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Development of fungi on *Lupinus angustifolius* L. and *Lupinus luteus* L.

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Testing for fungal, micromycetes and anthracnose in lupine varieties was conducted at the Voke Branch of Lithuanian Institute of Agriculture during a competitive trial of feeding lupine (*Lupinus* L.) in 2005 - 2007. Application of lupines for green manure in fruit and vegetables gardens enriches the soil with nitrogen and other nutrients, influences species composition and functional properties of microorganisms developing in plant rhizosphere. In rhizosphere and on the aboveground parts of lupines cultivated for green manure certain micromycete species, potential infection agents of plants grown in fruit and vegetable gardens, are recorded. Here belong some species of the *Fusarium* Link, *Pythium* Nees, *Thielaviopsis* Went, *Rhizoctonia* DC., *Phoma* Sacc., *Ascochyta* Lib., *Romuliaria* Sacc., *Septoria* Sacc., *Erysiphe* R. Hedw.ex DC. genera. Particularly hazardous to plants are fungi of the *Colletotrichum* (Corda) genus, which frequently infect lupines. The main routs of distribution of potentially hazardous fungi are seeds and phytopathogenic condition of soil in which plants grow. The phytopathogenic condition depends upon a few factors: resistance of lupines grown for green manure to various disease agents, proper treatment of seeds before sowing, selection of preplant and soil preparation that suppress the development of phytopathogens and stimulating plant growth powers.

***Key words*:** lupine (*Lupinus* L.), fungi, micromycetes, anthracnose

Ecological farming stimulates the interest in green manure, which enriches soil with valuable natural, easily available nutritious substances. Presently green manure is applied for fertilization of fruit and vegetable gardens, aiming to increase the nitrogen amount in the soil.

Presently in field rotation in Lithuania two species of lupines are grown, i. e. annual fodder *Lupinus luteus* and sideral *Lupinus angustifolius*. Strong roots, able to penetrate deep into soil and decompose soil components not available for other plants and, thus, enrich upper layers of soil, where roots of other plants usually develop, with the nutritious substances, characterize these lupines. Lupines, similarly as other leguminous plants, are characterized by symbiosis with nitrogen-fixing bacteria. Bacteria *Bacterium radicicola,* able to fix the air nitrogen, are detected on lupine roots. Nodular bacteria are less abundant on roots of these plants compared with clover and peas. It should be noted that nodular bacteria of lupines are resistant to acid reaction of soil. Minimum soil pH is 3.5. The great majority of bacteria belong to the group of slowly growing *Bradyrhizobium* nodular bacteria (Lapinskas, 1996). Lupine nodular bacteria are widespread in fields and meadows of lupines and other annual leguminous plants.

Due to processes performed by these bacteria the amount of pure nitrogen in soil under lupines can increase by 150–200 kg during the vegetation period. It depends upon environmental conditions, intensity of development and accumulation of root

phytomass. Seeds and phytomass of some lupines are rich in proteins, e.g. in seeds of *Lupinus luteus* proteins comprise 45 %, and in phytomass – 18–22 % of weight (Lazauskas, 1996).

However, enrichment of soil with nitrogen by lupines is impeded by widely spread fungal diseases; every year the disease agents destroy about 50 % and sometimes even more of lupine crops. It causes great economic losses, due to seed loss lupine crop areas can not be expanded and, therefore, they can not be rationally applied for fertilization of soil, especially in light textured soils, though lupines grow well in heavy textured soils and are also suitable for improvement of their fertility. Therefore, it is essential to fit lupines properly in plant rotation so that favourable sanitary conditions for lupine cultivation are ensured, development of other plant pathogens suppresses, and phytopathogenic condition of the arable soils improved. The main infection agents of lupines are of great interest to the specialists. A Ukrainian researcher N. M. Pidoplichko (1979) considers *Aphanomyces euteiches* Drechsler, various fungi of the *Fusarium* genus, and *Thielaviopsis basicola* (Berk. et Br.) Ferr. being the main agents of lupine root rot; fungi of the *Fusarium* genus and *Verticillium alboatrum* Reinke et Berthold – the main agents of lupine wilt; *Sclerotinia sclerotiorum* (Lib.) de Bary, *Diaporthe lupini* Harkn. – agents of stem rot and infections; *Ascochyta lupinicola* Petra., *Cercospora longispora* Peck, *Pleiochaeta setosa* (Kirchn.) S. Hughes, *Septoria kaznowskii* M. Nikol – blight agents; *Erysiphe communis* Grev., *Phyllactinia lupinicola* Roth. – powdery mildew agents; *Uromyces lupinicola* Bub., *Uromyces renovatus* P. Syd. et Syd. – rust agents (Lazauskas, 1996).

W. Branderburger (1985) mentions the following fungi infecting lupines grown in Europe: *Olpidium brassicae* (Wor.) Dang., *Pleiochaeta setosa* (Kirchn.) S. Hughes, *Aphanomyces euteiches* Drechsler, *Pythium sylvaticum* Campbe. et Hendrix, *P. intermedium* de Bary, *P. vexans* de Bary, *P. rostratum* Butler, *Chalaropsis thielavioides* Peyronel, *Thielaviopsis basicola* (Berk. et Br.) Ferr., *Rhizoctonia crocorum* (Pers.) Dc. ex Mérot., *R. solani* Kühn, *Botrytis cinerea* Pers; *Fusarium avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdt., *F. sambucinum* Fuckel, *Verticillium alboatrum* Reinke et Berthold, *V. dahliae* Kleb., *Phomopsis leptostromiformis* (Kühn) Bub.; *Erysiphe pisi* Dietrick, *Uromyces lupinicolus* Bub., *Uromyces renovatus* P. Syd. et Syd., *Ustilago lupini* Camara, *Ramularia lupini* J. J. Davis, *Stemphylium botryosum* Wallr., *Truncatella ramulosa* (van Beyma) Stey., *Ascochyta lupinicola* Petr., *Septoria kaznowskii* M. Nicol. The degrees of distribution and aggressiveness of the above-mentioned species highly differ and very much depend upon meteorological conditions and many other natural and anthropogenic factors. These fungi, as lupine disease agents, are also mentioned in works of many researchers (Strucinskas, 1996).

Conidial stage of *Glomerella cingulata* (Ston.) Sp. et Schr. such as *Colletotrichum gloeosporiodes* (Penz.) Penz et Saess., and *Colletotrichum acutatum* Simm. and Simmonds causing anthracnose are considered among the most active lupine infections. On *Lupinus angustifolius* this fungus was for the first time registered in 1939. Presently the disease anthracnose caused by this fungus is widely spread in various countries (Talhinhas *et al*., 2002; Pieczul, Rataj-Guranowska, 2004; Thomas, Adcock, 2004).

The aim of this work was to detect and identify micromycetes in the rhizosphere of lupines, on roots, aboveground parts of plants as well as plant remnants, which get into soil and, thus, determine its sanitary condition.

MATERIALS AND METHODS

In 2005–2007 lupines were cultivated in research facilities of the Vokė Branch of the Lithuanian Institute of Agriculture. The soil in experimental plots – sandy loam on carbonated fluvioglacial eluviated gravel

(JD_P), according to FAO UNESCO classification – Haplic Luvisolis (LVh) (Buivydaite *et al*., 2001). The soil was of the following agrochemical characteristics: pH_{KCL} 5.6–5.7; hydrolytic acidity – 2.9–3.8 mekv kg^{-1}, sum of sorptive bases – 6.4–7.2 mekv kg^{-1} of soil; humus – 1.97–2.1%, mobile phosphorus 232 mg kg^{-1} and potassium 187–205 mg kg^{-1} of soil. Summer barley was used as preplant for lupines. In autumn the field was ploughed, in spring it was fertilized with phosphorus and potassium fertilizers at a rate of $P_{40}K_{60}$ per ha. In experimental plots lupines of 2 Lithuanian varieties were grown: *Lupinus luteus* 'Augiai' and *Lupinus angustifolius* sideral 'Snaigiai'. Lupines were sown in April employing the sowing-machine 'Saxonija'. The seed rate was 1.3 mln (150–170 kg) per ha. After sowing the field was rolled. In order to destroy weeds the crops were sprayed with 2.5 kg ha^{-1} of herbicide.

During the trial period meteorological conditions varied, especially high variations were observed in the amount of precipitation (Table 1). The precipitation was more abundant in June (20 mm), during other months the average amount of precipitation was only 7-20 mm. In the June of 2007 the amount of precipitation reached 20 mm, in July and August – 70 and 7 mm, respectively. Therefore, the weather was wet during that period.

Separate years of the trial also differed regarding air temperature (Table 1). In 2005 July was sufficiently warm, average air temperature reached 19.1°C and was 2.2°C higher than the average many-year temperature. The amount of precipitation during the vegetation period was close to the many-year average. In the May of 2006 the average air temperature reached 12.4°C, in July and August – 20.8 and 17.6°C, respectively. It is by 1–4°C higher than the many-year average.

Meteorological conditions in 2005 and 2007 were most favourable for the development of micromycetes.

Soil samples were taken before lupine sowing and after harvesting. For mycological investigations plant samples were taken when plants formed rosette, at the beginning of budding, during plant flowering, during the period of pod formation, and when pods ripened. Samples were taken from each experimental plot.

For microbiological investigations samples were taken considering principles of selective sampling. From each experimental plot the noticed infected plants were taken. If they were not abundant, all plants were taken for the investigation, if abundant – from each plot plants were taken selectively; in each research site the sample of not less than 20 items. Samples were taken with sterile instrument, placed into separate sterile glass vessels or bags, hermetically sealed, and the label containing the main information about the sample attached. Samples were analyzed immediately or the following day, but not later than after 3 days keeping them in a refrigerator. Analysis of each sample was performed with three replications. While analyzing the lupine infections, more attention was given to systematic position of the disease agents and to their abundance because later the protection measures are chosen according to biological properties of the infection agent. Therefore, the main aim of the work was to isolate and identify micromycetes – infection agents. For this purpose the methods described in Rabie *et al*. (1997) and Samson *et al*. (Samson *et al*., 2000) were employed.

When visual observation of plants or their seeds revealed the presence of at least one infection agent, the methods of direct sowing or reprints were used. The surface of woody parts of the aboveground plant parts as well as roots was disinfected with 70 % ethanol for 2 min, then rinsed with sterile water (Andrews *et al*., 1997). The sample or its part was placed into a Petri dish with malt agar extract medium supplemented with chloramphenicol (50 mg/l) and kept for 5–7 days in a thermostat at a temperature of 26±2°C. A dilution method was used for the analyses of soil and samples heavily contaminated with

different microorganisms. The following procedure was performed: in sterile conditions 1 g or the tested sample was placed in 10 ml of sterile water, shaken for 10 min., and a series of dilutions were prepared from the obtained suspension (1 ml of the primary suspension was poured into 9 ml of sterile water, shaken, etc.). From each dilution series 1 ml of suspension was drawn into a sterile 9 cm diam. Petri dish to which 15 ml 48°C malt agar medium with chloramphenicol was added. The cultures were cultivated under the already mentioned conditions.

In order to obtain pure cultures, the isolated micromycete cultures were sown on standard Czapek, malt and corn extract media and cultivated for 5–7 days at a temperature of 26±2°C. Micromycetes were identified according to manuals (Arafa *et al*., 2002).

The infected plants were counted according to the methods described by Mathur and Kongsdal (2003).

The obtained data were assessed by the method of dispersion analysis, employing the ANOVA statistical data processing software (Tarakanovas, 2002).

RESULTS

Sandy loam soil prevailed in the site of lupine cultivation. Summer barley was used as a preplant. In autumn the field was ploughed and in spring – fertilized with phosphorus and potassium fertilizers ($P_{40}K_{60}$). These factors predetermined the micromycete species composition and intensity of their development in soil intended for lupine cultivation. From soil the following micromycete species were isolated: *Penicillium decumbens* Thom, *P. funiculosum* Thom, *P. lividum* Westling, *P. ochrochloron* Biourge, *P. piceum* Raper et Fennell, *P. purpurescens* Soop, *P. simplicissimum* (Oudem.), Thom, *P. capsulatum* Raper et Fennell, *Mortierella alpina* Peyronel, *M. vinacea* Dixon-Stew., *Botryosporium longibrachiatum* (Oudem.) Maire; *Phoma pomorum* Thüm, *Pythium sylvaticum* Campbe. et Hendrix, *Tielaviopsis basicola* (Berk. et Br.), Ferr., *Tilachlidium brachiatum* (Batsch et Fr.) Petch, *Trichoderma polysporum* (Link ex Pers.) Rifai, *T. viride* Pers. *Drechslera sorokiniana* (Sacc.) Subram. et Jain, *Rhizoctonia solani* Kuhn, *Mycelia sterilia*. It should be mentioned that in soil after such treatment micromycetes potentially able to damage lupine seedlings and roots were also recorded (*Pythium sylvaticum*, *Tielaviopsis basicola, Rhizoctonia solani, Drechslera sorokiniana*). The above-mentioned micromycetes successfully survived in nutrient-poor soil with plant remnants, and after addition of nutrients with fertilizers they started to function intensively.

Abundance and composition of micromycetes in soil changed after lupine sowing in April. After sowing the field was rolled and sprayed with herbicide gezagard. The number of micromycete propagules (cfu) per 1 g of dry soil where non-treated seeds of *Lupinus angustifolius* 'Snaigiai' were sown reached 81081, where seeds treated with vitavax were sown this number was 59978 cfu g^{-1}; in case of *Lupinus luteus* 'Augiai' these numbers were higher, but less differentiated – 99448 and 97614 cfu g^{-1}, respectively.

The data of lupine seed contamination with micromycetes is presented on Table 2.

Micromycetes of 21 species were isolated from the non-treated seeds of lupines 'Snaigiai' and 25 species from 'Augiai'. After seed treatment with fungicide vitavax the diversity of micromycete species significantly reduced; however, after seed sowing no evident reduction of micromycete propagules in soil of experimental plot was noticed. Somewhat lower amount of micromycete propagules was observed in soil under *Lupinus angustifolius* 'Snaigiai'.

As lupines pushed through, first infections of seedlings were soon noticed. Lupine roots were infected most frequently. Micromycetes isolated from roots of infected lupine seedlings are presented on Table 3.

Roots of *Lupinus angustifolius* seedlings were infected with micromycetes of some *Fusarium* species, *Aphanomyces euteiches,* and *Pythium sylvaticum*, other fungi were less frequent. Fungi from the *Fusarium* genus were isolated from roots of *Lupinus luteus* seedlings. Probably, they were the main agents of early root rot of lupine seedlings. Only solitary instances of *Sclerotinia sclerotiorum* were revealed. They were more abundant in crops of the control variant where non-treated seeds were sown. *Pythium sylvaticum* fungi and sterile white mycelium (*Mycelia sterilia*) were most frequently isolated from infected seedlings of *Lupinus luteus* grown from the treated seeds.

First symptoms of anthracnose in lupine crops were noticed already in the period of plant budding. However the disease became mostly evident during lupine blooming. At first lupines were infected only in separate sectors, which gradually expanded till they extended throughout the whole experimental plot. Lupines 'Snaigiai' were considerably more resistant to the anthracnose agents than 'Augiai'. In the years 2005 and 2007, when the weather was warm and even hot, all *Lupinus luteus* were infected (100%) already at the beginning of blooming; in 2006, as the weather was dry, the degree of lupine infection was markedly lower (Figure 1).

Table 1. Weather conditions during the vegetation period

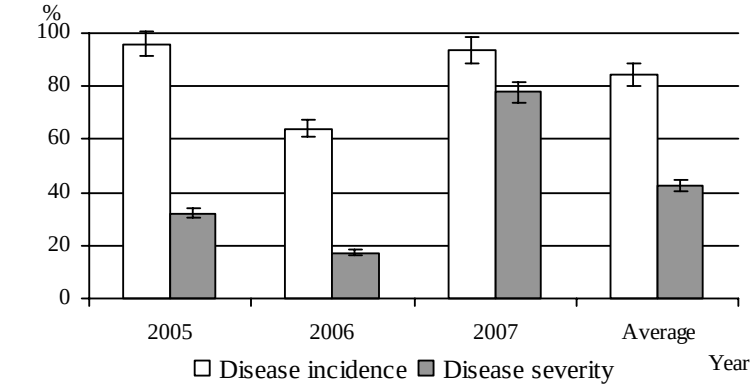
Month	Air temperature, °C				Precipitation, mm			
	2005	2006	2007	Long-term average	2005	2006	2007	Long-term average
May	12.1	12.4	13.6	12.5	45	15	20	60
June	15.0	16.5	17.7	15.7	20	7	20	77
July	19.1	20.8	17.0	16.9	23	15	70	78
August	16.7	17.6	18.9	16.3	67	51	7	68

Table 2. Fungal contamination of lupine seeds before sowing: a) *Lupinus angustifolius* L. and b) *Lupinus luteus* L.

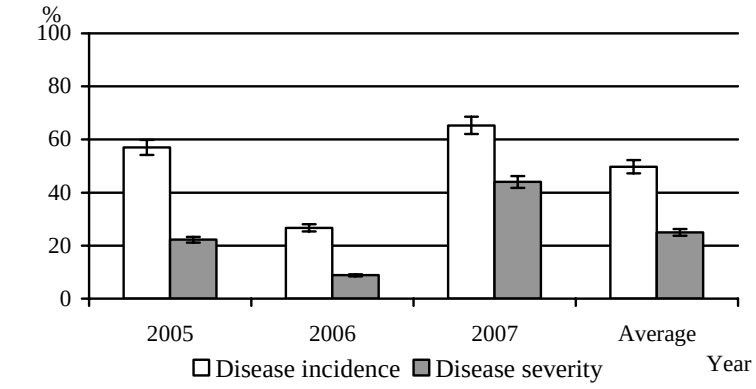
a) Isolated micromycetes	b) Isolated micromycetes
Alternaria alternata (Fr.) Keissl.	*Alternaria alternata* (Fr.) Keissl.
Fusarium equiseti (Corda) Sacc.	*Ascochyta lupinicola* Petr.
Fusarium oxysporum Schltdl.	*Fusarium equiseti* (Corda) Sacc.
Mucor racemosus Fresen.	*Olpidium brassicae* (Wor.) Dang.
Rhizomucor pusillus (Lindt) Schipper	*Penicillium chrysogenum* Thom
Rhizopus oryzae Went ex Prins. Geerl.	*Penicillium expansum* Link
Penicillium expansum Link	*Penicillium verrucosum* Dierckx
Penicillium verrucosum Dierckx	*Phoma medicaginis* Malbr. et Roum.
Phoma medicaginis Malbr. et Roum.	*Rhizomucor pusillus* (Lindt) Schipper
Ramularia lupini J. J. Davis	*Rhizopus oryzae* Went ex Prins. Geerl.
Thielaviopsis basicola (Berk. et Br.) Ferr.	*Rhizoctonia solani* Kühn
Mycelia sterilia	*Thielaviopsis bassicola* (Berk. et Br.) Ferr.
	Mycelia sterilia

Table 3. Micromycetes detected on lupine roots: a) *Lupinus angustifoliu* L. and b) *Lupinus luteus* L.

a) Isolated micromycetes	b) Isolated micromycetes
Fusarium avenaceum (Fr.) Sacc.	*Fusarium avenaceum* (Fr.) Sacc.
Fusarium culmorum (Wm. G. Sm.) Sacc.	*Fusarium equiseti* (Corda) Sacc.
Fusarium oxysporum Schltdl.	*Fusaruim oxysporum* Schltdl.
Aphanomyces euteiches de Bary	*Mucor hiemalis* Wehmer
Geotrichum candidum Link ex Pers.	*Mycelia sterilia*
Penicillium fellutanum Biourge	
Pythium sylvaticum Campbell et Hendrix	
Mycelia sterilia	



a)



b)

Fig. 1. Severity of (a) *Lupinus luteus* L. and (b) *Lupinus angustifoliu* L. stems anthracnose during the research period, %.

DISCUSSION

The applied methods did not allow isolating anthracnose agents from soil. Still the disease agents were easily detected in plant stem areas approximating to roots and on leaves. These are fungi of the *Colletotrichum* Corda genus, which for a long time had been ascribed to the *Ascomycetes* class, the *Sphaeriales* order. Presently Satton *et al*. (2001) ascribe them to the class of mitosporic *Coelomycetes*. On agar media fungi of this genus form very different colonies, usually from gray to brown in colour, the reverse of the colony is brown, sometimes with orange, red, or brown shades. Commonly they are parasites of

higher plants. Some authors (Domsch *et al*., 1980; Sutton; 1983; Talhinhas *et al*., 2002) state that *Colletotrichum* is frequently recovered from soil. However, in some cases the setae are lacking and previously such forms would have been placed in *Gloeosporium* (Barron, 1968). One sample of sterile mycelium we have isolated from soil might have been the propagules of these fungi because, according to von Arx (1957), in culture colonies of *Colletotrichum* may have sparse setae and produce pinkish, water-soaked colonies. Because of the parasitic nature of this genus and the slight morphologic distinctions between some species, it is difficult to identify a *Colletotrichum* isolate to species. A survey of the genus *Colletotrichum* and a concept of species are based on morphological characters.

During our investigation some fungal forms of the *Colletotrichum* genus were isolated from lupines. The most frequently detected form, according to morphological features, was closest to *Colletotrichum gloeosporioides* (Penz.) Penz. and Sass. (*Glomerella cingulata* (Ston.) Sp. et Schr.) species. Earlier this fungus was called *Vermicularia gloesporioides.* Experiments conducted in Russia demonstrated that the main way for anthracnose agent penetration into lupine plants was via the injuries of the epidermal surface at the soil level in the root neck zone caused by temperature drop, soil microorganisms or mechanical injury (Yakusheva, Soloviyanova, 2002). Basing on morphological and cultural features we tentatively ascribed 2 other forms to *Colletotrichum acutatum* Simmonds and *Colletotrichum dematium* (Pers. ex Fr.) Grove. Still for more precise identification of the fungi the combination of morphological and genetic identification methods is indispensable. These fungi are characterized by extensive adaptive properties that allow them to infect a wide range of garden and orchard plants in early stages of their vegetation. The propagules of these fungi get into soil with remnants of lupines infected with anthracnose, previously used as green manure, and thus cause real danger for the later cultivated plants. *Colletotrichum gloeosporioides* is a species-level complex of morphologically related forms, which infect a diverse range of plants. Significant variation in spore morphology and colony appearance exists and confusion with *Colletotrichum acutatum* has occurred. DNA sequencing of isolates identified as *C. gloeosporioides* from lupines in UK indicated greater homology to *C. acutatum* than *C. gloeosporioides* (Sweetingham *et al*., 1998). The disease is very harmful and introduces substantial threat to cultivation of lupine in countries with a wet climate. Mucous conidium can become active in the affected places after a rain. With drops of rain, the inoculum is diffused and infects adjacent healthy plants. The agent spends the winter together with post-harvest crop residues, in the form of minosclerotium, or in the form of fruit bodies developed in affected tissues.

Under the above-mentioned experimental conditions fungi of the *Fusarium* and *Pythium* genera could be regarded as the main agents causing lupine root rot. Fungi of these genera were isolated from lupine roots, less abundantly from the soil of the rhizosphere, and aboveground parts during all vegetation stages; they were also detected on seeds. Nevertheless, as soon as seeds started to germinate, development of *Fusarium* fungi on seedling roots was observed. During further stages of plant growth, the intensity of *Fusarium* fungi development as well as their species diversity increased. It significantly depended upon humidity of soil and environment together with temperature conditions. *Fusarium oxysporum* and *F. avenaceum* micromycetes were the first to appear on lupine roots. It can be due to previously cultivated summer barley in the rhizosphere of which these fungi, and especially *F. avenaceum,* usually prevail and, therefore, with plant remnants they easily get into lupine rhizosphere. Abundant evidence claim *F. oxysporum* as being among the main agents infecting lupine roots and

causing wilt (Mathur, Kongsdal, 2003). Basing on the obtained results *Fusarium equiseti*, *F. culmorum,* which were detected during the earliest stages of plant vegetation, *F. solani* (Mart.). Appel et Wollenw., *F. sambucinum* Fuckel, *F. proliferatum* (Matsush.) Nirenberg, *F. poae* (Peck) Wollenw., which appeared later, should be listed as major infection agents of lupine roots. It should be mentioned that fungi of some *Fusarium* species reacted differently towards excess or lack of humidity in soil and environment. More diverse species composition of *Fusarium* fungi was observed in the rhizosphere of *Lupinus luteus* than of *Lupinus angustifolius*.

During rainy years or periods more intensive development of the *Pythium* genus fungi (*Lupinus angustifolius* in particular) in the rhizosphere of lupines was observed. *Pythium sylvaticum* micromycetes dominated. Colonies on cornmeal agar produce cottony aerial mycelium. Fresh isolates usually show no sexual organs in single culture, but after long maintenance oogonia can be produced in single cultures, especially around the inoculum of female isolates. *P. sylvaticum* was originally isolated from soil, water in ponds and lakes, *Picea*, pea, flax, cress, strawberry, lettuce. *P. sylvaticum* produces toxins, it can become pathogenic to seedlings of apple, wheat, flax, pea, radish, lettuce, carrot, cucumber, and strawberry (Van Der Plaats-Niterink, 1981; Phan *et al*., 2007).

We succeeded to isolate *Pythium intermedium* de Bary from the rhizosphere of the investigated lupines. It was originally isolated from dead plant material, but it is a typical soil inhabitant; *Pythium vexans* de Bary fungi were isolated from the rhizosphere of lupines 'Augiai'. Usually fungi of these species have been recorded from both soil and plants in several countries. In Germany they were recorded on lupines and lucerne. *P. vexans* shows no or only weak parasitism on avocado, papaya, tomato, watermelon, morning-glory seedlings, squash, kidney bean, rice, pineapple, and cereals (Van Der Plaats-Niterink, 1981).

During wet years, from the rhizosphere of lupines 'Snaigiai' *Pythium rostratum* Butler fungi were isolated. They are slowly growing, typical inhabitants of humid poor soils. They are characterized by the intercalary often catenulate oogonia and monoclinous sessile or hypogynous antheridia. Several records are known from plants and roots of *Lupinus* from Germany. During dry year of 2006 these fungi were not recorded in the rhizosphere of lupines.

Lupinus angustifolius more often than other suffer from the causative agent of dry rot *Thielaviopsis basicola* Ferr. The affected plants remain undersized and dwarfish. The root system turns black and dies; frequently the stem base is affected. The disease can be spread on the stem as brown bands, then the leaves and stem turn yellow. Sporification of the fungus covers the injured places with white farinaceous coating that later becomes brown. With the help of chlamydospores, the fungus spends the winter on affected plant residues. Disease development is urged by increased temperature in combination with an unstable water regime in soil.

Under the experimental conditions lupines were also infected by *Verticillium alboatrum* Reinke et Berth fungi. They and *V. dahliae* Klebahn. have often been treated synonymously for a long time. The former fungus forms sclerotia and the basal hyaline conidiophores, whereas the latter form the resting thick- walled cell aggregates and basal dark conidiophores. These fungi cause lupine wilt. The disease manifests by fast withering of plants. Less susceptible forms of lupine are resistant to the pathogen, which is expressed by slower development of pathological process accompanied at first by yellowing, browning, and withering of leaves. Withering takes place owing to the blockage of main conducive vessels in of plant by the mycelium of the fungus, that impedes the inflow of water and nutrients. Besides, toxic products of the pathogens metabolism inhibit the plant. The pathogen

as an affected plant residue survives in the form of microsclerotia and chlamydospores.

Sometimes on stems of *Lupinus angustifolius* a light coating with sparse reddish-yellowish, gradually darkening spots could be observed, after some time semi-globular pycnidia with papillae at the top formed in the spots. Inside them were cylindrical, ovate lanceolate 5–12×15–2 µm conidia. Conidiophores filiform, of the same length as conidia, only sometimes a little longer. Later on lupine stems brownish-yellowish pycnidia formed. These were plants affected with *Phomopsis leptostromiformes* (Kühn) Bub. fungi. Such plants start to wither, their development is inhibited, and they often perish. The fungus actively develops at a temperature of 25–30°C, if it drops below 20°C or rises above 30°C the development of the fungi is significantly detained. Under lower temperature (15°C) the fungi do not develop but remain viable. The investigation revealed no significant impact of these fungi upon lupines.

In roots of lupines fungi of the *Olpidium* (A. Braun) Schröt. genus were frequently detected. However, fungi of this species-rich genus of the *Olpididiaceae* J. Schröt family, the *Spizellomycetales* phylum are little investigated. It is stated that *Olpidium brassicae* (Wor.) Dang. fungi make the most harm to lupines, though *Olpidium endogenum* (A. Braun.) Schröt is considered the type species of the genus.

When lupines are grown in plant rotation for green manure it is essential to ensure that lupines do not become the infection source for other plants and, thus, do not worsen phytopathological condition of soil. It is very important, therefore, to select most efficient measures for lupine seed treatment and to choose the most appropriate time for lupine application as green manure till the most harmful plant disease agents are not widespread.

CONCLUSION

Application of lupines for green manure in fruit and vegetables gardens enriches the soil with nitrogen and other nutrients, influences species composition and functional properties of microorganisms developing in plant rhizosphere. In rhizosphere and on the aboveground parts of lupines cultivated for green manure certain micromycete species, potential infection agents of plants grown in fruit and vegetable gardens, are recorded. Here belong some species of the *Fusarium* Link, *Pythium* Nees, *Thielaviopsis* Went, *Rhizoctonia* DC., *Phoma* Sacc., *Ascochyta* Lib., *Romuliaria* Sacc., *Septoria* Sacc., *Erysiphe* R. Hedw.ex DC. genera. Particularly hazardous to plants are fungi of the *Colletotrichum* (Corda) genus, which frequently infect lupines. The main routs of distribution of potentially hazardous fungi are seeds and phytopathogenic condition of soil in which plants grow. The phytopathogenic condition depends upon a few factors: resistance of lupines grown for green manure to various disease agents, proper treatment of seeds before sowing, selection of preplant and soil preparation that suppress the development of phytopathogens and stimulating plant growth powers.

References

Andrews S, Pardoel D, Harun A, Treloor T. 1997. Chlorine inactivation of fungal spores on cereal grains. *International Journal of Food Microbiol*., 35: 153–162.

Arafa MKM, Hassan MHA, Botro SA, El-Gantiry SMM. 2002. Fungal diseases of lupin – species of pathogenic fungi, their occurrence and control recommendations. *Assiut Journal of Agricultural Sciences*, 33(4): 67–79.

Barron GL. 1968. The Genera of *Hyphomycetes* from soil. The Williams et Wilkins Company. *Baltimore*, pp. 34-87.

Buivydaite VV, Vaicys M, Juodis J, Motuzas A. 2001. Lithuanian Soil Classification [Lithuanian]. Lithuanian Academy of Sciences. Vilnius, pp. 57-86.

Domsch KH, Gams W. 1980. Anderson T.N. Compendium of soil Fungi. Academic Press. London, pp. 254-859.

Lapinskas E. 1996. Biological nitrogen fixation and nitroginas [Lithuanian]. Lithuanian Institute of Agriculture. Dotnuva–Akademija, pp. 12-124.

Lazauskas J. 1996. Cultivation of lupine seed, feed, fertilizer and green [Lithuanian]. Lithuanian Institute of Agriculture. Dotnuva–Akademija, pp. 10-28.

Mathur SB, Kongsdal O. 2003. Common Laboratory Seed Health Testing Methods for Detecting Fungi. First edition. Copenhagen, Denmark, pp. 241-425.

Phan HTT, Ellwood SR, Adhikari K, Nelson MN, Oliver RP. 2007. The first genetic and comparative map of white Lupin (*Lupinus albus* L.): identification of QTLs for antracnose resistance and flowering time, and a locus for alkaloid content. *DNA Research*, 14: 59-70.

Pieczul K, Rataj-Guranowska M. 2004. *Colletotrichum* species causing lupins anthracnose in Poland. *Phytopathologia Polonica*, 34: 59–70.

Rabie CJ, Lubben A, Marais GJ, Jansen Vauren H. 1997. Enumeration of fungi in barley. *International Journal of Food Microbiology*, 35: 117–127.

Samson RA, Hoekstra EE, Lund F, Filtenborg O, Frisvad JC. 2000. Methods for the detection, isolation and characterization of food–borne fungi. In: Samson R. A., Hoekstra E. S. (eds.), Introduction to food-arhd airborne fungi. Sixth Edition, pp. 283–297.

Strucinskas MT. 1996. Lupine disease Lithuania [Lithuanian]. Vilnius, pp. 10-53.

Sutton BC. 1983. The *Coelomycetes. Fungi Imperfecti* with pycnidia, acervuli and stromata. Commonwealth Mycologicol Institute. Kev., pp. 45-78.

Sweetingham MV, Jones RAC, Brown AGP. 1998. Deseases and Pests. In: J. S. Gladstones *et al*. (eds.) Lupin as crop Plants, Biology. Production and utlization, pp. 263–289.

Talhinhas P, Sreenivasaprasad S, Neves-Martins J, Oliveira H. 2002. Genetic and morphological characterization of *Colletotrichum acutum* causing anthracnose of lupins. *Phytopathology*, 92(9): 986-996.

Tarakanovas P. 2002. Data transformation of biological experiments using a computer program ANOVA. *Zemdirbyste-Agriculture*, 77: 170-180.

Thomas GJ, Adcock KG. 2004. Exposure to dry heat reduces anthracnose infection of lupin seed. *Australasian Plant Pothology*, 33(4): 537–540.

Van Der Plaats-Niterink AJ. 1981. Monograph of the Genus *Pythium*. Centraal bureau voor Schimmel cultures *Baarn*,. *Studies in Mycology*, 21: 1–242.

Yakusheva AS, Soloviyanova NN. 2002. Some biological peculiarities of lupin anthracnose agent. Integrated systems of plant protection. The present and the Future. [Materials of the International Scientific Conference devoted to the 90th anniversary of the birth of the Corresponding-Member of the AS RB A. L. Ambrosov and the 65th anniversary of the birth of the Academician of the AAS RB V.F. Samersov Minsk-Prilukii, 15–17 July, 2002], 161–162.