# Occurrence and richness of arbuscular mycorrizal fungi in vineyards with grapevine decline and dieback symptoms

Aelton dos Santos Bezerra[1] Marcelo Betancur-Agudelo[1] Edenilson Meyer[1]
Karl Kemmelmeier[2] Sidney Luiz Stürmer[2] Cláudio Roberto Fonsêca Sousa Soares[3]
Paulo Emilio Lovato[1]*

[1]Departamento de Engenharia Rural, Universidade Federal de Santa Catarina (UFSC), 88040-900, Florianópolis, SC, Brasil. E-mail: paulo.lovato@ufsc.br.
*Corresponding author.
[2]Departamento de Ciências Naturais, Universidade Regional de Blumenau (FURB), Blumenau, SC, Brasil.
[3]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brasil.

**ABSTRACT**: *This research identified arbuscular mycorrhizal fungi (AMF) in rhizosphere soil of grapevines with Grapevine Death and Decline symptoms (GDD) or asymptomatic healthy (H) plants, and characterized the relationship of AMF communities with soil chemical attributes. The AMF spore number ranged from 287 to 432 spores 50 $cm^{-3}$ in soil with GDD plants, and from 357 to 464 spores 50 $cm^{-3}$ in H plants, with no differences among vineyards or between GDD and H plants within each vineyard. We detected 42 species and 17 genera, and most taxa belonged to Acaulosporaceae or Glomeraceae. Claroideoglomus etunicatum, Funneliformis mosseae, and Archaeospora trappei were the most frequent species in all vineyards. Soil chemical attributes were not determinant for the occurrence of most fungal species; although, Entrophospora infrequens, Diversispora sp1 and Diversispora sp2 were associated with a vineyard having high soil copper. Vineyards harbor highly diverse AMF communities, which are determined by location.*
**Key words**: *community structure, glomeromycota, soil factors, Vitis.*

## Ocorrência e riqueza de fungos micorrízicos arbusculares em vinhedos com sintomas de declínio e morte da videira

**RESUMO**: *Este trabalho teve como objetivo identificar fungos micorrízicos arbusculares (FMA) em solo rizosférico de videiras com sintomas de declínio e morte da videira (D) e em plantas saudáveis (S), e caracterizar a relação das comunidades de FMA com atributos químicos do solo. O número de esporos de FMA variou de 287 a 432 esporos 50 $cm^{-3}$ em solo em plantas D, e de 357 a 464 esporos 50 $cm^{-3}$ em plantas S, sem diferenças entre vinhedos ou entre plantas D e S dentro de cada vinhedo. Detectamos 42 espécies e 17 gêneros, sendo que a maioria dos táxons pertencia a Acaulosporaceae ou Glomeraceae. Claroideoglomus etunicatum, Funneliformis mosseae e Archaeospora trappei foram as espécies mais frequentes em todos os vinhedos. Os atributos químicos do solo não foram determinantes para a ocorrência da maioria das espécies de fungos, embora Entrophospora infrequens, Diversispora sp1 e Diversispora sp2 estivessem associadas a um vinhedo com alto teor de cobre do solo. Os vinhedos abrigam comunidades FMA altamente diversificadas, que são determinadas pela localização.*
**Palavras-chave**: *estrutura da comunidade, glomeromycota, fatores do solo, Vitis.*

## INTRODUCTION

Expansion and renewal of vineyards are constrained by factors such as relief, land costs, and pests and diseases, which can affect the emergence of grapevine decline and dieback (GDD) (BASSO et al., 2017). Different pathogens may cause early GDD (VALENCIA et al., 2015), which affect trunk, leaves, or fruits (BERTSCH et al., 2012). Plants affected by GDD have low vigor, internerval leaf chlorosis, and weak, uneven branches (MENEZES-NETTO et al., 2016). The plant vegetative stage affects the symptoms since grapevines are more susceptible to onset of GDD at the beginning of the fruiting stage

(AL-MAWAALI et al., 2013). As a result, grapevines are deficient in potassium, magnesium, phosphorus, sulfur, and often have high leaf copper (TERRA et al., 2003). GDD results in low plant stand, and plants die before the investment in vineyard installation is recovered. Research on this subject in Brazil is scant and mostly focused on pathogenic fungi in the country's southern region (GARRIDO et al., 2004; DAMBROS et al., 2016; MENEZES-NETTO et al., 2016). Among the procedures proposed to solve GDD problems, and the association between arbuscular mycorrhizal fungi (AMF) and grapevines is a possibility.

Mycorrhizas have a fundamental role in plant survival, growth, and development (SMITH

& READ, 2008). Arbuscular mycorrhizal fungi (AMF) are obligate symbiotrophs, associating with around 72% of plant species worldwide (BRUNDETT & TEDERSOO, 2018). AMF may reduce GDD occurrence and enhance plant growth (TROUVELOT et al., 2015). GDD may also affect AMF root colonization, suggesting a complex relationship of these symbiotic fungi with other GDD causes (SCHREINER, 2003; WASCHKIES et al., 1994). Mycorrhizas promote tolerance or resistance to pathogenic fungi through improved plant nutrition, compensation of root damage, competition for plant photosynthates or root colonization points, and activation of plant defense mechanisms (AZCON-AGUILAR & BAREA, 1996).

Measures to mitigate losses caused by GDD include use of resistant varieties or grafting. However, such practices are not enough to solve the problems. One possibility is inoculation with AMF isolated from vineyards, and a first step to develop this approach is to inventory the AMF communities associated with grapevines in field conditions (SILVEIRA, 2006). This research is a first step to identify AMF species occurring in areas with GDD to isolate them in single cultures for further testing under controlled conditions. We characterized AMF communities present in soils of grapevines with and without symptoms of GDD and investigate the effect of soil attributes on those AMF communities.

## MATERIALS AND METHODS

### Field sampling

Soil samples were collected in four vineyards, with different ages and management histories, in the Rio do Peixe Valley, state of Santa Catarina, Brazil, a region responsible for most of the state grapevine production (BACK et al. 2013).

Vineyards were located in the municipalities of Videira (V1 and V4), Pinheiro Preto (V2), and Tangará (V3) (Table 1). The region has two types of climate: humid mesothermal subtropical with hot summers (Cfa), and humid mesothermal subtropical with mild summers (Cfb), with annual rainfall ranging from 1.300 mm to 1.900 mm (PANDOLFO et al., 2002).

All vineyards have conventional management system (not organic), using black oats as winter cover crops. The soil was sampled according to the Tropical Soil Biology and Fertility methodology (MOREIRA et al., 2008). In each vineyard, 14 samples were collected, seven around plants with GDD symptoms (GDD), and seven from asymptomatic, presumably healthy (H) plants. The symptoms of decline observed in plants were physiological decay, apparently caused by fungi, viruses, and other agents. At each point with an H or GDD plant, a central plant was georeferenced, and soil subsample (0-20 cm deep) was collected from six points around each plant: three sub-samples were collected at a 3.0-m distance from the central plant, and three other sub-samples collected 6.0 m away from the central plant. Those six sub-samples from each plant were pooled into a composite sample with approximately 1.0 kg of soil, kept at 4 °C until processing. In total, 56 samples were collected in the vineyards, 28 of healthy plants, and 28 of plants that presented GDD symptoms.

### Soil fertility analysis

Soil fertility analyses followed the methods of EMBRAPA (1997). Active acidity was measured as pH in water 1:1 (v:v). Potassium (K), copper (Cu), and phosphorus (P) were extracted with Mehlich I solution and P was measured in UV-visible spectrometry in HCl 0.87 M and $(NH_4)6Mo_7O_{24}$ $4H_2O$ solutions, and K was measured by flame photometry. Exchangeable aluminum (Al), calcium

Table 1 - General information on four vineyards in the Rio do Peixe Valley, Santa Catarina,, Brazil.

| | -----------------------------------------------Municipality / Vineyards Identification----------------------------------------------- | | | |
|---|---|---|---|---|
| | Videira (V1) | Pinheiro Preto (V2) | Tangará (V3) | Videira (V4) |
| Rootstock/graft | VR 043-43/Bordô | VR 043-43/ Isabel Precoce | Paulsen-1103/ Isabel Precoce | Paulsen1103/Chardonnay |
| Age (years) | >8 | >8 | >30 | >10 |
| Soil classification | Nitossol | Nitossol | Nitossol | Nitossol |
| Area (ha) | 0.6 | 0.5 | 2.0 | 0.7 |
| Altitude (m) | 719 | 709 | 641 | 719 |
| Coordinates | S 27°03'53.3" W 51°09'54.8" | S 27°04'03.1" W51°10'42.9" | S 27°05'30.2" W51°12'59.2" | S 27°01'57.1" W51°08'02.7" |

(Ca), and magnesium (Mg) were extracted with KCl 1 mol L$^{-1}$ and quantified by atomic absorption spectrophotometry. Potential acidity (H$^+$+Al$^{3+}$) was determined with calcium acetate buffered to pH 7.0 and determined volumetrically with NaOH. Resin-extracted phosphorus (Pr) was extracted with an anion exchange resin, colored with ammonium molybdate, and PC reducing solution (1-amino-2naphthol-4sulfonic acid, sodium sulfite, and sodium metabisulfite), and measured by UV-visible spectrometry (TEDESCO et al., 1995).

*Morphological identification of AMF*

A 50-cm³ volume of soil from each field sample was used to extract AMF spores by wet sieving (GERDEMANN & NICOLSON, 1963), followed by centrifugation in a 60% sucrose solution (JENKINS, 1964). The supernatant was poured into stacked sieves of 180, 90, and 45 μm. Spores of each size class were placed on a microscope slide using polyvinyl alcohol-lactic acid-glycerol (PVLG) and PVLG + Melzer reagent as mounting media. Taxonomic identification of species was based on morphological descriptions available at the INVAM website (International Culture Collection of Arbuscular Mycorrhizal Fungi – http://invam.wvu.edu) and Blaszkowski (2012). We followed the classification proposed by REDECKER et al. (2013). AMF species identified in vineyards were registered in the National Genetic Resource Data Base (SisGEen - Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado), under number A9FA3BC.

*Trap cultures*

An aliquot of about 400g of each sample was used to establish trap cultures, according to MORTON et al. (1993). Each soil sample was placed in a 1.5 dm³ pot containing 40% soil collected in the field, 30% vermiculite, and 30% sterile sand, with *Urochloa decumbens* as the host plant. One month after seeding, plants were pruned to cause physiological disturbance and increase tillering. Plants were irrigated three times a week during the first two months, and after that establishment period, submitted to five-day periods without watering to stimulate AMF root colonization and sporulation. After five months, irrigation was suspended, and after another month, spores were extracted, mounted on slides, and identified as previously described.

*Analysis of AMF species richness*

Species richness was calculated as the number of AMF species identified from field samples and trap culture. The frequency of occurrence (F) was calculated by the equation F = (Ji/K)*100, where F is the frequency of species I, Ji is the number of samples in which the species was detected, and K is the total number of samples. Species frequency was classified as dominant (85% ≤ FO ≤ 100%), very common (50% ≤ FO < 85%), common (30% ≤ FO < 50%) and rare (FO < 30%) according to ZHANG et al. (2004).

*Statistical analyses*

Similarity among AMF communities from the four vineyards was estimated by the non-metric multidimensional scale (NMDS) based on Jaccard's index, as described in CLARKE (1993). The effects of the vineyard soil chemical attributes on AMF communities were analyzed by Canonical Redundancy Analysis – RDA (BORCARDT et al., 2011). Those analyses were performed using the Vegan package in the Program R studio 3.1.4 (OKSANEN et al., 2013).

**RESULTS**

Soil attributes varied among sites. Soil pH ranged from 5.1 to 6.5, and the V3 and V4 vineyards had values of 5.7 and 5.1 (Table 2), respectively, below the desirable value of 6.0 (Comissão de Química e Fertilidade do Solo, 2016). According to those regional criteria, exchangeable K was high or very high. Ca, Mg, Cu, organic matter, CEC, and Melich-extracted P were high for all areas, while resin-extracted P ranged from high to very high. The highest soil Cu concentration occurred in V3, the oldest vineyard.

Mean number of AMF spores ranged from 287 to 432 spores per 50 cm³ in soils with GDD plants and between 357 and 464 spores per 50 cm³ in asymptomatic plants (Figure 1). No differences in spore number were detected among vineyards nor between asymptomatic and GDD plants.

Morphological characterization of AMF spores yielded 42 species and 17 genera belonging to Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Gigasporaceae, Glomeraceae, and Paraglomeraceae (Table 3). *Entrophospora infrequens* was detected but not allocated to any family, following REDECKER et al. (2013). The highest number of AMF species occurred V1 and V4 vineyards, with 31 and 30 species, while V2 and V3 had the lowest numbers, with 26 and 28 species. The families with the highest species richness (66% of all morphotypes recovered) were Glomeraceae and Acaulosporaceae, represented

Table 2 - Soil chemical attributes in four vineyards in the Rio do Peixe Valley, Santa Catarina, Brazil.

| | V1 | V2 | V3 | V4 |
|---|---|---|---|---|
| pH in $H_2O$ | 5.7 | 6.1 | 6.5 | 5.1 |
| H+Al (mmolc $dm^{-3}$) | 55 | 42 | 28 | 69 |
| K(mg $dm^{-3}$) | 427 | 486 | 185 | 158 |
| P Melich (mg $dm^{-3}$) | 34 | 154 | 90 | 52 |
| P resin (mg $dm^{-3}$) | 38 | 130 | 53 | 46 |
| Al (mmol$_c$ $dm^{-3}$) | 0.31 | 0.04 | 0.00 | 1.10 |
| Ca (mmol$_c$ $dm^{-3}$) | 86 | 172 | 155 | 85 |
| Mg (mmol$_c$ $dm^{-3}$) | 37 | 45 | 41 | 30 |
| Cu Melich (mg $dm^{-3}$) | 9.0 | 23 | 65 | 10 |
| CEC (mmol$_c$ $dm^{-3}$) | 189 | 272 | 229 | 188 |
| Clay (g $kg^{-1}$) | 490 | 360 | 470 | 650 |
| Organic matter (g $kg^{-1}$) | 34 | 31 | 28 | 36 |

by 20 and 8 species. We detected 24 morphotypes in both trap cultures and field samples, and 15 morphotypes were reported only in field samples. Morphotypes detected exclusively in trap cultures were *Glomus* sp3 and *Glomus* sp4 from V4 and *Sclerocystis* sp1 from V1. The most frequent species were *Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Archaeospora trappei*, with global frequencies of 93, 89, and 84%, respectively. Those species were dominant or very common in soil

from both GDD- and H-plants. Thirty-seven species (88% of the total richness) were shared by soils with GDD and H-plants (Figure 2). Species associated exclusively with H-plants were *Acaulospora alpina*, *A. foveata*, *Rhizophagus fasciculatus*, and *Sclerocystis* sp1, while the only exclusive species in GDD-plants was *Oehlia diaphana* (Table 3).

The principal coordinate analysis (PCoA) indicated that vineyards separate AMF communities and that occurrence of AMF species within each
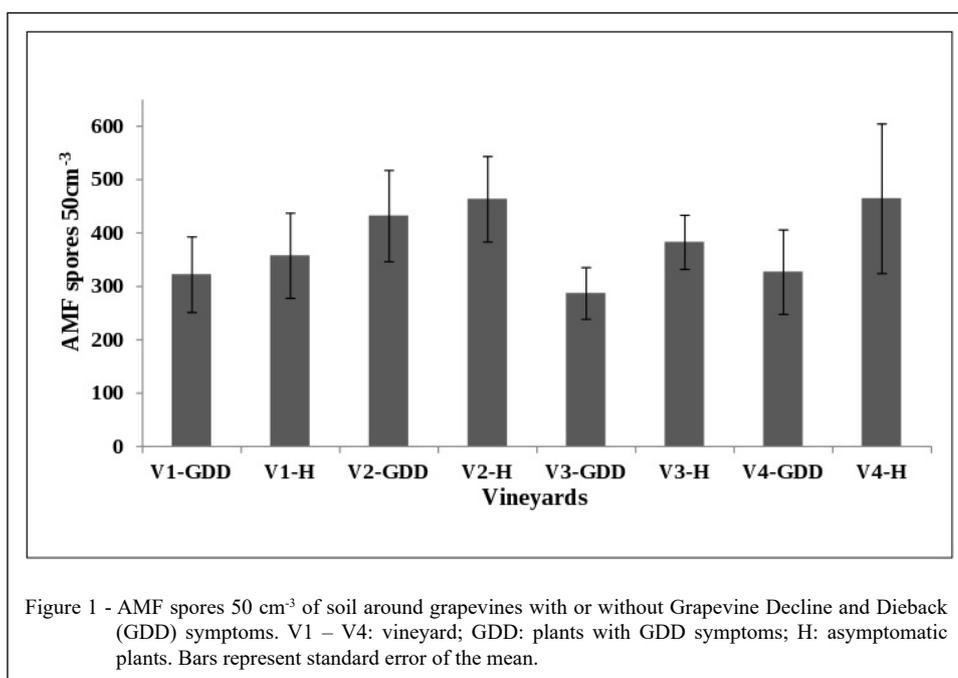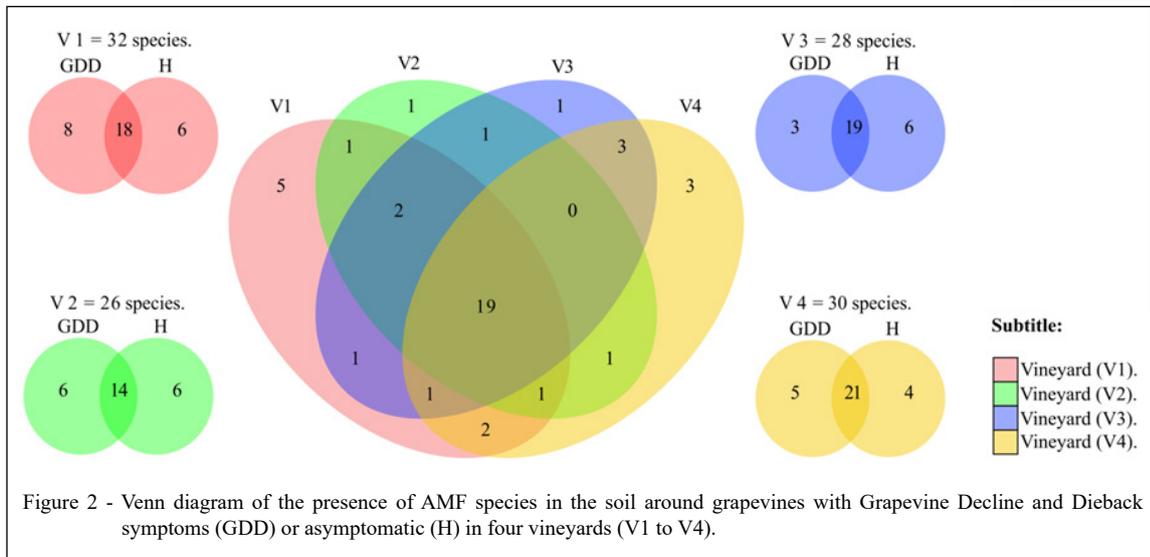


Figure 1 - AMF spores 50 $cm^{-3}$ of soil around grapevines with or without Grapevine Decline and Dieback (GDD) symptoms. V1 – V4: vineyard; GDD: plants with GDD symptoms; H: asymptomatic plants. Bars represent standard error of the mean.

Table 3 - Occurrence frequency and global frequency (F%) of arbuscular mycorrhizal fungus species in soil around grapevines with or without Grapevine Decline and Dieback (GDD) symptoms in four vineyards (V1 to V4).

| Species in field samples or trap culture | --Plants with GDD symptoms-- | | | | -------Asymptomatic plants------ | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Family / Species | V1 | V2 | V3 | V4 | V1 | V2 | V3 | V4 | F% |
| Family Acaulosporaceae | | | | | | | | | |
| *Acaulospora alpina* Oehl, Sýkorová and Sieverd | - | - | - | - | - | - | R | - | 1.8 |
| *Acaulospora foveata* Trappe & Janos | - | - | - | - | - | R | - | - | 1.8 |
| *Acaulospora mellea* Spain and N.C. Schenck | VC | C | R | D | VC | R | VC | R | 46.4 |
| *Acaulospora morrowiae* cf. Spain and N.C. Schenck | D | R | - | R | C | R | R | R | 25.0 |
| *Acaulospora reducta* Oehl, B.T. Goto and C.M.R. Pereira | - | R | - | - | - | - | R | - | 3.6 |
| *Acaulospora scrobiculata* Trappe | R | R | VC | R | C | - | R | R | 28.6 |
| *Acaulospora tuberculata* Janos and Trappe | R | - | - | - | - | - | - | - | 3.6 |
| *Acaulospora walkeri* cf. Kramad. and Hedger | - | - | - | - | - | - | R | R | 3.6 |
| Family Ambisporaceae | | | | | | | | | |
| *Ambispora leptoticha* (N.C. Schenck and G.S. Sm.) R.J. Bills & J.B. Morton | VC | - | - | - | - | R | - | - | 8.9 |
| *Ambispora reticulata* Oehl and Sieverd | - | - | - | R | - | R | - | VC | 12.5 |
| Family Archaeosporaceae | | | | | | | | | |
| *Archaeospora trappei* (R.N. Ames and Linderman) J.B. Morton and D. Redecker | D | D | D | D | VC | D | D | VC | 83.9 |
| Family Claroideoglomeraceae | | | | | | | | | |
| *Claroideoglomus claroideum* (N.C. Schenck and G.S. Sm.) C. Walker and A. Schüßler | R | - | VC | - | - | VC | R | - | 17.9 |
| *Claroideoglomus etunicatum* (W.N. Becker and Gerd.) C. Walker and A. Schüßler | D | D | VC | D | D | VC | D | D | 92.9 |
| Family Diversisporaceae | | | | | | | | | |
| *Diversispora* sp1 | D | D | VC | VC | D | VC | C | D | 75.0 |
| *Diversispora* sp2 | - | R | D | D | R | - | D | VC | 48.2 |
| *Diversispora* sp3 | - | - | R | R | - | - | D | - | 21.4 |
| Family Gigasporaceae | | | | | | | | | |
| *Cetraspora pellucida* (T.H. Nicolson and N.C. Schenck) Oehl, F.A. Souza and Sieverd | C | D | D | C | VC | R | D | R | 57.1 |
| *Gigaspora margarita* cf. W.N. Becker and I.R. Hall | R | R | - | D | R | - | R | D | 33.9 |
| *Dentiscutata heterogama* (T.H. Nicolson and Gerd.) Sieverd., F.A. Souza and Oehl | - | - | - | R | R | - | R | R | 5.4 |
| Family Glomeraceae | | | | | | | | | |
| *Dominikia aurea* cf. (Oehl and Sieverd.) Błaszk., Chwat, G.A. Silva and Oehl | R | R | R | - | - | R | - | - | 8.9 |
| *Funneliformis mosseae* (T.H. Nicolson and Gerd.) C. Walker and A. Schüßler | D | D | D | D | VC | D | D | D | 89.3 |
| *Glomus microaggregatum* Koske, Gemma and P.D. Olexia | C | R | - | C | - | - | - | VC | 21.4 |
| *Glomus ambisporum* cf. G.S. Sm. and N.C. Schenck | VC | VC | VC | D | VC | D | VC | C | 67.9 |
| *Glomus spinuliferum* Sieverd. and Oehl | - | - | - | R | R | - | - | - | 3.6 |
| *Oehlia diaphana* (J.B. Morton and C. Walker) Blaszk., Kozlowska & Dalpé | R | - | - | - | - | - | - | - | 1.8 |
| *Glomus microcarpum* Tul. & C. Tul. | R | - | R | R | - | - | R | - | 8.9 |
| *Rhizophagus clarus* (T.H. Nicolson and N.C. Schenck) C. Walker and A. Schüßler | - | - | - | C | - | - | - | R | 8.9 |
| *Rhizophagus fasciculatus* (Thaxt.) C. Walker and A. Schüßler | - | - | - | - | R | - | - | - | 1.8 |
| *Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, Stille and A. Schüßler | R | VC | D | VC | C | VC | VC | D | 62.5 |
| *Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva and Oehl | R | - | - | - | - | - | - | - | 1.8 |
| *Sclerocystis sinuosa* Gerd. and B.K. Bakshi | C | C | VC | R | VC | R | VC | - | 39.3 |
| *Sclerocystis coremioides* Berk. and Broome | - | - | - | - | - | - | R | R | 5.4 |
| *Glomus* sp1 | D | VC | C | D | D | VC | C | D | 71.4 |
| *Glomus* sp2 | VC | C | R | D | VC | VC | R | D | 57.1 |
| *Glomus* sp3[*] | - | - | - | - | - | - | - | R | 1.8 |
| *Glomus* sp4[*] | - | - | - | - | - | - | - | R | 1.8 |
| *Rhizophagus* sp1 | C | R | R | R | C | - | R | R | 21.4 |
| *Rhizophagus* sp2 | R | - | R | R | R | R | R | - | 16.1 |
| *Sclerocystis* sp1[*] | - | - | - | - | R | - | - | - | 1.8 |
| Incertae sedis | | | | | | | | | |
| *Entrophospora infrequens* (I.R. Hall) R.N. Ames and R.W. Schneid | C | - | D | D | VC | R | D | VC | 62.5 |
| Family Paraglomeraceae | | | | | | | | | |
| *Paraglomus occultum* (C. Walker) J.B. Morton and D. Redecker | D | R | C | R | C | R | - | R | 33.9 |
| *Paraglomus brasilianum* (Spain and J. Miranda) J.B. Morton and D. Redecker | - | - | R | - | R | - | - | - | 3.6 |

D = dominant (85% ≤ FO ≤ 100%), VC = very common (50% ≤ FO < 85%), C = common (30% ≤ FO < 50%), R = rare (FO < 30%), [*]recovered exclusively from trap cultures.

Figure 2 - Venn diagram of the presence of AMF species in the soil around grapevines with Grapevine Decline and Dieback symptoms (GDD) or asymptomatic (H) in four vineyards (V1 to V4).

vineyard did not differ between soils with H- and GDD-plants (Figure 3). The PERMANOVA demonstrated differences among vineyards (Table 4), confirming the PCoA findings.

Distance-based redundancy analysis (dbRDA) (Figure 4) showed that soil attributes were strongly correlated. Such was the case of organic matter with V4, K, and resin-extracted phosphorus (P.r) with V1 and V2, while Cu, pH, Ca, and Mg are related to V3 (Table 5). Part of the AMF species showed positive correlations with some soil attributes. Organic matter was positively related to the presence of



Figure 3 - Principal coordinate analysis (PCoA) using a similarity coefficient of Jaccard for the presence-absence data of arbuscular mycorrhizal fungal species in the soil around grapevines with (GDD) or without (H) Grapevine Decline and Dieback (GDD) symptoms in four vineyards (V1 to V4).
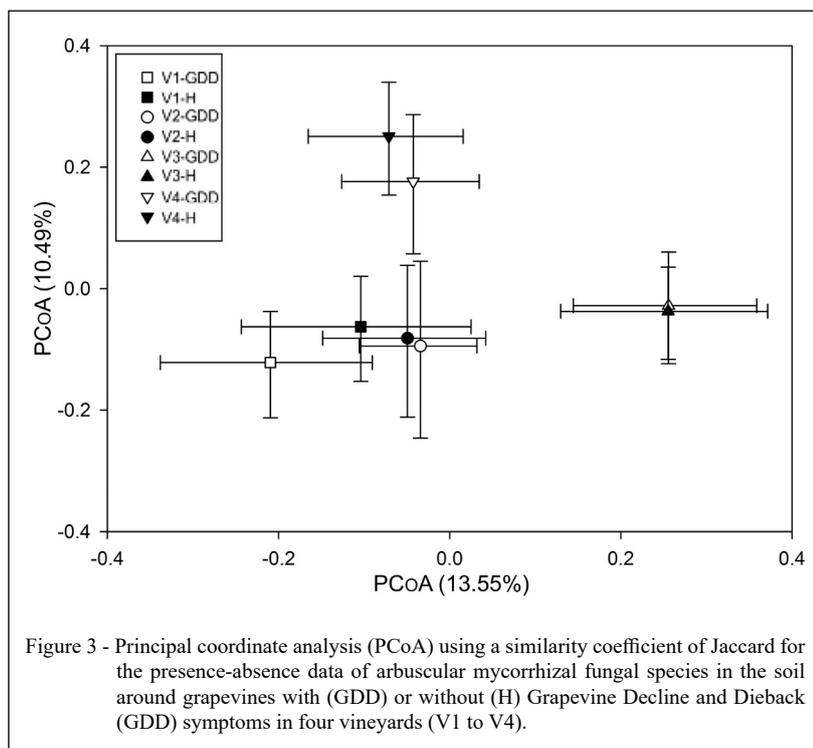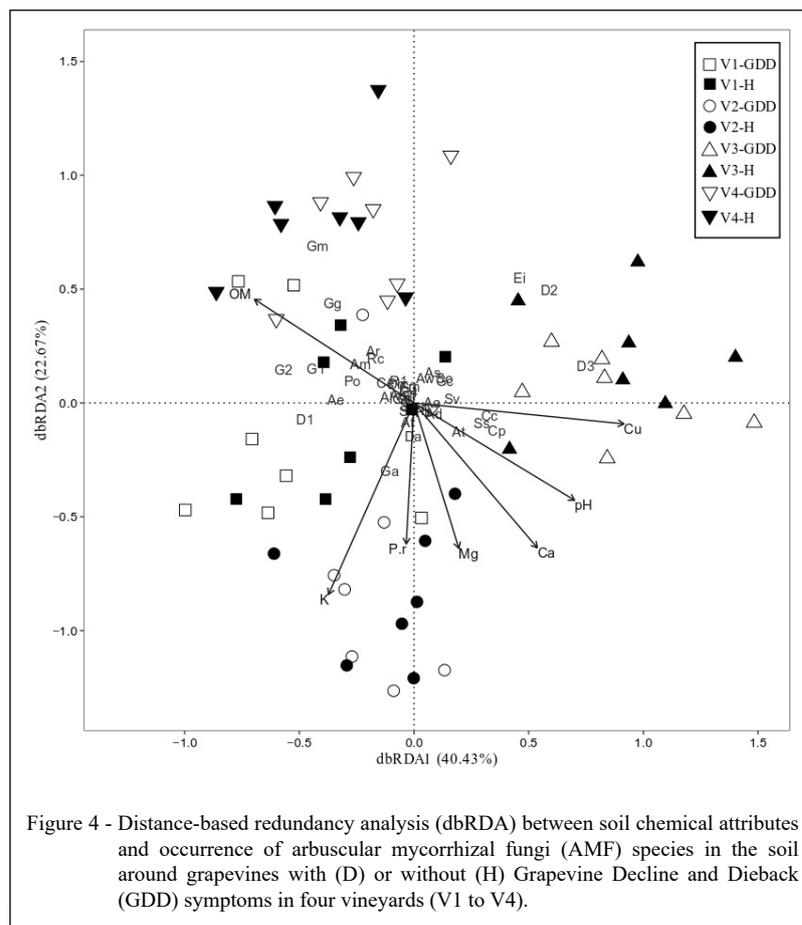
Table 4 - Permanova using Jaccard similarity coefficient for presence-absence data of AMF species in soil around grapevines with or without Grapevine Decline and Dieback (GDD) symptoms in four vineyards (V1 to V4).

| Source | Sum of squares | Degrees of freedom | Medium square | F | p |
|---|---|---|---|---|---|
| Vineyards | 2.9664 | 3 | 0.9888 | 6.9637 | 0.0001 |
| GDD or Asymptomatic | 0.0969 | 1 | 0.09699 | 0.6831 | 0.7757 |
| Interaction | 0.7634 | 3 | 0.25447 | 1.7921 | 0.0039 |
| Residual | 6.8156 | 48 | 0.14199 | | |
| Total | 10.642 | 55 | | | |

*Gigaspora margarita* and *Glomus microaggregatum*, while Cu concentration had a positive correlation with *Entrophospora infrequens*, *Diversispora* sp2, and *Diversispora* sp3. The variables Mg, Ca, pH, and Cu showed a negative correlation with *Gigaspora margarita* and *Glomus microaggregatum*, and K and P.r had a negative correlation with *Entrophospora infrequens*, *Diversispora* sp2, and *Diversispora* sp3.

## DISCUSSION

This research is the first systematic survey of AMF communities in vineyards with plants showing symptoms of GDD and asymptomatic plants in southern Brazil. We based our inventory of AMF communities solely on morphological identification of spores from field samples and trap



Figure 4 - Distance-based redundancy analysis (dbRDA) between soil chemical attributes and occurrence of arbuscular mycorrhizal fungi (AMF) species in the soil around grapevines with (D) or without (H) Grapevine Decline and Dieback (GDD) symptoms in four vineyards (V1 to V4).

cultures, which may have limitations. Some studies showed that molecular approaches revealed different species composition in comparison to analyses of spores, roots or soil (HEMPEL et al., 2007). However, assessment of AMF community using spore morphology may reveal a higher number of species than molecular approaches (VIEIRA et al., 2018). Our results evidenced that AMF community composition between GDD and H were highly similar, and *Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Archaeospora trappei* were the most frequent fungi recovered in both types of vineyards. These results suggested grapevine physiological conditions do not determine AMF community composition and structure, which are more affected by the vineyard location.

AMF spore numbers were higher than in vineyards in the Brazilian Northeast (FREITAS et al., 2011), in an arid climate, but they were lower than in Italian vineyards under Mediterranean climate (NAPPI et al., 1985). In V3-GDD plants, which had the lowest spore density, the value is 2.5 times higher than in organic and conventional vineyards in the same region, which had 115 and 45 spores 50 cm$^3$ (BETTONI et al., 2016). Several factors can impact AMF sporulation under field conditions, including moisture, soil factors, grapevine variety, and sampling season (JHA & SONGACHAN, 2020). We sampled vineyards in Autumn, which has a large thermal amplitude and is linked to physiological and nutritional changes in grapevines that seem to favor

AMF sporulation (RABATIN, 1979). Although, spore production may differ with grapevine varieties (KARAGJANNIDIS et al., 1997), we ruled out this factor since two rootstocks (VR 043-43 and Paulsen-1103) were evaluated and we detected no differences between them. Soil P and organic matter were high in all vineyards (Table 2), which can affect root colonization and C allocation to spore production (FREITAS et al., 2011). Sporulation usually increases after phosphate application, an effect associated with P tolerance of some AMF species (SYLVIA & SCHENCK, 1983), and organic matter also promotes sporulation in vineyards soils (FREITAS et al., 2011).

Regardless of vineyard location and plant health, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Archaeospora trappei* were the most frequent species. We attributed the dominance of these species to their life strategies as they can be considered r-strategists. *Claroideoglomus etunicatum* has an extensive production of small spores in a short time (PINHEIRO et al., 2019), *Funneliformis mosseae* rapidly colonizes ruderal habitats (SÝKOROVÁ et al., 2007), while *Archaeospora trappei* seems to have a sporulation-based strategy to survive in extreme environments (SPAIN, 2003; OEHL & CHISTIAN, 2014). These generalist species are usually ubiquitous and associate strongly with highly disturbed sites (OEHL et al., 2010), and their life strategies include traits such as fast spore germination and mycelium extension (CANO & BAGO, 2005). AMF species vary in carbon demand, which may define life

Table 5 - Eigenvalues explained variability and dbRDA analysis coordinates for chemical attributes of soil around grapevines with or without Grapevine Decline and Dieback (GDD) symptoms, in four vineyards (V1 to V4).

| Main Components | ------------------------Coordinates---------------------- | |
|---|---|---|
| | CAP1 | CAP2 |
| Eigenvalues [†] | 1.548 | 0.868 |
| Explained variability (%) | 40.43 | 22.67 |
| Explained accumulated variability (%) | 40.43 | 63.10 |
| ------------------------------------------------------------------Variable------------------------------------------------------------------ | | |
| P.r | -0.03 | -0.62[*] |
| pH | 0.70[*] | -0.43 |
| K | -0.37 | -0.84[*] |
| M.O | -0.69[*] | 0.45 |
| Ca | 0.54[*] | -0.64[*] |
| Mg | 0.19 | -0.64[*] |
| Cu | 0.91[*] | -0.10 |

[†]Components with eigenvalues > 1 explain > 10% of the total variance considered.

[*] Variables with significant projections on the main components (Value > 0.50).

strategies, interspecific variation, and occurrence of functional trade-offs (CHAGNON et al., 2013; HART et al., 2001). Changes in growth rates in the microbial community by nutrient availability may reflect changes to a predominance by either r-type or K-type AMF (BLAGODATSKAYA et al., 2007). Interestingly, *F. mosseae* and *A. trappei* were also detected in cohorts of AMF in grapevines from Italy (BALESTRINI et al., 2010), suggesting some host preference for those two fungal species.

The AMF species richness reported in our study is higher than in previous studies in vineyards in USA (17 species) (SCHREINER & MIHARA, 2009), and Italy (9 species) (BALESTRINI et al., 2010). A possible explanation for this is that we sampled four different vineyards in distinct geographical locations and used trap cultures to further detect AMF species. Alternatively, high AMF species richness could result from the presence of black oats used as a cover crop in all vineyards. GDD- and H-plants shared most AMF species (88% of the total species richness), and species richness in both GDD- and H-plants were high, regardless of the vineyard. That suggested that GDD-plants do not affect the AMF species associated with them and that AMF species composition associated with vineyards is affected mainly by soil attributes (CHENG & BAUMGARTEN, 2004) and plant age (SCHREINER & MIHARA, 2009). The lack of differences between H- and GDD-plants may have occurred because plants were under the same management conditions in both situations. The dominance of Glomeraceae and Acaulosporaceae was expected, as previously reported in southern Brazil (ÁVILA et al., 2007; SILVA et al., 2015) and the USA (SCHREINER & MIHARA, 2009). BOUFFAUD et al. (2016) used molecular techniques and found high variability in AMF abundance and root colonization, and a correlation with plant management. As our research was restricted to morphological techniques, diversity may have been underestimated compared to molecular techniques. Species associated exclusively with H-plants were *Acaulospora alpina*, *A. foveata*, *Rhizophagus fasciculatus*, and *Sclerocystis* sp1, while the only species associated exclusively with GDD-plants was *Oehlia diaphana* (Table 3). Although, all five species were rare in both types of vineyards, their presence might indicate some selectivity for specific physiological conditions of grapevines or may be limited to a soil patch providing favorable conditions for a given AMF species. That indicates that they could be promising for future research in inoculation programs of grapevine or be tested in field conditions to verify their influence on plants with GDD symptoms.

The PCoA (Figure 3) and PERMANOVA analysis (Table 4) indicated that AMF communities are more distinct between vineyards than between H- and GDD-plants within the same vineyard. Similar results were detected in Italy, where vineyards harbored sequences from six species groups in one site, while those from other site harbored *Glomus* group A, and locations shared few OTUs (BALESTRINI et al., 2010). Two types of natural filters interfered with an AMF community: the environmental filter, which selects species tolerant to environmental factors, and the host filter, which only allows colonization by compatible fungal partners (VÁLYI et al., 2016). Considering that rootstocks (host filter) did not affect AMF communities, our data suggested that some environmental filters (*e.g.,* cover crops) or historical processes (*e.g.*, dispersion) might be acting to shape AMF communities in vineyards of Santa Catarina. Moreover, our results suggested that the physiological state of grapevine plants (GDD- or H-) has little or no influence on the soil AMF community composition.

No clear pattern appeared when the relationship between AMF species, soil attributes, and vineyards was analyzed. Soil P concentration in all four vineyards (Table 1) is high (Comissão de Química e Fertilidade do Solo, 2016), and this was particularly evident for V2, which has a history of phosphate fertilization, (Figure 4). V3 vineyard has the highest levels of Cu, due to its age and cultivation history. In the region, frequent phytosanitary treatments are applied to control fungal diseases, and many use copper-based products. Therefore, vineyards with a long history have high levels of soil Cu (Casali et al., 2008); although, the highest Cu values (65 mg kg$^{-1}$ in V2) are not enough to cause toxicity to grapevines. Species associated with V2 are *Entrophospora infrequens*, *Diversispora* sp1 and *Diversispora* sp2., which suggested that they might have become adapted to high soil copper.

In conclusion, our results suggested that grapevines and, their associated cover crops, maintain a highly diverse AMF community, with high species richness and presence of most genera and families of Glomeromycota. Differences between vineyards were more related to geographical location than with rootstock or grapevine physiological state, i.e., GDD or healthy plants. Although, soil characteristics are an important determinant of AMF community assemblages (BALESTRINI et al. 2010; SCHREINER & MIHARA 2009), our results showed a limited role of soil factors in shaping AMF communities in vineyards. An exception would be the strong association of *E. infrequens*, *Diversispora* sp1, and sp2 with high levels of Cu in V2. These species could be isolated in single

culture for further research. Since the AMF community assembly was mainly affected by soil factors in each area, our research confirmed that AMF communities in vineyards are affected by characteristics of the plant's host (*e.g.*, rootstock, age) and management practices (*e.g.*, cover crops, fertilization regime). The rich AMF community associated with grapevine represents a biotechnological potential for inoculation programs aiming to reduce fertilizer input and prevent GDD emergence.

## CONCLUSION

Arbuscular mycorrhizal fungal communities differ among vineyards, but not between soil under plants with grapevine decline and dieback symptoms (GDD) or asymptomatic. Soil chemical attributes were not determinant for occurrence of most AMF species, but copper is associated with the presence of *Entrophospora infrequens*, *Diversispora* sp1, and sp2. *Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Archaeospora trappei* were dominant and are promising for use in production of AMF inoculated grapevine plantlets.

## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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