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UDK: 630*443:582.282 *Gremmeniella abietina* (497.1)
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GREMMENIELLA ABIETINA (LAGERB.) MORELET: DISTRIBUTION IN SERBIA AND MONTENEGRO, SIGNIFICANCE AND CONTROL

Abstract: Fungus *G. abietina* is one of the most dangerous pathogenic fungi occurring in conifer plantations and *Pinus* species are especially at risk. Among pines, the most susceptible species is Austrian pine, and particularly endangered plantations are between 8 and 25 years of age. This fungus was found in Serbia and Montenegro on Austrian pine, Scots pine and spruce, but it was only in mountainous regions (Копачник, Власина, Гоџ, Дурмитор). *G. abietina* forms both stages (*anamorph* and *teleomorph*) in its development. The imperfect form is far more significant for the infection process, i.e. the pycnidial stage and generally all infections are caused by conidia (pycnospores). Apothecia will be formed on the bark only two years after tree dying. In the severely infected plantations, all dead trees should be felled and removed, and the remaining trees should be treated with copper fungicides.

Key words: *Gremmeniella abietina*, Austrian pine, Scots pine, significance, control

GREMMENIELLA ABIETINA (LAGERB.) MORELET: РАСПРОСТРАЊЕЊЕ У СРБИЈИ И ЦРНОЈ ГОРИ, ЗНАЧАЈ И СУЗБИЈАЊЕ

Извод: Гљива *G. abietina* је једна од најопаснијих патогених гљива која се јавља у културама четинара, а посебно су угрожене *Pinus* врсте. Међу боровима најосетљивија врста је црни бор, а угрожене су културе у старости између 8 и 25 година. Ова гљива је забележена у Србији и Црној Гори на црном бору, белом бору и смрчи, али само у планинским крајевима (Копачник, Власина, Гоџ, Дурмитор). *G. abietina* у свом развоју образује оба стадијума (*anamorph* и *teleomorph*). За сам процес инфекције, далеко је већи значај несавршене форме, тј. пикнидиског стадијума и углавном све заразе се остварују конидијама (пикноспорама). Апотеције се образују на кори тек 2 године после сушења стабала. У јако зараженим културама, сва сува стабла треба посећи и уклонити, а преостала стабла треба третирати бакарним фунгицидима.

Кључне речи: *Gremmeniella abietina*, црни бор, бели бор, значај, контрола

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1. INTRODUCTION

Intensive afforestation of bare lands and deforested areas in the area of the former Yugoslavia was undertaken in the second half of the 20th century. The most frequent species in afforestation were *Pinus* species, and in Serbia especially Austrian pine and Scots pine plantations were established on large areas. The establishment of monocultures over large areas was followed by numerous problems already from the beginning. In pine plantations, among harmful biotic factors, especially important were pests and diseases caused by parasitic fungi (e.g. red pine needles, *Dothistroma* needle blight of pines, root rot, branch and shoot dying, etc.). During a detailed health checking of Scots pine and Austrian pine plantations, some dangerous pathogenic fungi were identified which had not been previously recorded in the area of Serbia. Among the new identified fungi, especially great damage is caused by the pathogenic fungus *Gremmeniella abietina*. Because of the *Gremmeniella abietina* significance and great damage caused in Austrian pine and Scots pine plantations, this paper presents its description, distribution, life cycle and the possibilities of control.

2. MATERIAL AND METHODS

The material was collected on several occasions from the diseased Austrian pine and Scots pine trees, from the areas of NP „Kopaonik”, NP „Durmitor”, Vlasina, Goč and mountain Ivica (Montenegro). The fungi were identified after microscopic analyses, based on the appearance of the fruiting bodies (pycnidia and apothecia), spore bearing organs (asci), reproduction organs (conidia and ascospores) and the appearance of the fungus pure culture.

The fungus was isolated from the infected needles and shoots of both Austrian pine and Scots pine on the nutritive media MEA (malt-extract agar) and PDA (potato-dextrose agar). The isolation method consisted of the following: first the diseased needles and shoots were immersed for 5 minutes in sodium hypochlorite with 1.5% active chlorine content; then they were cut into fragments of 2-3 mm and placed on the prepared nutritive media. After the appearance of the fungal colony on the media, the isolates were placed in thermostats and their growth was further monitored at the temperatures of 15 and 20°C. The isolation of this fungus often involves many difficulties, because the fungus has a very slow growth on the media and the media are often contaminated with other, first of all saprophytic, fungi.

The time of fruiting body opening (pycnidia and apothecia) and the release of reproductive organs (pyncospores and ascospores) were monitored during the period 1999-2006. Based on the above, we determined the time of ripening of fruiting bodies, the release of the spores and the potential time of infection.

At the beginning of May 1999, the experiments of chemical protection of Scots pine plantations were established at the site „Popov Do”. The trees were sprayed with the

suspension of copper fungicides (copper oxychloride-25 was applied in 1% concentration and Bakrocid in 0.7% concentration).

3. RESULTS AND DISCUSSION

3.1. Dispersal of the fungus *G. abietina* in Serbia and Montenegro

Fungi *G. abietina* was identified for the first time in the former Yugoslavia in 1979 in the area of NP Durmitor, in the vicinity of Žabljak, in mixed plantation of Austrian pine and Scots pine, aged between 25 and 30 years (Marinković, Karadžić, 1983). About ten years later (1988), this fungus was identified in Serbia in Scots pine and spruce plantations on Kopaonik (Karadžić, 1989). By the end of 1992, it was identified on Scots pine on the mountains Vlasina and Goč (tree age between 18 and 20 years). During 1998 *G. abietina* was identified in the Scots pine plantation in the area of Ivica (site „Popov Do”, plantation age 10 years). This fungus was identified again during 2002 in the area of Ivica in the Scots pine plantations established in 1992-93 (sites „Mala Barna” and „Ivički Vukodoli”). During 2006, fungus *Gremmeniella abietina* was identified in Scots pine plantation in the area of NP „Kopaonik”, (site „Samokovska Reka”, compartment 83, plantation age 50 years). The plantation area is 2.47 hectares. The tree age is 50 years, height 15-20 metres, diameter 15-30 cm. This Scots pine plantation was not tended, so there were numerous uprootings and broken trees. A severe infection by *G. abietina* was diagnosed on all trees, and in great many cases also the young shoots of the current vegetation were killed. During 2008, fungus *G. abietina* was identified in the forest of Scots pine in nearness Black lake (NP „Durmitor”).

3.2. Fungus description and significance

Gremmeniella abietina causes shoot dying and tree dying in Austrian pine and Scots pine plantations. It is widespread in North and Central Europe and North America on many *Pinus* species, and it was also identified on the species in the genera *Larix*, *Picea*, *Pseudotsuga* and *Abies*. The most susceptible of all tree species is *Pinus nigra* Arn., and especially endangered plantations are aged between 8 and 25 years. The other pine species damaged by *G. abietina* in Europe are *Pinus cembra*, *P. mugo*, *P. strobus* and *P. sylvestris*. The intensity of infection and the disease development by all means also depend on the degree of pathogenicity, as it was determined that there are several races of different virulence. Skilling *et al.* (1979) identified two strains of the fungus, i.e. North American and European, and they report that in pine plantations in USA the European strain is more virulent and causes much greater damage. Dorworth and Krywienczyk (1975) propose (based on physiological and immunological characteristics) the classification into three races: North American, European and Asian. The value of the damage which can be caused by this pathogen in plantations makes *G. abietina* one of the most serious quarantine diseases, which is clearly identified by import regulations in many countries.

This fungus forms both stages of development, i.e. the pycnidial stage and apothecial stage.

The pycnidial stage (anamorph) described under the name *Brunchorstia pinea* (Karst.) Höhn., is much more frequent in the field and is far more significant for the infection process. Pycnidia are formed at the base of the infected needles, on the bark of dead shoots and more rarely on cone scales. The pycnidia are black, size 0.4-1 mm, at the beginning subepidermal, and then they appear on the surface. The conidia are colourless, sickle-shaped curved, with a greater number of septa (from 3 to 7), size 30–64×2.5-4 µm. Morelet (1980) classified two varieties of this fungus based on conidial length, i.e. *B. pinea* var. *pinea* (with shorter conidia) and *B. pinea* var. *cembrae* (with longer conidia, up to 73 µm and 5-7 septa).

The perfect stage (teleomorph), i.e. apothecial stage forms two years after tree dying. Apothecia were found on the bark of killed shoots, on dead branches and cone scales. Apothecia are 0.5-1 mm in diameter, dark brown or black, with a short peduncle. The size of asci is 80-122×7-11 µm. It was determined that the asci formed in apothecia on the cones are smaller, i.e. 70-85×6.7-7.5 µm (Karadžić *et al.*, 2002). Ascospores are colourless, ellipsoidal or elongated, size 10-22×4-5.7 µm, 3-septate. The ascospores from asci formed in apothecia on the cones are somewhat smaller, i.e. 10-15×4-5.7 µm. According to Punithalingam and Gibson (1973), the size of ascospores is 15-22×3-5 µm, and according to Morelet (1980), it is 12-24×3-6 µm.

In Scots pine plantations on Kopaonik, it was observed for the first time that the fungus forms pycnidia and apothecia also on the scales of two-year old cones, which is a new data in literature. This data is very important because, by collecting seed cones, this dangerous quarantine disease can be spread to the new uninfected regions.

The pure culture of *G. abietina* isolated on malt-extract agar grows moderately fast, at the beginning it is white, woolly, and later on it changes its colour and turns grey-green, yellowish-green or, more rarely, green. In the culture, pycnidia with numerous conidia form already after a few days after isolation. The medium below the mycelium changes and turns dark green.

3.3. Disease symptoms and life cycle

Primary infections are caused by conidia (more rarely also ascospores), which are transmitted by rain splash and wind. Infection period lasts from the beginning of May to the end of November, but the critical period for infection is May-June. Infection is spread through the buds and bark of young shoots. During the first phase of infection, which lasts from May to September, fungus penetrates into the external dead cells of the bark, and during the second stage of infection (from December), the germ tubes penetrate into the living bark cells and cause more or less visible necroses. In some cases, plant by its defence reactions can stop the spreading of the pathogen, and in the opposite case, the shoot is girdled and killed. Incubation period lasts for 9 months, i.e. if infection occurs in June of the current year, the first visible symptoms of infection occur in March of the following year.



Figure 1. Scots pine infected with the fungus *Gremmeniella abietina* (loc. Black lake, N.P. Durmitor)
Слика 1. Бели бор заражен гљивом *Gremmeniella abietina* (лок. Црно језеро, НП Дурмитор)

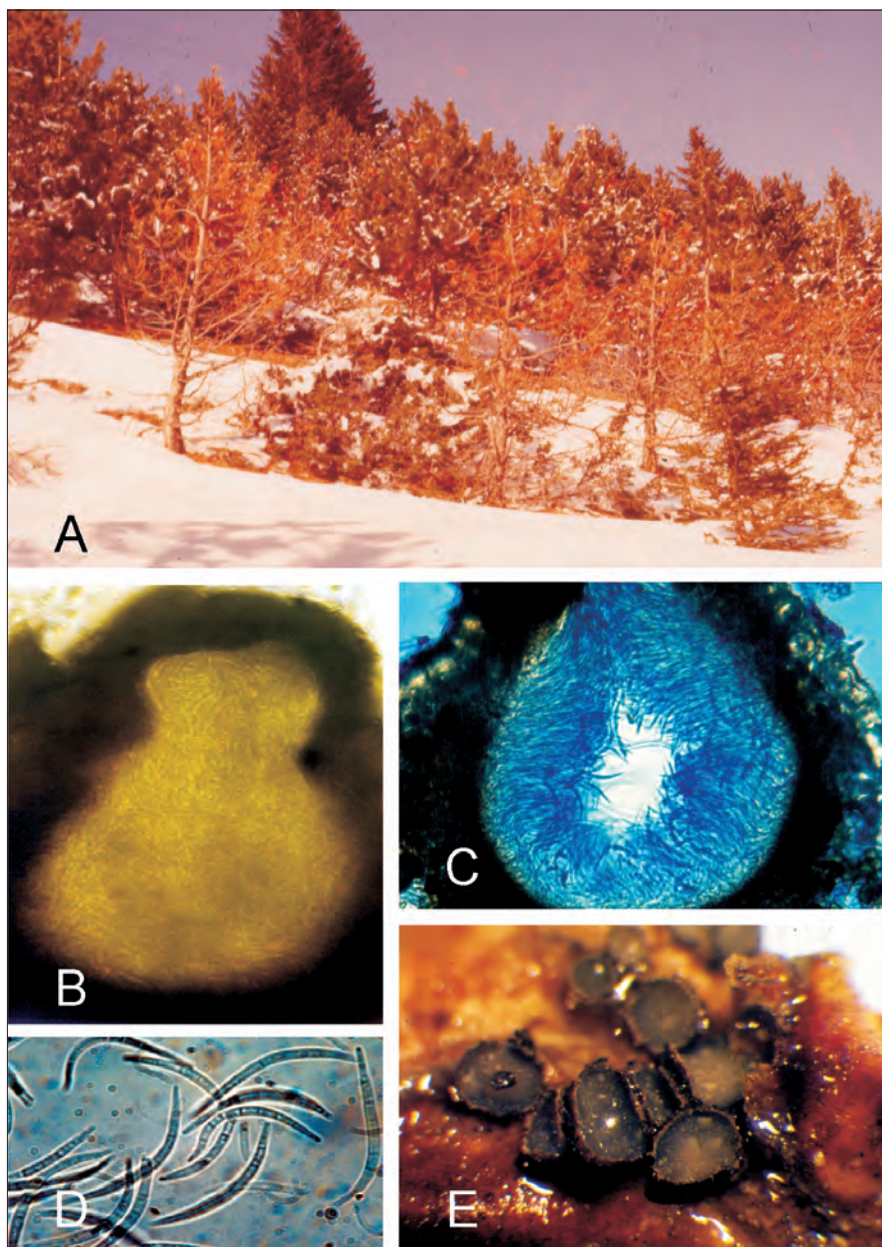


Figure 2. *Gremmeniella abietina* (Lagerb.) Morelet: A - Plantation of Austrian pine infected with fungus, B-C - perithecia vertical section; D - conidia, E - apothecia on the bark
Слика 2. *Gremmeniella abietina* (Lagerb.) Morelet: A - култура црног бора заражена гљивом, B-C - попречни пресек кроз пикнид, D - конидије, E - апотеције на кори

The symptoms of infection are expressed in several ways. During winter months, on the bud cross section, brown discolouration of the tissue starts from the bud base, and the infected buds fail to flush. The already formed shoots turn brown at the base, due to the necroses of the tissue (in vital trees, these necroses can heal later on). On the shoots with formed needles, the needles are decolourised starting from the base (during June, the needle bases turn orange or reddish, and gradually they turn brown to the tip, which is followed by needle-cast during summer, leaving the shoot without needles). The shoots die down starting from the tip. Black fruiting bodies of pin-head size - pycnidia appear on dead buds and shoots and at the base of the needles. During the vegetation period, in place of dead shoots, secondary shoots appear from dormant buds. From the infected shoots, the mycelium proceeds downwards along the branches in the stem and, if the younger trees are attacked, they die-back, while the development of the fungus in the old trees is stopped by the reaction of the host plant or saprophyte fungi. The trees die as the consequence of multiannual infections and shoot dying. The dead branches on the trees are usually broken by the wind and snow, and canker can be found on the mature trees. The fruiting bodies - apothecia form on the bark of dead shoots and branches not before two years after tree dying.

It was found also that some infected trees can recover, if high precipitation in summer (in which the fungus disperses to epidemic proportions) is followed by dry weather. In such cases, the crown turns green again, but only in the upper part, while the branches in the lower part are dead.

3.4. Control measures

To reduce the damage caused by the fungus *G. abietina* to a tolerable degree, it is necessary to undertake the following protection measures:

- in the establishment of new plantations (primarily Austrian pine and Scots pine), wet and cold sites should be avoided and the sites on which snow lasts long during winter;
- dense planting should be avoided and good air circulation should be ensured as much as possible to avoid air stagnation and high air humidity;
- disease-free planting material should be applied in plantation establishment and, if possible, the resistant provenances;
- all dead trees in the attacked plantations should be felled and eliminated;
- during the collection of seed cones in infected areas, care must be taken to prevent the spread of the disease to the new uninfected regions.

According to Butin and Siepmann (1980), good results in the suppression of this fungus were obtained by the application of Maneba, and tree spraying should be from June to September in 14-day intervals. Previous preliminary investigations in Serbia and Montenegro showed that the copper fungicides (for example copper oxychloride) have given the best results and protection. Protection is satisfactory if the treatment is carried out twice a year during the critical period of infection. However, such protection is possible

and economically justified only in the nurseries and young plantations (Karadžić *et al.*, 2002, Karadžić, 2006, Karadžić, Milijašević, 2008).

4. CONCLUSION

Fungus *G. abietina* is one of the most dangerous pathogenic fungi inhabiting conifer plantations and especially endangered species are the pines. Among pines, the most susceptible species is Austrian pine, and particularly endangered plantations are between 8 and 25 years of age. This fungus was found in Serbia and Montenegro on Austrian pine, Scots pine and spruce, but it was only in mountainous regions (Kopaonik, Vlasina, Goč, Durmitor). *G. abietina* forms both stages (anamorph and teleomorph) in its development. The imperfect form is far more significant for the infection process, i.e. the pycnidial stage and generally all infections are caused by conidia (pyncospores). Trees can be infected throughout the year, but the critical period for infection is May-June. Conidia are dispersed by raindrop splash and the infection enters the buds and the bark of the developing shoots. Incubation period lasts for 9 months, i.e. if the tree is infected in June of the current year, the first visible symptoms of infection will appear in March of the following year. The symptoms of infection are observed at the base of the buds (cross section), on the needles (at the beginning the base of the needles turns orange, and then the whole needle turns brown and falls off), and on the bark of young shoots. Soon after the appearance of the disease symptoms, fruiting bodies (i.e. pycnidia) appear on the necrotised tissues of the host. Apothecia will be formed on the bark only two years after tree dying. In the severely infected plantations, all dead trees should be felled and removed, and the remaining trees should be treated with fungicides. Previous preliminary investigations that the copper fungicides (for example copper oxychloride) have given the best results and protection. Protection is satisfactory if the treatment is carried out twice a year during the critical period of infection. However, such protection is possible and economically justified only in the nurseries and young plantations.

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***GREMMENIELLA ABIETINA* (LAGERB.) MORELET: РАСПРОСТРАЊЕЊЕ
У СРБИЈИ И ЦРНОЈ ГОРИ, ЗНАЧАЈ И СУЗБИЈАЊЕ**

Резиме

Гљива *G. abietina* је једна од најопаснијих патогених гљива која се јавља у културама четинара, а посебно су угрожене *Pinus* врсте. Међу боровима најосетљивија врста је црни бор, а посебно су угрожене културе у старости између 8 и 25 година. Ова гљива је забележена у Србији на црном бору, белом бору и смрчи, али само у планинским крајевима (Копаоник, Власина, Гоч, Дурмитор). Гљива *G. abietina* први пут је констатована у бившој Југославије 1979. год. на подручју НП Дурмитор, у непосредној близини Жабљака у мешовитој култури црног и белог бора, старој између 25 и 30 година (Маринковић, Караџић, 1983). Десетак година касније (1988. год.) гљива је констатована у Србији и то у културама белог бора и смрче на Копаонику (Караџић, 1989). Крајем 1992. год. забележена је на белом бору на Власини и Гочу (старост стабала између 18 и 20 година). У току 1998. год. *G. abietina* је забележена у култури белог бора на подручју Ивице (локалитет „Попов до”, старост култура 10 година). Ова гљива поново је констатована у току 2002. год. на подручју Ивице у културама белог бора подигнутим 1992-93. год. (локалитети „Мала Барна” и „Ивички вукодоли”). У току 2006. год. гљива *Gremmeniella abietina* је забележена у култури белог бора на подручју НП „Копаоник”, (локалите „Самоковска река”, одељење 83, старост култура 50 година). Површина културе је 2,47 ha. Старост стабала је 50 година, висина од 15-20 m, а пречник од 15-30 cm. У овој култури белог бора изостале су мере неге, тако да су констатоване и бројне извале и ломови стабала. Јака зараза од гљиве *G. abietina* констатована је на свим стаблима, а у великом броју случајева су били убијени и млади избојци из текуће вегетације. У току 2008. године јака зараза је примећена у природној састојини белог бора у непосредној близини Црног језера (НП „Дурмитор”).

G. abietina у свом развоју образује оба стадијума (*anamorph* и *teleomorph*). Плодоносна тела се образују на четинама, кори младих избојака и кори сувих грана. У културама белог бора на Копаонику први пут је констатовано да гљива формира пикниде и апотеције и на љуспицама 2-годишњих шишарица, што је нови литературни податак. Овај податак је веома важан због тога што се сакупљањем семених шишарица може ова опасна карантинска болест пренети у нова незаражена подручја. Пикнидски стадијум је описан под називом *Brunchorstia pinea* (Karst.) Höhn. За сам процес инфекције, далеко је већи значај несавршене форме, тј. пикнидског стадијума и углавном све заразе се остварују конидијама (пикноспорама). Заразе стабала су могуће у току целе године, али је критични период за инфекције мај-јун месец. Конидије се преносе кишним капима, а инфекције се остварују преко пупољака и коре младих избојака. Период инкубације траје 9 месеци, тј. ако су заразе остварене у јуну текуће године први видљиви симптоми заразе ће се јавити тек у марту следеће године. Симптоми заразе се уочавају у основи пупољака (на пресеку), на четинама (у почетку се појављује наранџаста боја у основи четина, а затим цела четина посмеђи и опада) и на кори младих избојака. Убрзо по појави симптома заразе на некротираним ткивима домаћина појављују се плодносна тела гљиве (тј. пикниди). Апотеције ће се образују на кори тек 2 године после сушења стабала.

Чиста култура гљиве *G. abietina*, изолована на малц-екстракт агару, расте умерено брзо, у почетку је бела, вунаста, а касније мења боју и постаје сиво-зелена, жућкасто-зелена или ређе зелена. У култури се већ после неколико дана по изолацији формирају пикниди са бројним конидијама. Подлога испод мицелије се мења и постаје тамно-зелена.

Да би се штете од гљиве *G. abietina* свеле на подношљиву меру, неопходно је предузети следеће мере заштите:

- приликом подизања нових култура (пре свега црног и белог бора), треба избегавати влажна, хладна места и места на којима се у току зиме дуго задржава снег;
- треба избегавати густу садњу и потребно је у највећој могућој мери обезбедити добру циркулацију ваздуха (прозрачност) да би се избегла висока стагнирајућа влажност ваздуха;
- приликом подизања култура треба користити здрав садни материјал и по могућности отпорне провенијенције;
- сва сува стабла у нападнутим културама треба посећи и елиминисати;
- приликом сакупљања семених шишарица у зараженим подручјима мора се водити рачуна да се преко шишарица ова опасна карантинска болест не пренесе у нова подручја.

Према Butin-у и Siepmann-у (1980) добри резултати у сузбијању ове гљиве су добијени коришћењем Манеба, с тим да се прскање стабала изврши у 14 дневним интервалима, од јуна до септембра. Истраживања спроведена у Србији и Црној Гори су показала да у јако зарженим културама сва сува стабла треба посећи и уклонити, а преостала стабла истретирати бакарним фунгицидима (нпр. бакарним оксихлоридом). Заштита је задовољавајућа, ако се третирање изврши два пута годишње у критичном периоду за инфекције. Међутим, таква заштита је могућа и економски оправдана само у расадницима и младим културама.