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# CHARACTERIZATION OF *ARMILLARIA* SPECIES IN VIRGIN BEECH FORESTS OF THE CARPATHIAN BIOSPHERE RESERVE

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***Characterization of Armillaria species in virgin beech forests of the Carpathian Biosphere Reserve. — T. Tsykun, V. Nikolaychuk, S. Prospero. —*** *Diversity and distribution of species of the wood-decaying genus Armillaria were investigated in the Uholsko-Shyrokoluzhanskyi protected forest massif of the Carpathian Biosphere Reserve. Armillaria gallica (Marxmüller & Romagnesi), A. cepistipes (Velenovsky), A. ostoyae (Romagnesi) Herink and Armillaria mellea (Vahl:Fr.) Kummer were identified for the first time in Transcarpathian Ukraine. Rhizomorphs of A. cepistipes and A. gallica were very frequent both in the soil and on the root collars of trees and their abundance decreased with increasing altitude of the plots. Species composition indicates that in the investigated forests the genus Armillaria plays an important role as wood decomposer.*

***Key words:*** *rhizomorphs, diversity, wood-decaying fungi, natural forests.*

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***Характеристика Armillaria spp. букових пралісів Карпатського біосферного заповідника. — Т. Цикун, В. І. Ніколайчук, С. Просперо. —*** *Видове різноманіття та розповсюдження дереворуйнівних грибів роду Armillaria було досліджено на території Угольсько-Широколужанського масиву Карпатського біосферного заповідника. Вперше було виявлено три види Armillaria gallica (Marxmüller & Romagnesi); Armillaria cepistipes (Velenovsky); Armillaria ostoyae (Romagnesi) Herink; Armillaria mellea (Vahl:Fr.) Kummer. Досліджено залежність розповсюдження ризоморфного міцелію даних грибів від абіотичних факторів.*

***Ключові слова:*** *ризоморфи, біорізноманіття, дерево-руйнуючи гриби, природні ліси.*

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## Introduction

The genus *Armillaria* (Basidiomycetes) is one of the major components of the wood-decaying mycoflora in forest ecosystem worldwide. *Armillaria* species differ in geographical distribution as well as in ecological behavior. All *Armillaria* species can survive saprotrophically by degrading woody substrates and typically produce, in the soil or under the tree bark, highly differentiated filamentous aggregations named rhizomorphs (Garraway *et al*. 1991). Several species can also act as primary or secondary pathogens causing white rot in infected trees. However, pathogenicity varies considerably among the different *Armillaria* species (Gregory *et al.* 1991).

Over 40 *Armillaria* species are currently known worldwide, with seven of them clearly defined in Europe (Watling, Kile and Burdsall, 1991). Two European species are exannulated, i.e. *A. tabescens* which occurs on hardwood and *A. ectypa* which is a rare species in peat bogs of high latitudes or altitudes (Guillaumin 1973, Rishbeth 1982, Zolciak *et al.* 1997). Both *A. ectypa* and *A. tabescens* behave mostly as saprotrophs. From the five European annulated species, three (*A. gallica, A. borealis*, and *A. cepistipes*) are reported to be mostly saprotrophs or weak pathogens, whereas two species (*A. mellea* and *A. ostoyae*) are known to be highly pathogenic (Guillaumin *et al.* 1993, Prospero *et. al* 2004).

In natural forest ecosystems, native pathogens are important in regulating plant species diversity and distribution (Castello *et al.* 1995). Soil-borne fungal pathogens, such as *Armillaria* species, selectively remove the less vigorous trees, thereby producing canopy gaps and woody substrates which help forest regeneration (Holah *et al.* 1997, Bendel *et al.* 2006). Artificial changes due to management practices may greatly alter the ecological balance by increasing the impact of pathogens on forest structure and composition (Castello *et al.* 1995, Jactel *et al.* 2009). For example, when native forests are converted to exotic plantations, indigenous *Armillaria* species can cause considerable tree mortality (e.g. Van der Pas 1981). Forest management may also modify the natural balance between different *Armillaria* species, sometimes resulting in pathogenic behavior by hitherto preferentially saptrotrophic species (Legrand *et al.* 1996). Therefore, understanding distribution, diversity, and ecological role of *Armillaria* species in natural ecosys-

tems could provide valuable information for the control of these fungi in managed forests.

In Transcarpathian Ukraine, the Carpathian Biosphere Reserve (CBR) offers a unique opportunity for studying biodiversity and natural processes in virgin or primeval forest ecosystems, i.e. forests that have never been significantly modified by human activity. In this study, we investigated the occurrence of *Armillaria* species in virgin beech forests in the territory of the Uholsko-Shyrokoluzhanskyi protected massif of the CBR. Up to now, little is known about the incidence and occurrence of *Armillaria* species in beech primeval forests in Ukraine, with most studies only referring to *Armillaria mellea sensu latu* (e.g. Dudka *et al.* 1997).

## Materials and Methods

### *Study site and sampling*

Our study was conducted in the Uholsko-Shyrokoluzhanskyi protected massifs of the CBR, where forests have never been managed and can, therefore, be considered natural (Shelyag-Sosonko 1997, Brändli *et al.* 2008, Rizun and Chumak 2008). In the Uholsko-Shyrokoluzhanskyi massif (10'383 ha), pure beech virgin forests occupy 88% of the total area and are included in the UNESCO's World Heritage list. The massif, which consists of two contiguous areas (Uholka and Shyrokyi Lug forestry), is located between 400 and 1350 m a.s.l., with the timberline at about 1150m a.s.l. The climate is characterized by an annual average temperature of +7 °C and an annual average precipitation of 948 mm (Brändli and Dowhanytsch 2003). In the Uholka area the annual temperatures are slightly higher and the vegetation period longer than in the Shyrokyi Lug area (Tasenkevich *et al.* 1982).

Systematic soil sampling was conducted by taking a cube of soil (15 cm side) on the four corners of 25 x 20 m rectangles located on a 1.5 x 1.5 km regular grid (total of 43 plots). In addition, the closest tree to each corner was checked for epiphytic rhizomorphs on the root collar. The soil samples were sieved through a 9 mm square mesh to separate the roots and rhizomorphs from the soil. All rhizomorphs were collected and brought to the laboratory for basic measurements. A total of 100 g of the sieved soil was taken for pH determination as described by Voznyuk and Kuzmich (1984). Additionally, in the Uholka forest *Armillaria* basidiocarps were collected in October 2009 along two forest trails of about 10 km length which partially covered the two sides of the Mala Uholka River's valley. Minimal distance between collected basidiocarps was 200 m and all collected basidiocarps were brought to the laboratory for species identification.

### *Armillaria isolation*

*Armillaria* was isolated from the rhizomorphs as described by Prospero *et al.* (2003). Three segments 1 cm in length of one rhizomorph (randomly selected) from each soil sample and one epiphytic rhizomorph (randomly selected) from each tree were dipped in 50% ethanol for 15–20 s. Then, they were surface sterilized in 30% $H_2O_2$ for 25–40 seconds and placed on a semi-selective agar medium (Maloy 1974). The isolation plates were incubated in the dark at 20–25 °C. After one to three weeks, pure cultures were transferred to Diamalt agar (15 g $l^{-1}$ Bacto Agar; 20 g $l^{-1}$ Diamalt, Hindelbank, Switzerland). All remaining rhizomorphs were frozen at -20 °C for genetic analyses. From the five *Armillaria* basidiocarps collected, spore prints were obtained. Spores from a spore print were then spread on the surface of 1% malt-extract agar and incubated 1–3 days in the dark at 24 °C. At least 12 germinating single spores from each basidiocarp were isolated and cultured using the method of Korhonen and Hintikka (1980).

### *Species identification*

Species identification was carried out by using two methods, i.e. interfertility tests and PCR-RFLP analysis of the ribosomal DNA.

*Interfertility tests*

Twenty-two unknown isolates were paired with three different haploid tester strains (Korhonen 1978) of the five annulated European *Armillaria* species (*A. borealis*, *A. cepistipes*, *A. ostoyae*, *A. mellea*, and *A. gallica*) as described in Harrington *et al.* (1992).

*PCR-RFLP analysis of the ribosomal DNA*

The evolutionary pressure for the conservation of the non-coding regions between the rDNA units is low. Therefore, sequences of these regions can exhibit high degrees of variability and are useful for studies at the level of species (Pérez Sierra *et al.* 2000).

DNA extraction. DNA was extracted from 50 mg of lyophilized (12 hours) rhizomorphs (one per soil sample and one per tree) and from 30 mg of lyophilized mycelium, which was obtained from three-week-old pure cultures. The extraction was performed using the CTAB method described in Gardes and Bruns (1993). The DNA was re-suspended in 50 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at 4 ºC until use.

PCR amplification. The IGS-1 region of the ribosomal DNA was amplified by PCR. The PCR reactions were performed in 50-µL volumes with the following final concentrations: 1X reaction buffer (Sigma), 4 mM $MgCl_2$, 100 µM dNTPs (Promega), 20 pmol of each primer (LR12R and O-1, Veldman *et al.* 1981, Duchesne and Anderson 1990), 2.5 U Taq DNA polymerase (Sigma), and about 50 ng of DNA template. The PCR program included an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 2 min, and one cycle of 72 °C for 30 min. The sizes of the PCR products were determined on 1.5% agarose gels.

RFLP analysis. For species identification, the PCR products of the IGS-1 region were digested with the four restriction enzymes *Alu* I, *Hinc* II (*Hind* II), *Mva1269* I (*Bsm* I), and *Nde* I (Fermentas) (Harrington and Wingfield 1995). The digest was performed according to the manufacturer's protocol using 10 µL of the PCR product. The sizes of the restriction fragments were determined on 3% agarose gels. Species identification was considered successful if the resulting pattern corresponded to one of the previously de-

scribed patterns (Table 1) for the five annulated European *Armillaria* species (Harrington and Wingfield 1995, Kim *et al.* 2000, Keča *et al.* 2006, Pérez Sierra *et al.* 1999).

Table 1. *Alu* I, *Hinc* II, *Nde* I, and *Bsm* l restriction patterns of the amplified IGS region of the rDNA of the five annulated European *Armillaria* species (Harrington and Wingfield 1995, Pérez Sierra *et al*. 1999)

| Species | Type | PCR product, bp | Fragment size (bp) after restriction digestion with | | | |
|---|---|---|---|---|---|---|
| | | | ***Alu* I** | ***Hinc* II** | ***Nde* I** | ***Bsm* I** |
| *A. borealis* | 1 | 920 | 310, 200, 104 | 920 | 920 | 550, 370 |
| | 2 | 920 | 310, 200, 135 | 920 | 920 | 550, 370 |
| *A. cepistipes* | 1 | 920 | 399, 200, 183 | 590, 330 | 920 | 920 |
| | 2 | 920 | 310, 200, 135 | 590, 330 | 920 | 920 |
| *A. ostoyae* | | 920 | 310, 200, 135 | 920 | 620, 300 | 550, 370 |
| *A. mellea* | 1 | 875 | 320, 155 | 920 | 600, 275 | 875 |
| | 2 | 875 | 320, 180, 155 | 920 | 600, 275 | 875 |
| *A. gallica* | | 920 | 399, 240, 183 | 590, 330 | 920 | 920 |

## Results

In the 43 plots, a total of 172 soil samples were collected and 172 trees were inspected for epiphytic rhizomorphs on the root collar. Rhizomorphs were present in 85% of the soil samples and on the root collar of 81% of the inspected trees. Total dry weight of *Armillaria* rhizomorphs in the soil samples was 203 g and the mean diameter of rhizomorphs was 1.2 mm. Consequently, the estimated total rhizomorph biomass in the soil was about 512 kg/ha. The simultaneous presence of rhizomorphs in a soil sample and on the root collar of the nearby standing tree was observed in 85% of the samples. Pearson's correlation tests showed that the presence of epiphytic rhizomorphs on the root collars significantly correlated with the presence of rhizomorphs in the soil ($r = 0.46$, $p < 0.0001$).

A slightly significant inverse correlation between abundance of rhizomorphs in the soil and altitude of the plots was detected. About 80% of all soil rhizomorph dry mass was found at altitudes between 500 and 910 m a.s.l. and in proximity of the timberline (1000 ± 150 m a.s.l.) rhizomorphs were less frequent. The abundance of rhizomorphs (dry weight) in the soil was also affected by the soil pH. Soil acidity optimum for rhizomorphs was pH 4.1–5.0 (80% of total rhizomorphs weight) and increasing acidity reduced the abundance of rhizomorphs.

In total, 269 isolates could be assigned to an *Armillaria* species. *Armillaria cepistipes* was the dominant species (60% of the specimens), followed by *A. gallica* (19% of the specimens). In 53% of the plots only *A. cepistipes* occurred, in 3% only *A. gallica*, and in 44% both species. These two species were found both in the soil and epiphytically on the root collars of trees at equal frequence (based on a chi-square test $x^2 = 0.08$, $p = 0.73$). Compared to *A. gallica*, *A. cepistipes* was more frequent at higher altitudes (14% at altitudes >1000 m a.s.l.) and in soils with higher pH (71% in pH classes ranging from 4.0 to 5.5). *Armillaria gallica* was preferentially found (91% of the specimens) in the pure beech forest of the Uholka area (Fig. 1.) at low altitudes (500–800 m a.s.l.). Almost 63% of the *A. gallica* soil rhizomorphs originated from acidic soils (pH 3.0–4.51).

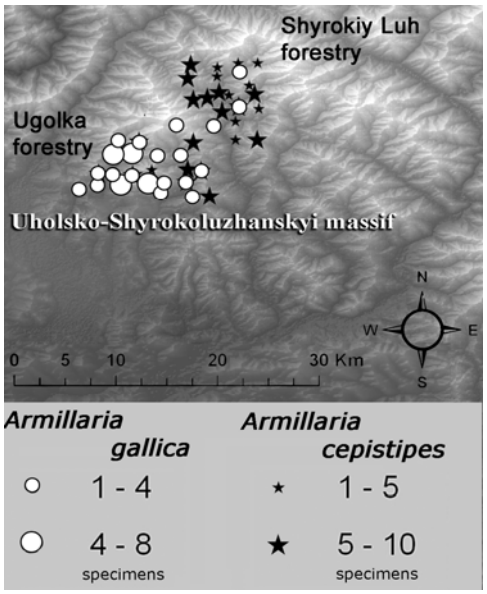


**Fig. 1.** Spatial distribution and incidence of *Armillaria gallica* and *Armillaria cepistipes* rhizomorphs in the Uholsko-Shyrokoluzhanskyi forest massif.

PCR-RFLP analysis of the IGS-1 amplicons and sequence analysis revealed heterogeneous interspecific variations (*A. cepistipes/A. gallica)* in about 20% of the specimens (Fig. 2.) and intraspecific variation in 40% of the *A. cepistipes* specimens (Table 2). Generally, fragment patterns obtained after restriction digestion of our samples with *Alu* I were similar (+/- 5 bp) to those previously reported for European *Armillaria* species (Table 1). However, a new *Alu* I pattern (584 and 194 bp) from an *A. cepistipes* haploid tester and from a diploid isolate was observed. Additional two patterns were also detected in *A. cepistipes* which seem to be an intraspecific combination of previously known *Alu* I digestion patterns and new patterns (Table 2).

One percent of the specimens were classified as *A. ostoyae* (five rhizomorphs) or *A. mellea* (one fruiting body). The five *A. ostoyae* rhizomorphs were collected at four different locations. Three rhizomorphs were found

in two plots on the root collar of three beech trees at altitudes ranging from 600 to 700 m a.s.l, whereas two rhizomorphs were found in the soil in two plots at 1000–1100 m a.s.l. and with a soil pH 4.01–5.0. Only one fruiting body of *A. mellea* was found in the Uholka forest in proximity to the CBR border.

**Discussion**

The high abundance of rhizomorphs in the soil and on the root collars of trees suggests that the genus *Armillaria* is an important component of the mycoflora in the virgin forests investigated, where it most likely plays an important role as a wood decomposer. This hypothesis is supported by the total absence of remarkable tree mortality caused by pathogenic fungi, and by the predominance of the preferentially saprotrophic *A. cepistipes* and *A. gallica* (Guillaumin *et al.* 1993, Rigling *et al.* 1998, Prospero *et al.* 2003). The incidence of *A. ostoyae* and *A. mellea*, which can behave as aggressive primary pathogens, is very low in the investigated territory.

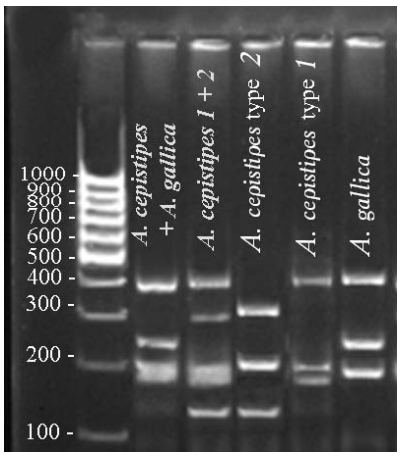


**Fig. 2.** *Alu I* restriction patterns of the Carpathian Armillaria isolates.

Table 2. *Alu* I, *Hinc* II, *Bsm* l, and *Nde* I restriction patterns of the amplified IGS region of the rDNA of *Armillaria* species from virgin Ukrainian beech forests

| Species | # isolates | PCR product (bp) | Fragment sizes (bp) after restriction digestion with enzymes* | | | |
|---|---|---|---|---|---|---|
| | | | ***Alu* I** | ***Hinc* II** | ***Bsm* I** | ***Nde* I** |
| *A. cepistipes* | 2 | 907 | (B5: 584, 194) | 584, | NRS*** | NRS |
| | 7 | | 584, 401, 182,194 | 323 | NRS | NRS |
| | 11 | | 584, 308, 135, 194 | 584, | NRS | NRS |
| | 32 | | 401, 182, 194 | 323 | NRS | NRS |
| | 45 | | (B2, B3: 308, 194, | 584, | NRS | NRS |
| | 65 | | 135) | 323 | NRS | NRS |
| | | | 401, 308, 194, 182, | 584, | | |
| | | | 135 | 323 | | |
| | | | | 584, | | |
| | | | | 323 | | |
| | | | | 584, | | |
| | | | | 323 | | |
| *A. ostoyae* | 8 | 907 | (C2, C5, C4: 308, | NRS | 620, | 547, |
| | | | 197,135) | | 287 | 360 |
| | | | | | NRS | 547, |
| | | | | | | 360 |
| *A. mellea* | 4 | 875 | (D2, D4, D5: 317, | NRS | 567, | NRS |
| | | | 175, 151) | | 308 | |
| *A. gallica* | 1 | 907 | (E4: 377, 257, 182) | 560, | NRS | NRS |
| | 52 | | (E5, E6: 401, 233, | 347 | NRS | NRS |
| | 3 | | 182) | 584, | NRS | NRS |
| | | | 401, 247, 182 | 323 | | |
| | | | | 584, | | |
| | | | | 323 | | |
| Isolates with a mixed *A. cepistipes* | 23 | 907 | 401, 308, 233, 182, | 584, | NRS | NRS |
| and *A. gallica* pattern | 29 | 907 | 194, 135 | 323 | NRS | NRS |
| | | | 401, 233, 194, 182 | 584, | | |
| | | | | 323 | | |

*Only fragments larger than 100 bp are shown.
**In brackets the name(s) and RFLP profiles of the haploid tester strain(s) for the specific species are given.
***NRS = No restriction sites present.

Previous studies showed that *A. ostoyae* has a very broad distribution in Europe (Guillaumin *et al.* 1993, Bendel *et al.* 2006) and behaves as an aggressive pathogen in managed coniferous stands (Mallett and Maynard 1998, Lung-Escarmant and Guyon 2004, Lushaj *et al.* 2009). In undisturbed stands and in beech or beech-fir stands, however, *A. ostoyae* may occur as a saprotroph or a secondary parasite (Legrand and

Guillaumin 1993, Tsopelas 1999). We found *A. ostoyae* rhizomorphs in pure beech forests, both in the soil (two plots) and epiphytically on the root collar (two plots) of trees, and their presence was never related to visible tree mortality. In the CBR's forests, *A. ostoyae*, like *A. cepistipes* and *A. gallica*, seems to preferentially behave as a saprotroph and rarely as a secondary/opportunistic parasite. Because of its pathogenicity toward conifers, *A. ostoyae* might be an important regulator of stand composition, leading forest succession in the direction of stable pure beech stands. However, detailed investigations are necessary to verify this hypothesis.

Although *A. cepistipes* and *A. gallica* are ecologically very similar, their distribution in the two forestry territories differed significantly. While *A. cepistipes* was the dominant species in almost all plots investigated, *A. gallica* was mainly restricted to the pure beech forests at low altitudes. *A. gallica* rhizomorphs were more frequent in the Uholka area where annual temperatures are slightly higher and the vegetation period longer than in the Shyrokyi Lug area. This distribution pattern of the two species supports previous findings indicating that *A. gallica* is generally confined to hardwood forests at low altitudes, while *A. cepistipes* can occur in both hardwood and coniferous forests (Guillaumin *et al.* 1993, Tsopelas 1999, Prospero *et al.* 2003, Keča *et al.* 2009). Our study suggests that soil pH may be a selective factor for the occurrence of either *A. cepistipes* or *A. gallica*. Eventually, *A. cepistipes* is more sensitive to soil acidity compared to *A. gallica* and it may have an advantage in occupying acidic soils in those forests where, according to the vegetation type and altitude, both species could theoretically occur. However, additional data would be necessary to better understand the factors affecting the distribution of these two species. In about 44% of the plots *A. cepistipes* and *A. gallica* co-occurred. The sympatrical co-existence of these two species was also observed in pure beech forests in Central France (Legrand *et al.* 1996), but it seems to be relatively rare. More frequently reported is the co-existence in the same stand of species characterized by a different ecological behavior, such as *A. cepistipes* and *A. ostoyae* (Prospero *et al.* 2003) or *A. gallica* and *A. mellea* (Baumgartner and Rizzo 2001).

In our study we observed heterogeneous intraspecific and interspecific variations in the IGS-1 region of the rDNA. In *Armillaria* species, heterogeneity in this conserved region was previously reported by other authors (Lochman *et al.* 2004, Keca *et al*. 2006, Hanna *et al.* 2007) but its origin is still unresolved. However, Hanna *et al.* suggested that the presence of different types of non-coding repeated regions of ribosomal DNA in the genome of an individual could be a consequence of still incomplete homogenization during the natural evolution process of species divergence. Considering that most of our isolates were diploid, the presence of isolates with combined *A. cepistipes* and *A. gallica Alu* I restriction patterns may indicate interspecific hybridization. Thus, in the investigated natural forests populations of *A. gallica* and *A. cepistipes* should still form interspecific crossings, as previously suggested by Keca *et al*. (2006) for populations in Serbia Montenegro. Mating tests combined with sequence analysis of ambiguous *Armillaria* isolates would be necessary to better understand the origin of this heterogeneity.

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**1.** *Baumgartner K., Rizzo D.M.* Ecology of *Armillaria* spp. in mixed-hardwood forests of California. // Plant Disease. – 2001. – Vol. 85. – P. 947–951.

**2.** *Bendel M., Kienast F., Bugmann H., Rigling D.* Incidence and distribution of Heterobasidion and *Armillaria* and their influence on canopy gap formation in unmanaged mountain pine forests in the Swiss Alps. // European Journal of Plant Pathology. – 2006. – Vol. 116. – P. 85–93.

**3.** *Brändli U.B., Dowhanytsch J.* Urwälder im Zentrum Europas. Ein Naturführer durch das Karpaten-Biosphärenreservat in der Ukraine. – Birmensdorf, Eidgenössische Forschungsanstalt WSL; Rachiw, Karpaten-Biosphärenreservat. Bern, Stuttgart, Wien, Haupt,. 2003 – P. 192.

**4.** *Brändli U.B., Dowhanytsch J., Commarmot B.* Virgin Forest of Uholka. Nature Guide to the Largest Virgin Beech Forest of Europe A UNESCO World Heritage Site. – Swiss Federal Resarch Institute WSL, Birmensdorf and CBR, Rakhiv, 2008. – P. 22.

**5.** *Castello J.D., Leopold D.J., Smallidge P.* Pathogens, patterns, and processes in forest ecosystems. // BioScience. – 1995. – Vol. 45 – P. 16–24.

**6.** *Duchesne L.C., Anderson J.B.* Location and direction of transcription of the 5S rRNA gene in *Armillaria*. // Mycological Research. – 1990. – Vol. 94 – P. 266–269.

**7.** *Dudka I.O., Heluta V.P., Hayova V.P. Merezhko T.O., Tykhonenko Yu. Ya., Andrianova T.V., Wasser S.P.* Fungi. // In: Diduh A.A. (Ed) Biodiversity of Carpathian Biosphere Reserve.(Ukrainian) – Kyiv – 1997. – P. 711 c.

**8.** *Garraway M.O., Hüttermann A., Wargo P.* Ontogeny and physiology. // In: Shaw C.G., Kile G.A. (Eds). *Armillaria* Root Diseases. – Forest Service Agriculture Handbook. Forest Service, United States Department of Agriculture, Washington D.C. – 1991. – No. 691. – P. 21–46.

**9.** *Gregory S.C., Rishbeth J., Shaw C.G.* III. Pathogenicity and virulence. // In: Shaw C.G., Kile G.A. (Eds). *Armillaria* Root Diseases. – Forest Service Agriculture Handbook. Forest Service, United States Department of Agriculture, Washington D.C. – 1991. – No. 691. – P. 76–87.

**10.** *Guillaumin J.J.* Etude du cycle caryologique de deux especes appartenant au genre Armillariella. // Annales de Phytopathologie. – 1973. – Vol. 5 – P. 317.

**11.** *Guillaumin J.J., Anderson J.B., Korhonen K.* Life cycle, interfertility, and biologicalspecies. In: Shaw C.G., Kile G.A. (eds), *Armillaria* Root Diseases. – Forest Service Agriculture Handbook. Forest Service, United States Department of Agriculture, Washington D.C. – 1991. – No. 691. – P. 10– 20.

**12.** *Guillaumin J.J., Mohammed C., Anselmi N., Courtecuisse R., Gregory S.C., Holdenrieder O., Intini M., Lung B., Marxmuller H., Morrison D., Rishbeth J., Termorshuizen A.J., Tirro A., Vandam B.* Geographical distribution and ecology of the *Armillaria* species in Western Europe. // European Journal of Forest Pathology. – 1993 – Vol. 23 – P. 321–341.

**13.** *Hanna J.W., Klopfenstein N.B., Kim M.S., McDonald G.I., Moore J.A.* Phylogenetic patterns of *Armillaria ostoyae* in the western United States. // Forest Pathology. – 2007 – Vol. 37 – P. 192–216.

**14.** *Harrington T.C., Worrell J.J., Baker F.A. Armillaria.* Methods for Research on Soilbovne Phyloputhogenic Fungi – American Phylopathological Society Press, Minnesota – 1992 – P. 81–85.

**15.** *Harrington T.C., Wingfield B.D.* A PCR-based identification method for species of *Armillaria*. // Mycologia. – 1995. –Vol. 87 – P. 280–288.

**16.** *Holah J.C., Wilson M.V., Hansen E.M.* Impacts of a native root-rotting pathogen on successional development of old-growth Douglas fir forests. // Oecologia – 1997. – Vol.111 – P. 429–433.

**17.** *Jactel H., Nicoll B.C., Branco M., Gonzalez-Olabarria J.R., Grodzki W., Långström B., Moreira F., Netherer S., Orazio C., Piou D., Santos H., Schelhaas M.J., Tojic K., Vodde F.* The influences of forest stand management on biotic and abiotic risks of damage. // Annals of Forest Science – 2009. – Vol. 66 – P. 701.

**18.** *Keča N., Bodles W.J.A., Woodward S., Karadžić D., Bojović S.* Molecular–based identification and phylogeny of *Armillaria* species from Serbia and Montenegro. // Forest Pathology. – 2006. – Vol. 36 – P. 41–57.

**19.** *Keča N., Karadžić D., Woodward S.* Ecology of *Armillaria* species in managed forests and plantations in Serbia. // Forest Pathology. – 2009. – Vol. 39 – P. 217 – 231.

**20.** *Kim M.S., Klopfenstein N.B., McDonald G.I., Arumuganathan K., Vidaver A.K.* Characterization of North American *Armillaria* species by nuclear DNA content and RFLP analysis. // Mycologia. – 2000 – Vol. 92 – P. 874–883.

**21.** *Korhonen K.* Interfertility and clonal size in the *Armillariella mellea* complex. // Karstenia. – 1978 – Vol. 18 – P.31–42.

**22.** *Korhonen K., Hintikka V.* Simple isolation and inoculation method for fungal cultures. // Karstenia. – 1980 – Vol. 20 – P.19–22.

**23.** *Legrand P., Guillaumin J.J. Armillaria* species in the forest ecosystems of the Auvergne (Central France). // Acta Oecologica. – 1993. – Vol. 14 – P.389–403.

**24.** *Legrand P., Ghahari S., Guillaumin J.J.* Occurrence of genets of *Armillaria* spp. in four mountain forests in Central France: the colonization strategy of *Armillaria ostoyae* // New Phytologist. – 1996. – Vol. 133. – P. 321–332.

**25.** *Lochman J., Serý O., Jankovský L., Mikes V.* Variations in ITS of ribosomal DNA of Czech *Armillaria* species determined by PCR and high performance liquid chromatography. // Mycological Research. – 2004. – Vol. 108 – P. 1153–1161.

**26.** *Lung-Escarmant B., Guyon D.* Temporal and spatial dynamics of primary and secondary infection by *Armillaria ostoyae* in a *Pinus pinaster* plantation. // Phytopathology. – 2004. – Vol. 94 – P. 125–131.

**27.** *Lushaj B.M., Woodward S., Keča N., Intini M.* Distribution, ecology and host range of *Armillaria* species in Albania // Forest Pathology (doi: 10.1111/j.1439–0329.2009.00624.x) – 2009.

**28.** *Mallett K.I., Maynard D.G. Armillaria* root disease, stand characteristics, and soil properties in young lodgepole pine. // Forest Ecology and Management . – 1998. – Vol. 105 – P. 37–44.

**29.** *Maloy O.C.* Benomyl-malt agar for the purification of cultures of wood decay fungi. // Plant Disease Reporter. . – 1974. – Vol. 58 – P. 902–904.

**30.** *Pérez Sierra A., Whitehead D.S., Whitehead M.P.* Investigation of a PCR-based method for the routine identification of British *Armillaria* species. // Mycological Research. – 1999. – Vol. 103 – P. 1631–1636.

**31.** *Pérez Sierra A., Whitehead D.S., Whitehead M.P.* Molecular methods used for the detection and identification of *Armillaria.* In: Fox R.T.V. (Ed). *Armillaria* Root Rot: Biology and control of Honey fungus. – Intercept LTD, Andover, England. – 2000. – P. 95–110.

**32.** *Prospero S., Rigling D., Holdenrieder O.* Population structure of *Armillaria* species in managed Norway spruce stands in the Alps. // New Phytologist. – 2003. – Vol. 158 – P. 365–373.

**33.** *Prospero S., Holdenrieder O., Rigling D.* Comparison of the virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. // Forest Pathology – 2004 –Vol. 34 – P. 1–14.

**34.** *Rigling D., Blauenstein H., Walthert L., Rigling A., Kull P., Schwyzer A., Heiniger U.* Rhizomorph producing *Armillaria* species in Norway spruce stands in Switzerland. // In: Delatour C, Guillaumin J.J., Lung-Escarmant B., Marçais B. (eds), *Proceedings of the 10th International Conference on Root and Butt Rots.* Carcans-Maubuisson. – Paris. – 1998 – P. 259–265.

**35.** *Rishbeth J.* Species of *Armillaria* in southern England. // Plant Pathology. – 1982. – Vol. 31. P. 9–17.

**36.** *Rizun V.B., Chumak V.O.* Continuum-cycle concept of zoo-assemblage of climax (virgin forest) ecosystem. (Ukranian) // Scientific Bulletin of the Uzhgorod National University. Biology 2008. – Vol. 24 – P. 24– 33.

**37.** *Shelyag-Sosonko Y.R., Popovitch S.Y., Ustimenko P.M.* Coenetic diversity. // In: Diduh A.A. (Ed), Biodiversity of the Carpathian Biosphere Reserve. – Interecocentr, Kiev. – 1997 – P.114–144.

**38.** *Tasenkevich L.O., Stoyko C.M., Tretyak P.R.* Uholsko-Shyrokoluzhanskyi massif. // In: Stoyko C.M, Tasenkvych L.O., Milkina L.I., Malinovsiy L.A., Tretyak P.R., Manko M.P., Bezusko L.G., Tsurick E.I., Melnyk A.S. (Eds) Flora and vegetation of the Carpathian Reserve. (Ukranian) –Naukova Dumka, Kiev – 1982. – P. 130–137.

**39.** *Tsopelas P.* Distribution and ecology of *Armillaria* species in Greece. // Forest Pathology. – 1999. – Vol. 29. – P. 103–116.

**40.** *Van der Pas J.B.* A statistical appraisal of *Armillaria* root rot in New Zealand plantations of *Pinus radiata*. // New Zealand Journal of Forestry Science. – 1981. – Vol. 11. – P. 23–36.

**41.** *Veldman G.M., Klootwijk J., Regt V.C. H. F. d., Rudi R. J.*, 1981. The primary and secondary structure of yeast 26S rRNA. // Nucleic Acids Reseach. – 1981. – Vol. 9 – P. 6935–6952

**42.** *Voznyuk S.T., Kuzmich P.K.* Land Improvement and Fundamental Hydrology. Textbook. (Ukranian). – Vysha Shkola, Lviv. – 1984. –P. 120

**43.** *Watling R., Kile G.A., Burdsall H.H. Jr.* Nomenclature, taxonomy, and identification. // In: Shaw C.G., Kile G.A. (Eds). *Armillaria* Root Diseases. *Armillaria* Root Disease. Agricultural Handbook – Forest Service Agriculture Handbook. Forest Service, United States Department of Agriculture, Washington D.C. – 1991. – No. 691. – P. 1–9.

**44.** *Zolciak A., Bouteville R.J., Tourvieille J., Roeckel–Drevet P., Nicolas P., Guillaumin J.J.* Occurrence of *Armillaria ectypa* (Fr.) Lamoure in peat bogs of the Auvergne – The reproduction system of the species // Cryptogamie Mycologie. – 1997. – Vol. 18. – P. 299–313.

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