

## ARBUSCULAR MYCORRHIZAE OF FIVE SUMMER GEOPHYTES FROM CLUJ COUNTY

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### Abstract

Phytobiont enhanced nutrient uptake through arbuscular mycorrhiza comes at a cost in form of carbon fixed through photosynthesis. Geophytes operate under resource efficiency constraint due to their particular life-cycle and have to balance it very well. This translates in highly controlled investment towards mycobiont, that could explain the wide range of colonization patterns according to plant species affordability to sustain the mutualism when carbohydrates are required to be retained in storage organ. Following microscopic examination of roots collected at anthesis from UASVM Cluj Agro-Botanical Garden, highest intensity for root colonization in root segments was found in *Dahlia variabilis* followed by *Helianthus tuberosus* and *Lilium candidum* and lowest in *Hemerocallis fulva* and *Gladiolus × grandiflorus*. Arbuscules richness varied strongly among species. This study documents for the first time the level of arbuscular mycorrhizae in some summer flowering geophytes from Cluj county, Romania. It is concluded that summer geophytes might be good model plants for ecology of endophytic relationship since are highly sensitive to any disbalance in carbon expenditure.

**Key words:** summer flowers, root endophyte, colonization, local climate

Geophytes can present different phenological particularities according to their life-cycle pattern: synanthous or hysteranthis and in addition according to their subvariants – with annual or perennial storage organs (Dafni A. *et al*, 1981; Kamenetsky R., 2013).

Life cycle is in close connection with resource allocation in geophytes. Resource allocation defines the relation between plant fitness and internal pattern of nutrient distribution which implies differential movement of substances between various organs, at different growth stages and under contrasting environmental conditions (Ruiters C. *et al*, 1993).

If taken as model, a geophyte with synanthous leaves and perennial storage organs such as *Lilium candidum*, needs large nutrient reserves to ensure flowering each year. In a favorable year the course of accumulation and consumption follows these general phases: expenses for leaf establishment until net production occurs, beginning of flowering and continuation of net production, the balance period between expenditure and production and finally expenditure exceeding production at mass flowering and seed setting (Dafni A. *et al*, 1981).

Great impact on plant nutritional status and fitness can be exercised by arbuscular mycorrhizae

which can bring many beneficial effects for ornamental plants including geophytes fact attested through numerous studies in experimental conditions (Crișan I. *et al*, 2017). Enhanced nutrient uptake for plants mediated by arbuscular mycorrhiza comes at a cost in form of carbon fixed through photosynthesis. Carbon source under the form of hexose/sucrose is taken by fungus at the interface represented by arbuscules, then converted to trehalose and glycogen – typical fungal carbohydrates. Triacylglyceride (TAG) is the main form of carbon stored by AM fungi and is almost exclusively made in the intraradical mycelium (Garg N.G., Kaur A., 2006).

At the same time, part of carbon fixed through photosynthesis is also directed to be stored in perennating organs. Starch is the most abundant reserve carbohydrate in geophytes but fructan and glucomannan also occur instead or in addition to starch (Ranwala A.P., Miller W.B., 2008).

This brings up the question how geophytes balance the need for carbon fixed through photosynthesis between symbiont and storage organs and what are the consequences for functionality of the association?

Comparative studies for arbuscular mycorrhiza (AM) root colonization in natural climatic conditions are almost inexistent in

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Romania. Thus, currently it is little known how plants from local pedo-climatic conditions interact with arbuscular mycorrhizae and how much they benefit from this symbiosis. Or, what are the prospects for these to benefit from supplementary inoculation.

The aim of this study was to investigate the level of root colonization by arbuscular mycorrhizae in five common grown summer geophytes in order to define the symbiosis status in local natural climatic conditions. This constitutes a preliminary study that documents for the first time the level of arbuscular mycorrhizae root colonization in summer geophytes from Cluj county.

## MATERIAL AND METHOD

Studied summer-flowering geophytes are grown in Agro-Botanical Garden UASVM Cluj-Napoca, Romania, located in temperate continental climate, at 363 m altitude, 8.1°C average annual temperature and average annual rainfall 635 mm. Soil type is clay loam.

Roots of five species were collected on 26.06.2018 from rhizosphere (top soil) in non-destructive way from plants at anthesis.

Roots were prepared for microscopic observation using staining technique of Vierheiling H. *et al* (1998) modified by Stoian V.H. *et Florian V.* (2009) through replacement of clearing strong base caustic potash with lye.

Immediately after collecting, the roots were washed with tap water and placed in 15% NaOH solution for two days. Then, roots were washed with tap water and introduced in staining solution

comprised by 5% ink, 5% vinegar (9°), and 90% H<sub>2</sub>O. After staining, roots were transferred to a solution of 5% vinegar.

Root samples (N=100) of 1 cm long each were mounted on glass slides and flatten by squash technique to be examined under Optika microscope at 100x-400x.

Colonization level was assessed using root segment estimation method (Trouvelot A. *et al*, 1986) and indicators were calculated using MycoCalc software (<https://www2.dijon.inra.fr>).

Indicators used for AM colonization: F% = frequency of mycorrhiza in the root system, m% = intensity of the mycorrhizal colonization in the root fragments, M% = intensity of the mycorrhizal colonization in the root system, a% = arbuscule abundance in mycorrhizal parts of root fragments, A% = arbuscule abundance in the root system.

In addition, dark septate endophyte presence was assessed using an intensity scale marked with "+" for each plant species.

## RESULTS AND DISCUSSION

The analyzed summer geophyte species belong to four botanic families and present different types of storage organs (*table 1*). Roots stained with slightly different intensity among species.

The highest arbuscular mycorrhizae colonization frequency was found in *Dahlia variabilis* (simple flower genotype) that presented all root segments analyzed with mycorrhizae, followed by *Hemerocallis fulva* and *Lilium candidum* with a frequency of 95%.

Table 1

**Arbuscular mycorrhizae colonization of five summer geophyte plants from Cluj county**

Botanic family	Species	Geophyte organ type	AM colonization frequency (F%)	AM vesicle/spore frequency (%)	DSE presence
Asteraceae	<i>Dahlia variabilis</i>	tuberous roots	100	60	+
Asteraceae	<i>Helianthus tuberosus</i>	tubers	85	25	++
Asphodelaceae	<i>Hemerocallis fulva</i>	tuberous roots	95	20	+
Liliaceae	<i>Lilium candidum</i>	scaly bulbs	95	10	+
Iridaceae	<i>Gladiolus × grandiflorus</i>	corm/bulbo-tuber	60	0	-

The lowest AM frequency was found in *Gladiolus × grandiflorus*. Structures typical for advanced stages of colonization such as spores and vesicles occurred also with different frequency. Thus, the two *Asteraceae* presented highest frequency of this structures, followed by *Hemerocallis fulva* and *Lilium candidum*. No such structures were identified in *Gladiolus × grandiflorus* root. Dark septate endophytes were

present in root segments of four out of five species analyzed, with higher intensity registered for *Helianthus tuberosus* (*table 1*, *figure 1*).

When considering the pattern of root colonization among high mycorrhizal species studied, it was noted that *Dahlia variabilis* presented typical *Arum* type spreading with well-developed branched arbuscules, *Hemerocallis fulva* typical *Paris* type spreading (*figure 1*) and by

contrast *Lilium candidum* presented a rather particular way of colonization with hyphae coils

having a development most similar with some less dense *Paris* proliferation (figure 2).

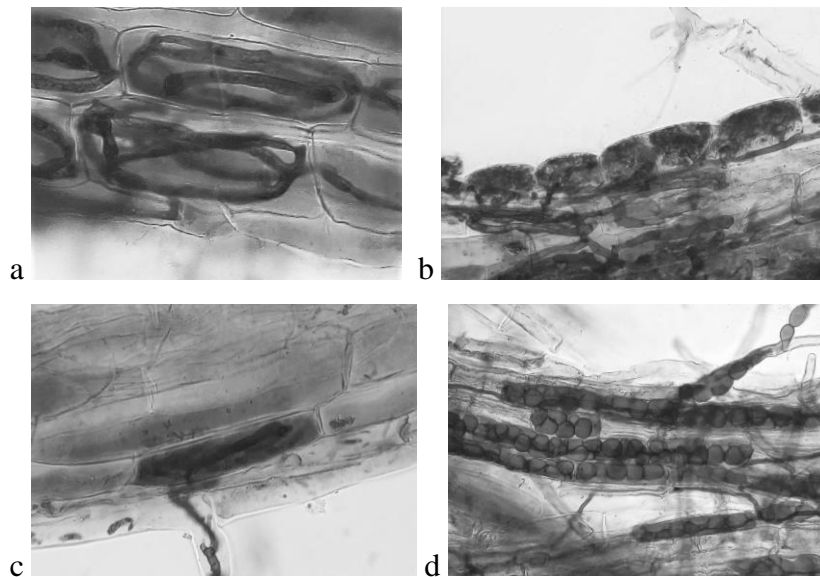


Figure 1 AMF *Paris*-type in *Hemerocallis* (a), and *Arum*-type in *Dahlia* (b), entry point in *Gladiolus* root (c), DSE microsclerotia cluster in *Helianthus* (d)

Regarding the colonization type, Barrett (1958) cited by Smith F.A. et Smith S.E. (1997) demonstrated that the plant not the endophyte determines host-fungus interaction. Thus, *Paris* type arbuscular mycorrhizae fungi isolated from *Hemerocallis* developed *Arum* type in the model plant *Zea mays*, for example. It has been suggested

that *Paris*-type is characteristic of plants in low-nutrient and high-stress conditions, while *Arum*-type is associated with fast growing plants, but co-occurrence of these types is also possible (Druva-Lusite I., Ievinsh G., 2010), as well as intermediate characteristics (Rodríguez-Rodríguez R.M. et al, 2013).

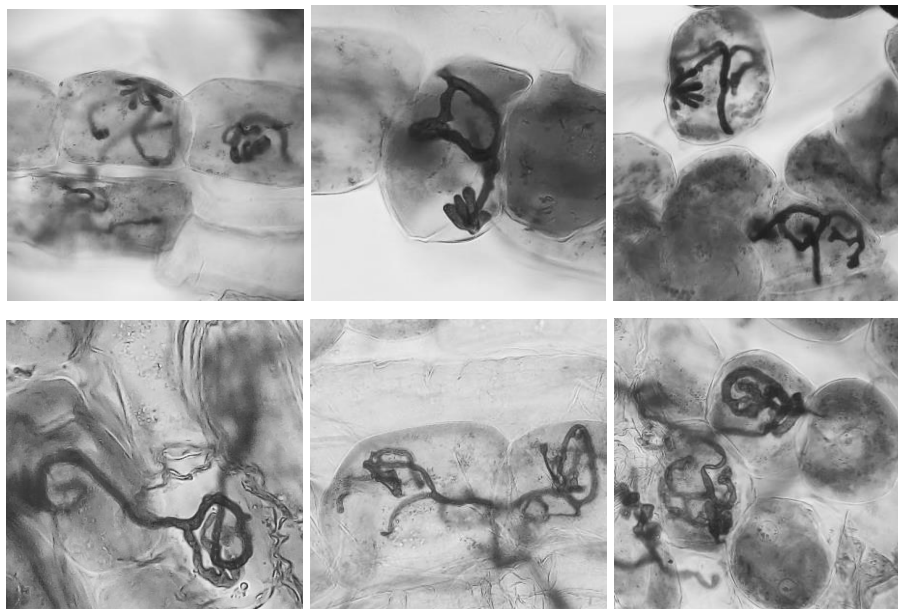


Figure 2 Particular type of fungal colonization in *Lilium candidum* root cells

Highest intensity for root colonization was found in *Dahlia variabilis* followed by *Helianthus tuberosus* and *Lilium candidum*.

Lowest level of colonization was found in *Hemerocallis fulva* and *Gladiolus × grandiflorus*.

When it comes to arbuscules abundance in mycorrhizal parts of root fragments, the hierarchical order changes with *Lilium candidum* having a = 48% situating at the top, while *Dahlia variabilis* with a = 33.37% second, and *Helianthus tuberosus* with only a = 10.3% among the last.

When looking at arbuscules abundance in the root system, the hierarchy is maintained just as for intensity of colonization, with *Dahlia variabilis* A = 16.85%, scoring the highest.

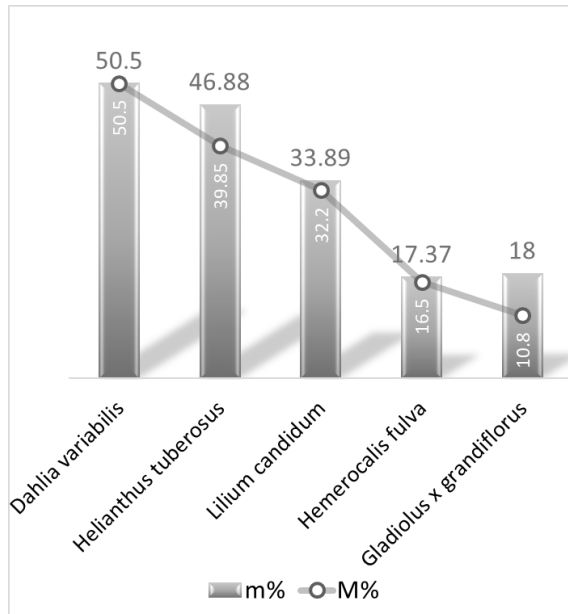


Figure 3 Intensity of endomycorrhizal colonization in root fragments (m%) and root system (M%)

In *Dahlia variabilis*, over a half of root segments analyzed had up and over 50% of the root length colonized (1 cm long segments) and situating in classes 3 and 4 on scale according to Trouvelot A. *et al* (1986), while arbuscule abundance was preponderantly in category “few” or “frequent”.

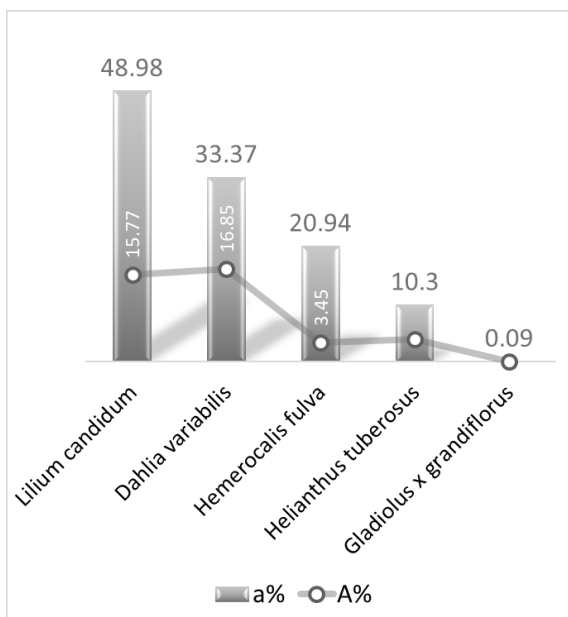


Figure 4 Arbuscule abundance in mycorrhizal parts of root fragments (a%) and root system (A%)

By comparison, root segments of the other species from same botanic family analyzed -

*Helianthus tuberosus* presented colonization distributed across all five classes but without scoring any highly abundant arbuscule areas.

For the species *Hemerocallis fulva*, majority of the root fragments belonged to classes 2 and 3, while for *Lilium candidum* belonged to classes 3 and 4.

High colonization classes caused by spreading of hyphae do not mean any advantage directly for the plant in absence of frequent arbuscules, the benefits remaining only potential.

But more difficult to explain perhaps remains the case of species with very low arbuscules and lack of advanced structures by anthesis such as spores or vesicles. *Gladiolus x grandiflorus*, compared to all other species analyzed, did not presented well spread hyphae inside the root instead many segments were non-colonized, while well-developed arbuscules were missing as well. The colonization frequency of 60% obtained in this study is similar with the one obtained by Kumar A. *et al* (2012) of 63.36% from plants grown as well in natural climatic conditions.

A study aimed to assess percentage of arbuscular mycorrhiza root colonization in four *Gladiolus* cultivars (‘Oscar’, ‘PM Rose’, ‘Priscilla’, ‘Wind Song’) at three growing stages found that in all cases it was lowest at early vegetative stage and highest at either 5-7 leaf stage for two of the cultivars (‘Priscilla’ and ‘Wind Song’) or at flowering (for the other two cultivars). Arbuscular mycorrhizae root length colonized was roughly 30-60% with arbuscules present with different abundance (Javid A., Riaz T., 2008). Thus, phenophase was proven to have importance in AMF colonization of *Gladiolus*, but in absence of vesicles and spores or digested arbuscules there is no evidence for possible exchange taking place at some stage before anthesis in plants from current study. A recent study indicated *Gladiolus gandavensis* as non-mycorrhizal (Marins J.F., Correnho R., 2017). Because this species was ancestor of cultivated gladioli, it might be possible to have low AM susceptibility among current hybrids due to this.

Results obtained in this study for root length colonization in *Lilium candidum* although higher (M = 32.2%, m = 33.89%) are still comparable with those obtained by Varshney A. *et al* (2002) from a pot experiment. They found a root length colonization in non-inoculated *Lilium* sp. plants (Asiatic hybrid ‘Gran Paradiso’) to be under 10% while in inoculated plants under different P regimes had a colonization between 20.8-25.3% accompanied also by improved plant characteristics. In natural conditions, Kumar A. *et*

al. (2012) found a colonization level in *Lilium rubescens* of 38.09%.

In this study arbuscule abundance in mycorrhizal parts of roots fragments for *Helianthus tuberosus* had low values of only A = 4.1%. Similarly, low values were obtained by Zubek S. *et al* (2011) for this species (A = 1%). However, relative mycorrhizal root length was higher in present study with M = 39.85% compared to M = 2.5% found by previous authors (Zubek S. *et al*, 2011). This shows that higher hyphae presence inside the roots in the current study, did not progress towards arbuscules formation. Di Barbaro G. *et al* (2017) studying *Helianthus tuberosus* plants grown in field found a high root colonization frequency of 97% and arbuscule abundance of 34% and vesicle abundance of 21%. High level of root colonization of *Helianthus tuberosus* (67-89% across experimental variants) was identified in a greenhouse experiment lasting 120 days involving application of different treatments that included microorganisms isolated from roots of same species (*Glomus* sp., *Klebsiella variicola*) in addition to Rock phosphate (Boonlue S. *et al*, 2017).

Due to nutrient efficiency constraint of geophytes caused by their life-cycle the balance of resource allocation between generative, vegetative and storage organs could cause highly controlled investments towards symbiont. This might explain wide range of colonization patterns according to plant affordability to sustain the mutualism when carbon paid towards symbiont is also required to be retained in storage organ.

It could be that suppression of arbuscules development or even resistance to colonization to be necessary for the plant by the time of anthesis, in natural conditions.

Thus, summer geophytes might be good model plants for resource allocation in endophyte relationship since could be considered highly sensitive to disbalance in carbon allocation.

## CONCLUSION

Exchange suppression or resistance to colonization might act in different phenophase according to priorities of the plant, with potential impact on predictability of symbiosis for this category of biotrophs.

Spores and vesicles were more abundant in *Asteraceae* species root segments.

Highest intensity for root colonization in root segments was found in *Dahlia variabilis* followed by *Helianthus tuberosus* and *Lilium*

*candidum* and lowest in *Hemerocallis fulva* and *Gladiolus × grandiflorus*.

Highest arbuscularity was identified in *Dahlia variabilis* and *Lilium candidum*.

## REFERENCES

- Crișan I., Vidican R., Stoian V., 2017.** Utilization of arbuscular mycorrhizae in the cultivation of ornamental plants. Research Journal of Agricultural Science, 49(4): 392-397.
- Boonlue S., Nacoos S., Kokaew U., 2017.** Efficiency of dual-inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on the growth and tuber inulin content of Jerusalem artichoke (*Helianthus tuberosus* L). SNRU Journal of Science and Technology. 9(2): 465-473.
- Dafni A., Cohen D., Noy-Mier I., 1981.** Life-cycle variation in geophytes. Annals of the Missouri Botanical Garden, 68(4): 652-660.
- Di Barbaro G., Andrada H., González Basso V., Alurralde A. L., Del Valle E., de Weht C. B., 2017.** Micorrizas arbusculares y hongos septados oscuros nativos en topinambur (*Helianthus tuberosus* L.) en Catamarca. Argentina. Rev. Cienc. Agr. 34(2): 98-106.
- Druva-Lusite I., Levinsh G., 2010.** Diversity of arbuscular mycorrhizal symbiosis in plants from coastal habitats. Environmental and Experimental Botany, 8: 17-34.
- Garg N.G., Kaur A., 2006.** Arbuscular mycorrhiza: Nutritional aspects, Archives of Agronomy and Soil Science, 52(6): 593 – 606.
- Javid A., Riaz T., 2008.** Mycorrhizal colonization in different varieties of gladiolus and its relation with plant vegetative and reproductive growth. International Journal of Agriculture and Biology, 10: 278-82.
- Kamenetsky Rina, 2013.** Biodiversity of geophytes In: Kamenetsky Rina, Hiroshi Okubo (Eds.) *Ornamental Geophytes: From Basic Science to Sustainable Production*. Taylor and Francis, p. 59.
- Kumar A., Bhatti S.K., Aggarwal A., 2012.** Biodiversity of Endophytic Mycorrhiza in Some Ornamental Flowering Plants of Solan, Himachal Pradesh. Biological Forum – An International Journal, 4(2): 45-51.
- Marins J.F., Carrenho R., 2017.** Arbuscular mycorrhizal fungi and dark septate fungi in plants associated with aquatic environments, Acta Botanica Brasilica. 31(2): 295-308 (S1, S2 supplementary material).
- Ranwala A.P., Miller W.B., 2008.** Analysis of nonstructural carbohydrates in storage organs of 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection. New Phytologist, 180: 421–433.
- Rodríguez-Rodríguez R.M., Herrera P., Furrázola E., 2013.** Arbuscular mycorrhizal colonization in Asteraceae from white sand savannas, in Pinar del Río, Cuba. Biota Neotrop. 13(3): 136-140.
- Ruiters C., McKenzie B., Aalbers J., Raitt A.M., 1993.** Seasonal allocation of biomass and resources in the geophytic species *Haemanthus pubescens* subspecies *pubescens* in lowland coastal fynbos. South Africa. A. Afr. J. Bot., 59(2): 251-258.

- Smith F.A., Smith S.E., 1997.** *Structural diversity in (vesicular)—arbuscular mycorrhizal symbioses*, *New Phytol.* 137: 373-388.
- Stoian V.H., Florian V., 2009.** *Mycorrhiza – Benefits, Influence, Diagnostic Method*. Bulletin UASVM Agriculture 66(1): 170-175.
- Trouvelot A., Kough J.L., Gianinazzi-Pearson V., 1986.** *Mesure du taux de mycorhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle*. In: V. Gianinazzi-Pearson and S. Gianinazzi (Eds.). *Physiological and Genetical Aspects of Mycorrhizae*. INRA Press, Paris, pp. 217-221.
- Varshney A., Sharma M. P., Adholeya A., Dhawan V., Srivastava P.S., 2002.** *Enhanced growth of micropropagated bulblets of Lilium sp. inoculated with arbuscular mycorrhizal fungi at different P fertility levels in an alfisol*. The Journal of Horticultural Science and Biotechnology. 77(3): 258-263.
- Vierheilig H., Coughlan A. P., Wyss U., Piché Y., 1998.** *Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal Fungi*. Appl Environ Microbiol., 64(12): 5004–5007.
- Zubek S., Błaszowski J., Seidler-Łożykowska K., Bąba W., Mleczko P., 2011.** *Arbuscular mycorrhizal fungi abundance, species richness and composition under the monocultures of five medicinal plants*. Acta Sci Pol-Hortoru. 12:127–141.

\*\*\* <https://www2.dijon.inra.fr/mychintec/>