УДК 57.063.8:630.332.2

G. A. Volchenkova, PhD student (BSTU); **V. B. Zvyagintsev**, PhD (Biology), assistant professor, head of department (BSTU); **A. V. Savitski**, student (BSTU)

SCREENING OF *PHLEBIOPSIS GIGANTEA* (FR.) JÜLICH STRAINS ACCORDING TO THEIR COLONIZATION ABILITY ON PINE STUMPS AFTER FELLINGS

Biological method, consisted in stump treatment with preparations on the basis of antagonistic fungus *Phlebiopsis gigantea* (Fr.) Jülich has a wide application in the complex of protective measures, restricting harmfulness of *Heterobasidion* spp. Efficacy of pine stump wood colonization with seven strains of *P. gigantea*, screened during laboratory experiments, was investigated for selection of effective belarusian strains of antagonist. According to conducted experiments two strains that have the best colonization ability in forming fruiting bodies on 76.0–92.6% treated stumps in six months were selected. These strains can underline development of native biological preparation.

Introduction. As a result of intensification of forest business activity and increase of anthropogenic load on biogeocenosis weakening of forest plantations and sharp reinforcement of hazardousness of phytopathogenic organisms are observed and diseases provoking as a result of their activity get epiphytotic character. Root rot, initiated by pathogenic fungi *Heterobasidion* spp. is the largest danger for coniferous plants of Belarus.

In the middle of the last century J. Rishbet put forward and proved a theory that mass spreading of pine fungus into a plant occurs because of germination of spores of the pathogen on fresh surfaces of stumps after cuttings [1]. Established fact let him make a conclusion that it is possible to decrease the number of infections and pathologic destruction of plants with the help of processing of stumps by chemical agents or artificial filling of newly cut stumps with fungi-antagonists. It is biological method based on antagonistic and competitive relationship between agents of bioprotection and pathogenic agents that was given the primary importance as application of this method in complex with other forestry-based measures permit to achieve the maximal forest protective and economic effect and avoid harmful influence on the environment [2].

As a result of numerous research a number of mycorrhizal and saprotrophic wood-decaying fungi that can be used as agents of biological protection were proposed. Among wide spectrum of tested antagonists saprotrophic wood-decaying fungus *Phlebiopsis gigantea* (Fr.) Jülich has the best characteristics [3]. Several biological preparations are developed on its base and have successful application in countries of Western Europe [4].

In connection with the increase of pine plants affected by pine fungus in the forests of Belarus and low effectiveness of forest protective measures the issue of the development of domestic biological agent on the basis of local strains *P. gigantea* is becoming urgent. The effectiveness of such agent mainly depends on individual characteristics of the using fungus strain. So, the study of antagonistic characteristics and selection of the most active isolates is an integral part in the process of creation of the biological preparation. Special attention is to be given to the fungus ability to adapt to wood substratum in natural conditions. Taking into the account above mentioned, together with laboratory research on the screening of the suitable strain, it is impossible to manage without field experiments that are the final stage on the way of search of the best agent of bioprotection.

Materials and methods. As a result of preliminary screening in 2011–2012 in laboratory conditions 7 strains of *P. gigantea* out of 46 selected isolates were chosen with faster lineal growth, better antagonistic activity towards *Heterobasidion annosum*, high wood-decaying ability and intensity of spore formation [5, 6]. In the autumn 2011 and in the spring 2012 three permanent experimental areas (PEA) for examination of the effectiveness of biological measures on limitation of harmfulness of pine fungus in Negorelskoe experimental forestry were established. Brief silvicultural and taxation characteristics of plants on PEA are the following:

– PEA No. 5 (area 60, division 8): age – 45 years, composition – 10P, type of forest – bracken pine forest, quality of locality – I, fullness – 0,8, area – 5,3 ha;

– PEA No. 6 (area 19, division 11): age – 45 years, composition – 10P, type of forest – bracken pine forest, quality of locality – I, fullness – 0,9, area – 3 ha;

– PEA No. 7 (area 60, division 9): age – 46 years, composition – 10P, type of forest – bracken pine forest, quality of locality – I, fullness – 0,9, area – 10 ha.

After improvement cuttings stump surfaces were processed with the helped of knapsack sprayer with spore suspension of different strains of *P. gigantean* in concentration of 10 mln. CFU per l [2]. On PEA No. 5 processing took place on 10^{th} of November in 2011, on PEA No. 6 – on 29^{th} of April in 2012, on PEA No. 7 – on 14^{th} of May in 2012.

On PEA No. 5 four strains of *P. gigantea* were tested, which were selected in 2011 (PG 10.6.2, PG 10.7.1, PG 10.8.3, PG 10.10.2). Each of four sections of tested area, where stump treatment was carried out, was divided into 2 subsections: on one subsection treated stumps were covered with forest litter, on the other – they were not. 118 pine stumps were treated altogether (also with strain PG 10.6.2 – 48 stumps, PG 10.7.1 – 27, PG 10.8.3 – 23, PG 10.10.2 – 20), 54 out of which appeared to be alive and the rest – stumps of trees, dried out to the moment of cutting.

A seven-section PEA No. 6 was used as an experimental object for the research of acclimation rate of six strains *P. gigantea* (PG 10.6.2, PG 10.7.1, PG 10.8.3, PG 10.10.2, PG 11.5.1, PG 11.15.3). On each section a part of stumps was on a portage and the other – under the canopy. Before treatment of stumps on a portage disks in thickness of 2–5 cm were cut from them. Each section included 4 variations of processing: newly cut stumps on a portage without covering; newly cut stumps on a portage covered with moss; newly cut stumps on a portage covered with sawn out disks; older stumps under forest canopy without covering. In total 325 stumps were treated on a portage and 207 stumps – under forest canopy.

On PEA No. 7 stump treatment was carried out with seven strains of *P. gigantea* (PG 10.6.2, PG 10.7.1, PG 10.8.3, PG 10.10.2, PG 11.3.1, PG 11.5.1, PG 11.13.1). Surfaces of stumps were renewed by sawing disks out, each section include four variations of processing: stumps without covering, stumps covered with moss, stumps covered with disks, stumps without sawing disks out without covering. In total 693 stumps of a pine were treated. In November 2012 visual stocktaking of stumps inhabited by the antagonist was carried out. Average acclimation rate of *P. gigantea* for the strain and also in accordance with the variations of processing ratio of the colonized stumps to the number of processed stumps on a relevant subsection was determined.

Main part. On PEA No. 5 calculation of stumps results of which are in table 1 was carried out a year later after inoculation.

Table 1

Acclimation rate of strains *P. gigantea* on pine stumps, % (PEA No. 5)

Strain	Variation of a test		Average acclimation rate
	Stumps without covering	Stumps covered with forest litter	
PG 10.6.2	62.5	30.0	44.4
PG 10.7.1	75.0	21.4	33.3
PG 10.8.3	57.1	50.0	55.6
PG 10.10.2	85.7	50.0	77.8
Average acclimation rate	69.2	28.6	48.1

In general on this experimental area acclimation rate of *P. gigantea* turned out to be rather low – only on 48.1% of stumps fruit bodies of fungus were formed which in all variations covered about 20% of butt-end surface of a wooden substratum. Strain PG 10.10.2 that colonized 77.8% of treated stumps showed the best acclimation rate. Such a low colonization ability of the tested strains most probably connected with carrying out of a late processing: on the day of inoculation the average daily air temperature was 2ºC and during the following 10 days it was as low as –3.8ºC at night.

On PEA No. 6 just in 3 months on the butt-end and side-end surface of stumps and even on forest litter intensive formation of typical extended fruit bodies of the antagonist was observed. It was easy to estimate effectiveness of carried out inoculation. The results of the undergone estimation are in table 2.

Table 2

Acclimation rate of strains *P. gigantea* on pine stumps, % (PEA No. 6)

Strain	Test variation				Average acclimation rate
	Stumps on a portage without covering	Stumps on a portage covered with moss	Stumps on a portage covered with disks	Stumps under the forest canopy without covering	
PG 10.6.2	94.4	100.0	94.4	64.3	89.7
PG 10.7.1	94.4	100.0	87.5	50.0	89.7
PG 10.8.3	100.0	73.3	85.7	80.6	84.6
PG 10.10.2	81.8	100.0	100.0	87.5	92.6
PG 11.5.1	95.7	95.7	86.4	63.9	82.7
PG 11.15.3	100.0	90.9	68.2	75.0	79.4
Average acclimation rate	95.5	93.6	85.8	73.4	84.6

Table 3

Acclimation rate of strains *P. gigantea* on pine stumps, % (PEA No. 7)

Strain	Test variation				Average acclimation rate
	Stumps without covering	Stumps covered with moss	Stumps covered with disks	Old stumps without covering	
PG 10.6.2	64.3	79.2	75.0	50.0	67.4
PG 10.7.1	78.3	65.4	93.9	66.7	77.4
PG 10.8.3	79.2	87.0	88.0	72.7	83.1
PG 10.10.2	74.3	73.9	91.3	60.0	76.0
PG 11.3.1	95.5	90.9	88.6	60.0	79.3
PG 11.5.1	79.3	75.9	83.3	50.0	75.0
PG 11.13.1	70.0	59.1	85.3	46.7	69.4
Average acclimation rate	76.1	75.7	87.1	58.1	75.8

Strains PG 10.10.2 (inhabited 92.6% of the processed stumps), PG 10.6.2 and PG 10.7.1 (89.7%) have the best acclimation rate in the condition of experimental area No. 6.

The results of processing on subsections also had variations. In spite of our expectations, on a portage, where stumps after spraying were not covered, there were the biggest number of stumps inhabited with antagonists (from 81.8 to 100.0% depending on a strain, on average on a variation 95.5%). However, in this case fungus adaptation of a butt-end of the substrate was carried out with the least intensity: fruit bodies of the antagonist on average only covered 63% of its area. Acclimation rate of *P. gigantea* in the condition of stumps covering with moss or disks is also high (93.6 and 85.8% correspondently), thus, there is the biggest average area of covering of the stump surface with mycelium of the fungus (88.0 and 77.7% of surface correspondently). Under the forest canopy, where the wood of the nourishing substrate was dried a little and was partially inhabited by the complex of other xylotrophic fungi, on average 73.4% of stumps were colonized and fruit bodies of the antagonist cover 67.4% of their butt-end surface.

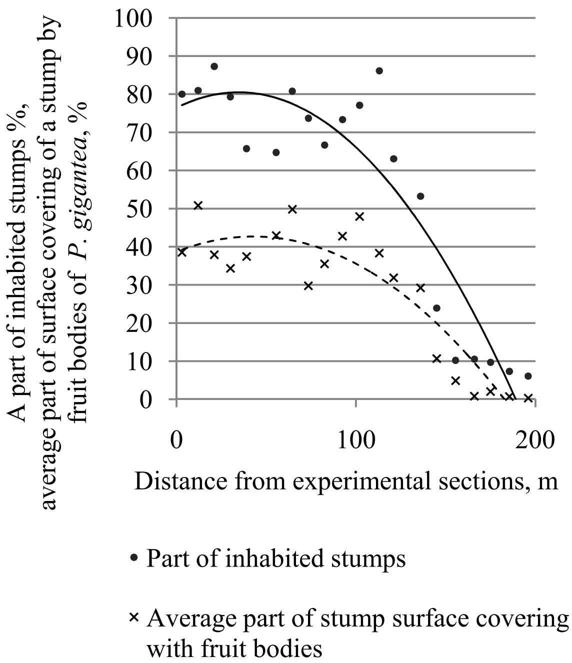
On PEA No. 7 as well as on the previous one, typical fruits bodies of *P. gigantea* were formed during six months. In table 3 there are the results of enumeration of the colonized stumps.

Average acclimation of the antagonist on this PEA turned out to be lower than on PEA No. 6 (75.8%). The best effectiveness of inoculation strains PG 10.8.3 (83.1%), PG 11.3.1 (79.3%) and PG 10.7.1 (77.4%) showed.

The results were also different on the variations of the processing: this time stumps covering with disks (on average 87.1% of stumps were colonized), which formed favorable temperature and humidity conditions for spores sprouting, preventing penetration of sun beams and drying off of the substrate surface, turned out to be more effective. Covering of stumps with disks and moss was also useful for more intensive growth of mycelium of *P. gigantea* on the surface of the wood: on average fruit bodies covered 77.3 and 79.6% of butt-end surface of the stump correspondently.

It should be mentioned that on the control sections under the conditions of PEA No. 6 and PEA No. 7, where processing of stumps was not carried out, there were also intensive formation of fruit bodies of *P. gigantea*. In not processed part of plants on PEA No. 6 73.1% of stumps was colonized by antagonist, on PEA No. 7 – on average – 55.4%. So, due to the quick formation of fruit bodies and intensive spore formation there is active spreading of the antagonist by air current.

With the aim of examination of the distance of spreading of spore material of *P. gigantea* on PEA No. 7 calculation of inhabited stumps in not processed part of plants at different distance from the border of the experimental section (Figure).



Variation of stumps colonization by *P. gigantea* on the control section in accordance with the distance from experimental sections

The results of the research show that the number of inhabited stumps on a control area is gradually decreasing away from the center. At the distance of 145 m occupation density is 23.9%, at the distance of 196 m – 6.1%. Average covering of the stump surface by fruits bodies changes as well (from 50.8% near the experimental area till 0.3% at the distance of 196 m).

Thus, spring improvement cuttings with further stumps processing with *P. gigantea* promote better acclimation rate of the fungus and also increase of infectious rate of the antagonist in planting that creates unfavorable conditions for pine fungus development.

Conclusion. All testing strains showed ability to colonize pine stumps surfaces. Stumps occupation density depends on individual peculiarities of the antagonist and the way of processing. Strains PG 10.10.2 (inhabited from 76.0 to 92.6%, on average 82.1% of stumps) and PG 10.8.3 (inhabited from 55.6 to 84.6%, on average74.4% of stumps) showed the best acclimation rate on natural substrate These strains are chosen for the further development of the biological agent.

Processing of the newly-cut stumps surfaces with moss and disks covering turned out to be the most effective. However, processing without covering is also very effective and taking into account its simplicity can be recommended for application in forestry.

Spring cuttings with application of *P. gigantea* permit fungus not only colonize processed wood substrate but also promote increase of infectious rate of the antagonist in planting due to the intensive spore growth of quick-growing fruit bodies.

References

1. Rishbeth, J. Observations on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations III. Natural and experimental infection of pines, and some factors afecting severity of the disease / J. Rishbeth // Ann. of Bot. – 1951. – Vol. XV, № 58. – P. 221–247.

2. *Heterobasidion annosum*: biology, ecology impact and control / Library of Congress Cataloging in Publication Data: edited by: S. Woodward [et al.]. – Cambriage: University Press, 1998. – 589 p.

3. Волченкова, Г. А. Развитие биологических методов ограничения вредоносности корневой губки / Г. А. Волченкова, В. Б. Звягинцев // Проблемы лесоведения и лесоводства: сб. науч. тр. / Ин-т леса Нац. акад. наук Беларуси. – 2011. – Вып. 71. – С. 445–455.

4. Pratt, L. E. Comparison of three products based on *Phlebiopsis gigantea* for the control of *Heterobasidion annosum* in Europe / J. E. Pratt, M. Niemi, Z. H. Sierota // Biocontrol Science and Technology. – 2000. – № 10. – P. 467–477.

5. Разработать и внедрить рекомендации по ограничению вредоносности корневой губки в сосновых лесных культурах и повышению их устойчивости и продуктивности: отчет о НИР (промежут.) / Белорус. гос. технол. ун-т; рук. темы В. Б. Звягинцев. – Минск, 2011. – 54 с.

6. Разработать и внедрить рекомендации по ограничению вредоносности корневой губки в сосновых лесных культурах и повышению их устойчивости и продуктивности: отчет о НИР (промежут.) / Белорус. гос. технол. ун-т; рук. темы В. Б. Звягинцев. – Минск, 2012. – 76 с.

Received 21.01.2012