ŽEMĖS ŪKIO MOKSLAI. 2006. Nr. 2. P. 24–36

Measures suppressing the development of fungi on *Lupinus luteus* L.

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During the period 2001–2003, in research facilities of the Vokė Branch of the Lithuanian Institute of Agriculture, investigations in order to determine the impact of various seed treatment preparations (Vitavax, Fundazol, Panoctine, Maxim, Maxim Star, Raxil) upon micromycetes detected on lupine seeds and in soil were performed. The prevailing soils were sandy loam on carbonated fluvioglacial eluviated gravel (JDp) Haplic Luvisols (Lvh). The Lithuanian variety 'Augiai' of *Lupinus luteus* was grown. Micromycetes were investigated on seeds, roots, aboveground parts of plants and in rhizosphere soil.

The biological efficiency of the preparations Maxim 2.0 l t^{-1}; Raxil 1.5 kg t^{-1}; Maxim Star 1.5 l t^{-1} which suppressed the development of *Colletotrichum gloeosporioides* and other fungi as well as stimulated the growth of lupines should be noted. Under the action of Vitavax, Panoctine and Maxim star the number of fungal species on seeds reduced to 1–2. Treatment of seeds with a chemical preparations had a different impact on the spreading of various groups of microorganisms in plant rhizosphere. The number of fungi colony forming units (cfu) decreased when seeds were treated with Vitavax, Fundazol and Maxim, while fungal species diversity decreased only under the action of Maxim. The number of *Streptomyces* and some mineral nitrogen assimilating bacteria decreased when seeds were treated with Panoctine, Maxim star and Raxil. In another trial where Vitavax 200 FF 2,0 l t^{-1} was used for seed treatment, lupines were sprayed with the following fungicides: Sportak 45% 1.0 l ha^{-1}; Ronilan DF 50% 1.0 kg ha^{-1}; Bravo 2.0 l ha^{-1}; Juventus 1.25 l ha^{-1}; Folicur BT 25.5% 1.0 l ha^{-1}. The applied fungicides did not protect the stems of lupines 'Augiai' from infection by *Colletotrichum gleosporioides* and other fungi. Roots of plants sprayed with the above-mentioned fungicides were somewhat less infected; the amount of *Alternaria alternata, Pythium intermedium, Thielaviopsis basicola* fungi reduced. The number of fungal species was lower on roots, stems and new yield seeds after application of the fungicide Bravo. Sportak 45% and Juventus were less efficient.

Key words: *Lupinus luteus*, micromycetes, infection, seed treatment preparations, fungicides

INTRODUCTION

When crops, vegetables, garden plants are cultivated under conditions of ecological farming, the issues of their nutrition as well of favourable conditions for their growth and development are essential. Nowadays green manure is frequently used for soil fertilization and therefore the areas under *Lupinus luteus* L. are increasing. Lupines enrich the soil with various substances and intensify the activity of biota developing in the soil.

A whole spectrum of interacting physical and chemical factors contributes to the varied nature of the soil habitat and hence determines the composition and activity of the soil biota of a particular site and time. The organic matter content of the soil, consisting of plant, animal, and microbial residues in various stages of decay, represents the main source of microbial nutrition. Most of soil heterotrophs are saprophytes utilizing dead organic matter. The breakdown of cellulose comprising the largest amount of plant residues is a good example of this saprophytic activity. A diverse soil microbial population produces nutrient mineralizing enzymes such as deaminases, phosphatases and sulphatases, and these microbes are particularly abundant in the rhizosphere where organically bound nitrogen, phos-

phorus, sulphur and other nutrients are continually released. Different types of soil organic matter tend to have a different composition of soil microbes. Lupines grown for green manure help to improve soil quality and change the conditions of micromycete development [26, 39, 23, 20, 18].

In any study involving soil biological activity, it is not sufficient simply to report the moisture content of the soil in question. This has little or no value in terms of how much of soil water is available to soil biota. A sandy soil may have a low water content, but most of this will be available to plant roots. Clay loam may have a higher water content but a roughly similar amount of water available to the plant roots. Microbial water relations are quite different from that of the plant. Although not all soil microbes are tolerant to water salts stress, some can grow under stresses much greater than most plants. Microbes are, in effect, permeable bags, without significant turgor and with identical water potentials, both inside and outside the cell. Microbial cells counterbalance the water potential stress for their soil environment partly through selective transport of soil salts. Soil water not only directly affects the growth and activity of the soil biota but also mediates effects through the supply of nutrients to the organisms in question. Maximum biological activity generally occurs at soil water potentials approximating to 40% of the soil water-holding capacity. Processes occurring after insertion of green mass of lupines into soil are strongly influenced by metabolites that form during the decomposition of the green mass. Here temporal, very specific communities of microorganisms and particularly micromycetes form. In these communities, the populations of the micromycete species, at the moment participating in the decomposition of the lupine biomass, prevail. While defining and describing such communities, attention is focused on the species composition, population density, nutrition requirements, and functional features that determine their relations with other biota and components of the environment. Considerable attention is currently being given to the strategies that heterotrophic soil microbes adapt to grow in soil under conditions of low and high availability of carbon and other nutrients [26, 39, 18].

Soil biological growth, particularly of plant root systems, can cause short-term soil acidification, because it tends to remove base cations such as calcium, sodium, potassium and magnesium in exchange for hydrogen ions. The microbial decomposition of organic matter also tends to increase soil acidity through the production of organic acids. Therefore, application of lupines for green manure changes the soil composition and consequently the conditions for microorganism development. Different groups of soil microbes have a well established pH optimum and ranges for growth; strains may somehow adapt to grow at pH values beyond the normal pH range of the species. Microorganisms parasitising plants are frequently sensitive to such conditions.

Temperature, a soil property of great biological significance, not only directly affects the rates of physiological reactions, but also has many indirect effects on soil biological activity through temperature-induced changes to other aspects of the soil physiochemical environment, such as diffusion rate, mineral weathering rates, redox potentials, water activity, etc. Temperature is an important ecological factor determining the intensity of lupine decay and the composition of microorganism communities during separate stages of the decay. When intensive decomposition of lupine green mass inserted into soil starts, the temperature considerably increases. Only a few degrees above the optimal temperature cause a dramatic fall in activity as a result of thermal denaturation of proteins and membranes (thermophilic soil organisms possess a great heat stability of these components). In most soils with mesophilic microbial community there is an approximate doubling of microbiological activity for each 10 °C rise in temperature between 0 °C and 30–35 °C [26, 27, 1].

Microbial biomass in soil is the driving force of most terrestrial ecosystems, because it is this biomass that largely controls the rates of turnover and mineralisation of organic substrates. The fungi, as a group, are the organotrophs primarily responsible for the decomposition of organic residues. In terms of biomass (not numbers), it is the fungi that generally dominate in soil microbiota. Fungi have a vast range of functions in soil, including their roles as plant symbionts, plant and animal pathogens, as oligotrophs, and carnivores. The most important ecological role of fungi in soil is the decomposition of organic matter from simplest sugars and amino acids to the most resistant polymers such as lignin and complex soil humic acids. In some cases the adaptation of a symbiotic strategy by a fungus has been associated with the loss of competitive saprotrophic ability, such as the loss of cellulolytic and ligninolytic enzymes, and with a stimulation of growth in the rhizosphere [2, 18]. The majority of soil-borne fungi belong to the *Deuteromycotina* group, also known as morphic fungi, *Deuteromycetes, fungi imperfecti*, mitosporic fungi, conidial fungi, called so because no sexual stage has been found for these fungi. These are fungi that disseminate by propagules not formed from cells where meiosis has occurred. Most of these propagules can be referred to as conidia, but some are derived from unspecialized vegetative mycelium. These fungi, which comprise more than half of the species of the entire soil fungi community, include *Penicillium, Aspergillus, Fusarium, Gliocladium, Scopulariopsis, Paecilomyces, Acremonium, Alternaria, Ulocladium, Drechslera, Cladosporium, Verticillium, Rhizoctonia* and many other genera. The majority of the *Deuteromycotina* are saprobic in soil, but again, many are parasitic on other fungi, higher plants, animals, and humans.

Fungi belonging to other systematic groups are also found in soil, e.g., *Mastigomycotina: Phytophthora, Pythium*. They reproduce asexually by motile zoospores

and sexually to produce thick-walled oospores. The *Zygomycotina:* fungi mostly ascribed to the *Mortierella, Mucor, Rhizomucor, Rhizopus, Absidia, Zygorhynchus* genera. They produced non-motile asexual spores in a specialized body or sporangium and hyphae without cross walls. They mainly exist in the spore form where there is a readily available substrate. The *Ascomycotina* fungi from the *Sordaria, Chaetomium, Colletotrichum, Sclerotinia, Erysiphe,* etc. genera comprising both septate, with hyphal cross walls, fungi and unicellular yeasts, produce sexual ascospores that are held by a sac or "ascus" contained by bodies that can take on a variety of closed (cleistothecia) and open (apothecia) forms. Many of the *Ascomycotina* fungi, such as *Erysiphe graminis,* are plant pathogens and tend only to exist in the spore form in the soil. A number of plant diseases are caused through attack by *Basidiomycotina* such as *Puccinia, Uromyces, Ustilago*; causative agents of cereal rust disease [31, 7, 24, 2, 4, 11, 33, 32, 44, 34, 19].

Attention is given to chemical substances of soil fertilization, pesticides used for soil and plant treatment, as well as to agrotechnical and agrochemical technologies applied for increasing plant productivity. The literature data show that soil microorganisms can accumulate chemical substances, change them into compounds unfavourable to the environment and its biota [5, 28, 6, 13, 30, 43, 22, 25, 36].

Fungicides of various chemical composition are used against fungi as plant disease agents. Soil fungi differently react towards these preparations. It has been determined that fungicides cause considerable changes in microorganism community in soil. The strength and consequences of this impact mostly depend upon the chemical composition of the applied compound. It is known that if the microorganism changes caused by anthropogenic factors last for 30–60 days, the soil reacts against their impact. The degree of disturbance by anthropogenic impacts was comparable with that of natural impacts, whereas its duration was significantly longer [41].

Presently synthesised fungicidal substances most often exert a selective effect upon saprophytic and parasitic fungi. The recommended rates of fungicides usually have no considerable impact upon the total number of saprophytic microorganisms in soil. Inhibition of soil enzymatic activity was noted only in case of 10 times larger rates of the test preparations [14].

Fungicides of different chemical composition are used against various plant diseases. For example, Vitavax is most frequently used against fungi of the genera *Rhizoctonia, Fusarium, Cercospora* [29, 35]. Fundazol actively suppresses the development of *Rhizoctonia solani* [42], and Sportak applied in the phase of booting for winter wheat protection reduces the spread of cercosporoses and other root diseases [15].

Many fungicidal substances are used as seed treatment preparations of cultivated plants. For example, application of Vitavax and Maxim star for flaxseed treatment reduces the number of damaged seedlings from 71.7% (control) to 26.2% and 11.7%, respectively. After application of Sportak, the number of damaged flax in the stage of "fir tree" reduced to 9.1%, while in the control variant the damage reached 56.9% [17]. Therefore, while choosing fungicides it is necessary to consider the biological properties of the protected plant as well as the impact of the preparation on soil microorganisms and plant disease agents.

The aim of the current work was to determine the impact of fungicides of different chemical composition upon the development of soil microorganisms on seeds, roots, aboveground parts of plants and in the rhizosphere of *Lupinus luteus.*

MATERIALS, CONDITIONS AND METHODS

In 2001–2003, lupines were cultivated in research facilities of the Vokė Branch of the Lithuanian Institute of Agriculture. The soil in experimental plots was sandy loam on carbonated fluvioglacial eluviated gravel (JDp) according to FAO-UNESCO classification – Haplic Luvisols (LVh) [10].

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. Spring barley was used as a 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 the experimental plots the Lithuanian variety 'Augiai' was grown. Lupines were sown in April, employing a Saxonia sowing-machine. The seed rate was 1.3 mln (150–170 kg) per ha. Lupine seeds were treated using the semi-moisturized method – 6 litres of water were used for 1 ton of seeds.

The seeds were treated with: carboxin 200 g l^{-1} + thiram 200 g l^{-1}, rates 2.0 l t^{-1} and 3.0 l t^{-1} (Vitavax 200 FF); benomyl 500 g kg^{-1} – 3.0 kg t^{-1} (Fundazol); guazatine acetate 350 g l^{-1} – 2.0 l t^{-1} (Panoctine); fludioxonil 25 g l^{-1} – 2,0 l t^{-} (Maxim); fludioxonil 18,75 g l^{-1} + cyproconazole 6.25 g l^{-1} – 1,5 l t^{-1} (Maxim star); tebuconazole 20 g kg^{-1} – 1.5 kg t^{-1} (Raxil).

During the years of lupine cultivation the meteorological conditions significantly varied (Table 1). In 2001, during sowing the temperature reached 12.7 °C, in May the amount of precipitation was 60 mm. The conditions were favourable for the lupine growth. July was warm, the average temperature was 21 °C, i. e. by 4.1 °C higher than the average many-year temperature; the amount of precipitation was 94 mm. Conditions favourable for fungi development and consequently disease spreading formed. In April and May of 2002, the amount of precipitation made only half of the many-year rate. July was warm (20,8 °C), the amount of precipitation in July and August made only half of the many year rate. Condi-

Table 1. **Metorological conditions during the vegetation period**

Month	Air temperature, °C				Precipitation, mm			
	average	2001	2002	2003	average	2001	2002	2003
April	5,7	8.5	8.0	4.9	45	58	31	40
May	12,5	12.3	15.3	13.6	60	61	31	74
June	15,7	14.3	16.7	15.2	77	39	69	65
July	16,9	21.0	20.8	19.7	78	94	33	92
August	16,3	17.8	19.9	16.8	68	52	32	105
September	11,6	11.9	12.3	12.3	65	82	59	22
Total precipitation					348	328	224	358

tions for fungi development were favourable. During the whole vegetation period of 2003 the air temperature was close to the many-year average, only in July it was by 2.8 °C lower than the average. Conditions for fungi development and spread were intermediate but worse than in 2001.

After sowing the field was rolled. To destroy weeds, the crops were sprayed with herbicide the Gesagard 2,5 kg ha^{-1}. Aiming to determine the efficiency of various fungicides for protection of maturing lupines, the areas where seeds treated with Vitavax 200 FF (rate 2.0 l t^{-1}) were sprayed with the following fungicides: prochloraz 450 l^{-}, rate 1.0 l ha^{-1} (Sportak 45%); vinclozolin 500g kg^{-1} – 1.0 kg ha^{-1} (Ronilan DF 50 %); chlorothalonil 500 gl^{-1} – 2.0 l ha^{-1} (Bravo); metkonazol 60 g l^{-1}– 1.5 l ha^{-1} (Juventus); tebuconazole 125 g l^{-1} + triadimefon 100 g l^{-1} – 1.25 l ha^{-1} (Folicur BT 22,5%). The control areas were not sprayed with fungicides.

Yellow lupines were cultivated according to agrotechnological recommendations for light-textured soils. Infection of seeds and seedlings with micromycetes was determined according to Якушева (2001), Mathur and Kongsdal (2003). A piece of visible infected lupine roots or stems was cut off with a sterile scalpel and placed on malt agar medium supplemented with chloramphenicol (50 mg/l). Fungi were cultivated for 7–10 days at a temperature of 26 ± 2 °C. For the isolation of fungi from seeds, with the help of sterilized forceps 10 seeds per dish were placed on the mentioned agar medium.

The dilution method was used for the analysis of soil and samples heavily contaminated with different microorganisms. The following procedure was performed: in sterile conditions 1 g of the test 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 Petri dish to which 15 ml of malt agar was added for isolation of fungi, and starch-ammonium agar was added for bacteria, including *Streptomyces*. The microorganisms were cultivated under 26 °C for 7 days. The analysis of each sample was performed in four replications. Colony-forming units of fungi per gram of dry soil (cfu/g d. s.) were calculated [45]. Pure micromycete strains were isolated on standard Czapek, malt and corn extract media and identified according to manuals [8, 3, 12, 37, 9, 38].

RESULTS AND DISCUSSION

Cultivation of lupines in light-textured soil greatly improves the soil fertility. In the Vokė Branch of the Lithuanian Institute of Agriculture, in case of favourable meteorological conditions and application of appropriate plant protection measures, the yield of about 32.5 t ha^{-1} of green mass is obtained; up to 4.81 t ha^{-1} of dry matter was accumulated. The average amount of lupine root mass reached 5.2 t ha^{-1}, or 1.2 t ha^{-1} of dry matter. In the trials when lupine green mass was used for fertilization, about 120 kg/ha^{-1} of biological nitrogen was inserted [22].

When lupine green mass is inserted into soil, active functioning of various microorganisms starts, rapidly changing the microbiological processes; their energy source is the decomposition of lupine green mass. Various microorganisms participate in these processes; their changes depend upon the composition of the decomposed substances, abundance and constant changing. During this research, attention was focused on microscopic fungi or micromycetes. Some of them are constantly present in soil and actively participate in all its metabolic processes, while others get into soil with seeds, organic fertilizers, with dust and other pollutants. The fate of micromycete propagules newly inserted into soil is not identical. Some of them easily integrate into general metabolic processes and can start dominating, others can make these processes favourable or hazardous to people, still others settle on roots and other parts of the cultivated plants and become parasites; part of micromycetes do not survive under conditions already created in soil by other microorganisms [1. 20].

In order to prevent parasitic and otherwise harmful micromycetes from getting into soil, lupine seeds before sowing were treated with seed treatment preparations of various chemical composition: Vitavax, Fundazol, Maxim, Maxim Star, Raxil; untreated seeds were used as a control (Table 2). It was revealed that the applied seed treatment reduced the contamination of lupine

Table 2. **Micromycetes isolated before sowing from seeds of yellow lupine 'Augiai' treated with various chemical substances**

Seed treatment preparation	Rate	Isolated micromycetes
Untreated Control	–	*Alternaria alternata* (Fr.) Keissl., *Ascochyta lupinicola* Petr., *Fusarium equiseti* (Corda) Sacc., *Olpidium brassicae* (Wor.) Dang., *Penicillium chrysogenum* Thom, *Penicillium expansum* Link, *Penicillium verrucosum* Dierckx, *Phoma medicaginis* Malbr. et Roum., *Rhizoctonia solani* Kühn., *Rhizomucor pusillus* (Lindt.) Shipper, *Rhizopus oryzae* Went et Prins. Geerl., *Thielaviopsis brasicola* (Berk. ct Br.) Ferr., *Mycelia sterilia*
Vitavax	2.0 l t^{-1}	*Olpidium brassicae* (Wor.) Dang., *Mycelia sterilia*
Vitavax	3.0 l t^{-1}	*Rhizopus stolonifer* (Ehrenb. ex Fr.) Vuill., *Mycelia sterilia*
Fundazol	3.0 kg t^{-1}	*Olpidium brassicae* (Wor.) Dang., *Penicillium expansum* Link, *Penicillium lanosoviride* Thom, *Rhizopus oryzae* Went et Prins. Geerl. *Mycelia sterilia*
Panoctine	2.0 l t^{-1}	*Penicillium lanosoviride* Thom, *Thamnidium elegans* Link ex Gray, *Mycelia sterilia*
Maxim	2.0 l t^{-1}	*Aspergillus fumigatus* Fresen., *Chaetomium globosum* Kunze, *Penicillium diversum* Raper et Fennell, *Penicillium expansum* Link, *Pythium intermedium* de Bary, *Ramularia lupine* J. J. Davis
Maxim Star	1.5 l t^{-1}	*Mycelia sterilia*
Raxil	1.5 l t^{-1}	*Cladosporium herbarum* (Pers.) Link ex G ray, *Fusarium equiseti* (Corda) Sacc., *Gónatóbotrys simplex* Corda, *Penicillium expansum* Link, *Penicillium ochrochloron* Biourge, *Penicillium velutinum* T. H. Beyma, *Rhizopus oryzae* Went ex Prins. Geerl., *Thielaviopsis brasicola* (Berk. et Br.) Ferr., *Mycelia sterilia*

seeds with micromycete propagules, but did not eliminate it. Maxim Star, Vitavax, and Panoctine were somewhat more efficient against micromycete infection. Not all fungi reacted equally to the seed treatment. On untreated seeds, *Alternaria alternata, Ascochyta lupinicola, Fusarium equiseti, Olpidium brassicae, Thielaviopsis basicola,* some species of the *Penicillium* and *Rhizopus* genera could be described as dominant, but they were not recorded on seeds treated with some of the above-mentioned preparations. For example, *Alternaria alternata, Ascochyta lupinicola, Fusarium equiseti, Thielaviopsis brasicola* survived only on seeds treated with Raxil; *Olpitrichum brassicae* was recorded on seeds treated with Vitavax (2.0 l t^{-1}) and Fundazol (3.0 kg t^{-1}). Under experimental conditions, *Aspergillus fumigatus, Chaetomium globosum, Penicillium expansum, Pythium*

intermedium reacted to Maxim (2.0 l t^{-1}). Sterile mycelium (*Mycelia sterilia*) was detected on both treated and untreated seeds. In some cases, after a longer period of time, it was identified as *Sclerotinia slerotiorum* and in other cases remained unidentified. *Colletotrichum gloeosporioides* had been isolated neither from treated nor from untreated lupine seeds, while the disease, anthracnose, caused by this fungus, reached 73.3% during later stages of plant development (July–August).

The impact of various seed treatment preparations upon soil microorganisms can be judged by the data of Table 3. It presents micromycetes isolated from the rhizosphere soil of lupines 'Augiai' grown from seeds treated with various seed treatment preparations. In rhizosphere soil of the control lupines, micromycetes typical of light-textured soils of Lithuania prevailed [44]. Among these, there are *Sclerotinia sclerotiorum* fungi characterized by a stronger pathogenicity, but during this research they were not recorded in the rhizosphere soil of lupines. Probably Vitavax suppressed their development and spread. *Verticillium album* micromycetes can be ascribed to those potentially pathogenic; *Acremonium strictum* under certain conditions can also cause lupine root rot. These fungi were isolated from the rhizosphere soil of lupines grown from seeds treated with Fundazol. In this soil, *Sclerotinia sclerotiorum*, *Verticillium album*, and *Pythium spp.* fungi were also recorded. So, the application of Fundazol did not eliminate the negative impact of potential pathogenic micromycetes, but stimulated the activity of many typical soil-borne micromycetes, reduced their number (Figure). In soil of the experimental plots under seeds treated with Panoctine (Table 3), micromycetes of the genus *Penicillium* dominated, potentially pathogenic micromycetes able to cause root rots *Fusarium oxysporum*, *Pythium sylvaticum*, *Venticillium album* were also isolated. A rather species-poor micromycete composition was revealed in the rhizosphere soil of experimental plots sown with lupine seeds treated with Maxim (2.0 l t^{-1}). Here micromycetes of the genus *Trichoderma* prevailed; they suppressed other micromycetes. Probably even a small amount of this preparation positively affected the physiological functions of *Trichoderma* fungi and induced their growth. Therefore, the viability and activity of other fungi slowed down. Meanwhile, from the rhizosphere soil of lupine seedlings grown from seeds treated with Maxim Star (1.5 l t^{-1}) micromycetes of 14 species were isolated; no evident pathogens were among them. The recorded *Fusarium oxysporum* and *Sclerotinia sclerotiorum* micromycetes are also ascribed to potentially pathogenic. *Mycelia sterilia* is abundant. In the rhizosphere soil of lupines grown from seeds treated with Raxil (1,5 kg t^{-1}) *Penicillium* and *Mortierella* fungi were most abundant. The amount of *Fusarium* micromycetes of *F. equiseti*, *F. graminearum*, *F. oxysporum* increased. *Thielaviopsis brasicola* micromycetes causing dry rot were recorded, although they are rarely detected in this stage of plant development. The highest number of micromycete propagules – colony forming units (cfu) – per one gram of dry soil (g d. s.) was recorded in the plot under seeds treated with Panoctine 229258 cfu / g d. s. (Figure, A–C), lower numbers were revealed in cases of Maxim Star – 142388; Raxil – 110254; Vitavax (rate 3,0 l t^{-1}) – 103939, in other cases the numbers did not exceed those of the control – 99448 cfu / g d. s. Only in case of Raxil the number of streptomycetes isolated from the rhizosphere soil of seedlings (10.5×10^6) was higher than in the control (9.0×10^6); the lowest number of streptomycetes was isolated in case of Vitavax treatment (3.9×10^6 cfu / g d. s.). A considerably higher number of bacteria was recorded in lupine rhizosphere soil when Maxim was

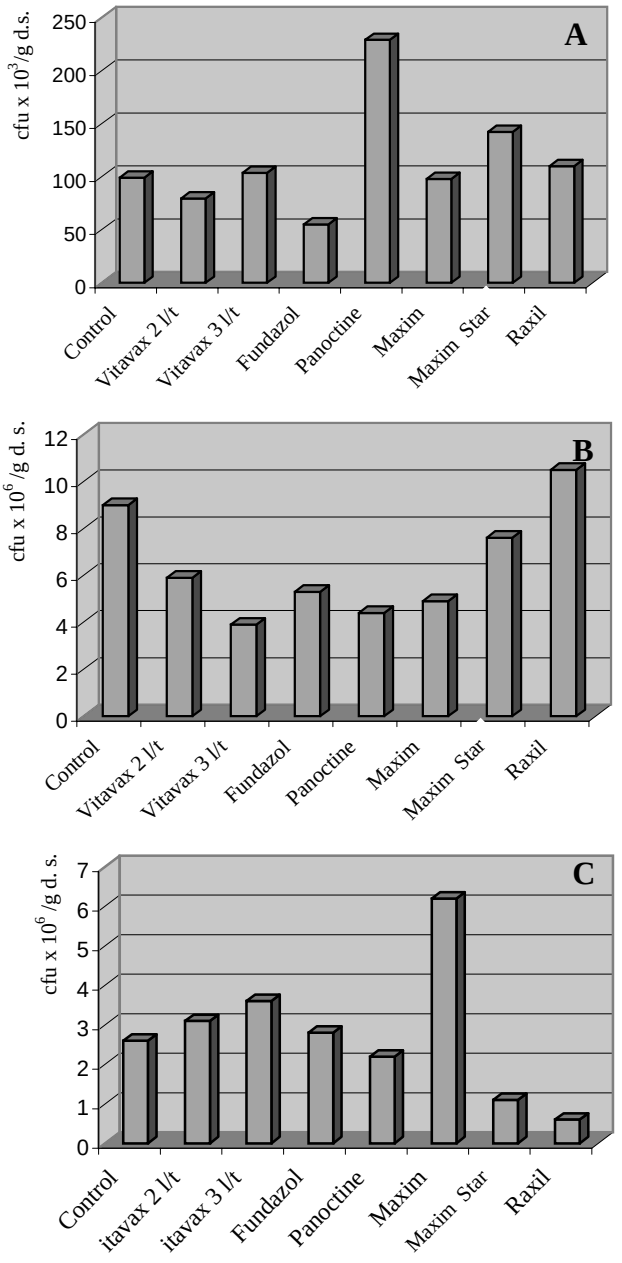

Figure. The number of colony forming units of microorganisms (A – fungi, B – actinomycetes, C – bacteria) in soil under treated lupine seeds

Table 3. **Micromycetes isolated from the rhizosphere soil of germinated yellow lupines 'Augiai'**

Seed treatment preparation	Rate	Isolated micromycetes
Untreated	–	*Acremonium charticola* (Lindau) W. Gams, *Acremonium* spp., *Mortierella hyalina* (Harz) W. Gams, *Penicillium lilacinum* Thom, *Penicillium ochrochloron* Biourge, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Talaromyces flavus* (Klöcker) Stolk et Samson, *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers., *Mycelia sterilia*
Vitavax	2.0 l t^{-1}	*Acremonium roseum* Petch, *Acremonium strictum* W. Gams, *Mortierella hyalina* (Harz) W. Gams, *Mortierella polycephala* Coem., *Oidiodendron echinulatum* G. L. Barron, *Penicillium adametzii* K. M. Zalessky, *Fusarium funiculosum* Thom, *Penicillium ochrochloron* Biourge, *Penicillium paxilli* Bainier
Vitavax	3.0 l t^{-1}	*Acremonium charticola* (Lindau) W. Gams, *Acremonium strictum* W. Gams, *Mucor globosus* A. Fisch., *Mucor hiemalis* Wehmer, *Penicillium canescens* Sopp, *Penicillium ochrochloron* Biourge, *Penicillium piscarium* Westling, *Rhizomucor pusillus* (Lindt) Schipper, *Verticillium album* (Preuss) Pidopl., *Zygorhynchus moelleri* Vuill., *Mycelia steriila*
Fundazol	3.0 l t^{-1}	*Acremonium charticola* (Lindau) W. Gams, *Acremonium strictum* W. Gams, *Mortierella hyalina* (Harz) W. Gams, *Mortierella humicola* Oudem., *Pythium* spp., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Trichoderma koningii* Oudem., *Trichosporiella cerebriformis* (G. A. de Vries et Kleine-Natrop) W. Gams, *Verticillium album* (Preuss) Pidopl., *Mycelia sterilia*
Panoctine	2.0 l t^{-1}	*Acremonium* spp., *Aspergillus versicolor* (Vuill.) Tirab., *Fusarium oxysporum* Schltdl., *Mortierella alpina* Peyronel, *Oidiodendron rhodogenum* Robak, *Penicillium chrysogenum* Thom, *Penicillium funiculosum* Thom, *Penicillium ochrochloron* Biourge, *Pythium sylvaticum* Campbell et Hendrix, *Trichoderma viride* Pers., *Verticillium album* (Preuss) Pidopl., *Mycelia sterilia*

Table 3 (continued)

Maxim	2.0 l t^{-1}	*Acremonium charticola* (Lindau) W. Gams, *Mucor spp.*, *Trichoderma viride* Pers.
Maxim Star	1.5 l t^{-1}	*Fusarium oxysporum* Schltdl., *Gliocladium radicicola* Pidopl., *Mortierella humicola* Oudem., *Mortierella hyalina* (Harz) W. Gams, *Mucor globosus* A. Fisch., *Mucor racemosus* Fresen., *Penicillium expansum* Link, *Penicillium funiculosum* Thom, *Penicillium ochrochloron* Biourge, *Rhizopus stolonifer* (Ehrenb. ex Fr.) Vuill., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Mycelia sterilia*
Raxil	1.5 kg t^{-1}	*Aspergillus terreus* Thom, *Chaetomium globosum* Kunze, *Fusarium equiseti* (Corda) Sacc., *Fusarium graminearum* Schwabe, *Fusarium oxysporum* Schltdl., *Mortierella hyalina* (Harz) W. Gams, *Mortierella stylospora* Dixon-Stewart, *Oidiodendron rhodogenum* Robak, *Penicillium lanosum* Westling, *Penicillium ochrochloron* Biourge, *Penicillium simplicissimum* (Oudem.) Thom, *Penicillium spinulosum* Thom, *Thielaviopsis brasicola* (Berk. et Br.) Ferr., *Mycelia sterilia*

Table 4. **Abundance (%) of streptomycete groups in soil after germination of 'Augiai' seeds treated with various preparations**

Streptomyces series	Seed treatment preparation								Total
	Conrol (untreated)	Vitavax 2 l t^{-1}	Vitavax 3 l t^{-1}	Fundazol 3 kg t^{-1}	Panoctine 2 l t^{-1}	Maxim 2 l t^{-1}	Maxim Star 1.5 l t^{-1}	Raxil 1.5 l t^{-1}	
Isolated strains	80	45	38	46	40	39	72	90	450
Albus	2.4	20	5.3	6.5	7.5	25.6	22.2	33.3	16.7
Albocoloratus	7.5	6.7	-	2.2	17.5	5.1	11.1	4.4	6.7
Achromogenes	57.5	33.3	71	80.4	45	64.1	23.6	54.4	52
Chromogenes	31.2	28.9	15.8	6.5	25	-	41.7	6.6	20.7
Flavus	-	4.4	-	2.2	-	2.6	-	-	0.9
Fradiae	1.2	2.2	5.3	-	5	2.6	-	-	1.6
Ruber	-	-	-	2.2	-	-	1.4	1.1	0.7
Violaceus	-	4.4	2.6	-	-	-	-	-	0.7

used (2.0 l t^{-1}) – 6.2 × 10^6, control – 2.6 × 10^6; the lowest numbers were registered in cases of Raxil – 0.6 × 10^6 and Maxim Star – 1.1 × 10^6 cfu / g d. s. treatment.

Differences in the abundance of streptomycete groups in soil under yellow lupine 'Augiai' treated with various preparations were noted (Table 4). In soil of the control, streptomycetes ascribed to five groups and in soil under seeds treated with Vitavax (2.0 l t^{-1}) streptomycetes of seven groups were recorded. In soil of the control variant, streptomycetes of the *Achromogenes* group dominated; they made 57.5% of all isolates, *Chromogenes* – 31.2%, *Albocoloratus* – 7.5%, *Albus* – 2.4%, *Fradiae* – 1.2%. Application of Vitavax (2.0 l t^{-1}) had a negative effect on the *Achromogenes* streptomycetes; their number reduced to 33.3%, and when Vitavax

Table 5. **Micromycetes detected on yellow lupine 'Augiai' dry seeds of a new yield**

Fungicide	Fungicide rate	Isolated micromycetes
Untreated seeds	–	*Alternaria alternata* (Fr.) Keissl. *Colletotrichum* spp. *Olpidium brassicae* (Wor.) Dang. *Penicillium expansum* Link., *Mycelia sterilia* Bacteria
Prochloraz 450 l^{-1} (Sportak 45%)	1.0 l ha^{-1}	*Alternaria tenuissima* (Kunze ex Pers.) Wiltshire *Cladosporium cladosporioides* (Fresen.) G.A.deVries *Fusarium* spp. *Mucor piriformis* A. Fisch. *Sclerotinia sclerotiorum* (Lib.) de Bary, *Stemphylium ilicis* Tengwall *Mycelia sterilia* Bacteria
Vinclozolin 500 g kg^{-1} (Ronilan DF 50%)	1.0 kg ha^{-1}	*Aspergillus niger* Tiegh. *Cladosporium cladosporioides* (Fresen.) G. A. de Vries, *Geomyces pannorum* (Link) Sigler et J. W. Carmich, *Olpidium brassicae* (Wor.) Dang. *Pythium intermedium* de Bary, *Mycelia sterilia*, Bacteria
Chlorothalonil 500 g l^{-1} (Bravo)	2.0 l ha^{-1}	*Alternaria radicina* Meier, Drechsler et E. D. Eddy *Cladosporium cladosporioides* (Fresen.) G. A. de Vries, *Olpidium brassicae* (Wor.) Dang., *Mycelia sterilia*, Bacteria
Metkonazol 60 g l^{-1} (Juventus)	1.25 l ha^{-1}	*Alternaria alternata* (Fr.) Keissl, *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire, *Cladosporium cadosporioides (Fresen.) G. A. de Vries,* *Olpidium brassicae* (Wor.) Dang, *Pythium intermedium* de Bary, Yeasts Bacteria
Tebuconazole 125 g l^{-1} + triadimefon 100 g l^{-1} (Folicul BT 22.5)	1.0 l ha^{-1}	*Alternaria alternata* (Fr.) Keissl, *Aspergillus niger* Tieghm. *Botrytis cinerea* Pers. et Fr., *Cladosporium cladoporioides* (Fresen.) G. A. de Vries, *Fusarium culmorum* (Wm. G. Gm.) Sacc., *Olpidium brassicae* (Wor.) Dang., *Rhizopus oryzae* Went ex Prins. Geerl, *Mycelia sterilia*, Bacteria

(3.0 l t^{-1}) was applied the number of this streptomycete group increased up to 71%. A considerable increase in the amount of *Achromogenes* streptomycetes in the rhizosphere soil was determined in the case of Fundazol (80.4%); the lowest amount of streptomycetes was recorded in the case of Maxim Star (23.6%). Therefore, seed treatment and chemical substances that get into seeds exert a certain influence on the other biots of the environment, change their functions, and influence the whole system.

Some seed treatment preparations positively acted on the further growth of lupines. For example, plants grown from seeds treated with Vitavax and Maxim were by 4–8 cm higher than the control plants.

Yellow lupines are very susceptible to various fungal diseases, therefore, in the variant where lupine

Table 6. **Micromycetes isolated from infected stems and roots of yellow lupines 'Augiai' 3 weeks after spraying with fungicides**

Fungicide	Fungicide rate	Isolated micromycetes	
		stems	roots
Control Untreated	–	*Colletotrichum gloeosporioides* (Penzig.) Sacc. *Colletotrichum* spp. *Fulvia fulvum* (Cooke) Cif. *Sclerotinia sclerotiorum* (Lib.) de Bary *Mycelia sterilia*	*Alternaria alternata* (Fr.) Keissl. *Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Pythium intermedium* de Bary *Rhizopus cohnii* Berl. et. de Toni *Thielaviopsis basicola* (Berk. et Br.) Ferr. *Mycelia sterilia*
Prochloraz 450 g l^{-1} (Sportak 45%)	1.0 l ha^{-1}	*Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Colletotrichum gloeosporioides* (Penzig.) Sacc. *Colletotrichum dematium* (Pers. ex Fr.) Grove *Penicillium stoloniferum* Thom *Mycelia sterilia*	*Colletotrichum* spp. *Fusarium oxysporum* Schltdl. *Mucor mucedo* Fresen. *Rhizopus oligosporus* Saito *Mycelia sterilia*
Vinclozolin 500 g kg^{-1} (Ronilan DF 50%)	1.0 kg ha^{-1}	*Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Colletotrichum gloeosporioides* (Penzig) Sacc. *Colletotrichum* spp. *Fulvia fulvum* (Cooke) Cif. *Sclerotinia sclerotiorum* (Lib.) de Bary *Mycelia sterilia*	*Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Fulvia fulvum* (Cooke) Cif. *Fusarium moniliforme* J. Sheld. *Fusarium* spp. *Penicillium expansum* Link. *Mycelia sterilia*
Chlorothalonil 500 g l^{-1} (Bravo)	2.0 l ha^{-1}	*Cladosporium herbarum* (Pers.) Link ex Gray *Colletotrichum gloeosporioides* (Penzig) Sacc. *Colletotrichum* spp. *Fulvia fulvum* (Corda) Cif.	*Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Mucor mucedo* Fresen. *Mycelia sterilia*
Metkonazol 60 g l^{-1} (Juventus)	1.25 l ha^{-1}	*Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Colletotrichum gloeosporioides* (Penzig) Sacc. *Glomyces* spp. *Pagidospora amoebophila* Drechsler *Tilachlidium brachiatum* (Batsch et Fr.) Petch *Mycelia sterilia*	*Cladosporium cladosporioides* (Fresen.) G. A. de Vries *Fulvia fulvum* (Cooke) Cif. *Hormomyces aurantiacus* Brandoni et Bisalputra *Mycelia sterilia*

Note. In all treatment variants bacteria were abundant.

seeds had been treated with Vitavax FF (carboxin 200 g l^{-1} + thiram 200 g l^{-1}), rate 2.0 l t^{-1}, at the beginning of June when first symptoms of anthracnose appeared, the plants were sprayed with the following fungicides: Sportak 45%, Ronilan DF 50%, Bravo, Juventus, and Folicur BT 22.5%. Three weeks after spraying, root and stem samples of lupines 'Augiai' were tested for the presence of micromycetes (Table 5).

Under conditions favourable for the development of fungi, three weeks after lupine spraying with Sportak 45%, *Colletotrichum gleosporioides* and *C. dematium* fungi were isolated from plant stems. On roots, fungi of this genus were developing slower, therefore, they were not identified for the species. *C. gleosporioides* also developed on lupine stems treated with Ronilan DF 50%. In this variant, on seeds these fungi were not recorded. The fungi were also detected on

lupine stems sprayed with the Juventus and Folicur fungicides, but they were not isolated from roots. *Cladasporium cladosporioides* fungi are rather resistant to the impact of fungicides, and they were constantly recorded on both stems and roots of lupines. Resistant to fungicides were *Cladosporium herbarum, Fulvia fulvum,* some fungi of the genera *Penicillium* and *Fusarium* (Table 6). Folicur had a weak impact upon *Verticillium alboatrum* and some *Fusarium* fungi that cause lupine root rot. In 2001 and 2003 the meteorological conditions were very favourable for the spreading of fungal diseases. Although fungicides were applied immediately after noticing the first symptoms of infection, the vast spreading of anthracnose and powdery mildew were not avoided. The causative agents of powdery mildew specifically reacted to the impact of fungicides. For example, the mycelium of *Erysiphe communis* evidently thickened, but in some places it totally disappeared. This fungus is often referred to as *Erysiphe trifolii* Grev. [16]. Usually fungi of this species are characterized by a white, web-like, powdered mycelium situated on both sides of a leaf. Under the influence of certain fungicides the mycelium thickens, while under the influence of other fungicides it becomes thinner or disappears; only single elliptical conidia survive, which remain viable, the colour of cleistothecia changes, the number of fulcra reduces, their branching changes.

Fungicides used in the above-mentioned concentrations changed the functional properties of fungi, usually suppressed them but did not stop the fungal action, and with improving environmental conditions the viability of fungi increased together with the hazard they caused to plants; the phytopathogenic potential of the environment increased.

Micromycetes isolated from seeds of a new yield are presented in Table 6. Only in seed samples of the control variant *Colletotrichum* spp. propagules were recorded. This fungus was not recorded on seeds of a new yield when fungicides had been used. However, the potentially pathogenic fungi *Alternaria alternata, A. radicina, Cladasporium clodosporioides, Olpidium brassicae* prevailed in this case, and after the application of some fungicides *Aspergillus niger* and *Botrytis cinerea* fungi prevailed. Seeds of yellow lupine intended for the next year sowing are not free from fungal infection; therefore, they should be appropriately stored and prepared for sowing.

The lupines 'Augiai' are usually supposed to be resistant to fusariosis [40]. It was only partly confirmed by the investigations. Three weeks after treatment with fungicides, in roots of both the control variant and in variants treated with Sportak 45%, Ronilan DF 50%, and Folicur, fungi of the genus *Fusarium* were detected (*Fusarium oxysporum, F. moniliforme, Fusarium* sp.). Propagules of the fungi of this genus were also detected on seeds of a new yield grown in experimental plots sprayed with Folicur (1.25 l ha^{-1}).

CONCLUSIONS

1. The reaction of various groups of microorganisms in lupine rhizosphere to the fungicides was different. The number of fungi decreased when seeds were treated with Vitavax, Fundazol and Maxim; the number of mineral nitrogen assimilating bacteria was reduced by Panoctine, Maxim Star and Raxil, while *Streptomyces* were suppressed by all the fungicides except Raxil.

2. Changes in the abundance of *Streptomyces* groups in soils under yellow lupines 'Augiai' treated with different seed treatment preparations were observed. In soil of the control variant where no seed treatment was applied, *Streptomyces* of five groups were recorded, and in the case of Vitavax (2.0 l t^{-1}) *Streptomyces* of seven groups were found. Fungal species diversity in lupine rhizosphere decreased only under the action of Maxim.

3. A single spraying of yellow lupines with the fungicides Sportak 45%, Ronilan DF 50%, Bravo, Juventus, and Folicur somewhat suppressed the functional abilities of fungi but did not block them completely. With improving the environmental conditions, the viability of fungi increased together with the hazard they caused to plants; the phytopathogenic potential of the environment increased. A lower diversity of fungal species was found on seeds of the new yield of plants treated with Bravo.

4. Obtaining healthy seeds of yellow lupines as well as reducing the phytopathogenic pollution potential of soil and environment are the issues of utmost importance, especially when the green mass of lupines and other agricultural plants is used for soil fertilization.

Received 7 February 2006

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GRYBŲ VYSTYMĄSI ANT *LUPINUS LUTEUS* L. SLOPINANČIOS PRIEMONĖS

Santrauka

2001–2003 m. Lietuvos žemdirbystės instituto Vokės filialo bandymų lauke, kuriame vyravo priesmėlio ant karbonatinio fliuvioglacialinio žvyro paprastojo išplautžemio dirvožemis (Idp) Haplic Luvisols (LVh), atlikti bandymai siekiant nustatyti įvairių beicų: vitavakso, fundazolo, panoktino, maksimo, maksimo star ir raksilo poveikį dirvožemyje aptinkamiems ir lubinus pažeidžiantiems mikromicetams. Auginta Lietuvoje išvestos ‘Augiai’ veislės geltonžiedžiai pašariniai lubinai. Mikromicetai tirti augalo šaknyno zonos dirvožemyje, ant augalo šaknų ir antžeminių dalių įvairiais vegetacijos tarpsniais bei sėklų.

Nustatytas preparatų maksimo 2,0 l t^{-1}; raksilo 1,5 kg t^{-1}; maksimo star 1,5 l t^{-1} efektyvumas slopinant *Colletotrichum gloeosporioides* ir kitų grybų vystymąsi ir skatinant lubinų augimą. Ant sėklų, beicuotų vitavaksu, panoktinu ir maksimu star, aptikta po 1–2 rūšis grybų. Sėklų beicavimas skirtingai veikė įvairių mikroorganizmų grupių gausumą dirvožemyje: grybų pradų sumažėjo panaudojus beicavimui vitavaksą, fundazolą ir maksimą, streptomicetų sumažėjo visuose bandymo variantuose (išskyrus naudojant raksilą), kitų bakterijų skaičių neigiamai veikė sėklų apdorojimas panoktinu, maksimu star ir raksilu. Dėl beicų poveikio lubinų daigų rizosferoje rūšių nesumažėjo (išskyrus variantą, kuriame sėklos beicuotos maksimu). Panaudoti fungicidai neapsaugojo ‘Augiai’ veislės geltonžiedžių lubinų stiebų nuo *Colletotrichum gleosporioides* ir kitų grybų pažaidos. Mažiau pažeistos buvo minėtais fungicidais nupurkštų augalų šaknys, sumažėjo *Alternaria alternata, Pythium intermedium, Thielaviopsis basicola* grybų. Ant sėklų, išaugusių ant fungicidais apdorotų augalų, aptikta mažiau grybų rūšių, ypač naudojant bravo. Spartako 45% ir juventus fungicidų poveikis buvo silpnesnis. Preparatų efektyvumas labai priklausė nuo meteorologinių sąlygų, ypač kritulių gausos.

Raktažodžiai: geltonžiedžiai lubinai, mikromicetai, beicai, fungicidai

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СРЕДСТВА ПОДАВЛЕНИЯ РАЗВИТИЯ ГРИБОВ НА *LUPINUS LUTEUS* L.

Резюме

В 2001–2003 гг. на опытных полях Вокеского филиала Литовского института земледелия, где преобладала супесчаная почва на карбонатном флювиогляциальном гравиальном простого размытия слое (Idp Haplic Luvisols LVh), проведены опыты с целью установить, как различные химические препараты (витавакс, фундазол, паноктин, максим, максим стар и раксил) действуют на микромицеты, встречаемые в почвах и паразитирующие на люпине. Выращивался желтоцветный кормовой люпин сорта „Augiai“, выведенный в Литве. Исследовались микромицеты в почве прикорневой зоны, на корнях и надземных частях растений в разные периоды вегетации, а также на семенах.

Выявлена способность препаратов максима 2,0 л $т^{-1}$; раксила 1,5 кг $т^{-1}$; максима стар 1,5 л $т^{-1}$ подавлять развитие *Colletotrichum gloeosporioides* и других грибов, при этом активируя рост люпина. На обработанных химическими препаратами витавакс, паноктин и максим стар семенах выявлены микромицеты одного–двух видов. Химические препараты оказывали различное влияние на численность разных групп микроорганизмов в почве. Сокращение микромицетов отмечено после обработки семян витаваксом, фундазолом и максимом, стрептомицетов – во всех вариантах опыта, за исключением применения раксила. Отрицательное воздействие на численность бактерий оказали паноктин, максим стар и раксил. Почти все исследованные химические препараты (кроме максима) не оказали влияния на разнообразие видов микромицетов, развивающихся в почве ризосферы люпина. Использованные фунгициды не защитили стебли желтоцветного люпина „Augiai“ от поражения *Colletotrichum gloeosporioides* и другими грибами. Обработанные вышеуказанными химическими препаратами корни люпина были менее поражены: сократилась численность *Alternaria alternata, Pythium intermedium, Thielaviopsis basicola.* На семенах растений, полученных из семян, обработанных фунгицидами (особенно браво), обнаружено меньше видов микромицетов. Воздействие фунгицидов спартак и ювентус было гораздо слабее. Эффективность воздействия препаратов заметно зависит от метеорологических условий, в особенности от количества осадков.

Ключевые слова: желтоцветный люпин, микромицеты, повреждения, фунгициды