Zbornik gozdarstva in lesarstva, 54, 1997, s. 109 - 124

GDK 411.16 : 172.8 (*Cenangium ferruginosum Fr.*) +181.22 +181.31

Prispelo / Received: 10. 10. 1997 — Izvirni znanstveni članek
Sprejeto / *Accepted*: 28. 10. 1997 — *Original scientific paper*

INFLUENCE OF MOISTURE, TEMPERATURE AND GROWTH MEDIUM ON CENANGIUM DIEBACK OF PINE *(CENANGIUM FERRUGINOSUM FR., ASCOMYCOTINA)*

Dušan JURC[*] , Maja JURC[**]

Abstract

C. ferruginosum culture was grown in vitro on MEA medium at 10, 20, 25, 30 and 35^0 C, on media with water activity modified to 0,98, 0,96 and 0,94 and on MEA, PDA and OA media at 23^0 C. The fungus grows at temperatures between 10^0 C and 25^0 C and on media with available water between 0,98 and 0,96. The optimal temperature for growth is 25^0 C, while at 30^0 C it doesn't grow at all. The best growth occurs on PDA medium, the second best on MEA medium and the lowest growth on OA medium. Composition of growth medium is an important factor and influences growth rate considerably. These results explain the ability of the fungus to invade the tissues of its host during colder periods of year and when the substrate is highly moistened. High summer temperatures are unsuitable for its growth.

Key words: Cenangium dieback of pines, *Cenangium ferruginosum Fr.*, temperature, moisture, growth medium.

VPLIV VLAGE, TOPLOTE IN HRANILNE PODLAGE NA SUŠICO BOROVIH VEJ (CENANGIUM FERRUGINOSUM FR., ASCOMYCOTINA)

Izvleček

Izolat C. ferruginosum smo gojili na hranilni podlagi MEA pri 10, 20, 25, 30 in 35^0 C, na gojiščih z vodno aktivnostjo uravnano na 0,98, 0,96 in 0,94 in na hranilnih podlagah MEA, PDA in OA pri 23^0 C. Gliva uspeva na temperaturah od 10^0 C do 25^0 C in na hranilnih podlagah z vodno aktivnostjo med 0,98 in 0,96. Optimalna temperatura za rast je 25^0 C, vendar pri 30^0 C ne uspeva več. Najbolje raste na hranilni podlagi PDA, manj na MEA in najmanj na OA. Sestava hranilne podlage je pomemben dejavnik, ki močno vpliva na hitrost rasti glive. Ugotovitve pojasnjujejo sposobnost glive, da prerašča tkiva gostitelja v hladnejših letnih obdobjih in ob visoki vlažnosti substrata. Visoke poletne temperature so neustrezne za njeno rast.

Ključne besede: sušica borovih vej, Cenangium ferruginosum Fr., toplota, vlaga, hranilna podlaga.

[*] Asist. mag., dipl. biol., Gozdarski inštitut Slovenije, Večna pot 2, 1000 Ljubljana, SLO
[**] Asist. dr., dipl. inž. gozd., Gozdarski inštitut Slovenije, Večna pot 2, 1000 Ljubljana, SLO

CONTENTS / *KAZALO*

1 INTRODUCTION

Austrian pine grows in very small scattered natural stands in the Alpine regions and also in central and southern parts of Slovenia. It is widely distributed as an allochtone tree species in the Karst region near the Adriatic coast, where it has been planted on more than 20.000 ha of degraded land beginning more than 100 years ago.

The epidemic of *Cenangium ferruginosum* Fr. in 1986 affected the greater part of the region and caused the felling of approximately 10.000 m^3 of Austrian pine (JURC 1987). Although only trees with more than 80 % of the crown dead were cut, numerous trees with dead branches in the crowns remained. Since that time the Cenangium dieback of pines has only appeared occasionally and locally.

Analysis of climatic factors for 1985, in comparison with 10 year averages, showed that the relative humidity had reached a 10 year minimum in all months from July to October, registered precipitations reached a 10 year minimum in September and October, while in August temperatures reached a 10 year maximum. This unusually dry, hot weather was proceeded by an unusually wet period. The first signs of dying in Austrian pine branches were noticed in December 1985. The largest mortality occured in 1986 on the southern and south-western slopes and in areas with shallow soil. All observations were in accordance with other well-described examples from the literature where drought stress is regarded as a decisive factor in *C. ferruginosum* dieback (JURC 1987).

C. ferruginosum is usually regarded as ubiquitus and opportunistic fungus which kills the bark and cambium of twigs and branches weakened by environment, other pests and pathogens, or natural senescence (BUTIN 1996, SINCLAIR / LYON / JOHNSON 1987). Outbreaks of Cenangium dieback occur only once in several years. The fungus is usually a competent saprobe on pine twigs killed by various agents. Its saprobic existence ensures a supply of spores and it seems likely that incipient infections begin each year in the summer and autumn and are held in check by host defenses unless these are overcome by environmental damage or by other pests (SINCLAIR / LYON / JOHNSON 1987). This common view of repeated yearly infections was overturned by the results of JURC (1996) who revealed the constant endophytic existence of *C. ferruginosum* in the healthy needles of Austrian pine.

The objectives of our laboratory study were to determine the temperature and available water requirements for the growth of *C. ferruginosum* in culture and to test its growth on different artificial nutrient media.

We hoped that these data would reveal the ecological factors which are decisive in fungal growth and are prerequisites for outbreaks of the disease. Such factors should enable the fungus to break its quiescent endophytic state and to spread quickly through weakened tissues of its host.

2 MATERIAL AND METHODS

2.1 COLLECTING SITE

A sampling of Austrian pine needles was performed in a natural stand of Austrian pine on Smolnik hill near the town of Jesenice (1075 m above sea level, y=54 343000, x=51 42100; projection G.-K., UTM MV43) in January 1995. This forest belongs to plant association *Pinetum subillyricum* Schmidt 1936 and grows on limestone.

2.2 SAMPLING PROCEDURE AND ISOLATIONS

The tree from which the samples were taken was about 60 years old. A branch was cut from the lower whorl of the southern part of the crown using tree loppers. Needles were transported in cold boxes at 4^0C in clean polyethylene bags and processed within 24 hours. Four needles of each age class, without any signs of damage, were washed in running tap water for one hour. From every needle three 0,3 cm long segments were taken - one from the base, one from the middle part and one from the tip of the needle. They were then surface-sterilised (1 min in 50 % ethanol v/v, 5 min in sodium hypochlorite with 2,6 % of active chlorine, 1 min in 50 % ethanol v/v). The segments were blotted dry and plated onto 2 % malt extract agar (Malt Extract, Biolife S.r.l., 20 g l^{-1}, Agar Bios Special LL, Biolife S.r.l., 20 g l^{-1}). Twelve segments per plate and 2 replicate plates per age class were plated out. Petri dishes were then incubated at 23^0 C and examined weekly for six weeks. Mycelial outgrowths from the segments were subcultured and identified.

The fungus used in all further experiments was isolated from the base segment of a four year old needle and it fit, in all details, to the description of *C. ferruginosum* anamorf (KUJALA 1950).

2.3 MEASURMENT OF FUNGAL GROWTH *IN VITRO*

1 % malt extract agar medium was used (MEA, Lab M Ltd, pH 4,5) to measure fungal growth. In all cases 25 ml of molten agar was poured into 9 cm diameter Petri dishes and inoculated with 4 mm diameter agar plugs of *C. ferruginosum* cultures taken from the growing margin of 14 day old colonies. The diameter of the colonies was measured in two directions at right angles to each other. All experiments were carried out in triplicates. The effect of temperature was examined by placing inoculated MEA agar plates in darkness at 10, 20, 25, 30 and 35^0 C. The effect of water availability (aw) was examined by modifying the media with glycerol to the values of 0,98, 0,96 and 0,94 aw (DALLYN / FOX 1980, MAGAN / LACEY 1984). Plates with the same aw were enclosed in clean polyethylene bags and incubated at 10, 20, 25, 30 and 35^0 C for 29 days.

To compare the growth rate on different growth media the fungus was incubated at 23^0 C on 2 % MEA (Malt Extract, Biolife S.r.l., 20 g l^{-1}, Agar Bios Special LL, Biolife S.r.l., 20 g l^{-1}), 3,9 % PDA (Potato dextrose agar, bioMérieux 51411, 39 g l^{-1}) and 3 % OA (oatmeal 30 g l^{-1}, Agar Bios Special LL, Biolife S.r.l., 20 g l^{-1})

3 RESULTS

Colonies of *C. ferruginosum* in culture have characteristics which make the growth tests less comparable and reliable. The growth of the mycelial colony is irregular and outgrowths can develop from some parts while in other parts the growth can be slower or halted. This is the reason for the great variation seen among some of the experiments. Photo 1 shows the morphology of *C. ferruginosum* cultures on MEA, OA and PDA media, while photo 2 shows slimy conidial masses on mycelium.

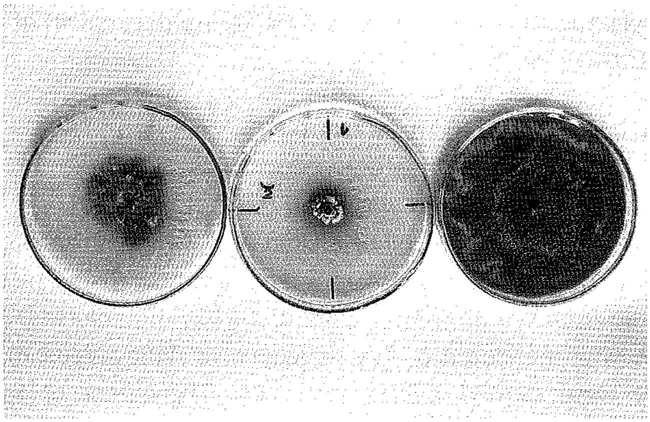


Photo 1: Differences in growth of 41 days old *C. ferruginosum* cultures on OA, MEA and PDA media (from left to right).

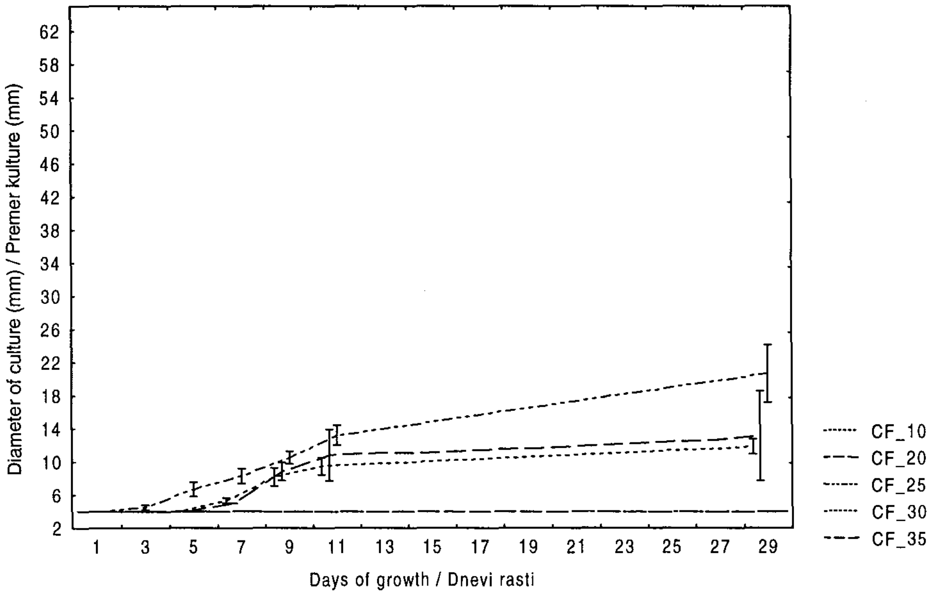
Fotografija 1: Razlike v rasti 41 dni starih kultur C. ferruginosum na gojišču OA, MEA in PDA (od leve proti desni).

Photo 2: Slimy conidial masses on culture of *C. ferruginosum* (all photos by D. Jurc).

Fotografija 2: Sluzaste mase konidijev na kulturi C. ferruginosum (vse fotografije: D. Jurc).

3.1 EFFECTS OF TEMPERATURE AND AVAILABLE WATER ON GROWTH OF *C. FERRUGINOSUM*

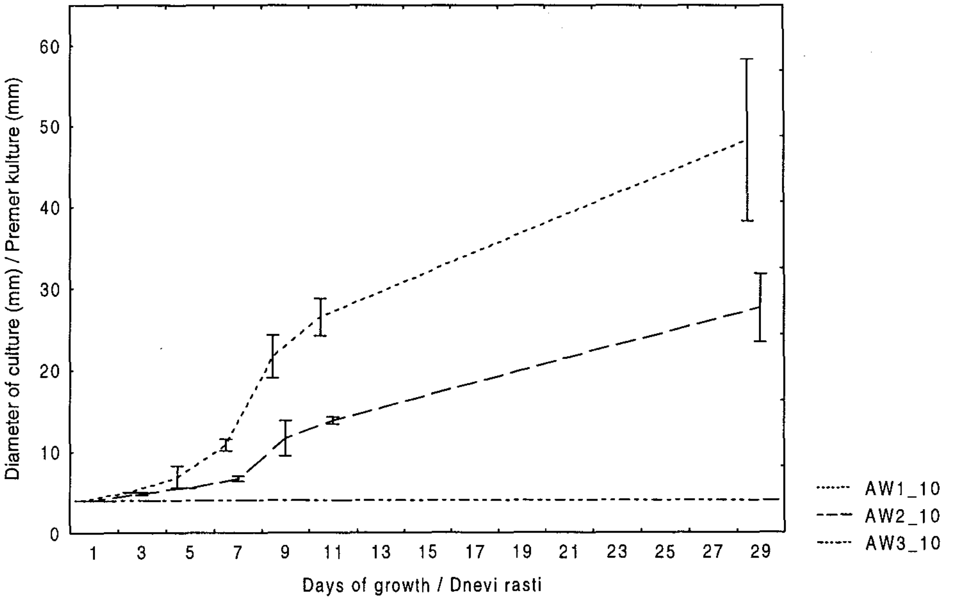
At 10^0 C and 20^0 C the growth of *C. ferruginosum* is similar, while the highest growth rate occurs at 25^0 C. High temperatures (30^0 C and 35^0 C) are not suitable for the growth of the fungus, in fact, at these temperatures, it doesn't grow at all (Graph 1).



Graph 1: Diameter (mean ± S.E.) of *C. ferruginosum* culture at temperatures 10, 20, 25, 30 and 35^0 C.

Grafikon 1: Premer (srednja vrednost ± S.E.) kulture C. ferruginosum pri temperaturah 10, 20, 25, 30 in 35^0 C.

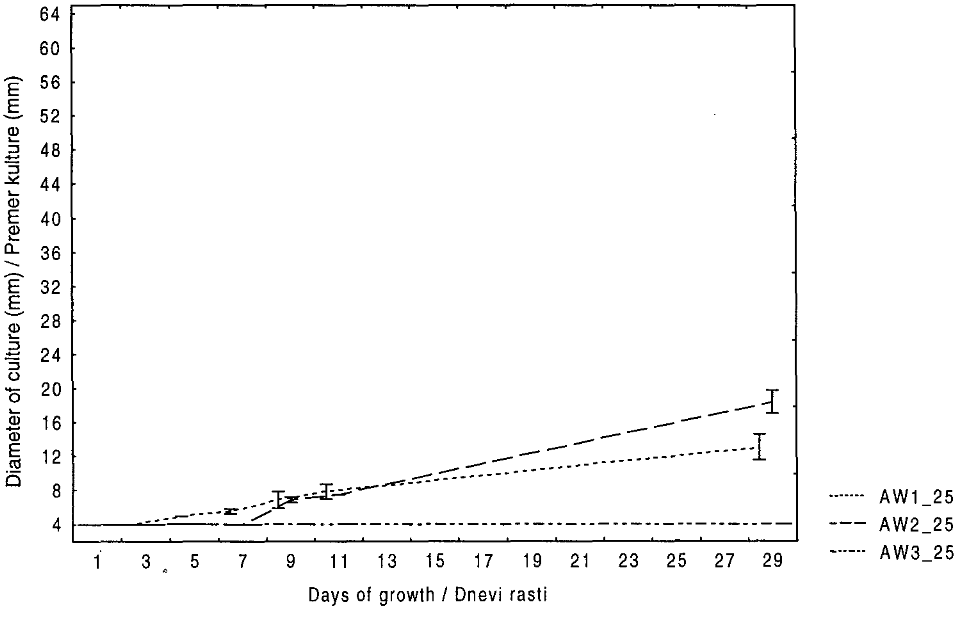
At 10^0 C *C. ferruginosum* grows well on relatively humid growth medium (aw1, aw2), it doesn't grow in relatively dry conditions (aw3) (Graph 2).



Graph 2: Diameter (mean ± S.E.) of *C. ferruginosum* culture on medium with different available water (aw1 = 0,98, aw2 = 0,96, aw3 = 0,94), at 10^0 C.

Grafikon 2: Premer (srednja vrednost ± S.E.) kulture C. ferruginosum na podlagi z različnimi količinami dostopne vlage (aw1 = 0,98, aw2 = 0,96, aw3 = 0,94), pri 10^0 *C.*

Growth rate of the fungus at 25^0 C and higher aw (aw1, aw2) is less than that at 10^0 C. As at 10^0 C the fungus doesn't grow at aw3 (Graph 3).



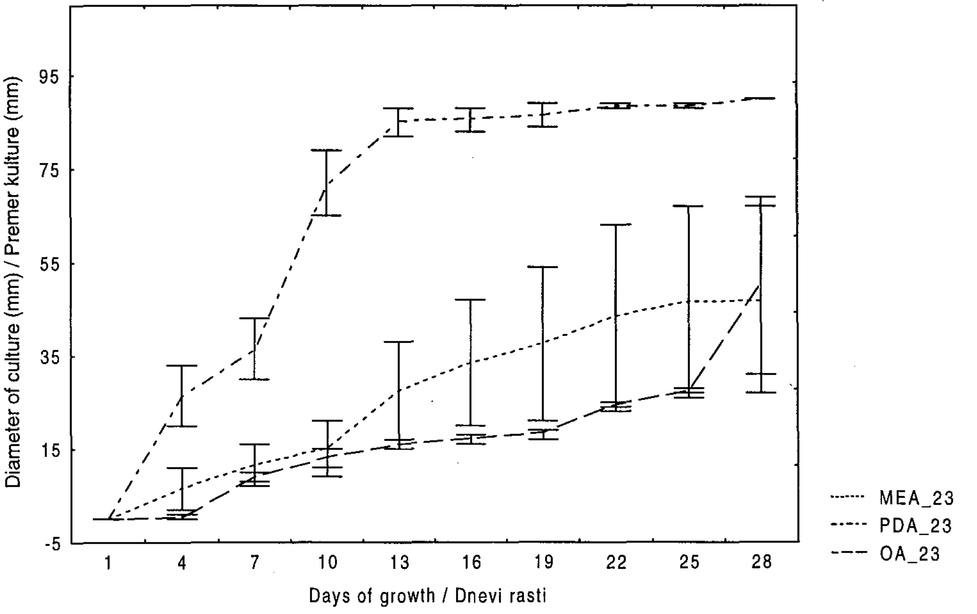
Graph 3: Diameter (mean ± S.E.) of *C. ferruginosum* culture on the medium with different available water (aw1 = 0,98, aw2 = 0,96, aw3 = 0,94), at 25^{0}C.

Grafikon 3: Premer (srednja vrednost ± S.E.) kulture C. ferruginosum na podlagi z različnimi količinami dostopne vlage (aw1 = 0,98, aw2 = 0,96, aw3 = 0,94), pri 25^0 C.

At 30^0 C and 35^0 C there is no growth on any medium no matter what the aw.

3.2 DIFFERENCE IN GROWTH ON MEA, PDA AND OA

The growth of *C. ferruginosum* cultures on different growth media is presented in Graph 4. The growth on MEA medium is irregular, often with rather quick outgrowths of parts of a colony, which leads to a high level of variability in the results. In culture, the fungus forms conidiomata which begin to appear on the14th day of subculturing on PDA and OA and on the 20th day on MEA.



Graph 4: Diameter (mean ± S.E.) of *C. ferruginosum* culture on the MEA, PDA and OA at 23^0 C.

Grafikon 4: Premer (srednja vrednost ± S.E.) kulture C. ferruginosum na podlagi MEA, PDA and OA pri 23^0 C.

The growth rate of the fungus is highest on PDA medium, while on MEA it is higher than on OA up to the end of the experiment, when the growth rate is equalised.

4 DISCUSSION

The biology and pathogenicity of *C. ferruginosum* has been thoroughly investigated in numerous works. It is defined as a facultative parasite of many pine species, although it rarely occurs in fir and spruce species (KUJALA 1950, LORENZ 1967, SMERLIS 1973). Its occurrence and ecological role is mostly regarded as saprobic and, as such, it cannot be harmful to its host if it grows under favourable climatic and site conditions (KOBAYASHI / MAMIYA 1963, LUKOMSKI 1968). The interpretation of its definition as a facultative parasite is based on numerous examples of its epidemic spreads after prolonged droughts, severe winters or other ecological disturbances in previous seasons, (LENGYEL 1963, PETRAK 1961, DONAUBAUER 1974, TORRES 1972, CAPRETTI / PANCONESI / PARRINI 1987, JURC 1987, SINCLAIR / LYON / JOHNSON 1987) such as epidemics of *C. ferruginosum* after attacks of harmful insects (DOMINIK 1976, LORENZ 1966, 1967).

C. ferruginosum fructifications are found on pine branches. If branches with immature ascomata are kept in moist conditions numerous conidiomata with masses of conidia can be found on the bark (JURC, D., unpublished observation). Stromatic tissues are formed in the bark and ascomata are formed on the scars of dropped needles, in bark cracks, or over entire branch surfaces. Millar (1981) supposed that infection occurs through, or in the vicinity of, the terminal bud in the succulent terminal region. Mechanical damage from insects as possible entrance points are also mentioned. Gremmen's (1959) statement that this fungus is a pioneer organism of branches still attached to the tree and that it only seems to inhabit branches suffering from primary parasitic attack, e.g. after damage by *Gremeniella abietina* or after insect damage (*Cecidomya brachyntera*), is also well-accepted.

C. ferruginosum is frequently isolated from Scots pine. Its endophytic occurrence in needles of *P. sylvestris* in plantations was reported by Rack and Scheidemann (1987). In parallel research of healthy and diseased needles they established that the fungus is more frequently isolated from the top and bottom of the crown, although there was no difference in the frequencies of isolation between old and young trees, and none among different age classes of the needles. Helander et al. (1994) found that the highest number of *C. ferruginosum* infections occur in the base segments of needles of Scots pine. They suppose that the fungus either systematically colonizes the needles beginning with the twigs and moving through the petioles into the needle base or that colonisation starts from spores invading the needle base and proceeding systematically into the twigs.

We presume that the relatively high *C. ferruginosum* infection rate of the needles enables this fungus to invade the twigs of drought stressed Austrian pine trees and that its constant presence as an endophyte explains why the epidemics can occur suddenly over such large areas, as occured in Slovenia in 1986. Our results explain the ability of the fungus to invade the tissues of its host during colder periods of year and when the substrate is highly moistened. High summer temperatures are unsuitable for its growth. Suitable conditions for its spread from the needles to the twigs occurs more often in the wet and relatively warm winter conditions of a submediterranean climate than in Nordic countries where the epidemic dying of pines doesn't occur. The optimal humidity for growth of this fungus is high and even at 10^0 C it still grows considerably. The symptoms of *C. ferruginosum* dieback of twigs occurs in Slovenia during the winter months (December through March) when the average monthly temperatures varies between 3^0 C to 7^0 C and when more than half of the yearly precipitation falls (1.643 mm). A prerequisite condition for the outbreak cf a Cenangium dieback of pines is a previous drought which modifies or damages the host. The nature of these damages are not defined but are inevitably connected with host defense mechanisms.

There are no comparative literature data about the growth of *C. ferruginosum* on different nutrient sources. Based on our data it has been established that the substrate is an important factor and influences the growth rate of the fungus considerably.

5 POVZETEK

Namen laboratorijskih poskusov je bil določiti toplotne in vlažnostne razmere za rast *C. ferruginosum* v kulturi in ugotoviti rast glive na različnih hranilnih gojiščih. Predvidevali smo, da bodo ti podatki odkrili tiste ekološke dejavnike, ki so odločujoči za rast glive in so pogoj za izbruh bolezni. Ti dejavniki omogočajo glivi, da prekine svoje mirujoče endofitno stanje in hitro preraste oslabela tkiva gostitelja.

Rast *C. ferruginosum* je pri 10^0 C in 20^0 C podobna, najhitrejša je pri 25^0 C. Visoke temperature (30^0 C in 35^0 C) niso ustrezne in pri teh temperaturah gliva ne raste (grafikon 1).

Pri 10^0 C *C. ferruginosum* dobro raste na relativno vlažni hranilni podlagi (aw1, aw2), ne raste pa na relativno suhi podlagi (aw3) (grafikon 2)

Pri 25^0 C in višji dostopni vlagi (aw1, aw2) je rast manjša kot pri 10^0 C. Gliva ne raste na aw3 (grafikon 3).

Pri 30^0 C in 35^0 C gliva ne raste ne glede na količino dostopne vode.

Najhitreje raste na gojišču PDA. Na gojišču MEA je rast večja kot na gojišču OA vse do konca poskusa, ko se izenači.

Domnevamo, da relativno močna, stalna okuženost zdravih iglic s *C. ferruginosum* omogoča tej glivi razrast iz iglic v vejice črnih borov, ki so oslabljeni zaradi suše. Njena stalna endofitna prisotnost je razlog, da epidemije nastopijo nenadno na tako velikih območjih, kot v Sloveniji v letu 1986. Naši rezultati pojasnjujejo sposobnost glive, da okuži tkiva gostitelja med hladnimi letnimi obdobji in takrat, ko je podlaga dovolj vlažna. Visoke poletne temperature so neustrezne za njeno rast. Ustrezne razmere za njeno razraščanje iz iglic v vejice so pogostejše v vlažnem in relativno toplem zimskem obdobju submediteranskega podnebja kot pa v hladnem podnebju nordijskih držav, kjer se epidemije bolezni ne pojavljajo. Optimalna vlažnost za rast glive je visoka, pri njej znatno raste še pri 10^0 C. Simptomi sušice borovih vej se pojavljajo v

Sloveniji v zimskih mesecih (od decembra do marca), ko so poprečne mesečne temperature med 3^0 C in 7^0 C in ko pade več kot polovica letnih padavin (1.643 mm). Pogoj za izbruh sušice borovih vej je predhodna suša, ki spremeni oziroma poškoduje gostitelja. Narava teh poškodb ni znana, vendar so neobhodno povezane z obrambnimi mehanizmi gostitelja.

V literaturi niso opisani primerljivi podatki o rasti kulture *C. ferruginosum* na različnih hranilnih podlagah. Na osnovi naših podatkov ugotavljamo, da je hranilna podlaga pomemben dejavnik, ki močno vpliva na rast glive.

6 ACKNOWLEDGEMENTS

The work was funded by The Ministry of Agriculture and Forestry, The Ministry of Science and Technology of the Republic of Slovenia (No. V9-6913-040) and The British Council of Slovenia (ALIS link No.28 1996/99).

7 REFERENCES

BUTIN, H., 1996. Krankheiten der Wald- und Parkbäume. Diagnose - Biologie - Bekämpfung.- Georg Thieme Verlag, Stuttgart,New-York, 261 s.

CAPRETTI, P. / PANCONESI, A. / PARRINI, C., 1987. Dieback of Aleppo and Maritime pine in plantations in northern Maremma, Italy.- Monti e Boschi. 38, s. 42-46.

DALLYN, H. / FOX, A., 1980. Spoilage of materials of reduced water activity by xerophilic fungi.- In: Microbial Growth and Survival Extremes of Environment (ed. G. H. Guld & J. E. L. Corry), London, Academic Press, s. 129-139.

DOMINIK, J., 1976. Insect pests and fungus diseases in young plantations and thickets of *Pinus ponderosa, P. jeffreyi, P. flexilis* and *P. resinosa* in the experimental forest at Rogów.- Sylwan 120, s. 14-18.

DONABAUER, E., 1974. Dieback of Pines in Austria caused by *Scleroderris lagerbergii* and *Cenangium ferruginosum*.- In 100 Jahre Forstliche Bundesversuchsanstalt. Vienna, Austria, Forstliche Bundesversuchsanstalt. s. 67-98.

GREMMEN, J., 1959. A Contribution to the Mycoflora of Pine Forests in the Netherlands.- Nova Hedwigia. B1, 3n., 4, s. 267-287.

HELANDER, M. L. / SIEBER, T. N. / PETRINI, O. / NEUVONEN, S., 1994. Endophytic fungi in Scots pine needles: spatial variation and consequences of simulated acid rain.- Can. J. Bot. 72, s. 1108-1113.

JURC, D., 1987. Zanimivosti naše mikoflore. 2 Povzročitelj sušice borovih vej.- Proteus, Prorodoslovno društvo Slovenije, Ljubljana, s. 183-187.

JURC, M., 1996. Endofitne glive in njihove značilnosti v iglicah črnega bora (*Pinus nigra* Arn.). (Endophytic fungi and their characteristics in the needles of Austrian pine (*Pinus nigra* Arn.)).- Dissertation thesis, University of Ljubljana, Department of biology, 198 s.

KUJALA, V., 1950. Über die Kleinpilze der Koniferen in Finland.- Communicationes Instituti Forestalis Fenniae, Helsinki, 38, 121 s.

KOBAYASHI, T. / MAMIYA, Y., 1963. A Cenangium causing dieback of Japanese Pines.- Bull. For. Exp. Sta., Meguro, Tokyo, No. 161, s. 133-150.

LENGYEL, G., 1963. Dieback of *Pinus nigra var. austriaca* in Hungary, 1960-1962. Erdész.- Kutatás., Budapest 59, s. 55-75.

LORENZ, I., 1966. The condition affecting the infection and development of Pine dieback (*Cenangium ferruginosum* Fr.).- Arch. Forstw. 15, s. 725-734.

LORENZ, I., 1967. Studies on the biology and pathogenicity of *Cenangium ferruginosum* Fr.- Arch. PflSch., Berl. 3, s. 143-153.

LUKOMSKI, S., 1968. Studies on biology and harmfulness of the fungus *Cenangium ferruginosum* Fr. ex Fr.- Prace Instytutu Badawczego Lesnictwa, Warszawa. No. 352, s. 55-96.

MAGAN, N. / LACEY, J., 1984. Effect of temperature and pH on water relations of field and storage fungi.- Trans. Br. mycol. Soc., 82, s. 71-81.

MILLAR, C. S., 1981. Infection processes on conifer needles.- In Microbial Ecology of the Phylloplane, ed. J.P. Blakeman, London, Academic Press, s. 185-209.

PETRAK, F., 1961. Critical remarks on the mortality of Pines in Austria.- Sydowia 15, s. 152-158.

RACK, K. / SCHEIDEMANN, U., 1987. Über Sukzession und pathogene Eigenschaften Kiefernnadeln bewohnender Pilze.- Eur. J. For. Path., 17, s. 102-109.

SINCLAIR, W. A. / LYON, H. H. / JOHNSON, W. T., 1987. Diseases of trees and shrubs.- Comstock Publishing Associates, Cornell University Press, Ithaca London, 575 s.

SMERLIS, E., 1973. Pathogenicity tests of some discomycetes occurring on conifers.- Canadian Journal of Forest Research 3, s. 7-16.

TORRES, J., 1972. *Cenangium ferruginosum* as a factor in natural selection.- Boletín de la Estación Central de Ecología 1, s. 29-30.