

SPRING-AUTUMN ARBUSCULAR MYCORRHIZA COLONIZATION DYNAMIC IN *Iris germanica* L. FROM URBAN MICROCLIMATE

Ioana CRIȘAN, Roxana VIDICAN, Andrei STOIE, Ștefania Alexandra SIMEA

University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca,
Manastur Street No. 3-5, 400372, Cluj-Napoca, Romania

Corresponding author email: andrei.stoie@usamvcluj.ro

Abstract

Nowadays arbuscular mycorrhiza (AM) is widely recognized as one of the most successful plant-fungi partnerships. Although the evidence for ecosystem services provided by these microorganisms is comprehensive, surprisingly few studies document arbuscular mycorrhiza in cities. The aim of this research was to describe arbuscular mycorrhiza (*Glomeromycota*) colonization pattern across a genotypic and seasonal gradient for *Iris germanica*. Colonization parameters were assessed for 2160 root segments from six *Iris germanica* cultivars collected in spring and autumn (two contrasting phenophases). The results showed that *Iris germanica* presents Paris AM morphotype. Phenophase exercised a significant influence over AM colonization parameters explaining around 70% of variance while the influence of the cultivar was non-significant and explained between 13-18% of variance. Towards autumn AM frequency decreased and average of 18.36% relative to spring while intra-radicular AM spores and vesicles increased on average of 22.73% relative to spring. This study suggests that plant-fungi interaction is controlled by plant metabolic state and decrease of frequency coincides with the debut of leaf senescence and implicitly a reduction of the carbon flux in plant, to which AM fungi react by sporulating.

Key words: urban microbiome, soil, rhizosphere, fungi, ornamentals.

INTRODUCTION

Iris germanica L. is a perennial ornamental and medicinal plant and the most cultivated species from this genus (Crișan et al., 2019). This species has been used as model plant for root studies (Meyer et al., 2011).

Green areas in urban environment play an important role in improving quality of life (Vargas-Hernández et al., 2018) not only due to their aesthetic and recreational value (Xu et al., 2016) but because can provide key ecosystem services such as nutrient cycling (Francini et al., 2018) or reduction of urban heat island effect, a meteorological phenomenon of current concern due to negative impact on human well-being (Aram et al., 2019; Maheng et al., 2019). Ecosystem services provided by green spaces in cities are dependent on the activity of organisms from soil that in turn are conditioned by vegetation type, distribution and soil properties (Francini et al., 2018). Because urban landscape is characterized by profound changes of ecological conditions, fragmented habitats, low biodiversity and shift towards non-native plant species (Anastasiu et al.,

2017), can be affirmed that challenges related to the health of urban ecosystems are unique and unlike those from natural ecosystems.

Arbuscular mycorrhiza (AM) is a symbiosis currently established by more than 70% of flowering plants with *Glomeromycota* fungi, in which characteristic exchange structures form inside root cortex. Fossil evidence suggests that this symbiosis has been a successful partnership since land colonization by first plants (Brundrett & Tedersoo, 2018).

Beneficial effects of arbuscular mycorrhiza are diverse and range from improving soil quality to increasing plant tolerance to various biotic and abiotic stressors. Following AM colonization, plant development can be improved both under normal as well as under stressful conditions chiefly due to enhanced nutrient uptake (Begum et al., 2019). Soil fungal webs have high capacity to store nutrients (Francini et al., 2018), and in particular arbuscular mycorrhizal fungi (AMF) can contribute to sustainable development of urban ecosystems by tackling emerging environmental challenges that plants face in urban conditions (Shi et al., 2011). Gardening

and landscaping are currently among the top sectors that employ commercial mycorrhiza application and there is increasing number of companies that produce inoculants driven by the growing interest for using them (Chen et al., 2018). However, to date there is a lack of studies documenting arbuscular mycorrhiza in urban environments, as well as the factors that can influence mycorrhizal symbiosis in cities (Chaudhary et al., 2019). Thus, although the knowledge of beneficial effects of arbuscular mycorrhizal fungi for soil and plant health are comprehensive (Chen et al., 2018) there are aspects that require more attention. Research on particularities and biology of arbuscular mycorrhizal symbiosis in urban environments can provide the knowledge base necessary to optimize their use or elaborate mycorrhiza-friendly management practices for greening cities.

The aim of this research was to obtain a comprehensive description of AM colonization pattern across a genotypic and seasonal gradient for *Iris germanica* from conditions of Cluj-Napoca. Two objectives were defined: 1) Identification of the influence of phenophase and cultivar on AM endophytic root colonization parameters, 2) Analysis of relationship existing between the colonization parameters to explain mycorrhiza continuity.

MATERIALS AND METHODS

Cluj-Napoca is the largest city from Transylvania region of Romania, situated in transitional temperate-continental climate (Criveanu, 2002), and experiences the effects of the atmospheric urban heat island (Herbel et al., 2016). Climatic conditions are suitable for cultivation of frost hardy *Iris* species (Crişan et al., 2016). Experimental plot was established in a green space from western part of Cluj-Napoca city at decimal degrees coordinates (DD): 46.766569, 23.545294. Plot used in this experiment was cleared of wild vegetation. Because no treatments were applied to this plot at least in last 15 years, it was considered suitable for studying natural occurring AM colonization dynamic in urban conditions.

The experiment was established in July 2017 and starting with spring of 2018 root samples were collected to assess natural AM

colonization dynamic. Climatic data between planting, first and second root collecting indicates that highest temperatures were registered in August 2017 (37°C), after planting the rhizomes and lowest in March 2018 (-17°C) right before plants entered in vegetation. Highest precipitation levels were registered in June 2018 after flowering. Compared to multiannual average temperature on 11 years (2006-2016) which was 9.99°C (<https://en.tutiempo.net>), average annual temperature registered in Cluj-Napoca city was 5.11% higher in 2017, and 12.11% higher in 2018. Compared to multiannual average for the sum of precipitation registered between 2006-2016 (<https://en.tutiempo.net>) which was 652.57 mm/m², the sum of precipitation was 37.49% lower in 2017 and 19.73% lower in 2018 (Figure 1). These values indicate that experimental year was slightly hotter and drier compared to average values from last decade.

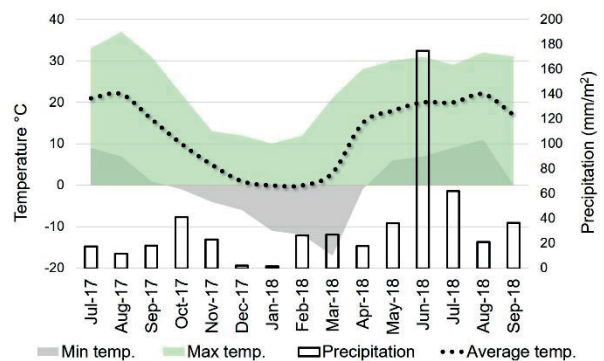


Figure 1. Monthly temperature and precipitation levels during planting and last sampling (<https://en.tutiempo.net>, <https://wunderground.com>)

Soil type in Cluj-Napoca city is luvisol (Stănilă et Dumitru, 2016). Analysis of soil sample (depth 0-20 cm) from experimental plot was conducted at an authorized agrochemical laboratory from Cluj-Napoca (OSPA Cluj). Results are presented in Table 1.

Table 1. Soil physicochemical properties

Analysis	Method	Result
pH	potentiometric	8.05
Humus	Walkley-Black	3.24%
Nitrogen	Kjeldahl	0.129%
Phosphorus	Colorimetric	224 ppm
Potassium	Flame photometry	304 ppm
CaCO ₃	Scheibler	10.4 me/100 g
Granulometric	Kacinski	clay-loam

According to analysis, the soil has clay-loam texture (coarse sand 11.45, fine sand 32.96, silt I 9.15, silt II 13.20, clay 33.24), has slight alkaline pH with moderate carbonate content, moderate humus level, low nitrogen content but very good/high phosphorus and potassium levels, compared to reference thresholds from literature (Davidescu et al., 1981).

The biologic material was represented by rhizomes of six *Iris germanica* L. cultivars with different flower colours (Figure 2), imported from Holland. Specimens from each cultivar studied can be found at Agro-Botanical Garden UASVM Cluj-Napoca, Romania (Table 2).

Table 2. *Iris germanica* cultivars and accession number

Cultivar	Flower	IPEN ¹ code
'Black Dragon'	black	XX-0-CLA-1757
'Blue Rhythm'	blue	XX-0-CLA-1758
'Sultan's Palace'	red	XX-0-CLA-1759
'Lime Fizz'	yellow	XX-0-CLA-1761
'Pinafore Pink'	pink	XX-0-CLA-1762
'Pure As The'	white	XX-0-CLA-1763

¹International Plant Exchange Network

Rhizomes (with leaves and roots trimmed), were planted in six randomized blocks with three rhizomes per replicate, resulting in a total of 108 rhizomes planted (18 rhizomes/cultivar).



Figure 2. *Iris germanica* cultivars in bloom (original)

Next vegetative season after planting, when plants were already well-established, roots were collected for assessment of natural AM colonization. Sampling was conducted on 20.04.2018 and 14.09.2018, when plants were in two distinct phenophases. At the time of root collecting in April (IV) plants were in shoot growth and leaf extension phenophase, while in September (IX) plants were at the debut of leaf senescence. Roots were taken immediately to the lab for processing on the same day. Root

samples were stained using the method by Vierheilig et al. (1998) but with the modification that two clearing stages were applied: first clearing with 5% KOH + 10% NaOH, and the second one with 15% NaOH. Stained roots were mounted on glass slides and squashed with the cover class. Observation was conducted at magnification 100× - 400× under Optika brightfield microscope equipped with condenser 0.90 N.A. Microscopic evaluation was performed on sets of 30 root segments of 1 cm long each in six replicates for each of the six cultivars both in spring and autumn. In total were analysed 2160 root samples (30 segments × 6 replicates × 6 cultivars × 2 samplings). Arbuscular mycorrhizal indicators as defined by Trouvelot et al. (1986) were obtained using MycoCalc software (<https://www2.dijon.inra.fr>). Arbuscular mycorrhiza (AM) colonization parameters calculated were: F% = frequency of mycorrhizal colonization, m% = intensity of the mycorrhizal colonization in the colonized root fragments, M% = intensity of the mycorrhizal colonization in the root system. In addition, were counted all AM spores and vesicles in each root segment and was calculated the frequency of AM vesicles and spores inside roots for each cultivar in spring and autumn. Statistical analysis was conducted by applying two-way ANOVA, Duncan test and Pearson correlation.

RESULTS AND DISCUSSIONS

Microscopic analysis of stained root fragments revealed that arbuscular mycorrhizal colonization in *Iris germanica* presented a typical *Paris* morphotype (Figure 4). Similar morphotype was described before in another species from same genus: *Iris stolonifera* (Zubek et al., 2011).

Average values of colonization parameters varied between cultivars within range 66.95-78.06 for the frequency of colonization, 15.10-10.28 for the intensity of colonization in root fragments, 8.16-10.39 for the intensity of colonization in root system. Analysis of the influence exercised by experimental factors on mycorrhizal colonization revealed that phenophase had a highly significant influence over two AM parameters: frequency of colonization (F %) and intensity of mycorrhizal colonization in root fragments (m %).

Phenophase explained around 70% of the overall variance observed for colonization parameters ($\eta^2 = 0.69-0.75$). It was also observed that the cultivar gradient and interaction between phenophase and cultivar (C×P) did not exercise a significant influence

on AM root colonization parameters. Cultivar explained between 13-18% of variance while the interaction between cultivar and phenophase explained 12-17% of the overall variance registered for AM colonization parameters (Table 3).

Table 3. Influence of phenophase and cultivar on arbuscular mycorrhiza (AM) colonization indicators: frequency (F %) and intensity (m %, M %) in *Iris germanica* roots

AM	Factors	Mean square	F	p	Sig.	η^2	s ²
F%	Phenophase (P)	3707.47	12.02	<0.001	***	0.69	336.60
	Cultivar (C)	192.47	0.62	0.682	n.s.	0.18	
	Interaction (C×P)	143.12	0.46	0.801	n.s.	0.13	
m%	Phenophase (P)	957.83	14.32	<0.001	***	0.75	74.53
	Cultivar (C)	32.81	0.49	0.782	n.s.	0.13	
	Interaction (C×P)	31.11	0.46	0.800	n.s.	0.12	
M%	Phenophase (P)	302.01	6.15	0.015	*	0.70	47.58
	Cultivar (C)	11.84	0.24	0.942	n.s.	0.14	
	Interaction (C×P)	14.60	0.30	0.912	n.s.	0.17	

Sig. = significance, η^2 = partitioning of variance, s² = variance
Two-way ANOVA; $p > 0.05$ (n.s.), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

Table 4. Combined effect of cultivar and month of root sampling on arbuscular mycorrhiza frequency (F %) and intensity of colonization (m %, M %) in *Iris germanica* roots

Experimental factors		Frequency of colonization (F %)			Intensity of colonization (m %)		
Month	Cultivar	Mean	±SD	±SE	Mean	±SD	±SE
April (IV)	‘Black Dragon’	75.00 abcd	13.62	5.56	9.09 abc	3.84	1.57
	‘Blue Rhythm’	82.22 cd	7.79	3.18	10.08 abc	4.19	1.71
	‘Sultan’s Palace’	84.44 d	7.20	2.94	9.55 abc	3.25	1.33
	‘Lime Fizz’	70.56 abcd	27.19	11.10	8.36 ab	4.53	1.85
	‘Pinafore Pink’	79.45 bcd	13.07	5.33	10.60 abc	9.16	3.74
	‘Pure As The’	77.22 bcd	9.29	3.79	7.09 a	3.15	1.29
Sept. (IX)	‘Black Dragon’	61.11 ab	25.36	10.35	16.54 abc	13.06	5.33
	‘Blue Rhythm’	63.33 abc	20.33	8.30	17.73 abc	6.67	2.72
	‘Sultan’s Palace’	71.67 abcd	22.49	9.18	11.02 abc	4.96	2.02
	‘Lime Fizz’	68.89 abcd	17.08	6.97	15.41 abc	7.32	2.99
	‘Pinafore Pink’	61.11 ab	19.05	7.78	19.60 c	8.60	3.51
	‘Pure As The’	56.67 a	13.82	5.64	18.23 bc	16.69	6.81
Overall	<i>Iris germanica</i>	70.97	18.35	2.16	12.77	8.63	1.02

Values followed by same letters have non-significant differences, Duncan test, $p < 0.05$
±SD (standard deviation), ±SE (standard error of mean)

By analysing the influence of phenophase on colonization indicators, it was observed that the frequency of colonization decreased and average of 18.36% at the start of leaf senescence relative to leaf growth phenophase. Results indicate there was no significant difference for frequency of colonization nor for intensity of colonization in root fragments between cultivars either in spring or autumn. This shows that all cultivars displayed similar susceptibility to colonization. The cultivar with

white flowers ‘Pure As The’ registered significant differences between the two phenophases both for frequency and intensity of colonization.

Highest colonization frequency was identified in spring for cultivar ‘Sultan’s Palace’ (F % = 84.44) and lowest frequency in autumn for ‘Pure As The’ (F % = 56.57). The lowest colonization intensity in root fragments was registered for cultivar ‘Pure As The’ in spring (m % = 7.09) and the highest intensity for

‘Pinafore Pink’ in autumn (m % = 19.60); this difference is statistically significant (Table 4). For *Iris germanica* cultivars, the frequency of root fragments with intra-radicular spores and vesicles increased an average of 22.73% in autumn relative to spring. In spring the frequency of root fragments with intra-radicular spores and vesicles ranged between 3.89-7.22% and in autumn ranged between 3.33-8.33%. Although in both

phenophases and for all six cultivars the frequency of root fragments with spores and vesicles maintained fewer than 10%, in autumn increased the number of spores and vesicles per cm root. Thus, in spring there were no more than 5 spores and vesicles per cm root, while in autumn the cultivars studied had up to 3% root fragments with more than 10 spores and vesicles per cm root (Figure 3).

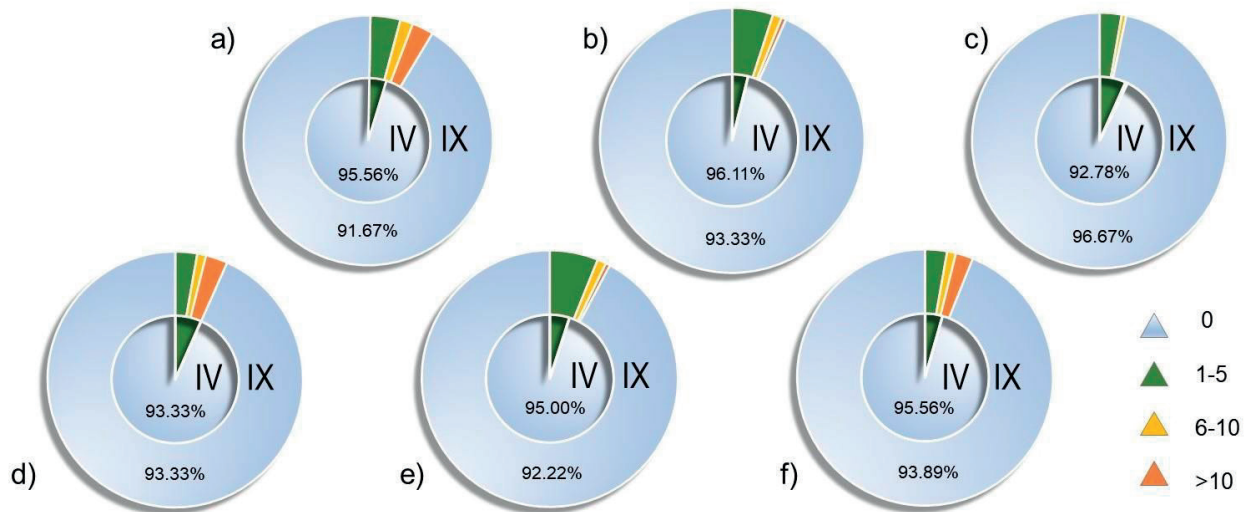


Figure 3. Occurrence (%) of root fragments with intra-radicular AM spores and vesicles: blue - fragments without spores and vesicles, green - fragments with 1-5 spores and vesicles per cm, yellow - fragments with 6-10 spores and vesicles per cm, orange - more than 10 spores and vesicles per cm root, in six *Iris germanica* cultivars: (a) ‘Black Dragon’, (b) ‘Blue Rhythm’, (c) ‘Sultan’s Palace’, (d) ‘Lime Fizz’, (e) ‘Pinafore Pink’ (f) ‘Pure As The’, in April (IV) and September (IX)

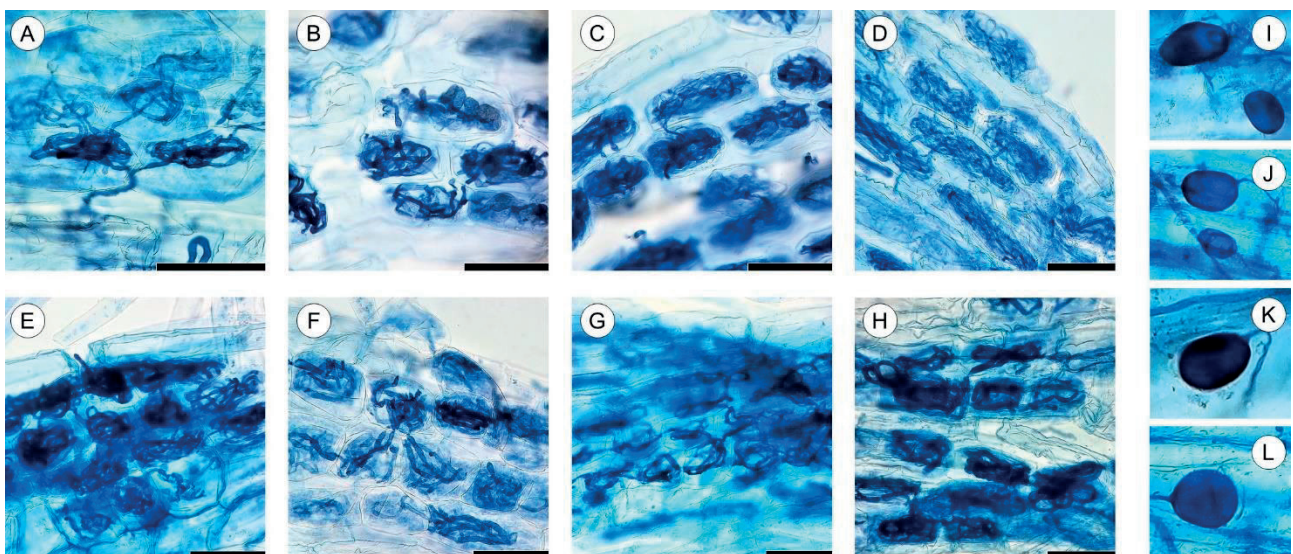


Figure 4. Arbuscular mycorrhiza (AM - *Glomeromycota*) in *Iris germanica* stained roots: (a-h) hyphae displaying cell to cell spreading (coils) - *Paris* morphotype, scale bar = 50 μ m; (i-l) intra-radicular AM spores and vesicles

Correlation coefficients between colonization indicators were analysed for two reasons: firstly, to define the relationship between

colonization parameters at a given phenophase and secondly in an attempt to identify the existence of endomycorrhiza continuity

between the two phenophases. Correlation matrix is presented in Figure 5. It was found a significant positive correlation between AM frequency colonization in April and September ($r = 0.346^*$). Between the intensity of colonization in root fragments (m %) and root system (M %), there was a highly significant positive correlation both in April and September and having identical value ($r = 0.945^{***}$). Correlation between frequency of colonization in root fragments (m %) and intensity of colonization in root fragments (m %) that was not significant at phenophase of shoot and leaf growth ($r = 0.211$) became significant in autumn ($r = 0.478^{**}$).

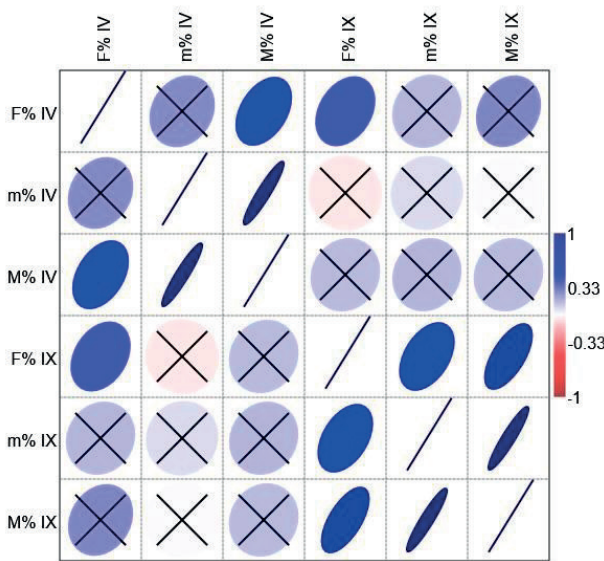


Figure 5. Pearson correlation matrix for AM colonization indicators: frequency (F %) and intensity (m %, M %) in April (IV) and September (IX), crossed $p > 0.05$ (n.s.)

Iris germanica plants presented AM intra-radicular spreading corresponding to typical *Paris* morphotype regardless of season or cultivar. Far fewer studies have been conducted on *Paris* morphotype compared to *Arum*, and as a result the AM biology and development under this morphotype are not well understood. There is evidence that *Paris*-type develops slower (Cavagnaro et al., 2001). Differences in colonization morphotypes between plant species can be firstly attributed to root histologic structure (Dickson, 2004) and secondly to fungi species colonizing the plant (Dickson et al., 2007). *Paris*-morphotype is considered more common in forest floor herbaceous plants which grow in partial shade, evergreen plants as well as long-lived woody

plants. Previous authors point out the interesting fact that *Paris*-type plants appear to have lower photosynthetic rates than *Arum*-type plants. Furthermore, recent experimental data based on stable isotope enrichment factors suggests that plants which develop *Paris*-morphotype could exhibit partial-mycoheterotrophy, thus implying transfer of photosynthates from one green plant to another through *Arum-Paris* type network (Giesemann et al., 2019). These aspects come to highlight the importance of morphotype for particularities existing within this partnership, which have been insufficiently explored in the past. The low frequency of intra-radicular spore and vesicles registered both in spring and autumn for *Iris germanica* could be due to occurrence of *Paris* morphotype. The frequency of spores and vesicles (<10%) can be considered low because *Arum*-type geophyte species in the same pedo-climatic conditions were shown to reach much higher frequency of spores and vesicles such as in *Dahlia variabilis* (60%) or *Liatris spicata* (75%) (Crişan et al., 2018; Crişan et al., 2019).

It is proposed that because in *Paris* morphotype the hyphae are spreading solely from cell to cell (Dickson, 2004) and since after a few days the intracellular mycorrhiza structures are digested by the plant cell (Bonfante & Genre, 2010), repeated dissolution of mycorrhiza structures could also limit the time interval necessary for intra-radicular spores and vesicles to form resulting in lower levels, as observed here. However, the biological and ecological significance of a lower count of intra-radicular spores remains to be determined. Formation of mycorrhiza storage structures, represented by spores and vesicles is a key stage in life cycle of arbuscular mycorrhizal fungi (Müller et al., 2017) and limited ability of a plant species such as *Iris germanica*, due to *Paris*-type morphotype to form intra-radicular spores could probably act in time as selective factor on AM community. This particularly when considering propagation strategies of different AMF taxa, such as ability to initiate colonization mostly through spores, or in addition through vesicles and mycelia fragments or colonized root fragments (Klironomos & Hart, 2002). Although spores are formed also by extra-radicular mycelia and

identity of AM taxa exercises influence on the abundance of intra-radicular spores or formation of vesicles (Błaszowski, 2012), undoubtedly intra-radicular spores and vesicles increase the quantity of infective AM propagules in rhizosphere. Thus, it could prove useful to explore the influence of colonization morphotype across *Arum-Paris* continuum on formation of spores and vesicles in relation with AMF taxa exhibiting different propagation strategies. This could be a way to identify if the presence of a particular morphotype possesses any selective influence, such as for example a *Paris*-type host “disadvantaging” in time the AMF species that rely mostly on spores to initiate re/colonization. It could be the case that plant species such as *Iris germanica* could rely to a higher degree on exogenous sources of infection such as presence of propagules in the surrounding soil to maintain a certain level of colonization during entire growth season. Regarding the other AM parameters, results indicated a potential dependency of AM colonization level registered at the beginning of leaf senescence on colonization level from shoot growth phenophase, reflected by a significant positive correlation between AM frequency at the start and the end of growth season. Firstly, this could highlight the importance of colonization initiated in spring when plants develop new roots. Secondly this could indicate mycorrhiza continuity within root system during entire growth season. A previous study involving another ornamental geophyte plant - *Gladiolus grandiflorus*, in experimental conditions demonstrated that both vegetative and reproductive parameters of the plants were correlated with AM colonization at early growth stage (Javaid & Riaz, 2008), further indicating the importance of early colonization. The significant influence of phenophase on AM colonization parameters could be explained by the control of plant metabolic state over the symbiose, higher AM frequency occurring in spring when *Iris germanica* plants experience accelerated growth, compared to autumn. Results are in consensus with previous findings that showed the existence of a variation in AM colonization levels between seasons or growth stages as described for *Allium tricoccum* (Hewins et al.,

2015), *Acaena elongata* (Vasquez-Santos et al., 2019) and *Asphodelus fistulosus* (Cavagnaro et al., 2001). Study of the relationship between AM colonization parameters in *Iris germanica* from this study has put in evidence a stable interdependence between some AM parameters. Previous investigations in other plant species also found evidence of an existing interdependence between parameters assessing different AM structures inside roots. Thus, in *Paris*-type plant *Asphodelus* sp. was found a significant association between entry points in root and hyphae coils in cortex (Cavagnaro et al., 2001), while pairwise significant positive correlations for AM total colonization, hyphal colonization and vesicles presence were identified in plant species *Zoysia tenuifolia* and *Desmodium triflorum* from a urban lawn (Han et al., 2019).

The results of this research attempt to bring a contribution to the understanding of AM biology for the least studied morphotype: *Paris*, in a common ornamental from urban environment. Based on the observations from this study it is considered that *Iris germanica* is a suitable model plant for studies on this colonization morphotype. Urban green spaces can harbor fungal biodiversity which can have implications for sustainable management of green areas and help plants cope better with stressors typical for urban environments. Thus, it is beneficial to use in landscaping plants which can establish arbuscular mycorrhiza while colonization indicators can prove useful in evaluating soil mycorrhizal potential in urban areas.

CONCLUSIONS

This study showed that phenophase exercises a significant influence on AM colonization frequency and intensity while the influence of the cultivar is non-significant. It is proposed that *Iris germanica* could be a good model plant for research on AM *Paris* morphotype. Based on the results and literature review, it is concluded that interdependence between some AM parameters exists, but the biologic significance for symbiosis and plant remains to be determined.

REFERENCES

- Aram, F., Higuera Garcia, E., Solgi, E., Mansournia, S. (2019). Urban green space cooling effect in cities. *Heliyon*, 5, e01339.
- Anastasiu, P., Petronela, C., Nagodă, E., Lițescu, S., Negrean, G. (2017). Nature reclaiming its territory in urban areas. Case study: Vacaresti nature Park, Bucharest, Romania. *Acta Horti Bot. Bucurest.*, 44, 71–99.
- Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N., Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in Plant Science*, 10, 1068.
- Błaszowski, J. (2012). *Glomeromycota*. Polish Academy of Sciences: Kraków, Poland.
- Bonfante, P. & Genre, A. (2010). Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature Communications*, 1, 1–11.
- Brundrett, M.C. & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, 220, 1108–1115.
- Cavagnaro, T.R., Smith, F.A., Lorimer, M.F., Haskard, K.A., Ayling, S.M., Smith, S.E. (2001). Quantitative development of Paris-type arbuscular mycorrhizas formed between *Asphodelus fistulosus* and *Glomus coronatum*. *New Phytologist*, 149, 105–113.
- Chaudhary, V.B., Sandall, E.L., Lazarski, M.V. (2019). Urban mycorrhizas: predicting arbuscular mycorrhizal abundance in green roofs. *Fungal Ecology*, 40, 12–19.
- Chen, M., Arato, M., Borghi, L., Nouri, E., Reinhardt, D. (2018). Beneficial Services of arbuscular mycorrhizal fungi - from ecology to application. *Frontiers in Plant Science*, 9, 1270.
- Crișan, I., Stoie, A., Cantor, M. (2016). Overwintering of some hardy Iris species in Agrobotanical Garden UASVM Cluj-Napoca. *Agriculture Science and Practice Journal*, 99, 6–14.
- Crișan, I., Vidican, R., Stoian, V., Șandor, M., Stoie, A. (2018). Arbuscular mycorrhizae of five summer geophytes from Cluj county. *Scientific Papers Agronomy Series*, 61, 61–66.
- Crișan, I., Vidican, R., Stoie, A., Stoian, V. (2019). Root colonization by micromycetes in ten Asteraceae species from Cluj County. *Journal of Horticulture, Forestry and Biotechnology*, 23, 51–57.
- Crișan, I., Vidican, R., Olar, L., Stoian, V., Morea, A., Ștefan, R. (2019). Screening for changes on *Iris germanica* L. rhizomes following inoculation with arbuscular mycorrhiza using Fourier transform infrared spectroscopy. *Agronomy*, 9, 815.
- Criveanu, H. (2002). *Agrometeorology*. Editura Risoprint: Cluj-Napoca, Romania.
- Davidescu, D., Calancea, L., Davidescu, V., Lixandru, G., Țârdea, C. (1981). *Agrochemistry*. Editura Didactica și Pedagogica, Bucharest, Romania.
- Dickson, S. (2004). The Arum–Paris continuum of mycorrhizal symbioses. *New Phytologist*, 163, 187–200.
- Dickson, S., Smith, F.A., Smith, S.E. (2007). Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza*, 17, 375–393.
- Francini, G., Hui, N., Jumpponen, A., Kotze, D.J., Romantschuk, M., Allen, J.A., Setälä, H. (2018). Soil biota in boreal urban greenspace: Responses to plant type and age. *Soil Biology and Biochemistry*, 118, 145–155.
- Giesemann, P., Rasmussen, H.N., Liebel, H.T., Gebauer, G. (2019). Discreet heterotrophs: green plants that receive fungal carbon through Paris-type arbuscular mycorrhiza. *New Phytologist*, 226, 960–966.
- Han, X., Xu, C., Wang, Y., Huang, D., Fan, Q., Xin, G., Müller, C. (2019). Dynamics of arbuscular mycorrhizal fungi in relation to root colonization, spore density, and soil properties among different spreading stages of the exotic plant three flower beggarweed (*Desmodium triflorum*) in a *Zoysia tenuifolia* lawn. *Weed Science*, 67, 689–701.
- Herbel, I., Croitoru, A., Rus, I.M., Harpa, G.V., Ciupertea, A.-F. (2016). Detection of atmospheric urban heat island through direct measurements in Cluj-Napoca city, Romania. *Hungarian Geographical Bulletin*, 65, 117–128.
- Hewins, C.R., Carrino-Kyker, S.R., Burke, D.J. (2015). Seasonal variation in mycorrhizal fungi colonizing roots of *Allium tricoccum* (wild leek) in a mature mixed hardwood forest. *Mycorrhiza*, 25, 469–483.
- Javaid, 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.
- Klironomos, J.N. & Hart, M.M. (2002). Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. *Mycorrhiza*, 12, 181–184.
- Maheng, D., Ducton, I., Lauwaet, D., Zevenbergen, C., Pathirana, A. (2019). The sensitivity of urban heat island to urban green space - a model-based study of city of Colombo, Sri Lanka. *Atmosphere*, 10, 151.
- Meyer, C.J., Peterson, C.A., Steudle, E. (2011). Permeability of *Iris germanica*'s multiseriate exodermis to water, NaCl, and ethanol. *Journal of Experimental Botany*, 62, 1911–1926.
- Müller, A., Ngwene, B., Peiter, E., George, E. (2017). Quantity and distribution of arbuscular mycorrhizal fungal storage organs within dead roots. *Mycorrhiza*, 27, 201–210.
- Shi, L., Diao, Z., Liu, R. (2011). Colonization and community structural features of AM fungi in urban ecosystem: a review. *Ying Yong Sheng Tai Xue Bao*, 22, 1939–1943.
- Stănilă, A.-L. & Dumitru, M. (2016). Soils Zones in Romania and Pedogenetic Processes. *Agriculture and Agricultural Science Procedia*, 10, 135–139.
- Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V. (1986). Estimation of vesicular arbuscular mycorrhizal infection levels. *Proceedings of the 1st European Symposium on Mycorrhizae*, Dijon, France.
- Vargas-Hernández, J.G., Pallagst, K., Zdunek-Wielgołaska, J. (2018). *Urban green spaces as a component of an ecosystem*. In Handbook of Engaged

- Sustainability; Marques, J., Ed.; Springer: Cham, Switzerland.
- Vázquez-Santos, Y., Castillo-Argüero, S., Martínez-Orea, Y., Sánchez-Gallen, I., Vega-Frutis, R., Camargo-Ricalde, S.L., Hernández-Cuevas, L.V. (2019). The reproductive phenology of *Acaena elongata* and its relation with arbuscular mycorrhizal fungi. *Symbiosis* 79, 129–140.
- Vierheilig, H., Coughlan, A.P., Wyss, U.R.S., Piché, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology*, 64, 5004–5007.
- Xu, L., You, H., Li, D., Yu, K. (2016). Urban green spaces, their spatial pattern, and ecosystem service value: The case of Beijing. *Habitat International*, 56, 84–95.
- Zubek, S., Nobis, M., Błaszowski, J., Mleczko, P., Nowak, A. (2011). Fungal root endophyte associations of plants endemic to the Pamir Alay Mountains of Central Asia. *Symbiosis*, 54, 139–149.
- ***Mycocalc software, <https://www2.dijon.inrae.fr> (accessed on Apr. 20, 2020).
- ***Cluj-Napoca climate data, Available online: <https://www.wunderground.com> (accessed on Apr. 20, 2020).
- ***Cluj-Napoca climate data, Available online: <https://en.tutiempo.net/climate> (accessed on Apr. 20, 2020).