Optimizing conditions for sporulation of European mistletoe hyperparasitic fungus (*Phaeobotryosphaeria visci*): effect of light and different media

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SUMMARY

*European mistletoe (*Viscum album*), the hemiparasitic shrub, infects a wide range of woody species. It adversely affects the height and diameter growth and it is associated with increased mortality of its hosts. Currently there are no effective control methods against it. Therefor, we started to study a specific hyperparasitic fungus (*Phaeobotryosphaeria visci*), which can completely destroy European mistletoe by infecting its branches, leaves and berries. An important aspect of the initial phase of mycopesticide candidate is culturing of the organism on artificial or non-synthetic media, followed by the optimization of spore production. We focused to determinate the growth media and light conditions needed for sporulation of* P. visci*. We also tested the viability and pathogenecy of the spores, because these are important features for further processing and applications. The cultures were grown on seven different media (potato dextrose agar, sugar free potato dextrose agar, cellophane covered potato dextrose agar, oatmeal agar, V8 Juice agar, S medium and SNA medium) under constant dark, constant light (400-750 nm) and 12 h of alternating dark and light illumination. The best primary agar media were oatmeal and sugar free potato dextrose agar under permanent illumination, while constant dark inhibited the conidial production. The viability and virulence of harvested conidia were normal, and the symptoms of the disease appeared 7-14 days after the inoculation on mistletoe leaves. We will continue our experiments studying the effect of near UV (280–400 nm) light. Use of variable photoperiods supplemented with near UV and different media could help us to optimize the spore production and create a fast and cheap mass production technique.*

Keywords: photoperiod, oatmeal agar, biological control, bioherbicide, mass production

INTRODUCTION

European or White berry mistletoe (*Viscum album* L.) of Santalaceae is an evergreen, perennial, epiphytic, hemiparasitic shrub (Zuber 2004), which is able to infect more than 450 woody species (Barney *et al.*, 1998). This hemiparasite is widely distributed in Europe and it was introduced to North America and Canada at the beginning of the 20th century (Hawksworth *et al.*, 1991). In addition to the detrimental effects caused by the global warming, air pollution and other abiotic stress factors mistletoe causes increasing damages in many forests and orchards. It adversely affects the quality and quantity of the wood, reduces fruiting, and predisposes their hosts to be attacked by other agents, such as insects or different fungi (Hawksworth 1983). Successful control methods of mistletoe could be the pruning of infected branches, but this technique causes disband of canopy and huge stress to the host.

We started to look for an effective control method by studying *Phaeobotryosphaeria visci* (Kalchbr.) A.J.L. Phillips & Crous [Syn.: *Botryosphaeria visci* (Kalchbr.) Arx & E. Müll.; anamorph: *Sphaeropsis visci* (Fr.) Sacc.]. Many species of Botriosphaeriaceae are common plant pathogens and saprobes found on variety of mainly woody hosts. The species *Phaeobotrioshaeria* form brown aseptate ascospores with small apiculus at either end. The anamorph pycnidia are more common with oval and aseptate conidia, which are greenish, while the older cells are brown (Phillips *et al.*, 2008). This plant pathogen causes leaf spot disease of the European mistletoe and it seems to have potential as a tool of biological control against European mistletoe (Varga *et al.*, Karadžić 2004, Fischl 1996). Three to six weeks after the inoculation the fungal infection spreads to all over the whole leaves, branches and berries; a few months later the whole shrub becomes dark yellow and necrotic (Stojanovic 1989).

The idea of biological control against mistletoe was conceived in the early 1970s but the detailed research began only in 2008 at the University of Pannonia. Laboratory studies with *Phaeobotrioshaeria visci* suggest that it is a promising biological control candidate. An important aspect of the initial phase of studies of this mycopesticide is culturing of the organism on artificial or non-synthetic media, followed by optimization of spore production. Mass production on agar can provide sufficient inoculum for laboratory and field tests, but more importantly, these studies provide valuable knowledge for subsequent optimization of mass production (Masangkay *et al*., 2000).

The most often and commonly used techniques for spore induction is the application of different light and photoperiods (Leach 1962, 1971) as well special media (Booth 1971). Kim *et al.* (2005) reached successful pycnidial and conidial production for a *Sphaeropsis* species using oatmeal agar, fluorescent lamp (near UV) and different photoperiods (12h light/12 h dark), while Palmer *et al.* (1987) applied effectively continuous visible light on *Sphaeropsis sapinea* growing on potato dextrose agar.

The specific culturing conditions needed for conidial production for *Phaeobotrioshaeria visci* are currently unknown. Rapid and intense mycelial growth was achieved on potato dextrose agar (PDA), oatmeal agar (OA), ¼ PDA+V8 Juice agar but no spore formation was detected on these media under dark condition (Varga *et al*., 2012b). First, we tested the application of different media and photoperiods, which were used earlier efficiently on other species of the genus *Sphaerosis*. After the spore induction experiments we tested the viability and pathogenecy of the spores as these are important features for further processing and applications.

MATERIALS AND METHODS

Fungal isolates and culture maintenance

Diseased European mistletoe leaves were collected in Ajka, Hungary in August 2010 from silver maple (*Acer saccharinum* L.). Monospore culture was prepared as detailed in Varga et al (2012a). The analysed strain was maintained on potato dextrose agar (4 g potato extract, 20 g glucose, 15 g agar) at 20°C and was replaced once a month. Plates were inoculated with a 5 mm diameter core of mycelium and agar excised from the growing edge of 7-day-old monoconidial stock culture.

Effect of agar media and light conditions on conidial production

The spore inducing effects of media were tested on seven different types of agar. These media were:

(1) Potato dextrose agar (20 g glucose, 4 g potato extract, 20 g agar L^{-1});

(3) Cellophane covered potato dextrose agar (20 g glucose, 4 g potato extract, 20 g agar L^{-1} covered with a semi-permeable plastic membrane);

(2) Sugar free potato dextrose agar (4 g potato extract, 20 g agar L^{-1});

(4) Oatmeal agar (100 g steamed oatmeal, 20 g agar L^{-1});

(5) ¼ PDA+V8 Juice agar (1 g potato extract, 5 g glucose, 150 ml V8 vegetable juice (Campbell South Co.), 3 g $CaCO_3$, 20 g agar L^{-1});

(6) S medium (20 g sucrose, 30 g $CaCO_3$, 20 g agar L^{-1}) and

(7) SNA medium (KH_2PO_4 1g, KNO_3 1g, $MgSO_4$ x 7 H_2O 0.26 g, KCl 0.5 g, glucose 0.2 g, sucrose 0.5 g, agar 20 g L^{-1}).

Plastic Petri-dishes (90 mm diameter) contained 20 ml from these media in six repeat per photoperiod treatment. Potato dextrose agar (PDA), sugar free potato dextrose agar (SF PDA), cellophane covered potato dextrose agar (CC PDA), oatmeal agar (OA) and ¼ PDA+V8 Juice agar (V8) plates were inoculated directly with a 5 mm diameter core of mycelium and agar excised from the growing edge of 7-day-old monoconidial stock culture. Dishes were sealed with parafilm and incubated in dark thermostat on 25°C for 7 days. S and SNA medium were inoculated with 7-days-old agar free mycelia discs which were initially grown on cellophane covered PDA in dark thermostat on 25°C prior the inoculation. The seven days old isolates were placed in a thermostat (Binder ATM line^{TM} KBV) under constant dark or under constant light and under 12 h dark/12 h light photoperiod for 14 days at 20°C. A standard 865 daylight coloured (400-750 nm, 6400 K) light tube was used for the illumination.

Pycnidia were harvested 21 days after the inoculation with 5 ml of sterile deionized water and the surface of the colonies were scraped with a sterile rubber spatula. Conidia were extracted with braying of pycnidia and counted with the aid of haematocytometer under a light microscope (× 100). Sporulation was expressed in two ways: the number of conidia per cm^2 of colony and the number of conidia per colony. The morphology of cultures and the zonation of sporulation were evaluated visually.

All plates were evaluated and averaged. One treatment contained six Petri-dishes where the number of conidia was counted on four plates while remaining plates were evaluated visually (pycnidia and conidia germination).

Pathogenicity test

The harvested conidia were used for a pathogenicity tests. Healthy mistletoe leaves were inoculated with 2 × 50 µl of spore suspension (2.5×10^4 db ml^{-1}) 4x. Pathogenicity test was made following Varga *et al.* (2012b). The results were evaluated visually.

Data analysis

The data processing was carried out in Microsoft Office Excel 2010 and all statistical analyses were performed using the statistical program R, version 2.15.1. (R Development Core Team 2012), for editing R scripts the Tinn-R code editor was used (Faria 2011) while graph plotting was carried out with the package "sciplot" (Morales 2011).

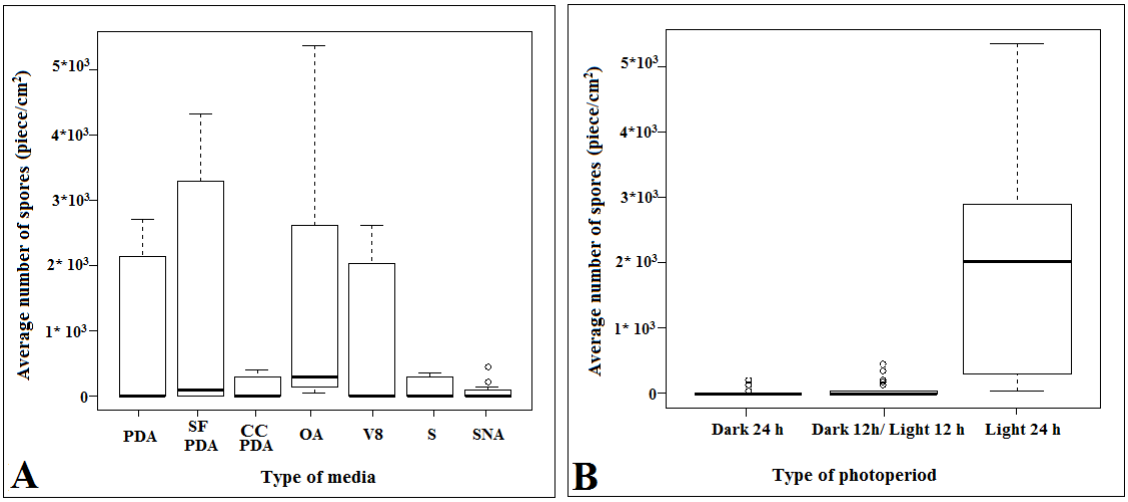
For characterization of relationships a two-way analysis of variance (ANOVA) type I (sequential) sum of squares was used. In this case the first factor was the "light condition" and the second factor was the "type of media". To determine the difference between factor levels a Dunnett-Tukey-Kramer pairwise multiple comparison test was performed. To estimate the factor level treatment contrast was used. After the analysis all accepted statistical models were checked pre-eminently with help of diagnostics plots.

RESULTS

Effect of different media

The Dunnett-Tukey-Kramer pairwise multiple comparison tests confirmed that the spore production was significantly higher on oatmeal agar (OA) and sugar free potato dextrose agar (SF PDA); there was no statistical difference among these media ($p<0.001$). The number of spore formed on cellophane covered potato dextrose agar (CC PDA) was extremely low, as well on S and SNA media. Sporulation on potato dextrose agar (PDA) and ¼ PDA+V8 Juice agar (V8) was lower than on sugar free potato dextrose agar (SF PDA), but this difference is statistically significant only at 0.05 significance level (*Figure 1 [A]*).

Figure 1: **Effects of different media (A) and photoperiods (B) on spore production of *Phaeobotrioshaeria visci***



PDA= potato dextrose agar, SF PDA= sugar free potato dextrose agar, CC PDA= cellophane covered potato dextrose agar, OA= oatmeal agar, V8= ¼ PDA+V8 Juice agar, S= S medium, SNA= SNA medium

Effect of different photoperiod

The two-way ANOVA showed significant difference between the number of spores and light conditions ($F_{2,63}$ = 260.63; $p<0.001$), but there was no statistical difference ($p<0.001$) in spore production between continuous dark (24 h) and alternating light/dark (12h–12 h) treatments. Spore production was very low, or there was absolutely no sporulation on most of the plates under these photoperiods. While these treatments did not induce higher spore number, the continuous illumination stimulated significantly higher ($p<0.001$) spore production. There was different extent of sporulation on all media influenced by continuous light (*Figure 1 [B]*).

Interactions among media and photoperiods

The quantity of formed spores can be seen on *Table 1* and *Figure 2*.

Table 1

Influence of agar medium and light conditions on conidial production of *Phaeobotrioshaeria visci* after incubation for 21 days

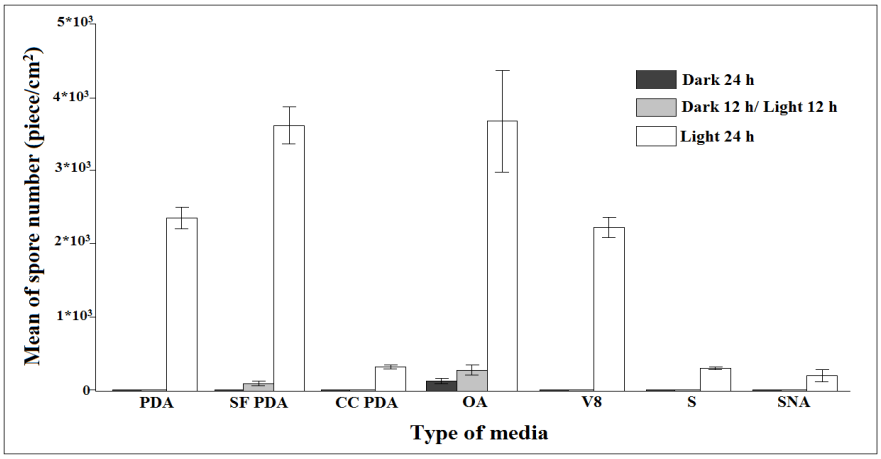
Medium	Number of conidia (piece/plate and piece/cm²)					
	Light conditions					
	Dark 24h		Dark12h/ light12h		Light 24h	
PDA	0	0	0	0	14.9×10^4	2347.5
SF PDA	0	0	0.62×10^4	97.7	22.9×10^4	3604.00
CC PDA	0	0	0	0	2.1×10^4	327.25
OA	0.8×10^4	130.7	1.8×10^4	283.5	23.2×10^4	3659.25
V8	0	0	0	0	14.1×10^4	2216.75
S	0	0	0	0	1.9×10^4	305.25
SNA	0	0	0	0	1.3×10^4	207.00

PDA= potato dextrose agar, SF PDA= sugar free potato dextrose agar, CC PDA= cellophane covered potato dextrose agar, OA= oatmeal agar, V8= ¼ PDA+V8 Juice agar, S= S medium, SNA= SNA medium

The significantly highest ($p<0.001$) conidial density after 21 days of incubation was formed on oatmeal agar (OA) and sugar free potato dextrose agar (SF PDA), when colonies were exposed to constant illumination

resulting in number of spores 22–23 × 10^4 piece/plate. Spores formed only on oatmeal agar in all illumination conditions, while they appeared on sugar free potato dextrose agar after alternating illumination and continuous light too. Conidial production was low due to these combinations (0.6–1.8 × 10^4 piece/plate); the number of conidia was almost 30 times higher on oatmeal agar after permanent illumination, than after imperfective dark.

Figure 2: **Average value of spore number with SEM (standard error of mean)**



PDA= potato dextrose agar, SF PDA= sugar free potato dextrose agar, CC PDA= cellophane covered potato dextrose agar, OA= oatmeal agar, V8= ¼ PDA+V8 Juice agar, S= S medium, SNA= SNA medium

In case of the interaction of constant light and media there was no statistically significant difference between the number of spores on potato dextrose agar (PDA) and ¼ PDA+V8 Juice agar (V8). Although the quantity of spores formed was significantly lower ($p<0.001$) than formed on oatmeal agar (OA), the amount of spores was relatively high (14–14 × 10^4 piece/plate).

The other combinations resulted in low spore production without significant difference ($p<0.001$), the average value of spores was 1–2 × 10^4 piece/plate. The lowest level of sporulation were noticed on S and SNA media in continuous light. Moreover, there was no sporulation on these media in permanent dark.

Morphological differences and pathogenicity tests

Morphological and sporulation differences of 21-days-old *Phaeobotrioshaeria visci* cultures affected by different photoperiods and media can be seen on *Fig. 3* and *Fig. 4.*

Figure 3: **Impact of the interaction among photoperiods and different media (PDA= potato dextrose agar, SF PDA= sugar free potato dextrose agar, OA= oatmeal agar, V8= ¼ PDA+V8 Juice agar) to sporulation of *Phaeobotrioshaeria visci***

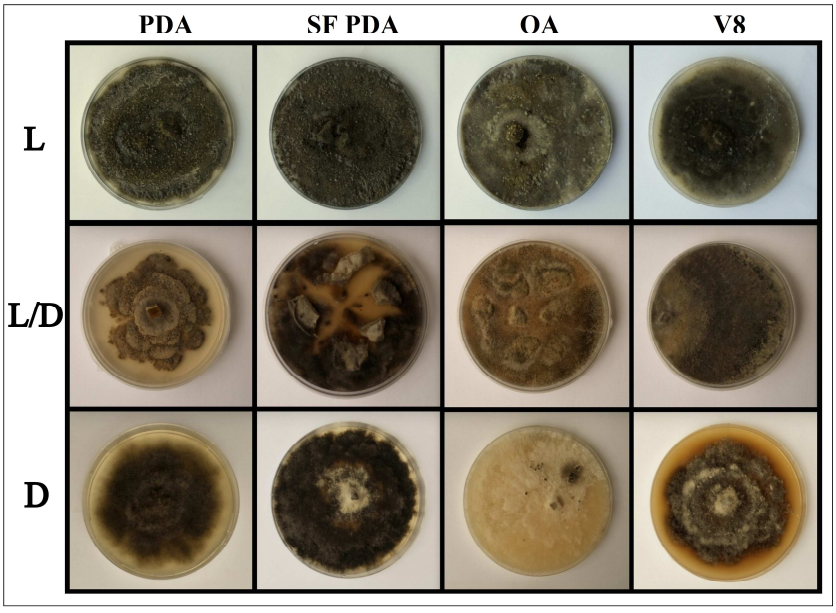
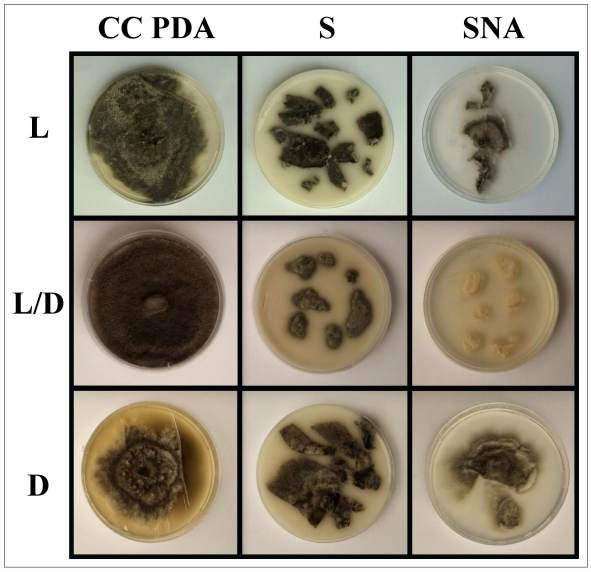


Figure 4: **Impact of the interaction among photoperiods and different media (CC PDA= cellophane covered potato dextrose agar, S= S medium, SNA= SNA medium) to sporulation of *Phaeobotrioshaeria visci***



Each media influenced the macromorphology of the cultures. The zonation, or the rosette-like appearance was not so typical in old cultures than in younger (7–14-days-old) ones, but this was observed only with some media (cellophane covered potato dextrose agar, ¼ PDA+V8 Juice agar). The filamentous habit was typical on oatmeal agar, where the colour of mycelia remains white for a longer period and becomes darker later, than on other media. Contrary to this the mycelia become dirty gray or black after 2-4 days of inoculation on S medium, and the cultures cease to grow any further. The situation is similar on SNA medium, but the mycelia remain always lighter than on S media.

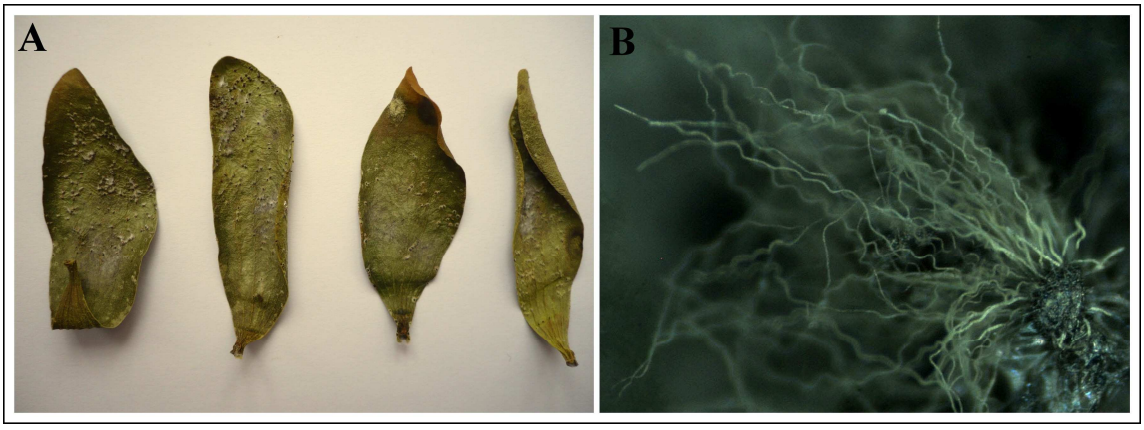
Regarding the illumination, the zonation of mycelial growth was not as pronounced, as in cultures of some other fungal species affected by the alternation of light and dark. Mycelia were mostly lighter under permanent dark and alternating illumination. The colonies always become dark and the pigmentation increased under continuous light.

The zonation of sporulation was not noticed under any photoperiod. The first pycnidia appeared beside the inoculation point, then they covered sporadically the surface of plates. Sporulation was observed in all cases on older plates, while none of the media induced earlier sporulation.

The pathogenicity test of harvested conidia was successful (*Figure 5 [A]*). One week after the inoculation the infected leaves became chlorotic and pycnidia formed after 10–14 days. Conidiogenesis started few days after the appearance of newly formed pycnidia.

The visual examination of pycnidia was carried out on 28 days old cultures on all media. The conidia started to germinate on all media by this time. The hymenium of the mature pycnidia was breached by germinating conidia, while the surface of the pycnidia was covered with fresh hyphae. Germination of harvested conidia began right after harvesting, when spores came to water and spore suspension for pathogenicity test was made. The proportion of the germinating spores was approximately 10% in the one day old spore suspension, which rose very fast.

Figure 5: **Viability and pathogenicity tests of formed spore: symptoms of *Phaeobotrioshaeria visci* on mistletoe 2 weeks after inoculation (A) and visually evaluation of conidia germination (× 45) of 28-days-old pure cultures (B)**



DISCUSSION

Only few previous studies have addressed the sporulation and mycelial growth of *Phaeobotrioshaeria visci* under *in vitro* laboratory conditions. Stojanovic (1989) observed sporulation on potato dextrose agar under permanent dark that we did not notice under similar conditions. Furthermore, he observed sporulation on prune extract agar (prune 30 g, sucrose 20 g, 15 g agar L^{-1}), that was also missing in our experiments.

The most appropriate medium and light condition for spore production of *Phaeobotrioshaeria visci* were oatmeal agar and sugar free potato dextrose agar under constant illumination with daylight tube at 20°C for 14 days. The maximal number of conidia (22–23 × 10^4 piece/plate) was produced in these conditions. Sporulation was inhibited by constant dark, but minimal spore production was observed on oatmeal agar (0.8 × 10^4 piece/plate). The alternating dark and light illumination did not result in considerable rise of spore number; however, the sporulation was also low on sugar free potato dextrose agar (1.8 × 10^4 piece/plate) in similar conditions. For species of *Sphaeropsis* the use of oatmeal agar gave the fastest mycelial growth and the highest number of conidia under 12 dark/12 h light conditions, while no sporulation was observed under permanent dark (Kim *et al.*, 2005, Xiao 2006). Based on these and our results oatmeal agar seems the most suitable to induce sporulation.

On the glucose free potato dextrose agar the high number of conidia was most probably due to low sugar concentration of the medium. In certain fungal species sporulations is stimulated *in vitro* by the depletion of the medium with nutrients. Such an association is well known in fungi that sporulate poorly on sugar-rich media. Sporulation of some facultative parasites on necrotic tissue is apparently associated with low sugar content in these tissues (Rotem *et al.*, 1978, Hawker 1966). Presumably the process of sporulation takes place in the same way in *Phaeobotrioshaeria visci*, thus sugar free potato dextrose agar can also be used efficiently. With cellophane covered potato dextrose agar we wanted to model circumstances analogues to sugar free conditions. Previously this method was successfully used with a species of *Microdochium* by Browne and Cook (2004). In our case the quantity of formed spores was only 2 × 10^4 piece/plate, much lower than in *Microdochium*, indicating the inappropriateness of this method for *P. visci.*

The other media did not proved to be as efficient as expected. The V8 Vegetable Juice is one of the most common media used effectively for spore induction in species of *Drechslera* (Raymond and Bockus 1982, Babadoost and Johnston 1998). This media (and constant light), similarly as with potato dextrose agar, induced only moderately high spore number (14 × 10^4 piece/plate), and this was equivalent with the number of spores produced in species of *Dreshlera* on this media. Furthermore S media, which is widely used for sporulation of species of *Alternaria* (Shahin and Shepard 1978, Masangkay *et al.*, 2000) also proved to be inproper to induce sporulation in *Phaeobotrioshaeria visci.* Compared to *Alternaria* – which formed large number of conidia on S medium – *P. visci* produced only 1.9 × 10^4 spores on a Petri–dish. Similarly with this medium, the quantity of conidia was also low on SNA medium. The use of these media which are suitable to induce sporulation in species of *Fusarium* (Paparu *et al.*, 2006) are not recommended for *P. vsci.*

Exposure to permanent dark inhibited conidial production, while 12 h of alternating light and dark did not result in significantly higher spore number. The positive effect of constant illumination was noticed on all agar media as this treatment enhanced dramatically the conidia production. Besides using daylight tubes, one of the most popular and successful methods is the use of near UV tubes (Rotem *et al.*, 1978, Booth 1971). Using different photoperiods of daylight, dark and near UV (280–400 nm) could be more effective, than using continuous light. Therefore, in order to optimize the spore production and to create a fast and cheap mass production system, we will continue experiments with different combinations of photoperiods and agar media.

ACKNOWLEDGEMENT

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REFERENCES

Babadoost, M. and Johnston, M. R. (1998): Sporulation of *Drechslera graminea* on barley straw extract agar. Mycologia, 90 (1): 63–68.

Booth, C. (1971): Fungal culture media. : Booth, C. (ed). Methods of Microbiology. London, Academic Press, 49–94.

Faria, J.C. (2011): Resources of Tinn-R GUI/Editor for R Environment.UESC, Ilheus, Brasil.

Fischl, G. (1996): A fagyöngy (*Viscum album* L.) levélfoltossága. Növényvédelem, 32 (4): 181–183.

Hawker, L. E. (1966): Environmental influences on reproduction. In: Ainsworth, G. C. and Sussman A. S. (eds.) The Fungi. Academic Press, New York. 2: 435–469.

Hawksworth, F. G. (1983): Mistletoes as forest parasites. In: Calder, M. and Bernhardt, P. (eds.) The biology of mistletoes. Academic Press, Sydney. 317–333.

Hawksworth, F. G., Scharpf, R. F. and Marosy, M. (1991): European mistletoe continues to spread in Sonoma County. Calif. Agric., 45: 39–40.

Karadžić, D., Lazarev, V. and Milenković, M. (2004) The most significant parasitic and saprophytic fungi on common mistletoe (*Viscum album* L.) and their potential application in biocontrol. Bulletin Faculty of Forestry, Univ. of Bajna Luka, Serbia, 89: 115–126.

Kim, Y. K., Xiao, C. L. and Rogers, J. D. (2005): Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. Mycologia, 97 (1): 25–32.

Leach, C.M. (1962): Sporulation of diverse species of fungi under near ultraviolet radiation. Can. J. Bot., 40: 151–161.

Leach, C.M. (1971): A practical guide to the effects of visible and ultraviolet light on fungi. In: Booth, C. (ed). Methods of Microbiology. Academic Press, London. 609–644.

Masangkay, R. F., Paulitz, T. C., Hallett, S. G. and Watson, A. K. (2000): Characterization of Sporulation of *Alternaria alternata* f. sp. *sphenocleae*. Biocontrol. Sci. Techn., 10 (4): 385–397.

Morales, M. (2011): Sciplot: scientific graphing functions for factorial designs. R package version 1.0–9.

Palmer, M. A., Stewart, E. L. and Wingfield, M. J. (1987): Variation among isolates of *Sphaeropsis sapinea* in the north central United States. Phytopath., 77: 944–948.

Paparu, P., Dubois, T., Gold, C. S., Niere, B., Adipala, E. and Coyne, D. (2006): Colonisation pattern of nonpathogenic *Fusarium oxysporum*, a potential biological control agent, in roots and rhizomes of tissue cultured *Musa* plantlets. Ann. Appl. Biol., 149: 1–8.

Phillips, A. J. L., Alves, A. , Pennycook, S .R., Johnston, P. R., Ramaley, A., Akulov, A. and Crous, P. W. (2008): Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia, 21: 29–55.

R Development Core Team (2012): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/.

Raymond, P. J. and Bockus, W. W. (1985): An *in vitro* technique for profuse sporulation of *Drechslera tritici-repentis*. Phytopath., 72: 934.

Rotem, J., Cohen, Y., and Bashi, E. (1978): Host and environmental influences on sporulation in vivo. Annu. Rev. Phytopathol., 16: 83–101.

Shanin, E. A. and Shepard, J. F. (1978): An efficient technique for inducing profuse sporulation of *Alternaria* species. Phytopath., 69: 618–620.

Stojanovic, S. (1989): The investigation of *Sphaeropsis visci* (Salm.) Sacc. and *Colletotrichum gloeosporoides* (Sacc.) Penz., parasite on European mistletoe (*Viscum album* ssp. *typicum* Beck). Zastita Bilja, 40: 493–503.

Varga, I., Taller, J., Baltazár, T., Hyvönen, J. and Poczai, P. (2012a): Leaf-spot disease on European mistletoe (*Viscum album*) caused by *Phaeobotryosphaeria visci*: potential candidate for biological control. Biotechnol. Lett., 34 (6): 1059–1065.

Varga, I., Baltazár, T. and Pejchal, M. (2012b): Optimisation of crowing conditions of European mistletoe hyperparasitic fungus (*Phaeobotryosphaeria visci*): Effect of different media and antibiotics. VII. Medzinárodná Vedecká Konferencia, *in press*.

Xiao, C. L. (2006): Postharvest Fruit Rots in d'Anjou Pears caused by *Botrytis cinerea*, *Potebniamyces pyri*, and *Sphaeropsis pyriputrescens*. Plant Health Progress (online), doi:10.1094/PHP-2006-0905-01-DG.

Zuber, D. (2004): Biological flora of Central Europe: *Viscum album* L., Flora, 199 (3): 181–203.