to the formula: $P = (n / N) \times 100$, where n — number of affected plants, N — number of assessed plants.

In our tests we estimated anthracnose *Colletotrichtum gloesporoides* (Penz.) Penz & Sass. resistance of the new narrow-leafed forage and the effect of anthracnose resistance on seed yield.

One-way analysis of variance (ANOVA) with the use significance of differences (LSD_{05}) was determined at the significance level of $P \leq 0.05$.

Results and Discussion. As mentioned earlier, climate conditions are considered as the most important factors causing the spread of anthracnose caused by *Colletotrichum gloeosporioides* in lupine crops, which can be infected both internally and externally [10]. Studies have shown that anthracnose remains viable in seeds for a maximum of 3 years [9]. The development of anthracnose agents requires an average daily temperature of 18—24°C, light rain for three days, and wind, as the spores of the fungus spread in an air-droplet manner as well as requires young plant tissues which are vulnerable [11].

The weather conditions during the experimental period were varied. As a result, the spread of lupine anthracnose was different, too. During the period of 2019—2020 no anthracnose was spotted in the treatments of narrow-leafed forage lupine at the seedling stage due to the early lupine sowing and low air temperature (8.0—15.3^{0}C) during the emergence — stem growth stage. Under natural conditions the first disease symptoms can manifest themselves during the stem growth period, but most often they are identified at later growth stages of lupine. Experimental evidence suggests that the disease severity, incubation period and the number of pathogen's generations are different under natural conditions and depend on the lupine species, genotype, variety, and earliness. Furthermore, infection development is markedly affected by the weather conditions and plant growth stage at which the disease appears. The greatest harm is done when anthracnose starts to spread at bud formation — flowering stage or shiny pods stage. When lupine has reached the end of milk maturity — beginning of wax maturity, the disease does not cause any serious damage to the seed yield, but the seed gets infected. Table 1 shows the monthly average daily temperature and precipitation.

1. Average monthly daily temperature and amount of precipitation

Month	Year			Multiannual average
	2019	2020	2021	
Temperature, °C				
May	13.3	10.3	11.2	12.5
June	21.1	19.4	19.5	15.7
July	17.1	17.6	22.1	16.9
Precipitation, mm				
May	28.6	77.8	14.7	60.0
June	27.5	68.4	55.0	77.0
July	49.9	66.8	93.5	78.0

In recent years, the beginning of lupine vegetation in Lithuania is the first — second ten days of May. In this period, in Lithuania is usually both cold and rainy, as in 2020—2021, average air temperature 10.3—11.2°C (multi-annual average 12.5°C), precipitation amount reached 77.8—147 mm (multi-annual average 60 mm) or hot and dry weather like 2019 when average monthly temperature reached 13.3°C, only 28.6 mm of precipitation fell, which is not suitable for the development and spread of the anthracnose pathogen in the early phases of lupine development (BBCH 11—25). Unfavorable meteorological conditions pathogen development and delay plant infection. The weather circumstances were exceedingly adverse during the intensive growth of lupines and the formation of generative plant components (BBCH 30—81) in June 2019. The average daily temperature was 21.1°C (compared to the long-term average of 15.7°C), and precipitation was 27.8 mm (compared to the long-term average of 77 mm). When the lupines reached the stage of green maturity at the end of the third decade of June and the beginning of the first decade of July, favorable conditions for

Fig.1. Lupinus angustifolius variety 'VB Ainiai' and damage to Colletotrichtum gloesporoides (Authors' photos)

the spread of anthracnose were produced (BBCH 81—87). In June 2020, a higher air temperature of 19.4°C (the average multi-year temperature was 15.7°C) and ideal humidity levels prevailed. July was cooler (17.7°C) and more humid than typical. The conditions for the growth and development of lupines were favorable. When the lupines reached green maturity (BBCH 87), the first signs of anthracnose appeared. The month of June 2021 was warmer and drier than usual (the average daily air temperature was 19.5°C only 55 mm of precipitation fell (long-term average 77 mm)). Plants are poor. The absence of precipitation was also observed in the second and third decades of July, when the average air temperature reached 22.1°C (the long-term average is only 16.9°C). Anthracnose symptoms appeared when the lupines reached (BBCH 77), the full pod filling stage.

Looking at the three-year results (Fig. 2 and 3), all the Lithuanian lupin genotypes were more resistant to *Colletotrichtum gloesporoides* and produced a higher yield of grains than the control 'Boruta'. On average, 'VB Derliai' and H1820 were the most resistant to *Colletotrichtum gloesporoides*. The average infection with the pathogen was 6.8% ± 0.230 and 6.9% ± 0.237 (respectively). These genotypes yielded on average 2.8 t/ha ± 0.142 over three years. The genotypes 'VB Vilniai' (12.7% ± 0.430), 'VB Rausviai' (11.6% ± 0.321) and 'VB Pilkiai' (11.7% ± 0.345) were less resistant to *Colletotrichtum gloesporoides*. The yield was accordingly 2.0 t/ha ± 0.114. The remaining

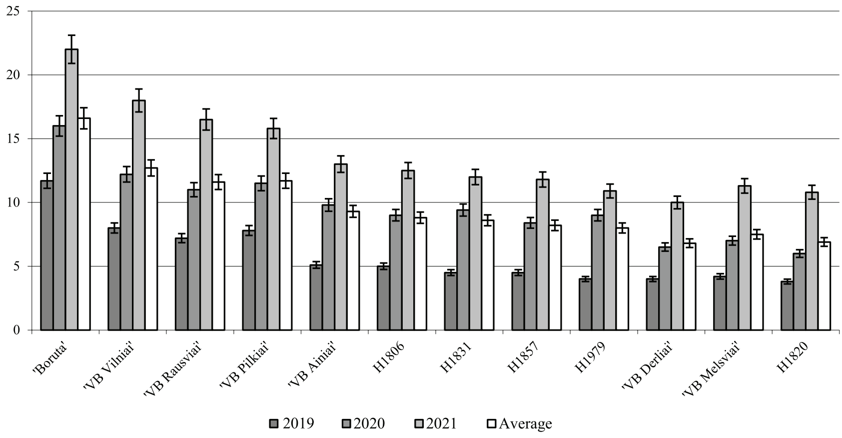

Fig. 2. Estimation of Colletotrichum disease prevalence (%) on different genotypes of Lupinus angustifolius (LSD$_{05}$ = 1.227; ± SE — standard error)

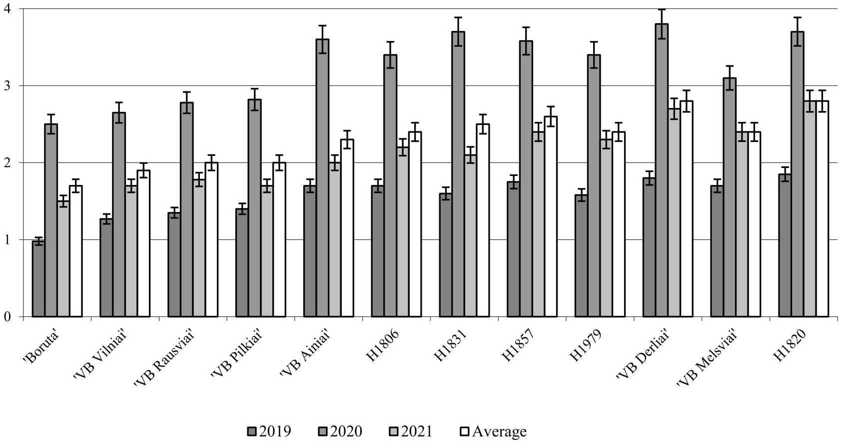

Fig. 3. Yield of Lupinus angustifolius, t/ha (LSD$_{05}$ = 0.061; ± SE — standard error)

genotypes ('VB Ainiai', 'VB Melsviai', H1806, H1831, H1857 and H1979) were on average resistant to *Colletotrichtum gloesporoides* (9.3—7.5%) and produced a grain yield (2.6—2.3 t/ha).

Fungal diseases spread on lupine plants every year. Disease spread depends on variety genetics, meteorological conditions, previous crops, and weediness of the field. Lupine anthracnose is one of the most harmful diseases which affect all lupine species at any plant growing stage [10]. Lupine grain yield depends on the plant growth stage at which anthracnose affects the plants. The selected narrow-leaved lupine genotypes show a high resistance to this fungal disease. Conditionally resistant to anthracnose are genotypes in which affected plants were less than 2.5%. In the world lupine gene bank, there are no varieties fully resistant to fungal and viral diseases. But lupine varieties that are partially resistant at a low disease epitophe undergo less infection in the vegetation period. The tested genotypes produced a high seed yield.

The selected and tested narrow-leaved lupine genotypes showed a fast growth rate in all stages and thus a rather high resistance to anthracnose and high seed yield. The vegetation period of narrow-leaved genotypes is short (88—92 days) and they can be grown naturally in the whole country without applying special agroengineering measures. Narrow-leaved genotypes have a high value in genetic, breeding and farming aspects.

CONCLUSIONS

Breeding for disease resistance is one of the most challenging tasks in the breeding work. A rapid mutation of pathogen's populations, its ability to continuously form a large number of races differing in virulence and aggressiveness are the chief obstacles in the development of resistant lupine varieties. When limited genefund is used in breeding work lupine anthracnose can occur in the varieties differing in genetic potential. From the presented data we can see that the development of the infection is influenced by human factors and pathogen's characteristics, genotype, as well as the weather conditions. The world lupine genefund does not contain any varieties completely resistant to fungal or viral diseases. It is likely that the varieties characterised by a partial resistance do not lose this character for a longer period and in the years of weak epiphytoty such varieties either do not catch the infection or are insignificantly affected, like breeding lines H1806. H1831, H1857, H1979 and H1820.

Conflict of interest: authors declare no conflict of interest.

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Вплив стійкості литовських генотипів проти антракнозу (*Colletotrichum* spp.) у люпину вузьколистого (*Lupinus angustifolius* L.)

Селекція на стійкість проти хвороб є одним з найскладніших завдань у селекційній роботі. Швидка мутація популяцій патогена, його здатність постійно утворювати велику кількість рас, що відрізняються за вірулентністю та агресивністю, є головними перешкодами у створенні стійких проти хвороб сортів люпину. У конкурсному сортовипробуванні 2019—2021 рр. було випробувано дванадцять генотипів люпину вузьколистого кормового, створених методами індивідуального добору, які характеризуються високою стійкістю проти антракнозу (*Colletotrichtum gloesporoides* (Penz.) Penz & Sass.) — 7—9 балів, швидким темпом росту та високою врожайністю насіння 1,3—3,8 т/га. Дослідження є цінним матеріалом з точки зору генетичних, селекційних та агрономічних характеристик, який буде використано в подальшій селекційній роботі, а найбільш перспективні будуть передані в державне сортовипробування.

вузьколистий кормовий люпин; індивідуальний добір; сорт; стійкість проти антракнозу

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