Dziadas, M., Kolasiński, M., & Bykowska, J. (2026). Mycorrhizal inoculation under reduced fertilisation modulates aroma precursors in must and volatile composition in wines of Vitis vinifera 'Danmarpa Polonia'. *OENO One*, *60*(3).
https://doi.org/10.20870/oeno-one.2026.60.3.9906

SUPPLEMENTARY DATA

Materials and methods

1. Propagation and Mycorrhizal Inoculation

1.1. Rooting Procedure

Before the start of the growing season, canes were collected from mother plants and used to prepare three-node hardwood cuttings with an average thickness of 11.87 mm. The cuttings were placed - 10 per container - into 25 dm³ pots filled with perlite. These containers were placed in a plastic tunnel equipped with a misting system that maintained air humidity at a level of 90–95 %. The temperature inside the tunnel ranged between 20 - 26 °C. In October, after the growing season, an average of 24.12 roots per 20.15 cm segment was recorded. The average length of the developed canes was 84.96 cm. The 200 strongest cuttings were selected for further cultivation. These were overwintered in frost-free conditions. In April, after dormancy, they were pruned above the first bud. Some of them were subjected to a mycorrhizal treatment and then planted in appropriate combinations.

1.2. Mycorrhizal Inoculation

After overwintering, 40 rooted cuttings per treatment were initially prepared for inoculation. The rooted portions were immersed in a hydrogel solution containing live hyphae of ectomycorrhizal fungi, arbuscular (endomycorrhizal) fungi, or a mixture of both types, depending on the treatment. The ectomycorrhizal inoculum contained fungal isolates obtained from grapevine roots collected from natural habitats and from vines established in a deciduous forest, together with selected ectomycorrhizal basidiomycetes, including Boletus edulis, Boletus erythropus, and Xerocomus chrysenteron, according to the producer's information. The arbuscular mycorrhizal inoculum contained Rhizophagus intraradices (formerly Glomus intraradices), Glomus claroideum, Glomus etunicatum, Glomus fasciculatum, Gigaspora sp., and Entrophospora sp. Control plants were immersed in pure hydrogel without fungal inoculum.

Prior to field establishment, uniform and healthy vines were selected from the initial plant material. A total of 24 plants per treatment were used for the final field experiment, arranged as three biological replicates of eight vines each in a randomised block design. The selected vines were planted in the field in mid-April.

1.3. Fertilisation Protocol

A multi-component fertiliser, Azofoska (INCO GROUP, Poland), was used, containing three primary macronutrients in the ratio of 1 (N) : 0.5 (P_2O_5) : 1.4 (K_2O). Additionally, the fertiliser included magnesium (MgO), sulphur (S), and micronutrients such as iron, manganese, copper, zinc, boron, and molybdenum. Baseline soil chemical characteristics, including total nitrogen content, were determined in 2019.

In the first year after planting, no fertilisation was applied. In the second year, a dose of 15 g m^{-2} was applied in the fungal treatment combinations and in combination E, corresponding to 50% of the full mineral fertilisation dose. In combination D, representing the full mineral fertilisation control, a dose of 30 g m^{-2} was applied. In the following years of cultivation, fertilisation was increased to 30 g m^{-2} in the reduced-fertilisation treatments, including the fungal treatment combinations and combination E, and to 60 g m^{-2} in combination D, representing 100% mineral fertilisation.

Fertilisation doses were selected according to the producer's recommendations for young grapevine cultivation. The reduced-fertilisation treatments, including the fungal inoculation treatments and combination E, received 50% of the dose applied in the fully fertilised control treatment (combination D).

1.4. Cultivation System

The plants were trained using the single Guyot system with a fruiting cane bearing 6 - 8 buds. Fruit for volatile-compound analysis was harvested in the third and fourth years after planting, corresponding to the 2021 and 2022 growing seasons.

1.5. Soil characteristic

Site	Soil layer [cm]	Agronomic soil category	pH [-]	Measurement uncertainty [-]	Soil reaction [-]	Soil quality class
Poznań–Marcelin	0–20	Light soil	6.1	0.23	Slightly acidic	III

1.6. Soil macronutrient content

Site	P_2O_5 [mg kg^{-1}]	P [mg kg^{-1}]	K_2O [mg kg^{-1}]	K [mg kg^{-1}]	Mg [mg kg^{-1}]	Total N [g kg^{-1}]
Poznań–Marcelin	30	13.1	35	29	4.3	1.1

The total nitrogen content was classified as moderate, whereas the concentrations of the remaining macronutrients were classified as high.

2. Vinification Procedure

Grapes were harvested at technological maturity and transported the same day to the laboratory in plastic containers. Technological maturity was assessed in the field using uniform visual and organoleptic criteria applied across all treatments. These criteria included berry softening, typical berry colour development, absence of pronounced green or herbaceous taste, development of characteristic varietal aroma, and browning of seeds. All treatment combinations were harvested on the same date and processed under the same vinification conditions. From each replicate, 5 kg of grape berries were crushed, treated with 100 mg L^{-1} sodium metabisulfite ($Na_2S_2O_5$), and inoculated with Saccharomyces cerevisiae (strain CECT 10835, Lallemand Inc.) at a dose of 10 mg L^{-1}. The musts were fermented in 5 L glass vessels at room temperature (19–21 °C) until complete sugar consumption (dry wine). The wines were racked twice, stored at 10 °C in glass containers, and analysed after 6 months. No additional SO_2 adjustment was performed before storage, and headspace volume was minimised by filling the glass containers as completely as possible.

3. Solid-Phase Extraction (SPE) of Non-Polar Compounds

A 100 mL aliquot of must or wine was spiked with 13.5 µg of $^2H_7$-geraniol and 20 µg $^2H_7$-geranyl-β-D-glucopyranoside as internal standards and stirred for 5 min. The sample was then loaded onto a 500 mg reversed-phase C18 SPE cartridge previously conditioned with 25 mL of methanol followed by 25 mL of water. The cartridge was washed with 150 mL of water. Non-polar volatile compounds were eluted with 25 mL of pentane/dichloromethane (2:1, *v/v*), whereas the glycosidically bound fraction was subsequently eluted with 25 mL of methanol. The pentane/dichloromethane eluate was concentrated to approximately 500 µL using a rotary evaporator, and 1 µL of the extract was injected into the GC–MS system in splitless mode. The methanolic extract containing the glycosidically bound fraction was subjected to enzymatic hydrolysis.

4. Enzymatic hydrolysis of glycosidically bound volatile compounds

The methanolic extract containing the glycosidically bound fraction was evaporated to dryness using a rotary evaporator. The residue was dissolved in 5 mL of a 2% (*w/v*) AR-2000 enzyme solution (DSM, the Netherlands) prepared in tenfold-diluted citrate–phosphate McIlvaine buffer at pH 4.5. The stock buffer was prepared by mixing 9.08 mL of 0.2 M Na_2HPO_4 with 10.92 mL of 0.1 M citric acid. The resulting 20 mL of buffer was subsequently diluted with ultrapure water to

a final volume of 200 mL. The pH was verified and, when necessary, adjusted to 4.5. Enzymatic hydrolysis was carried out for 24 h at 36 °C. After incubation, the sample was loaded onto a 500 mg reversed-phase C18 SPE cartridge previously conditioned with 25 mL of methanol followed by 25 mL of water. The cartridge was washed with 150 mL of water, and the released non-polar volatile aglycones were eluted with 25 mL of pentane/dichloromethane (2:1, *v/v*). The pentane/dichloromethane eluate was concentrated to approximately 500 µL using a rotary evaporator, and 1 µL of the extract was injected into the GC–MS system in splitless mode.

5. GC–MS Analysis

Analysis was performed using an Agilent 7890A GC system coupled to an Agilent 5975C VL MSD quadrupole mass spectrometer with a triple-axis detector and diffusion pump. Liquid extracts were injected using an Agilent 7683B autosampler in splitless mode at 260 °C with a split/splitless inlet liner. Separation was achieved using a Supelcowax-10 column (30 m × 0.25 mm × 0.25 µm, Supelco, Bellefonte, PA). The oven temperature program was: 40 °C for 1 min, ramped at 5 °C min^{-1} to 200 °C, then 20 °C min^{-1} to 230 °C, held for 9.5 min. Total runtime: 44 min. Transfer line temperature was 240 °C, ion source 230 °C, quadrupole 150 °C. Spectra were acquired in electron ionization (EI, 70 eV) full scan mode (*m/z* 33 - 333 Da). The detector operated with a gain factor (GF = 1) at EMV 1760 V. Carrier gas: helium, flow rate 0.8 mL min-1 (32.4 cm s^{-1}).

5.1. Compound identification, semi-quantification and statistical analysis

Compounds were identified by comparing retention times and mass spectra with authentic standards when available. When authentic standards were not available, compounds were tentatively identified by comparison of mass spectra with spectral libraries and by comparison of retention behaviour with literature data.

Semi-quantitative values were calculated as concentration equivalents using the corresponding internal standards. Free volatile compounds were quantified relative to $^{2}H_{7}$-geraniol, whereas glycosidically bound compounds released after enzymatic hydrolysis were corrected using $^{2}H_{7}$-geranyl-β-D-glucopyranoside. The analytical approach was therefore interpreted as semi-quantitative and used for relative comparison among treatments. Data analysis, compound deconvolution and quantification were performed using AMDIS software (v.2.65). Compound libraries (*.msl, *.isl) were created for targeted analysis and signal-to-noise ratio calculation.

Data from the 2021 and 2022 growing seasons were analysed separately. For each treatment within each growing season, three independent field-derived biological replicates were included (n = 3). Means and standard deviations were calculated directly from the three biological-replicate values. Data from different growing seasons were not pooled, and means of seasonal means were not used.

For each compound and growing season, treatment effects were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test at $p < 0.05$. To control the false discovery rate, the 33 compound-level ANOVA p-values obtained within each growing season were adjusted using the Benjamini–Hochberg procedure. Principal component analysis was performed separately for each growing season and sample fraction using only the three independent biological replicates per treatment.

Principal component analysis (PCA) was performed using Statistica software (version 13.3). The analysis was based on the correlation matrix, which corresponds to standardisation of variables before PCA. This approach was selected because the analysed volatile compounds differed markedly in their semi-quantitative concentration equivalents, and standardisation prevented compounds present at higher abundance from disproportionately influencing the ordination. PCA was used only as an exploratory unsupervised multivariate tool to visualise treatment-related differences in volatile compound profiles.