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# Growth and Root Colonization of Grapevines Inoculated with Different Mycorrhizal Endophytes

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***Abstract.*** **Grapevine seedlings (*Vitis berlandieri* Planch. × *Vitis riparia* Michx., 420 A) were inoculated with the mycorrhizal fungi *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerd., *G. fasciculatum* (Thaxter *sensu* Gerd.) Gerd. & Trappe, *G. monosporum* Gerd. & Trappe, *G. occultum* Walker, and *Glomus* sp. E3 in three sterile soils. *G. monosporum* and *G. occultum* were the most infective endophytes. *G. monosporum, G. occultum*, and *Glomus* sp. E3 increased shoot dry weight and, to a lesser extent, root length. Some of the efficient symbionts in pot culture were not naturally present in the experimental soils. Significant growth responses could be observed also with endophytes inoculated at low concentration.**

Vesicular–arbuscular mycorrhizal (VAM) fungi are commonly present in vineyard soils throughout the world and infect roots of many species and hybrids of the genus *Vitis* L. (5, 10). These fungi can enhance grapevine growth by taking up nutrients, primarily P, and translocating them to the host plant (5, 7).

The capacity of different VAM fungal strains to increase plant growth differs widely and may depend on soil characters (9). The choice of efficient endophytes is of primary importance for inoculation programs, especially in unsterile soils. In such soils, the efficiency of the introduced endophyte must be greater than that of the indigenous ones if the inoculation is to be useful.

No results have been reported, to the best of our knowledge, about the comparative efficiency of VAM fungi in stimulating grapevine growth. This paper reports the effects of the inoculation with different VAM fungal strains on grapevine growth in three sterilized soils. Some of the strains were isolated in viticultural soils and some were of other origins.

Seeds of the rootstock 420 A were surface-sterilized with $AgNO_3$ (two 1-min rinses followed by 10 washings in sterile water) and germinated in sterile sand. Mixed inoculum (spores, mycelium, and infected roots) was obtained from 6-month-old pot cultures, grown with *Trifolium pratense* L., of the following VAM endophytes [specimens deposited at the Herbarium Cryptogamicum (HC) of the Dept. of Plant Biology, Turin Univ.]: *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerd. (HC/Fungi E04), *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerd. & Trappe (HC/Fungi E08), *G monosporum* Gerd. & Trappe (HC/Fungi E09), *G. occultum* Walker (HC/Fungi E07), and *Glomus* sp. E3 (HC/Fungi E10). Inoculum potentials per unit dry soil weight for each endophyte were assessed with the most probable number (MPN) method (12), using five replicates at five inoculum dilutions and *T. pratense* as the indicator plant.

Rootstock seedlings were transplanted 15 days after emergence in 500-$cm^3$ plastic pots filled with a steam-sterilized mix of 1 soil : 1 silica sand (v/v). At the same time, 3 g of inoculum was placed beneath the roots of each plant. The following vineyard soils were used (pH and P content of the mix assessed after sterilization): a clay loam from Albugnano, with a pH of 7.5 and 12 ppm Olsen P (Typic Dystrochrept); a sandy loam from Grugliasco, with a pH of 6.9 and 7 ppm Olsen P (Dystic Eutrochrept); a sandy loam from Roasio, with a pH of 5.3 and 26 ppm P (Typic Hapludalf). Among the endophytes tested, *G. monosporum* and *G. occultum* were naturally present in the Albugnano soil and *G. occultum* in the Grugliasco soil (13).

Plants were grown in a glasshouse with photosynthetic photon flux (PPF) reaching a maximum of 700 $\mu mol \cdot s^{-1} \cdot m^{-2}$, a photoperiod ranging from 11 (transplanting date) to 14 hr (last harvest date), 22° (day)/18°C (night) air temperature, and 70% RH. Pots were kept at field capacity and each was given 10 $cm^3$ per week of Hoagland solution lacking P. They were harvested at 1-month intervals, for a total of three harvests. At each harvest, dry shoot weight, root length, and percent colonization, using the gridline intersect method (6), were assessed. For each soil type, five single plant replicates were used per treatment, arranged in a completely randomized design. Percent colonization data were submitted to angular transformation. For each soil type, data were analyzed by analysis of variance and averages separated by Duncan's multiple range test.

The inoculum potential was largest with *G. monosporum* in the Grugliasco and Albugnano soils. No large differences among endophytes could be observed in the Roasio soil, and E3 had the least MPN values in all soils (Table 1).

In the Grugliasco soil, only *G. monosporum* colonized roots extensively after 1 month of growth; the other endophytes showed a lagging development up to the second harvest and, at the end of the experiment, *G. monosporum* and *G. occultum* had the largest percentage of infection (Table 2). In the Albugnano soil, a similar pattern of fungal development was observed, but *G. occultum* was more infective at the first two harvests than in the Grugliasco soil. In both soils, E3 propagules remained dormant for an extended time; only after 3 months of growth could root colonization be observed. In the Roasio soil, root colonization was very low for all endophytes at the first harvest. By the end of the experiment, colonization reached a similar level for all fungi, except for E3, the growth of which remained quite low.

No significant differences in shoot or root growth were observed until the last harvest. Then, an increased dry weight and root length were observed after inoculation with *G. monosporum, G. occultum*, and E3 in the Grugliasco soil. The effect of inoculation with E3 was larger than that of the other two efficient endophytes (Table 3). In the Albugnano soil, *G. monosporum* and E3 enhanced dry shoot weight, and the former also en-

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Table 1. Inoculum potential of the endophytes tested in the experimental soils as expressed by the mean propagule number (MPN).

| Endophytes | Soil | | |
|---|---|---|---|
| | Gruglfasco | Albugnano | Roasio |
| *Glomus caledonium* | 4.64 | 1.59 | 3.25 |
| *Glomus fasciculatum* | 1.59 | 3.25 | 1.11 |
| *Glomus monosporum* | 20.80 | 6.37 | 3.25 |
| *Glomus occultum* | 4.64 | 1.59 | 1.11 |
| *Glomus* sp. E3 | 0.27 | 0.48 | 0.48 |

Table 2. Percent root infection at the three harvests following innoculation with the endophytes tested in the experimental soils.

| | Root infection (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Soil type | | | | | | | | |
| | Grugliasco | | | Albugnano | | | Roasio | | |
| | Harvest no. | | | Harvest no. | | | Harvest no. | | |
| Endophyte | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| *Glomus caledonium* | 0.0 b[z] | 19.2 a | 14.7 b | 5.9 b | 2.0 c | 21.0 b | 0.0 a | 0.0 b | 43.0 a |
| *Glomus fasciculatum* | 0.0 b | 2.3 b | 5.6 b | 3.1 b | 6.5 c | 22.8 ab | 3.9 a | 4.2 b | 32.4 a |
| *Glomus monosporum* | 23.4 a | 24.3 a | 50.6 ab | 17.2 a | 41.2 a | 58.9 a | 5.8 a | 31.4 a | 33.0 a |
| *Glomus occultum* | 2.5 b | 2.7 b | 53.1 a | 10.8 ab | 26.0 b | 44.2 a | 0.0 a | 12.7 ab | 29.3 ab |
| *Glomus* sp. E3 | 0.0 b | 0.0 b | 17.0 b | 0.0 b | 0.0 c | 37.2 ab | 0.0 a | 9.2 b | 1.1 b |

[z]Means in each column separated by Duncan's multiple range test, 5% level.

Table 3. Dry shoot weight and root length 3 months after inoculation with different endophytes in the experimental soils.

| | Soil type | | | | | |
|---|---|---|---|---|---|---|
| | Grugliasco | | Albugnano | | Roasio | |
| Endophyte | Dry wt (mg) | Root length (cm) | Dry wt (mg) | Root length (cm) | Dry wt (mg) | Root length (cm) |
| *Glomus caledonium* | 31.2 bc (±10.4)[z] | 10.6 b (±2.5) | 26.2 b (± 2.4) | 15.0 b (± 1.3) | 36.2 cd (± 4.9) | 12.5 a (± 3.0) |
| *Glomus fasciculatum* | 15.2 c (± 1.7) | 12.8 b (±1.9) | 28.2 b (± 3.3) | 31.7 b (± 5.3) | 42.2 bc (± 5.0) | 11.7 a (± 2.6) |
| *Glomus monosporum* | 39.0 b (±11.1) | 17.8 a (±6.3) | 127.2 a (±35.4) | 62.7 a (±18.6) | 26.2 d (± 2.7) | 11.1 a (± 2.4) |
| *Glomus occultum* | 41.2 b (± 4.5) | 19.1 a (±3.3) | 36.4 b (± 9.7) | 24.1 b (± 5.5) | 60.0 a (±15.5) | 31.9 a (±14.3) |
| *Glomus* sp. E3 | 62.2 a (±13.0) | 18.3 a (±4.1) | 74.4 a (±14.9) | 31.5 b (±10.0) | 53.2 ab (± 7.7) | 13.7 a (± 1.8) |
| Uninoculated | 22.0 c (± 4.3) | 12.8 b (±1.8) | 27.4 b (± 2.0) | 17.1 b (± 4.6) | 39.4 bcd (± 5.2) | 11.5 a (± 0.9) |

[z]Means (±SE) in each column separated by Duncan's multiple range test, 5% level.

hanced root length. In the Roasio soil, *G. occultum* increased dry shoot weight but not root length.

When inoculated on grapevine plants in different sterilized soils, VAM endophytes had different effects on plant growth. Some of them increased it (*G. monosporum, G. occultum, Glomus* sp. E3), whereas the others had no effect. Thus, grapevine growth enhancement was dependent on the presence of particular mycorrhizal strains, as has been observed on other crops (2). Although all the efficient fungal strains enhanced dry shoot weight, not all of them increased root length. It is well-known, however, that mycorrhizal plants have a lower root : shoot ratio than non-mycorrhizal ones (8); this may explain the lack of correspondence between shoot and root growth enhancement.

The VAM strains indigenous to the experimental soils often increased host growth, indicating their suitability to the plant and soil type. Among the species not naturally present in the experimental soils, *Glomus* sp. E3 increased host growth by as much or more than the indigenous species.

The strain E3 previously was reported to increase growth of barley, clover, onion, and strawberry (3, 9, 11, 14); it enhanced host growth in a wide range of soil pH (9) and P concentrations (14). The question arises why this endophyte, very active in pot culture, was not present in the vineyard soils. A possible explanation is that the growth of an endophyte in the roots may be limited by that of other fungal species (1), so E3 may be "crowded out" by other endophytes in natural conditions. Further, growth enhancement caused by E3 has been reported mostly from plants in pots, where factors such as soil temperature, water availability, and soil volume exploitable are very different from those in natural soil. Thus, E3 may be better adapted to pot than to field conditions.

VAM inoculum concentration is a key factor for growth enhancement, because low concentration may mean slow root colonization (4). In our experiment, endophytes inoculated at low concentration showed a lagging infection for 1 or 2 months, although the results after 3 months were largely independent of this factor. Growth responses were obtained from low concentration inoculations of E3 in the Grugliasco and Albugnano soils and *G. occultum* in the Roasio soil. Fungal species with a large growth enhancement : inoculum concentration ratio are good candidates for routine inoculation, as VAM inoculum production is currently cumbersome and costly.

The results of this study suggest that some VAM endophytes are more effective than others in enhancing grapevine growth, and that fungal species not naturally present in the soil can be efficient in increasing plant growth in pots. The behavior of each species may suggest an inoculation strategy for vines cultivated in pots, e.g., during propagation, which, however, cannot be extrapolated easily to the vineyard or vine nursery, where plant roots and VAM mycelium are exposed to different environmental conditions.

### Literature Cited


1. Abbott, L.K. and A.D. Robson. 1984. Colonization of the root system of subterrancan clover by three species of vesicular-arbuscular mycorrhizal fungi. New Phytol. 96:275–281.
2. Abbott, L.K. and A.D. Robson. 1984. The effect of VA mycorrhizae on plant growth, p. 113–130. In: C.L. Powell and D.J. Bagyaraj (eds.). VA mycorrhiza. CRC Press, Boca Raton, Fla.
3. Clarke C. and B. Mosse. 1981. Plant growth responses to vesicular-arbuscular mycorrhiza: XII. Field inoculation responses of barley at two soil P levels. New Phytol. 87:695–703.
4. Daft M.J. and T.H. Nicolson. 1972. Effect of *Endogone* mycorrhiza on plant growth: IV. Quantitative relationships between the growth of the host and the development of the endophyte in tomato and maize. New Phytol. 71:287–295.
5. Gebbing H., A. Schwab, and G. Alleweldt. 1977. Mykorrhiza der Rebe. Vitis 16:279–285.
6. Ciovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.
7. Hayman, D.S. 1982. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can. J. Bot. 61:944–963.
8. Hayman D.S. and B. Mosse. 1971. Plant growth responses to vesicular-arbuscular mycorrhiza: I. Growth of *Endogone*-inoculated plants in phosphate-deficient soils. New Phytol. 70:19–27.
9. Hayman, D.S. and M. Tavares. 1985. Plant growth responses to vesicular-arbuscular mycorrhiza: XV. Influence of soil pH on the symbiotic efficiency of different endophytes. New Phytol. 100:367–378.
10. Menge, J.A., D.J. Raski, L.A. Lider, E.L.V. Johnson, N.O. Jones, J.J. Kissler, and C.L. Hemstreet. 1983. Interactions between mycorrhizal fungi, soil fumigation and growth of grapes in California. Amer. J. Enol. Vitic. 34:117–121.
11. Mosse B. 1972. The influence of soil type and *Endogone* strain on the growth of mycorrhizal plants in phosphate deficient soils. Rev. Ecol. Biol. Sol. 9:527–537.
12. Porter W.M. 1979. The "Most probable number" method for enumerating propagules of vesicular-arbuscular fungi in soil. Austral. J. Soil Res. 17:515–519.
13. Schubert, A. and M.C. Cravero. 1985. Occurrence and infectivity of vesicular-arbuscular mycorrhizal fungi in north-western Italy vineyards. Vitis 24:129–138.
14. Schubert, A. and D.S. Hayman. 1986. Plant growth responses to vesicular-arbuscular mycorrhiza: XVI. Effectiveness of different endophytes at different levels of soil phosphate. New Phytol. 103:79–90.