# EFFECTS OF BUD LOAD AND PHOSPHORUS CONTAINING FOLIAR SPRAYS ON GRAPEVINE MYCORRHIZATION

Ádám Donkó[1], Gábor Zanathy†[2], Zsolt Erős-Honti[3], Zsolt Zsófi[4], Szabolcs Villangó[4], Judit Göblyös[5], György Dénes Bisztray[2] and Dóra Drexler[1]

[1] Hungarian Research Institute of Organic Agriculture (ÖMKi)
H-1033 Budapest, Miklós tér 1
[2] Szent István University, Faculty of Horticultural Science, Institute of Viticulture and Oenology, Department of Viticulture
H-1118 Budapest, Villányi út 29-43
[3] Szent István University, Faculty of Horticultural Science, Department of Botany and Soroksár Botanical Garden
H-1118 Budapest, Villányi út 29-43
[4] Eszterhazy Karoly University, Research Institute for Viticulture and Enology
H-3300 Eger, Kőlyuktető Pbox. 83
[5] Eszterhazy Karoly University, GYKRC
H-3200 Gyöngyös Mátrai út 36
E-Mail: adam.donko@biokutatas.hu

The symbiotic interaction with mycorrhizal fungi is of admirable importance for grapevines: it increases water and nutrient uptake and thus helps to cope with biotic and abiotic stresses. Beside its above mentioned roles, the fungus has special importance for the phosphorus uptake of the plant. Based on field trials carried out with grapevines grown on the sandy soil of Szigetcsép (Kunság wine region, Hungary) we studied the effect of different bud loads combined with foliar phosphorus sprays on the degree of mycorrhizal colonization. For the determination of endomycorrhizal colonization, fine root samples of the interspecific wine grape cultivar 'Viktória gyöngye' were stained with aniline blue and investigated under light microscope. The foliar phosphorus sprays resulted in a reduced level of mycorrhizal colonization in case of the control bud load, but the foliar P spray treatments had a positive effect on the percentage of the arbuscules in case of the highly bud-loaded and non-trimmed stocks. Control blocks sprayed with phosphorus showed a lower water balance ($\psi$m) compared to the unsprayed control vines.
**Keywords:** endomycorrhiza, grapevine, bud load

**Auswirkungen von Schnittstärke und phosphorhaltigen Blattdüngern auf den Mykorrhizapilzbefall der Weinrebe.** Die symbiotische Wechselwirkung mit Mykorrhizapilzen ist für Weinreben von großer Bedeutung: Sie erhöht die Wasser- und Nährstoffaufnahme und hilft so, mit biotischen und abiotischen Belastungen fertigzuwerden. Zusätzlich hat der Pilz eine besondere Bedeutung für die Phosphoraufnahme der Pflanze. Auf der Grundlage von Feldversuchen mit auf dem sandigen Boden von Szigetcsép (Kunság-Weinregion, Ungarn) angebauten Weinreben wurde der Einfluss unterschiedlicher Schnittstärken in Kombination mit phosphorhaltigen Blattdüngern auf den Grad der Besiedelung durch Mykorrhizapilze untersucht. Für die Bestimmung der Besiedelung durch Endomykorrhizapilze wurden feine Wurzelproben der interspezifischen Rebsorte 'Viktória gyöngye' mit Anilinblau gefärbt und unter dem Lichtmikroskop untersucht. Die phosphorhaltigen Blattdüngern führten bei der Kontrollvariante zu einer Reduzierung der Mykorrhiza-Besiedelung, wirkten sich aber bei den ungeschnittenen Varianten und bei den Varianten mit hoher Schnittstärke positiv auf den Anteil der Arbuskeln aus. Die mit phosphorhaltigen Blattdüngern behandelten Kontrollvarianten zeigten eine geringere Wasserbilanz ($\psi$m) im Vergleich zu den ungespritzten Kontrollreben.
**Schlagwörter:** Endomykorrhiza, Weinrebe, Schnittstärke

Similarly to other host plants, mycorrhizal symbiosis, i. e. the mutual interaction between fungi and the root of vascular plants, also has significant importance in case of grapevines. The fungal partner (mycobiont) supports the water and nutrient uptake of the host plant, while the mycobiont gets carbohydrates from the plant (Smith and Read, 1997). This symbiosis is essential for the optimal development of the host plants. The mycorrhiza forming ability of the grapevine was first recorded by Stahl (1900). This mutualism is of special importance for the grape since the plant has fewer rootlets that cannot intensively net the soil (Eissenstat, 1992). Grapevines establish endomycorrhizal interaction with *Glomeromycetes*: the hyphae penetrate the cell wall of the root cells, and invaginate the cell membrane thus forming intracellular fungal structures. Within the cells of the host plant, dichotomously-branching hyphal endings (arbuscules) and in case of certain species, storage hyphal endings (vesicles) develop. The establishment of arbuscules considerably increases the nutrient translocation surface (interface) between the fungus and the root cell, so it facilitates the transfer of nutrients between them (Schreiner, 2005). Therefore, the effectiveness of the endomycorrhizal interaction is not revealed merely by the degree of colonization (i. e. the number of the hyphae living within the roots), but the number of arbuscules observed in the colonized root fragments (Pinkerton et al., 2004; Schreiner, 2005). Moreover, mycorrhizal fungi have the ability to colonize different plants at the same time to form an interconnected physiological system, called common mycelia network (Francis and Read, 1984; Selosse et al., 2006). This develops a nutrient and water transport system between grapes and different cover crop species (e. g. *Vulpia myuros* var. *hirsuta* cv. Zorro (Zorro fescue) (Baumgartner, 2003; Cheng and Baumgartner, 2005). The nutrient uptake of mycorrhizal plants is also influenced by the soil characteristics, the soil cultivation method and the nutrient supply. Defoliation of the grapevines leads to a decreasing production of carbohydrates which may lead to a decreasing mycorrhizal colonization (Pinkerton et al., 2004). The yield also closely correlates with the arbuscule number: in an investigation on the rootstock effect, the varieties with lower yield (e. g. 101-14 Mgt) proved to have more arbuscules (thus more intensely functioning mycorrhizae) than those with higher yields (e. g. Teleki-Fuhr SO4). The latter kind of rootstocks usually use more carbohydrates at ripening, so there are fewer carbohydrates available for the mycorrhizal fungi (Schreiner, 2003). The symbiosis has an admirable effect on water and nutrient thus also the phosphorus (P) uptake of the grapevine (Possingham and Obbink, 1971; Karagiannidis et al., 1995; Ryan and Graham, 2002; Schreiner, 2005). Increasing the P supply can lead to the moderation of the degree of mycorrhizal colonization (Gebbing et al., 1977; Koide and Schreiner, 1992; Linderman and Davis, 2001). In soils with abundant phosphorus content, the carbohydrate demand of the mycorrhiza is not proportional to the benefits offered by the fungus. Therefore, the degree of mycorrhizal colonization will be lower here compared to soils with phosphorus deficiency (Baumgartner, 2003). Experimental results in vineyards of Oregon resulted in reduced levels of the mycorrhizal colonization due to foliar P sprays (Schreiner and Lindermann, 2005). According to Schreiner (2010), however, the small negative effect of foliar P spray treatments on the mycorrhizal colonization will likely have little impact on the vine physiology and fruit quality. The climatic and edaphic conditions and the trimming system may also influence the plant/fungus-interaction (Sieverding, 1991; Bhardwaj et al., 1997; Pinkerton et al., 2004; Schreiner and Lindermann, 2005). Nevertheless, the relationship between the impacts of the different bud load and canopy management on mycorrhizal colonization has not been studied. Therefore, the aim of our open field study was to investigate the effect of different bud load and canopy management treatments, combined with foliar P spray treatments, on the mycorrhizal colonization of grapevine roots over a two-year period.

## MATERIALS AND METHODS

The experiment was carried out in the Kunság wine region, on sandy soil, near Szigetcsép, in the training vineyard and winery of the Corvinus University of Budapest, Hungary. The investigated variety was the interspecific, disease resistant 'Viktória gyöngye', grafted on T-K 5BB rootstocks. The vineyard was planted in 1997, the growing space is 3 x 1 m, using single-curtain training system. Four different treatments were set up in the vineyard in four replications in a randomized block design. Each replication contained 25 vines (n = 100 vines/treatment). The treatments were as follows:

**C**) control (low bud load: 9 buds/vine (3 buds/m$^2$))
**C+P**) control with P-spraying
**HL**) heavy bud load: 32 buds/vine (10.7 buds/m$^2$)
**HL+P**) heavy bud load with P-spraying (10.7 buds/m$^2$).

For foliar P treatments Fosfonin Flow foliar fertilizer (Sumi Agro Hungary Ltd.; Budapest, Hungary) was applied three times: at the end of the flowering (BBCH 69), at berry touch (BBCH 77), and at the phenophase of ripening (BBCH 83) (2011: 14$^{th}$ of June, 17$^{th}$ of July, 12$^{th}$ of August; 2012: 11$^{th}$ of June, 14$^{th}$ of July, 16$^{th}$ of August), at the dose of 1.5 l/ha. The foliar fertilizer contained $P_2O_5$ (72.7 % w/w), $K_2O$ (8.3 % w/w) and $KH_2PO4$ (81.0 % w/w).

Root samples were taken in spring (at the end of flowering) and autumn (before harvest) of the years 2011 and 2012. At each occasion 16 grapevines were sampled per treatment. Four holes were dug on the four sides of the vines, and the fine roots (diameter: 1 to 2 mm) were collected from the soil depth range of 0 to 50 cm. The roots were cleaned in distilled water and fixed in ethanol (70 %). Before evaluation, the roots were cut into 1 cm long pieces (samples), and then the samples were pooled in one Petri-dish per treatment. Randomly 30 samples per treatment were taken; they were stained with aniline blue (Gianinazzi and Gianinazzi-Pearson, 1991), and investigated under light microscope using the method of Mcgonigle et al. (1990) and Schreiner (2003). If in the sample arbuscules, vesicles, or hyphae were found, we counted it as colonized, so we determined the mycorrhizal colonization (%). Moreover, if the root segment contained arbuscules we calculated arbuscular frequency (%). For 2012 the midday water potential (Ψm) of the vines was measured on 9$^{th}$ and 23$^{th}$ August with a Scholander pressure chamber instrument (SPKM 4000, Skye Instruments Ltd.; Llandrindod Wells, UK) (Scholander et al., 1964). We collected from every second vine one (in all eight) sunlit, mature and undamaged leaves from each treatment (Zsófi et al., 2009). All results were statistically analysed with One-Way Anova and Fisher's exact test using the software SPSS 22.

Table 1: Arbuscular-mycorrhizal colonization of the roots in the years 2011 and 2012

| Treatment | | Mycorrhizal colonization (%) | Arbuscular frequency (%) | Mycorrhizal colonization (%) | Arbuscular frequency (%) |
|---|---|---|---|---|---|
| | | 2011 spring | | 2011 autumn | |
| C | control with low bud load [1)] | 71 ab | 38 a | 50 a | 38 a |
| C+P | control with P-spraying | 61 a | 33 a | 80 b | 34 ab |
| HL | heavy bud load [2)] | 73 b | 20 b | 71 c | 27 b |
| HL+P | heavy bud load [2)] with P-spraying | 76 ab | 28 ab | 50 a | 31 ab |
| | Sign. | * | * | * | * |
| | | 2012 spring | | 2012 autumn | |
| C | control with low bud load [1)] | 49 | 28 a | 62 a | 32 a |
| C+P | control with P-spraying | 48 | 18 b | 67 a | 22 b |
| HL | heavy bud load [2)] | 52 | 8 c | 36 b | 13 c |
| HL+P | heavy bud load [2)] with P-spraying | 50 | 18 b | 43 b | 26 ab |
| | Sign. | n.s. | * | * | * |

[1)] low bud load: 9 buds/vine = 3 buds/m$^2$; [2)] heavy bud load: 32 buds/vine = 10.7 buds/m$^2$

* = $P < 0.05$; n.s. = no significant difference

## RESULTS

In the root samples collected in the spring of 2011 (Table 1) we found significantly higher arbuscular frequency (%) of the control plants (C) compared to both the sprayed control (C+P) and the heavy load treatments (HL and HL+P). In the case of the mycorrhizal colonization (%) the C+P treatment showed the least effect. The results of autumn 2011 show that compared to the control (C), foliar P-spraying (C+P) had a negative effect on the degree of arbuscular colonization. However, in case of the highly loaded blocks (HL and HL+P), the P treatment increased the percentage of arbuscules (Table 1).

For the mycorrhizal colonization we detected the highest rate in the C+P treatment. In 2012 at the first sampling we just found significant differences in case of the arbuscular frequency (%). The results of autumn 2012 revealed that the degree of arbuscular colonization of the control blocks (C) was significantly higher than that of sprayed control (C+P) and heavy-loaded (HL) stocks. Similar to the results of 2011, the arbuscular colonization of the high-loaded and P-treated stocks (HL+P) was higher than that of the untreated high-loaded blocks (HL). The indices of the mycorrhizal colonization (%) were the lowest in the case of the C+P and HL blocks (Table 1). Among the low bud-loaded (3 buds/m2) blocks (C and C+P) the C+P treatment showed a lower water balance ($\psi$m), compared to the unsprayed vines (C) (Table 2).

Table 2: Midday water potential ($\psi_m$) of the vines

| Treatment | | $\psi_m$ (MPa) |
|---|---|---|
| 09.08.2012 | | |
| C | control with low bud load [1)] | -1.24 a |
| C+P | control with P-spraying | -1.36 ab |
| HL | heavy bud load [2)] | -1.46 b |
| HL+P | heavy bud load [2)] with P-spraying | -1.431 b |
| Sign. | | * |
| 23.08.2012 | | |
| C | control with low bud load [1)] | -0.925 a |
| C+P | control with P-spraying | -1.09 b |
| HL | heavy bud load [2)] | -1.1 a |
| HL+P | heavy bud load [2)] with P-spraying | -1.18 ac |
| Sign. | | * |

[1)] low bud load: 9 buds/vine = 3 buds/$m^2$
[2)] heavy bud load: 32 buds/vine = 10.7 buds/$m^2$
* = $P < 0.05$

## DISCUSSION

In 2011 the results showed significant differences between the control (C), sprayed control (C+P), and heavy load treatments (HL and HL+P) (Table 1). The effectiveness of the plant-fungus interaction is not revealed by the mere degree of the mycorrhizal colonization, but it is indicated by the number of arbuscules in the colonized root fragments (Pinkerton et al., 2004; Schreiner, 2005). The high bud load treatments (HL and HL+P) resulted in a lower level of arbuscular colonization than the low bud load treatments (C and C+P). The reason is most probably the increased carbohydrate demand of the plants, due to the increased canopy and number of bunches. This may have resulted in a decrease in the carbohydrate amount available for the fungal partner. An increased P supply can lead to the decrease of mycorrhizal colonization (Gebbing et al., 1977; Lindermann and Davis, 2001), however, Schreiner (2010) found that foliar P sprays may have little effect on the degree of the mycorrhizal colonization of the grapevine. In our experiment, similarly to the observations of Schreiner and Lindermann (2005), foliar P sprays resulted in a reduced level of the colonization in case of the control bud load (C+P). Our result shows that in case of the high bud loaded stocks the foliar sprays had positive effect on the percentage of arbuscules. Most probably the nutrient content of the sprayed fertilizer compensated the high nutrient demand of the overloaded vines. It could have affected beneficially the plants' physiological parameters, like the intensity of carbohydrate synthesis. Drought reduces the photosynthetic activity of both the berries and the leaves (Kounduras et al., 2008; Eibach and Alleweldt, 1984). In case of drought, the role of mycorrhizal fungi is even more important compared to such situations when enough water is available (Dell'amico et al., 2002). A higher colonization rate is beneficial for the water balance of plants under drought conditions (Possingham and Obbink, 1971; Karagiannidis et al., 1995; Ryan and Graham, 2002; Schreiner, 2005). We found that the heavy loaded blocks (HL and HL+P) had a lower water balance ($\psi$m) than the control (C) (Table 2).

However, the unfavourable conditions (overloaded stocks, not trimmed canopy) could have been the main reason for the lower water balance, but the decreased level of the arbuscules could have contributed to this phenomenon (Baumgartner, 2003; Cheng and Baumgartner, 2005; Schreiner, 2005).

## CONCLUSION

The here presented results are derived from a field trial with a sample area representing the soil type and the climatical conditions of the largest wine region of Hungary, the Kunság wine region. By studying the effect of extreme bud load and canopy management treatment our aim was to investigate how the mycorrhizal interaction of the vine responds to the changing leaf area/ photosynthetic ability combined with P foliar fertilization. Under field conditions we found that heavy bud load (10.7 buds/$m^2$) resulted in a lower colonization, which might be explained with the fewer carbohydrates available for the fungi. Furthermore, we recorded that foliar P spraying can lead to the decrease of arbuscular frequency and midday water potential ($\psi$m) in case of physiologically optimal bud loaded grapevines with controlled (trimmed) canopy, but the P treatment increased the arbuscule richness of the overloaded and non-trimmed stocks.

ACKNOWLEDGEMENT

Our work was supported by TÁMOP grants (TÁMOP-4.2.2/B-10/1-2010-0023-, TÁMOP- 4.2.1.B-09/1/KMR).

## REFERENCES

Baumgartner, K. 2003: Why and How. Encouraging beneficial AM fungi in vineyard soil. Practical Winery and Vineyard 14: 57-60

Bhardwaj, S., Dudeja, S.S., Khurana, A.L. 1997: Distribution of vesicular-arbuscular mycorrhizal fungi in natural ecosystems. Folia Microbioogica. 42 (6): 589-594

Cheng, X. and Baumgartner, K. 2005: Overlap of grapevine and cover crop roots enhances interactions among grapevines, cover crops, and arbuscular mycorrhizal fungi. In: Christensen, P. and Smart, D. (eds.): Proceedings of the soil environment and vine mineral symposium, 29-30 June 2004, San Diego, CA, pp. 171-174. – Davis (CA): Am. Soc. Enol. Vitic., 2005

Dell'Amico, J., Torrecillas, A., Rodriguez, P., Morte, A. and Sanchez-Blanco, M.J. 2002: Responses of tomato plants associated with the arbuscular mycorrhizal fungus Glomus clarum during drought and recovery. J. Agric. Sci. 138: 387-393

Eibach, R. und Alleweldt, G. 1984: Einfluß der Wasserversorgung auf Wachstum, Gaswechsel und Substanzproduktion traubentragender Reben. III. Die Substanzproduktion. Vitis 23: 11-20

Eissenstat, D.M. 1992: Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15(6/7): 763-782

Francis, R. and Read, D.J. 1984: Direct transfer of carbon between plants connected by vesicular arbuscular mycorrhizal mycelium. Nature 307: 53-56

Gebbing, H., Schwab, A. und Alleweldt, G. 1977: Mikorrhiza der Rebe. Vitis 16: 279-285

Gianinazzi, S. and Gianinazzi-Pearson, V. (1991): Cytology, histochemistry and immunocytochemistry as tools for studying structure and function in endomycorrhiza – In: Norris, J.R., Read, D.J. and Varma, A.K. (eds.): Techniques for mycorrhizal research. vol. 23, pp. 569-601. San Diego: Acad. Press, 1991

Karagiannidis, N., Nikolaou, N. und Mattheou, A. 1995: Wirkung dreier VA-Mykorrhizapilze auf Ertrag und Nährstoffaufnahme von drei Unterlagen und einer Tafeltraubensorte. Vitis 34: 85-89

Koide, R.T., Schreiner, R.P. 1992: Regulation of the vesicular-arbuscular mycorrhizal symbiosis, Ann. Rev. Plant Physiol. Plant Mol. Biol. 43: 557-581

Kounduras, S., Tsialtas, T., Zioziou, E. and Nikolaou, N. 2008: Rootstock effects on the adaptive strategies of grapevine (Vitis vinifera L. cv. Cabernet Sauvignon) under contrasting water status: Leaf physiological and structural responses. Agric. Ecosyst. Environm. 128: 86-96

Linderman, R.G. and Davis, E.A. 2001: Comparative response of selected grapevine rootstocks and cultivars to inoculation with different mycorrhizal fungi. Am. J. Enol. Vitic. 52: 8-11

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. and Swan, J.A. 1990: A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115: 495-501

Pinkerton, J.N., Schreiner, R.P., Ivors, K.L. and Vasconcelos, M.C. 2004: Effects of *Mesocriconema xenoplax* on *Vitis vinifera* and associated mycorrhizal fungi. J. Nematol. 36(3): 193-201

Possingham, J.V. and Obbink, J.G. 1971: Endotrophic mycorrhiza and the nutrition of grape vines. Vitis 10: 120-130

Ryan, M.H. and Graham, J.H. 2002: Is there a role for arbuscular mycorrhizal fungi in production agriculture. Plant and Soil 244: 263-271

Scholander, P.F., Hammel, H.T., Hemmingsen, E.A. and Bradstreet, E.D. 1964: Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. National Acad. Sci. USA. 52: 119-125

Schreiner, R.P. 2003: Mycorrhizal colonization of grapevine rootstocks under field conditions. Am. J. Enol. Vitic. 54(3): 143-149

Schreiner, R.P. 2005: Mycorrhizas and mineral acquisition in grapevines. In: Christensen, P. and Smart, D. (eds.): Proceedings of the soil environment and vine mineral symposium, 29-30 June 2004, San Diego, CA, pp. 49-60. – Davis (CA): Am. Soc. Enol. Vitic., 2005

Schreiner, R.P. 2010: Foliar sprays containing phosphorus (P) have minimal impact on 'pinot noir' growth and p status, mycorrhizal colonization, and fruit quality. HortScience 45(5): 815-821

Schreiner, R.P. and Linderman, R.G. 2005: Mycorrhizal colonization in dryland vineyards of the Willamette Valley, Oregon. Small Fruits Rev. 4(3): 41-55

Selosse, M.A., Richard, F., He, X. and Simard, S.W. 2006: Mycorrhizal networks: des liaisons dangereuses? Trends Ecol. Evol. 21(11): 621-628

Sieverding, E. 1991: Vesicular-Arbsucular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn. 371: 52-54

Smith, S.E. and Read, D.J. 1997: Mycorrhizal symbiosis, 2nd ed. – London: Acad. Press, 1997

Stahl, E. 1900: Der Sinn der Mycorrhizenbildung. Jahrb. Wissenschaftliche Botanik 34: 539-668

Zsófi, Zs., Gál, L., Szilágyi, Z., Szűcs, E., Marschall, M., Nagy, Z., and Bálo, B. 2009: Use of stomatal conductance and pre-dawn water potential to classify terroir for the grape variety Kékfrankos. Austr. J. Grape Wine Res. 15: 36-47

Received August, 26th, 2015