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RESEARCH ARTICLE

Seasonal changes in the mycorrhizal symbiosis of *Rhododendron tomentosum* Harmaja in the Ukrainian Polissia

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Abstract

This study focuses on the mycorrhizal associations of *Rhododendron tomentosum* Harmaja with ericoid mycorrhizal fungi, typical for species in the Ericaceae family. The development of these symbiotic relationships varies seasonally and by habitat. Mycorrhizal colonization was studied across different phenological stages in natural populations of *R. tomentosum* in the Bilokorovytsky forestry, Zhytomyr region (Ukraine). Samples were taken at four stages of the growing season, analyzed morphologically, and quantitatively assessed. The results revealed that colonization peaks at the beginning and end of the vegetation period while declining during flowering and seed maturation. Additionally, we observed both ericoid mycorrhizae and dark septate endophytes (DSE) coexisting within the roots, suggesting that the symbiotic relationship is complex and influenced by multiple factors. These findings contribute to a deeper understanding of mycorrhizal dynamics in *R. tomentosum* and highlight the need for further research on seasonal and environmental influences on these associations.

Keywords: *Rhododendron tomentosum*, Ericaceae, Ukrainian Polissia, ericoid mycorrhiza, dark septate endophytes, seasonal variation, symbiosis, boreal-subarctic species

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Introduction

Rhododendron tomentosum Harmaja, known as marsh Labrador tea (*Ledum palustre* L.), is an evergreen shrub typically distributed on raised bogs. Until 1990, this species was classified under the genus *Ledum* L., but as a result of subsequent taxonomic investigations, *Ledum* was nested as a subgenus within the genus *Rhododendron* L. of the family Ericaceae (Kron & Judd, 1990; Harmaja, 1991).

Rhododendron tomentosum is a glacial relict, boreal-subarctic species. In Ukraine, it is primarily distributed in Polissia, with sporadic occurrences in the Prykarpattia (Ciscarpathian) and Carpathian regions. A single site has been documented in the Zakarpattia (Transcarpathian) region. Here, the species inhabits waterlogged and wet pine or, less commonly, mixed forests and thrives in peat bogs (Sokolov & Zamotaev, 1993).

This species, like all members of the genus *Rhododendron*, is characterized by the

presence of ericoid mycorrhiza (Vitolinya, 1972; Peterson et al., 1980; Piercey et al., 2002; Usuki et al., 2003; Zhang et al., 2009; Tian et al., 2011). Plants forming this type of mycorrhiza usually grow on soils with minor mineral nutrients. *Rhododendron tomentosum* is a typical mycotrophic species (Treu et al., 1995). Researchers studying the mycorrhiza of this species in Eastern Europe, southern India, and China (Vohnik & Albrechtová, 2011; Rose & Senthilkumar, 2016; Liu et al., 2024) have noted variations in its development depending on the habitat, season, and phenological phase. However, there is limited data on the mycorrhizal development of Ericaceae species growing in Ukraine, and *Rhododendron* species remain underexplored in this context. This makes studying the seasonal development of mycorrhiza in *R. tomentosum* within the Ukrainian Polissia of particular scientific interest.

Ericoid mycorrhiza is composed of dense coils of fungal hyphae in the outer cell layers of roots and hyphae on the root surface, sometimes referred to as a 'mycodermis', which extend over long distances in the surrounding soil. The formation of mycorrhiza involves colonization of epidermal cells by fungal hyphae, followed by the development of a complex of branched hyphae in each colonized cell (Peterson et al., 2004; Smith & Read, 2008). The duration of hyphal complexes in each cell varies. Over time, cytoplasmic degradation occurs in both the host plant and fungal cells – a process some researchers describe as phagocytosis. The breakdown of the symbiotic association may partly depend on the lifespan of hair roots, typical for *Rhododendron* species, or the developmental stage of the host plant.

Symbiotic fungi that form ericoid mycorrhiza belong to the phylum Ascomycota. Among the most common species are *Hymenoscyphus ericae* (Read) Korf & Kernan, its anamorph *Scytalidium vaccinii* Dalpé, Litten, & Sigler, and several species of the genus *Oidiodendron* Robak (anamorphs of *Myxotrichum setosum* (Eidam) Orr, Kuehn & Puenkett and *Gymnascella dankaliensis* (Castellani) Currah). The first species occurs in heathlands (Read, 1991) and alpine mountain zones (Hambleton & Currah, 1997; Thormann et al., 1999). Species of *Oidiodendron*, such as *O. maius* Barron, first isolated from rhododendron grown in culture (Douglas et al., 1989) were later described as common for the forest ecosystems (Perotto et al., 1996; Hambleton & Currah, 1997; Thormann et al., 1999).

In addition to typical ericoid mycorrhiza on the roots of Ericaceae representatives, including rhododendrons, so-called dark septate endophytes (DSE) are often observed (Vohník & Albrechtová, 2011). Unlike mycorrhizal fungi, endophytic fungi are not known to form specialized nutrient transport interfaces during root colonization. The presence of simple intracellular or intercellular hyphae resembling finger-like structures on root cell surfaces is generally referred to as 'endophytic'. DSE belong to the group of ascomycetous anamorphic fungi, which colonize root tissues intracellularly and intercellularly and often form clusters of spherical or oval cells known as microsclerotia.

Although these fungi are identified in roots by forming dematiaceous (darkly pigmented) hyphae and microsclerotia, some studies suggest that their dark walls are integrated structures with mycorrhizal hyaline septate hyphae in roots (Haselwandter & Read, 1982; Newsham et al., 2009). Therefore, earlier research might have underestimated their abundance and significance (Smith & Read, 2008). Evidence exists of the presence of both ericoid mycorrhiza (ErM) and DSE in *R. groenlandicum* (Oeder) Kron & Judd, a close relative of *R. tomentosum*, collected near Quebec and Guelph, Canada (Massicotte et al., 2005). However, data on the physiological or ecological interactions between these symbiotic types are lacking. Available information suggests that the occurrence and intensity of DSE and ErM development depend on altitude and latitude. Plants growing at higher altitudes or further north exhibit increased DSE presence in their roots, whereas ErM presence diminishes.

This study examines the morphological features of mycorrhiza in *R. tomentosum* during different developmental periods of the host plant to determine the influence of seasonality on specific symbiotic associations.

Table 1. Indicators of mycorrhizal infection on the roots of *Rhododendron tomentosum* at different phenological stages.

Characteristics	Start of the vegetation season	Flowering	Seed maturation	End of the vegetation season
Occurrence frequency (%)	90 ± 1.3	63 ± 2.5	78 ± 1.8	100 ± 1.5
Degree of mycorrhization (points)	3.2 ± 0.5	2.5 ± 0.7	2.1 ± 0.5	3.8 ± 0.8
Presence of external hyphae (%)	84 ± 7.4	7 ± 0.1	60 ± 5.2	92 ± 12.2

Material and methods

The study was conducted in the Bilokorovytskyi Forestry of Korosten district, Zhytomyr region, Ukraine. Root samples for analyzing anatomical structure and morphological characteristics of mycorrhiza were collected in natural coenoses of *R. tomentosum*. These habitats predominantly included *Pinus sylvestris* L. in the upper tier and a medium tier represented by *Vaccinium myrtillus* L., *V. vitis-idaea* L., *Pteridium aquilinum* (L.) Kuhn, *Rhododendron luteum* Sweet., and *Carex sylvatica* Huds., with a litter layer composed of *Dicranum scoparium* Hedw. and pine needles.

Root samples were collected from five individuals in the study area, washed in running water, and fixed in 70% alcohol. Root maceration and staining with aniline blue were performed following Kobel's methodology (Betehtina & Utkina, 2008). According to previous studies (Rose & Senthilkumar, 2016), fungal colonization of host plant root cells begins in late summer, peaking in autumn. During winter, fungal mycelium within the cells is digested, and no new infection occurs. The epidermis containing digested hyphae is shed, leaving root cells nearly free of mycorrhiza in spring.

To capture seasonal dynamics, samples were collected four times a year: in early vegetative growth (March 21–29), in flowering period (May 21–27), during seed maturation (September 3–11), and at the end of the vegetative period (November 3–11). A Primo Star light microscope (Carl Zeiss, Jena, Germany) equipped with a Canon PowerShot A640 digital camera was used for sample analysis and capturing the images.

The frequency of mycorrhizal infection (in %) was quantified following Selivanov (1987) – the proportion of microscopic fields of view showing mycorrhizal presence was calculated. The intensity of root cell colonization by hyphal clusters was assessed using a mycorrhization index (score). Additionally, the occurrence of external hyphae was analyzed as a percentage relative to the microscopic field of view to investigate structural development during specific vegetative periods.

Statistical analysis of the reliability and one-way analysis of variance (ANOVA) with Tukey's test for post hoc comparisons of the obtained results was conducted using Microsoft Excel. Differences between the experimental indicators were considered statistically significant when $P > 0.05$.

Results and discussion

The results of the study demonstrated that the frequency of mycorrhizal occurrence in *R. tomentosum* varies depending on the season. The lowest values were observed during the flowering period, while the highest occurred at the beginning and end of the vegetation period (Table 1). During seed maturation and flowering, the mycorrhizal frequency reached its minimum values.

The degree of mycorrhization was highest at the start (3.2 points) and the end (3.8 points) of the vegetation period and lowest during the seed maturation (2.1 points). Despite the lower intensity of mycorrhizal infection during this phase, it was compensated by a more substantial development of external hyphae. This is evidenced by a 50% increase in external hyphae presence compared to the flowering period and a 15% higher occurrence frequency.

At the beginning of the vegetation period, roots were densely covered with two types of hyphae: (a) light-colored and loosely structured; (b) dark-colored, straight, and septate. Cells contained hyphal coils structures (Fig. 1). During flowering, there was less cellular filling with hyphal coils compared to the previous phase, and external hyphae were almost absent (Fig. 2). In the seed maturation phase, two types of hyphae reappeared on root surfaces, but fewer hyphal coils were present in endodermal cells compared to the previous two periods. Light-colored external hyphae displayed conidia-like structures, and dark hyphae exhibited pronounced budding and septation, indicating that the fungi forming this symbiosis belong to the Ascomycota phylum (Fig. 3).

At the end of the vegetation season, hyphae of both types were present on the root surface, while root cortex cells showed the lowest mycorrhizal colonization of the entire season (Fig. 4).

According to some reports (Haselwandter, 1987; Read, 1996), such a distribution of mycorrhizal infection may be linked to climatic characteristics, as humidity and drought can regulate mycorrhizal infection development. For instance, Australian researchers (Cairney & Ashford, 2002) found that ericoid mycorrhizal fungi function year-round, except during the dry season. Bell & Pate (1996) and Hutton et al. (1994) identified seasonal fluctuations in the number of hair roots and mycorrhizal infection in several ericoid plant species in southwestern Australia. They also discovered that the length of hair roots decreases in summer and reappears in autumn (April) in all the species they studied.

To align the obtained data with literature references, the results were analyzed in relation to weather indicators (Fig. 5). For this purpose, the diagrams plotted precipitation and temperature values throughout the observation period and the research indicators during the vegetative season. All mycorrhizal indicators showed an inverse correlation with temperature and precipitation. In other words, mycorrhiza develops better at lower temperatures, while high humidity, resulting from increased precipitation during the warm period, does not positively affect its development.

Considering the obtained data and analyzed literature, it can be assumed that ericoid-type mycorrhiza exhibits distinct seasonality. However, this issue requires further study.

The presence of two types of hyphae on the surface of *R. tomentosum* roots also aligns with research on rhododendrons collected in Europe (Vohník & Albrechtová, 2011). The authors have established that hyphae of dark septate endophytes (DSE) and ericoid mycorrhiza (ErM) were present in six studied *Rhododendron* species.

Conclusions

Studies of the seasonal changes in the mycorrhiza of *R. tomentosum* in the Ukrainian Polissia showed that depending on the vegetative phase, mycorrhiza development undergoes certain quantitative and qualitative changes. It was found that the highest degree of mycorrhizal development occurs at the beginning and end of the vegetation period, which may be related to both climatic conditions and the plant's phenological phases. The plant seemingly ceases its 'collaboration' with fungi during the growing season.

One hypothesis suggests that the plant requires substantial resources during active growth phase, thus limiting the fungi's carbon supply. Conversely, during the dormancy phase, the plant restores its carbon supply from reserves accumulated during the growing season, receiving mineral nutrients from the fungi, in turn, to survive the dormancy period. This hypothesis, however, requires further investigation.

The coexistence of two types of symbionts observed during this study indicates that ericoid mycorrhiza (ErM) alone does not fully support the plant's vital functions. Other types of symbionts, including dark septate endophytes (DSE), are also involved. However, the balance between these types of symbiosis at different stages of the plant's development and whether one type dominates during specific periods remain open questions for future investigations.

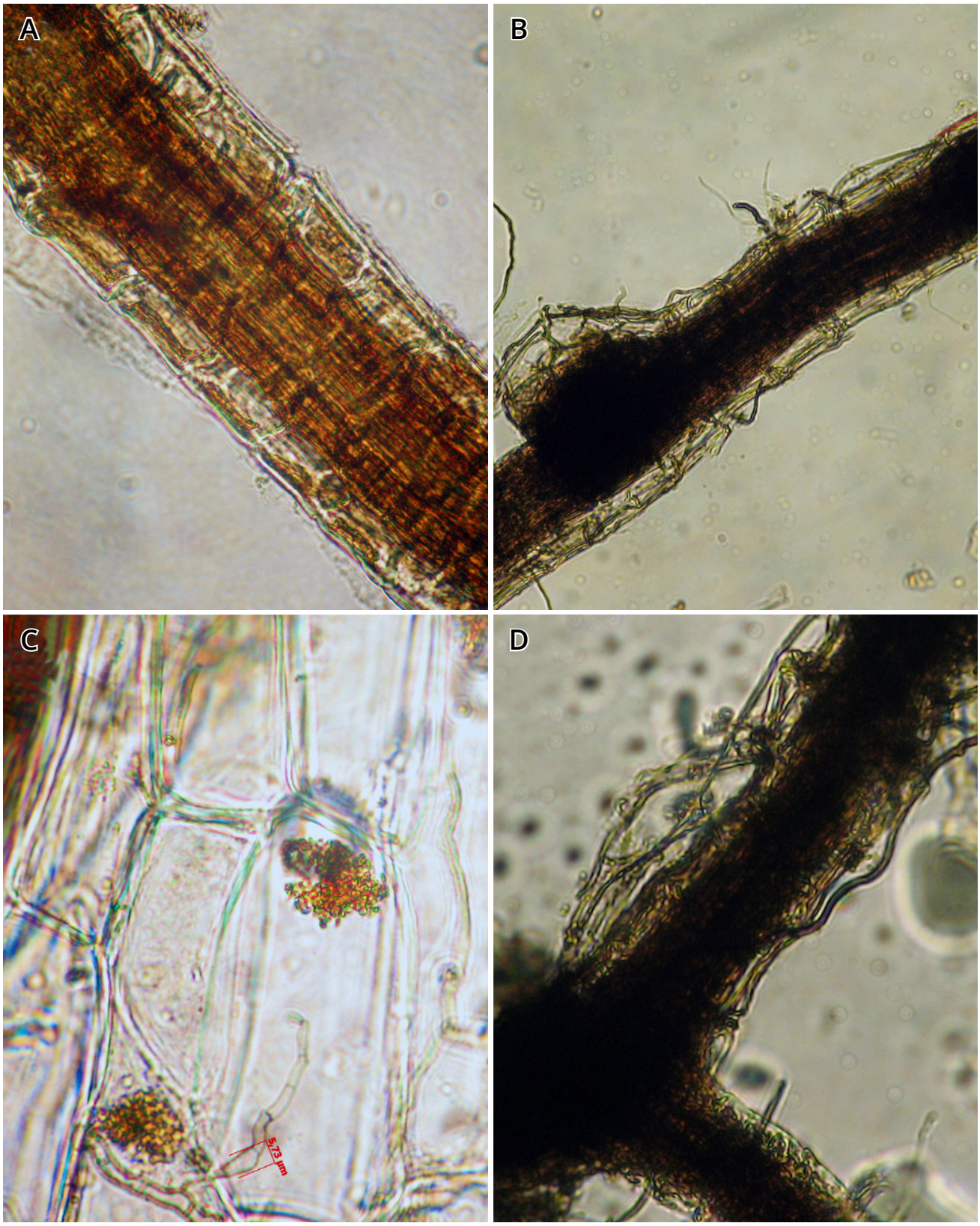

Figure 1. Colonization of *Rhododendron tomentosum* cells by mycorrhizal fungi at the beginning of the vegetation period: **A** – hyphal coils in the endodermal cells of the root; **B, D** – different types of hyphae on the root surface; **C** – hypha penetrating a root cell.

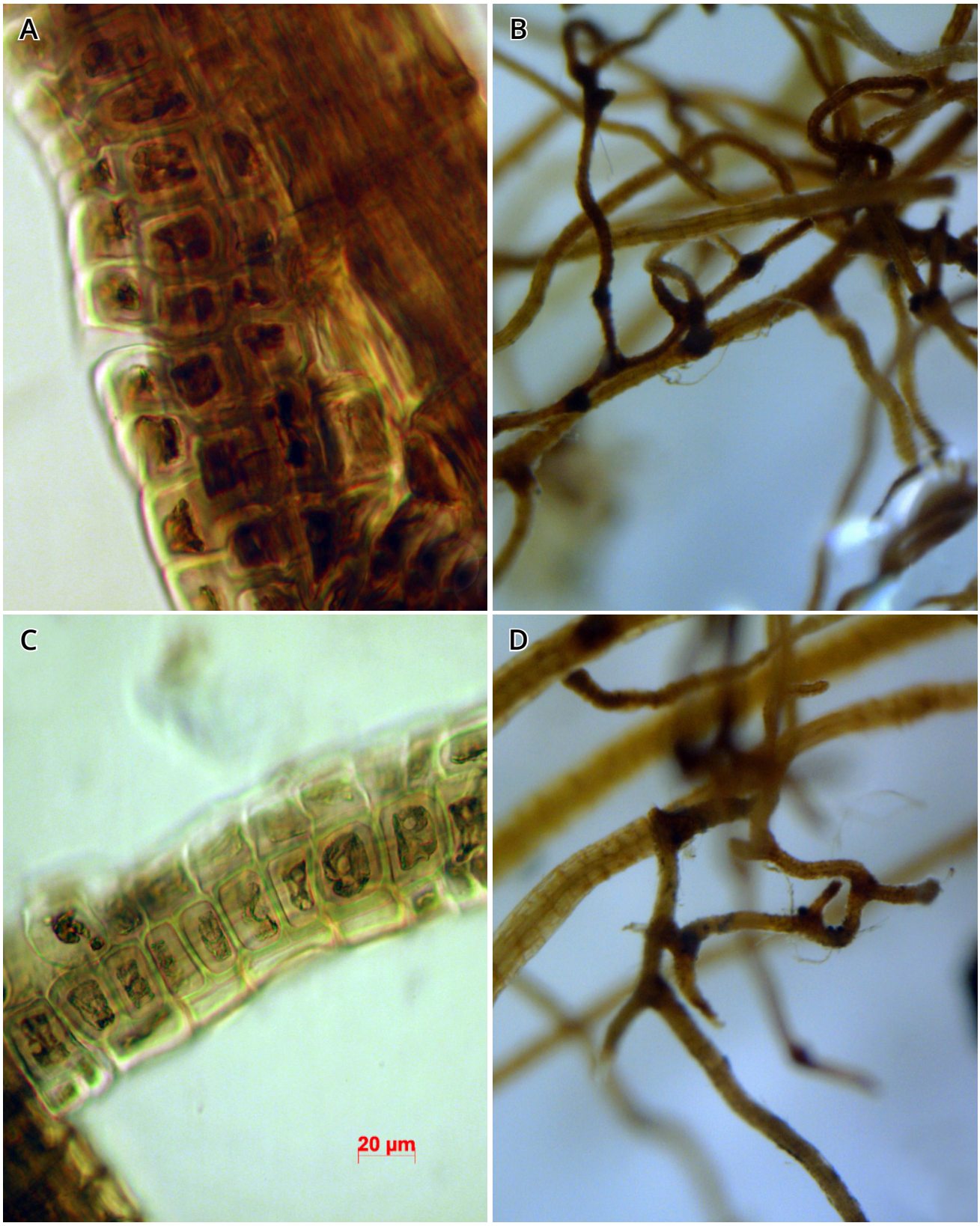

Figure 2. Colonization of *Rhododendron tomentosum* cells by mycorrhizal fungi during the flowering: **A, C** – root cells with hyphal coils inside; **B, D** – single hyphae on the root surface.

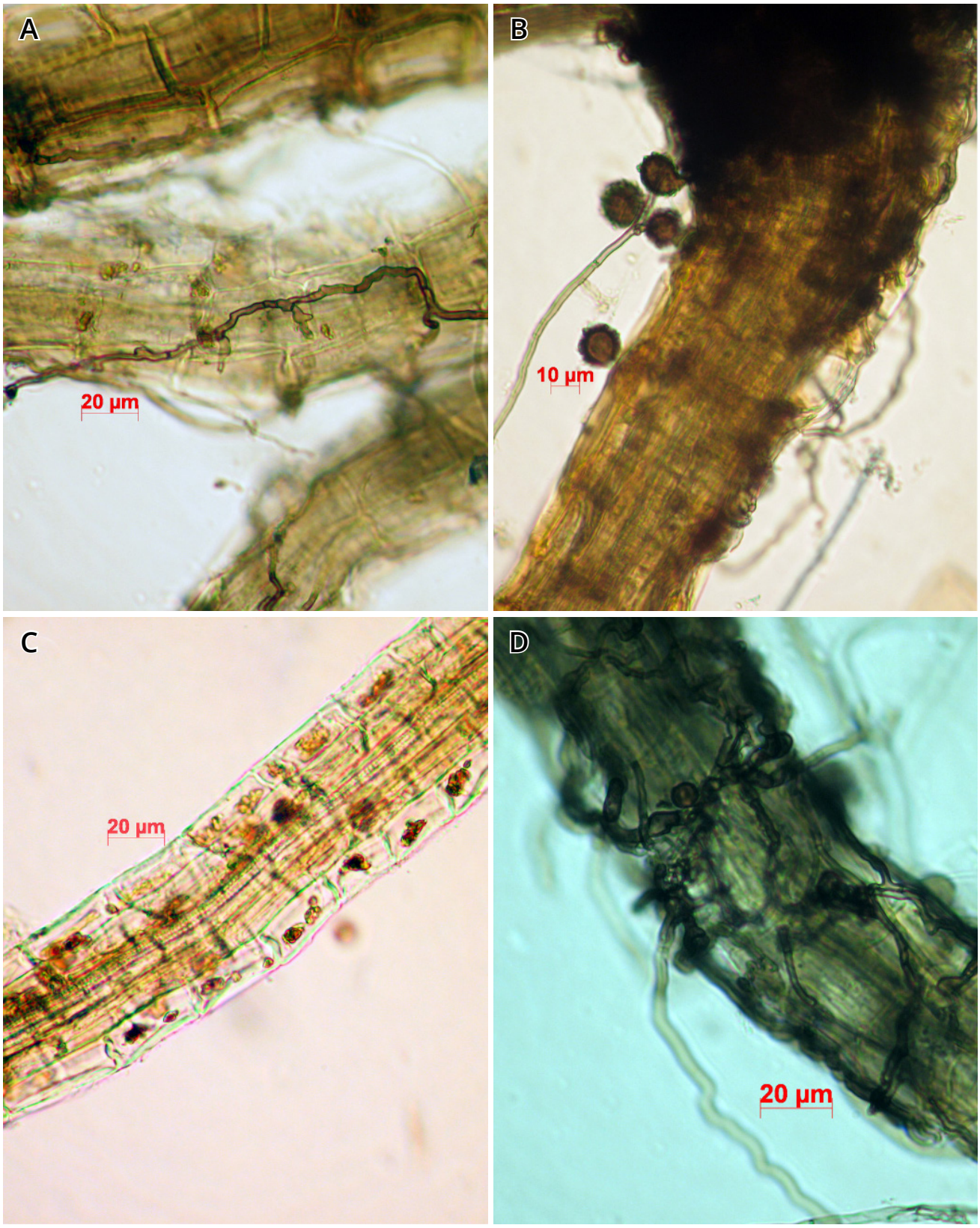

Figure 3. Colonization of *Rhododendron tomentosum* cells by mycorrhizal fungi during the seed maturation: **A, D** – prominent budding and septation of external hyphae on the root surface; **B** – conidial structures on the root surface; **C** – hyphal coils in root cortex cells.

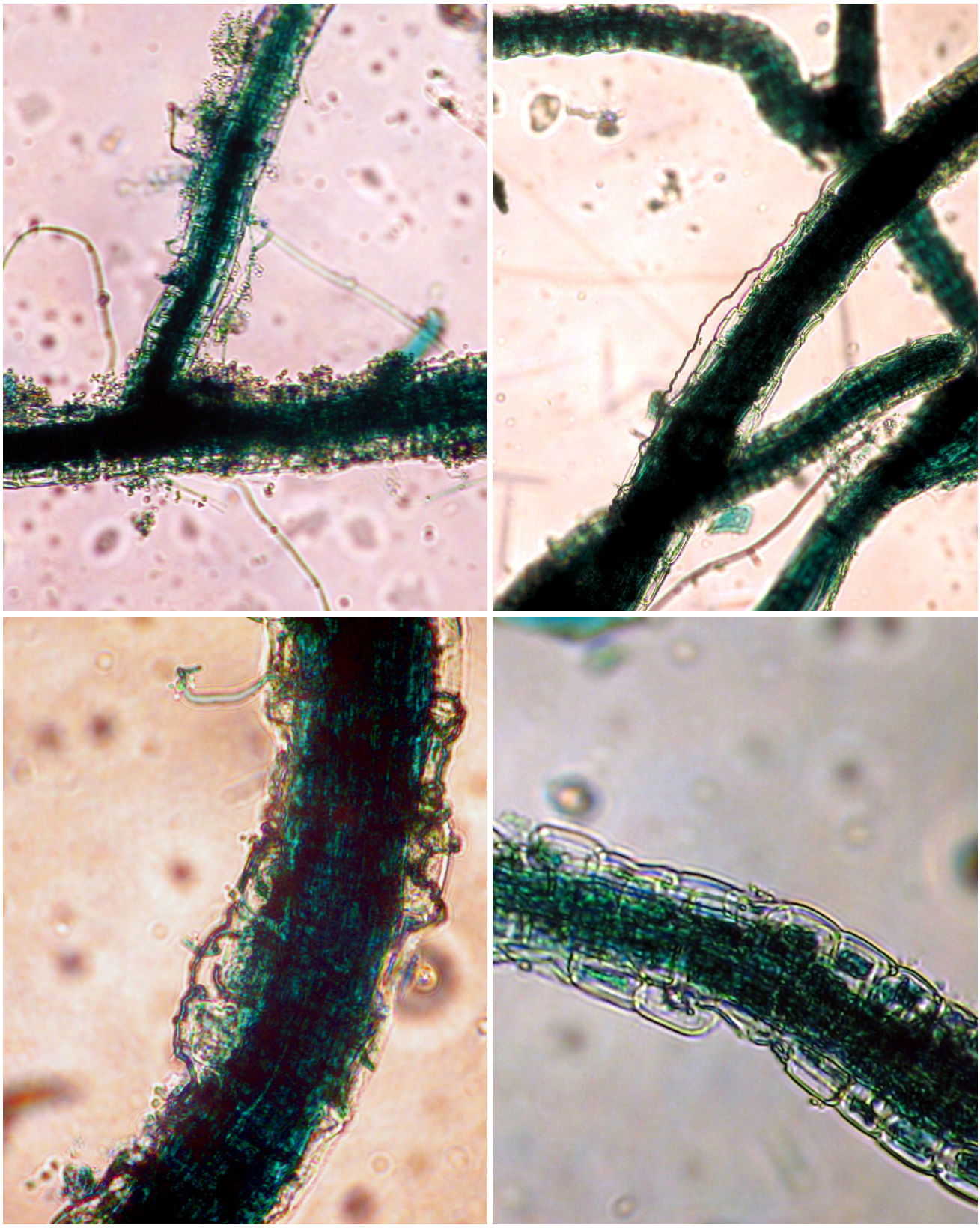

Figure 4. Colonization of *Rhododendron tomentosum* cells by mycorrhizal fungi at the end of the vegetative period: **A, C** – loose mycelium on the root surface; **B, C** – dark, septate mycelium on the root surface: **D** – hyphal coils in root cortex cells.

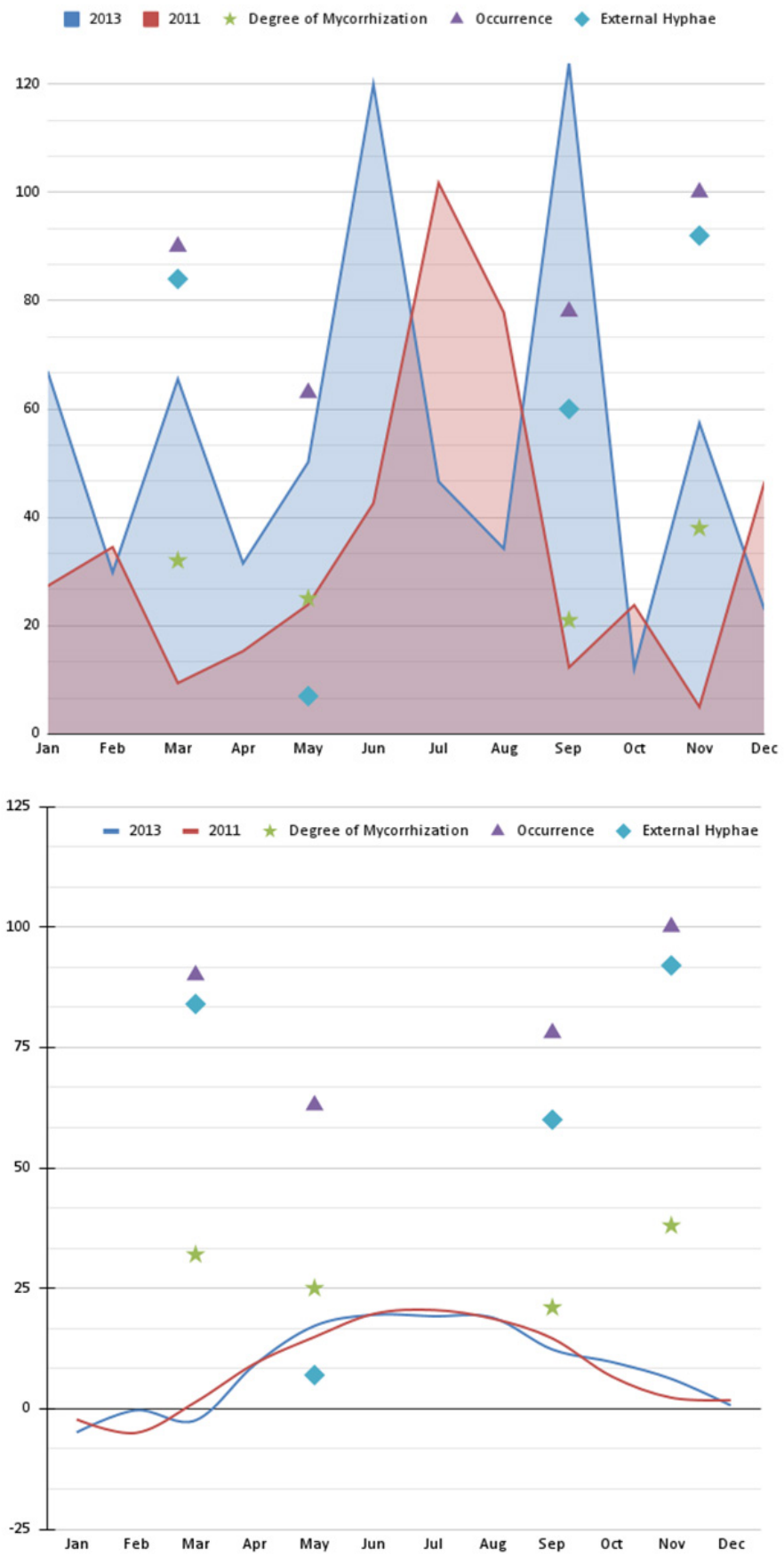

Figure 5. Influence of climatic factors on mycorrhiza development on *Rhododendron tomentosum* roots: **A** – the influence of precipitations; **B** – the impact of temperature. To facilitate comparison with other parameters, the mycorrhization degree values have been scaled by a factor of 10.

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Сезонні зміни мікоризного симбіозу *Rhododendron tomentosum* Harmaja в Українському Поліссі

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Представлене дослідження зосереджене на мікоризних асоціаціях *Rhododendron tomentosum* Harmaja з ерикоїдними мікоризними грибами, характерними для родини Ericaceae. Мікоризну колонізацію вивчали на різних фенологічних етапах у природних популяціях *R. tomentosum* у Білокоровицькому лісництві Житомирської області. Зразки відбирали на чотирьох стадіях впродовж періоду вегетації та аналізували морфологічно і статистично. Результати дослідження показали, що колонізація досягає максимуму на початку та в кінці вегетаційного періоду, а під час цвітіння та дозрівання насінин вона значно знижується. Крім того, у коренях спостерігали одночасну присутність ерикоїдної мікоризи і темно-септованих ендофітів, що вказує на складний характер симбіотичних взаємин. Отримані дані сприяють кращому розумінню динаміки мікоризних асоціацій *R. tomentosum* та підкреслюють необхідність подальших досліджень щодо сезонних і екологічних впливів на ці взаємини.

Ключові слова: *Rhododendron tomentosum*, Ericaceae, Полісся України, ерикоїдна мікориза, темно-септовані ендофіти, сезонні зміни, симбіоз, бореально-субарктичні види