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Phytochemical and Growth Patterns in *Cistus albidus* L.: Seasonal Impacts of Inoculation and Fertilization

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Abstract

Seasonal dynamics and cultivation practices jointly regulate plant growth and secondary metabolism, yet their relative contributions and modes of interaction remain insufficiently resolved in many medicinal species. In this study, the effects of organic fertilization and arbuscular mycorrhizal fungus (AMF) inoculation on vegetative development, flowering behaviour, and phytochemical profiles of *Cistus albidus* L. across one complete vegetative cycle under controlled cultivation conditions have been examined. Four treatment groups (control, fertilization, inoculation, and combined fertilization + inoculation) were evaluated with respect to growth parameters, reproductive output, total phenolic content (TPC), antioxidant capacity, and polyphenolic composition using UHPLC–MS. Across the vegetative cycle, seasonal progression emerged as the dominant regulator of both growth and metabolic traits, exerting strong control over leaf morphology, specific leaf area, flowering dynamics, and the temporal organisation of individual secondary metabolites. Fertilization primarily stabilised vegetative growth and reduced inter-individual variability, whereas AMF inoculation imposed consistent growth constraints, reduced reproductive output, and increased phenotypic and metabolic variability. Despite the absence of growth promotion typically associated with functional mycorrhizal symbiosis, inoculated plants exhibited elevated antioxidant capacity and increased concentrations of selected phenolic compounds, particularly during autumn. Phytochemical regulation was class-specific rather than uniform. Flavan-3-ols and flavonols displayed high seasonal plasticity and context-dependent responsiveness to inoculation, whereas phenolic acids, ellagitannins, and the secoiridoid glycoside were structured predominantly by harvest time and developmental stage. Total phenolic content and antioxidant capacity were only weakly coupled, reflecting opposing seasonal trajectories among individual compounds and highlighting the importance of matrix-level organisation rather than bulk metabolite accumulation. These results indicate, cultivation practices influenced *C. albidus* primarily by modulating growth regulation and developmental allocation, while secondary metabolism was reorganised selectively within a strong seasonal framework. This integrative response provides a conceptual basis for interpreting growth–defence–signalling trade-offs in medicinal plants under controlled cultivation.

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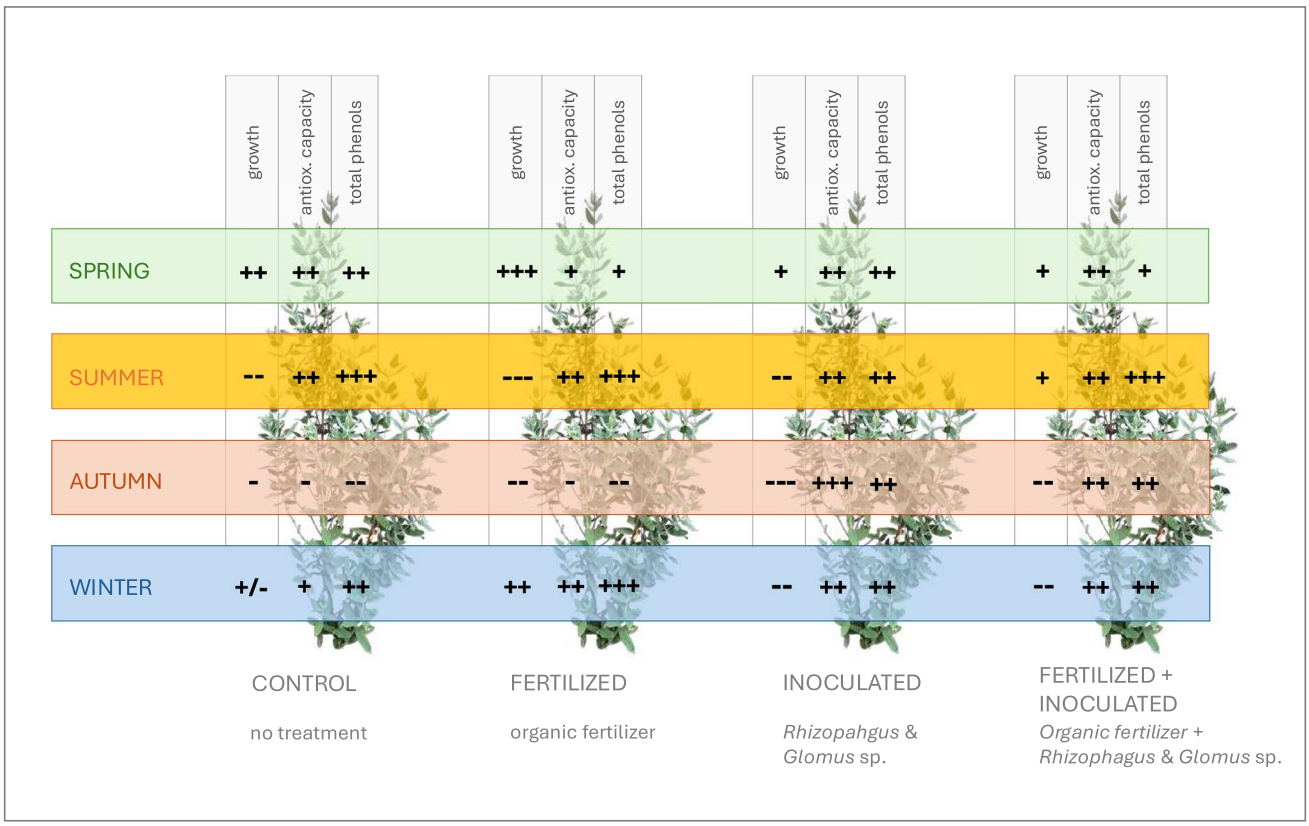
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Graphical Abstract



Keywords *Cistus albidus* L. · Mycorrhization · Fertilization · Polyphenols · Seasonality

Introduction

Phenolic compounds, which are ubiquitous in plants, are essential secondary metabolites known for their diverse biological functions and therapeutic potential, including antioxidant, anti-inflammatory, and antimicrobial activities (Rana et al. 2022). Environmental stresses can significantly impact plant metabolism, either by inhibiting or promoting the production of secondary metabolites (Xiao et al. 2019). The effects of various climate change components, such as high temperatures, elevated carbon dioxide levels, drought stress, and enhanced ultraviolet-B radiation, on secondary metabolites, especially phenolic compounds, are under continuous investigation, with evidence steadily increasing (Qaderi et al. 2023). Understanding these effects is crucial, as secondary metabolites play significant roles in the adaptability and success of certain plants. Furthermore, optimizing the biosynthesis of phenolic compounds in medicinal plants is of significant interest for enhancing their therapeutic efficacy.

Cistus albidus L., commonly known as white rockrose, is a Mediterranean shrub with a long history of traditional use. It is valued for its medicinal properties, including antimicrobial and anti-inflammatory activities and potential effects on the central nervous system (Raus de Baviera et al. 2023). The main constituents of this species are terpenes and polyphenols, with more than 200 identified compounds (Raus de Baviera et al. 2023). The increasing interest in *C. albidus*, which is obtained mainly from wild collection, makes it necessary to consider its cultivation.

C. albidus is highly stress-tolerant, and recent work has focused on the mechanisms underlying this resilience (Pérez-Llorca et al. 2019; Casadesús et al. 2022, 2021; Munné-Bosch et al. 2003; Müller et al. 2014), with polyphenols implicated as key contributors to stress regulation.

Strategies to enhance phenolic biosynthesis include abiotic/biotic elicitation (Tariq and Ahmed 2024; Ahlawat et al. 2023) and mycorrhizal inoculation (Avio et al. 2017; Zhang et al. 2013). Nevertheless, the effects of AMF inoculation remain debated. While many studies report growth and stress tolerance gains via improved nutrient uptake (Chandrasekaran 2020), others suggest a stress-like effect, reduced growth coupled to elevated secondary metabolism,

especially in contexts where functional symbiosis is limited (Cosme et al. 2018; Veiga et al. 2013).

Cistus species have been reported to form both ectomycorrhizae (ECM) and AMF (Lorente et al. 2021; Ortuño et al. 2018), whereas another study reported evidence of ECM symbiosis only (Comandini et al. 2006). Although effective associations between *Cistus* species and specific AMF taxa, such as *Glomus iranicum*, have been described under Mediterranean conditions, this species was not included in the inoculum used in the present study, and such reports are therefore discussed here only for comparative purposes (Ortuño et al. 2018). It should be noted that the application of arbuscular mycorrhizal fungi does not necessarily imply the establishment of a functional symbiosis, as mycorrhizal associations can span a continuum from mutualistic to neutral or even parasitic depending on environmental and physiological context (Johnson et al. 1997; Smith et al. 2011). In several Mediterranean shrub species, including members of the genus *Cistus,* the outcome of AMF inoculation has been shown to be highly context-dependent, influenced by soil conditions, nutrient availability, native microbial communities and host developmental stage (Comandini et al. 2006; Hoeksema et al. 2010; Albornoz et al. 2024). Consequently, plant responses to AMF inoculation may reflect early signalling events, rhizosphere-mediated effects, or stress-related physiological adjustments rather than fully established mutualistic interactions, particularly during initial phases of plant–fungus contact or under non-limiting nutrient conditions (Werner and Kiers 2015; Moreno et al. 2025).

The present study explores the seasonal effects of organic fertilization and AMF inoculation on growth, total phenolic content (TPC) and antioxidant capacity of *C. albidus* over one vegetative cycle. The primary objective was to identify cultivation practices that enhance phytochemical traits relevant to medicinal use (Raus de Baviera et al. 2025); secondarily, we aimed to clarify whether AMF inoculation acts predominantly through growth-promoting symbiosis or through stress-associated physiological responses under the tested conditions.

Table 1 Experimental treatments of the four groups (CONT, FERT, INOC, and FERT+INOC) with n = 100 *C. albidus* plants each

Sample	Treatment	Concentration per individual plant
Control (CONT)	No treatment	–
Fertilization (FERT)	Organic fertilization	0.3 mL
Inoculation (INOC)	Fungus inoculation (spores, mycelia)	0.1 mg
Combination (FERT+INOC)	Organic fertilization + fungus inoculation (spores, mycelia)	0.3 mL (FERT) + 0.1 mg (INOC)

Materials and Methods

Plant Material

The plant material was obtained from an experimental culture located in Murcia, Spain (37° 36’ 21.64" N; 1° 47’ 36.13" W). For this experiment, 400 two-year-old *C. albidus* plants with uniform growth trends were selected. The plants were grown in a southwest orientation 475 m above sea level in polyethylene culture bags that contained substrate (Mediterranean Rambla soil) without any previous treatment. This soil has the same characteristics as the soil of the mother plants from which the seeds of the experimental culture originated (Table S1).

Once the culture was established, it was divided randomly into four groups of 100 plants each to provide different treatments and evaluate the effects of different culture conditions: control plants (CONT), fertilized plants (FERT), inoculated plants (INOC) as well as inoculated plants that received additional fertilization (FERT+INOC) (Table 1). Fertilization was performed using organic fertilizer (Table S2) at least 14 days before each harvest. The plants in the last two groups (INOC and FERT+INOC) were inoculated with a mixture of different arbuscular fungi of the genera *Rhizophagus* and *Glomus* to determine the responses of the experimental plants to biotic stimuli. The mixture contained *R. irregularis, G. aggregatum, G. mosseae* and *G. etunicatum* in equal proportions. These fungi belong to the *Glomeromycetes* class, with the total administration of spores and mycelia divided into two applications of 0.2 mg per plant eight weeks before the first harvest to promote potential interactions between the inoculated fungi and the plant root system (Hart and Reader 2002). However, the extent of root colonization and the functional establishment of mycorrhizal symbiosis were not directly assessed in this study. Since the CONT plants should serve as a control, they were completely untreated and thus grown under conditions similar to those in the wild.

Throughout the duration of this study, all the plants were provided sufficient water by maintaining the soil moisture content constant at 35.0 ± 0.7%.

Flowering was determined based on a daily count from February to July of both years, 2021 and 2022.

Leaves were collected from healthy plants during one cultivation cycle in spring (May 2021), summer (July 2021), autumn (November 2021) and late winter (March 2022). Leaves were always collected in the early morning before sunrise to ensure the maximum content of flavonoids, as shown in a previous study by Gori et al. (Gori et al. 2020). Three to six healthy and completely developed leaves were selected from the same position (2^{nd}–4^{th} node levels of leaves down from the apices) of fully developed

branches (with 5 or more leaf nodes) per plant and cleaned with pressurized air. Leaves with visible malformations, damage or abnormal coloration were discarded. A total of 200 leaves were harvested from each treatment group and in each season. Half of the leaves were assigned to the growth analysis, with 90 uniformly growing leaves selected per treatment group. Leaves destined for the growth analysis were measured using ImageJ software (version 2.3.0/1.53q) to obtain a leaf profile and area after being scanned at 600 dpi resolution using a flatbed scanner (TS 5100 series; Canon, Tokyo, Japan). An electric balance (ML 204; Mettler Toledo Company, Greifensee, Switzerland; measurement accuracy 0.0001 g) was used to obtain the fresh weight and dry weight of the samples after they were dried in an oven at 70 °C to a constant mass.

The plant heights were measured in July 2021 and 2022. The mean growth of the lateral and principal shoots was measured between the shoot apical meristem and first leaf node of three representative shoots per plant of five plants per treatment group during the growth season between December 2021 and the end of May 2022.

Extraction Procedures

The plant material used for the phytochemical analysis, consisting of 100 fresh leaves per treatment group, was ground using a mechanical mill to a maximum size of 2 mm for further processing.

C. albidus aqueous extracts were produced using distilled water at a temperature of 50 °C. The preparations were gently agitated for approximately 4 h at a plant-to-solvent ratio of 1:5. The filtered extract (using a Büchner device) was then centrifuged to remove insoluble solid components, and only the supernatant was used. The extracts were concentrated by rotary evaporation and stored at -80 °C until one day before use, at which time the temperature was increased to 4 °C. Three independent extracts were prepared for each condition.

Extract Characterization

Total Phenol Content and UHPLC Analysis

The TPC of the *C. albidus* extracts was determined spectrophotometrically using the Folin–Ciocalteu assay as described by Álvarez-Martínez et al. (Álvarez-Martínez et al. 2021). The molecular composition of the extracts was analyzed via an ultrahigh-pressure liquid chromatography system (UHPLC) with a Nexera UHPLC system (Shimadzu Scientific Instruments, Inc., Tokyo, Japan) composed of a DGU-405 degasser, an LC-40D × 3 binary pump, a SIL-40C autosampler, a CTO-40C column oven and a CBM-40 controller. The UHPLC system was coupled to an LCMS-8050 triple quadrupole mass spectrometer from Shimadzu equipped with electrospray ionization (ESI) mode (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). Chromatographic separation was performed with a Poroshell 120 SB-C18 column (4.6 × 150 mm, 2.7 µm) (Agilent Technologies, Inc., Palo Alto, CA, USA), as described by Barrajón-Catalán et al. (Barrajón-Catalán et al. 2011).

For the identification of the main compounds, a homemade library of polyphenolic compounds was used. Therefore, the retention times and MS/MS data of the peaks in the samples were compared with those of authentic standards or data reported in the literature (Barrajón-Catalán et al. 2011; Lukas et al. 2021; Fernández-Arroyo et al. 2010). The interpretation of the spectra and identification of the main compounds were conducted using LabSolutions software (v. 5.109) from Shimadzu.

Trolox Equivalent Antioxidant Capacity Assay

In the Trolox equivalent antioxidant capacity (TEAC) assay, the antioxidant capacity was determined by evaluating the effects of the extracts on the free radical $ABTS^{\cdot+}$ (2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid)). This assay was performed as described by Barrajón-Catalán et al. (Barrajón-Catalán et al. 2011).

Statistical Analysis

Plants with average growth and no abnormalities were selected to determine all growth parameters, the antioxidant capacity, and concentrations of phenolic compounds. The statistical analysis was performed using the Python SciPy library. Analysis of variance was performed, and Duncan's multiple range test was conducted to compare the means. Bartlett tests were performed to assess differences between the treatment groups and determine the homogeneity of variance.

The Shapiro–Wilk test was used to assess the normality of the data. Where appropriate, depending on the independent variables, either one-way or two-way ANOVA was performed. In addition, Tukey's honestly significant difference (HSD) post hoc test with a 0.05 significance level was conducted (Hsu 1996). Since neither the normal distribution nor the homogeneity of the variances were given, a Kruskal–Wallis test was used to assess differences between groups. If statistical significance was observed, the Mann–Whitney U test was performed for each pairwise comparison of the data with the subsequent Bonferroni correction.

Pearson's correlation analysis was performed with interaction terms, considering the correlations with other variables to assess the linear relationships between the

treatments and the various vegetative parameters. The significance of the correlation was evaluated using a two-tailed *p* value with a significance level set at 0.05. The results are reported as the correlation coefficients (r) along with the corresponding *p* values.

The results are presented as the means ± standard errors (SEs). The results of the statistical analysis are provided in tables in the Supplementary Information.

Results

Leaves

The temporal progression of seasons exerts a critical influence on the phenotypic development of leaf characteristics, as shown in Table 2, manifesting distinct patterns across various experimental groups.

In the spring season, peak leaf development was observed across all the treatment groups, with the leaves of the fertilized group displaying the largest mean leaf area (8.55 ± 0.23 cm^2). The significant differences among all the groups suggested an effect of the treatment on leaf development, with a marked increase in leaf expansion due to fertilization. The control group presented a mean leaf area of 7.42 ± 0.15 cm^2, followed by the inoculated (5.77 ± 0.17 cm^2) and fertilized + inoculated groups (5.24 ± 0.09 cm^2), with the latter two exhibiting a significant reduction in the mean leaf area. The maximum leaf area for all the treatment groups was recorded in spring, with a subsequent decline observed as the seasons transitioned into summer and autumn. The lowest leaf surface areas were noted during the summer for FERT and CONT, whereas FERT+INOC presented the greatest value (5.68 ± 0.09 cm^2), followed by INOC. Conversely, the inoculated groups experienced minimal growth in autumn. The mean values observed in autumn were generally lower than those observed in spring and winter across groups, whereas the winter leaves showed a slight increase, indicating the commencement of a new growth cycle in late winter, as this species exhibits continuous growth throughout the year with slowing growth in summer and winter.

As shown in Fig. 1, significant differences in leaf area were detected between all groups in spring and summer, whereas no differences between INOC and FERT+INOC were observed in autumn and winter, and in winter, no differences were detected between CONT and FERT. Observations conducted in winter revealed no significant differences between the control and fertilized treatment groups. Conversely, significant differences were noted for FERT+INOC and INOC compared with CONT and FERT, underscoring the significant effect of inoculation (with or without fertilization) on the leaf area in winter. Across harvest times, only FERT was significantly different from all other groups in spring. However, the fertilized plants presented significant differences at all harvest times, indicating a distinct effect of fertilization, depending on the season. Interestingly, no differences were detected between FERT in summer and FERT+INOC and INOC in autumn and winter. During summer, FERT+INOC had the most pronounced positive effect on leaf area, with significant differences observed in comparison to all other treatments, except for INOC in spring. However, both the combined treatment and inoculation alone significantly influenced the leaf area compared with that of untreated or solely fertilized plants at each harvest time.

In terms of leaf perimeter, although no statistically significant differences were observed among the treatment groups during spring, summer and autumn, the results indicated that

Table 2 Results for leaf parameters measured over one vegetative cycle: leaf area (cm^2), perimeter (cm) and fresh weight per leaf (mg) from leaves harvested in four seasons (data are presented as the means ± SEs of n = 90 plants). The data were analyzed via one-way ANOVA with Tukey's HSDpost hoc test. Means in the same column followed by different letters are significantly different ($p < 0.05$)

	SPRING		SUMMER		AUTUMN		WINTER	
Leaf area (cm^2)								
CONT	7.42	± 0.15^{a}	3.51	± 0.06^{a}	4.28	± 0.10^{a}	4.57	± 0.10^{a}
FERT	8.55	± 0.23^{b}	3.22	± 0.06^{b}	3.53	± 0.06^{b}	4.74	± 0.09^{b}
INOC	5.77	± 0.17^{c}	4.16	± 0.08^{c}	2.62	± 0.06^{c}	3.27	± 0.06^{c}
FERT+INOC	5.24	± 0.09^{d}	5.68	± 0.09^{d}	2.83	± 0.06^{c}	2.87	± 0.07^{d}
Leaf perimeter (cm)								
CONT	11.66	± 0.10^{a}	8.55	± 0.14^{a}	8.58	± 0.13^{a}	9.16	± 0.09^{bc}
FERT	11.35	± 0.13^{a}	8.15	± 0.15^{a}	8.81	± 0.12^{a}	10.28	± 0.12^{ab}
INOC	10.69	± 0.16^{a}	8.91	± 0.17^{a}	7.83	± 0.10^{a}	8.1	± 0.09^{bc}
FERT+INOC	10.1	± 0.10^{a}	5.68	± 0.12^{a}	7.97	± 0.11^{a}	7.63	± 0.09^{c}
Fresh weight per leaf (mg)								
CONT	157.19	± 3.14^{a}	115.33	± 4.23^{a}	133.33	± 1.01^{a}	153.00	± 5.77^{a}
FERT	153.72	± 3.60^{a}	142.31	± 3.09^{b}	153.17	± 2.74^{b}	150.31	± 2.96^{b}
INOC	80.67	± 1.54^{b}	73.69	± 3.94^{c}	64.92	± 2.93^{c}	124.58	± 2.57^{c}
FERT+INOC	65.07	± 1.62^{c}	93.81	± 4.00^{d}	87.2	± 2.57^{d}	96.77	± 3.26^{d}

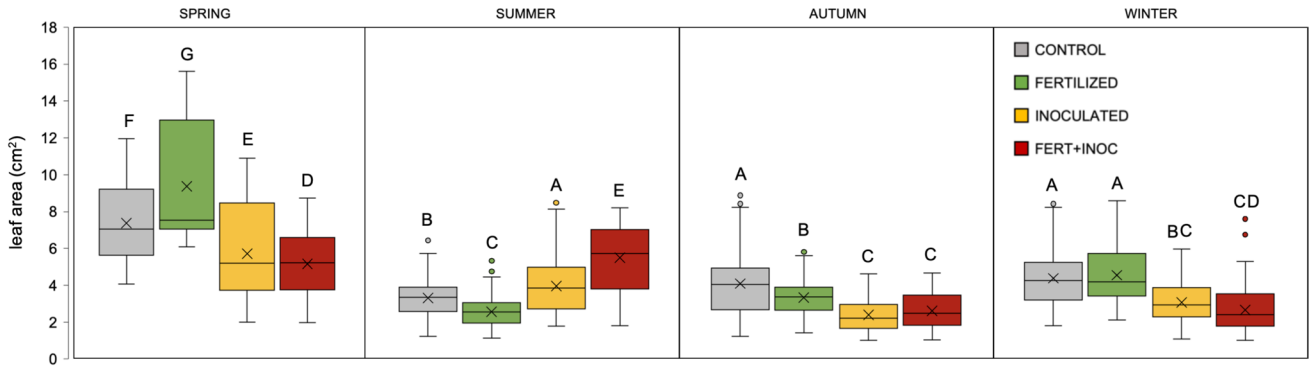


Fig. 1 Boxplots of leaf area according to treatment and season. Each box represents the interquartile range (Q1-Q3), the horizontal line indicates the median, and whiskers show the data range with outliers. The data (n = 90 leaves per group) were analysed by two-way ANOVA (treatment × season) followed by Tukey's HSD post hoc test. Different letters indicate significant differences ($p < 0.05$) among all treatment × season combinations. Identical letters, including those repeated across panels, denote groups that are not significantly different

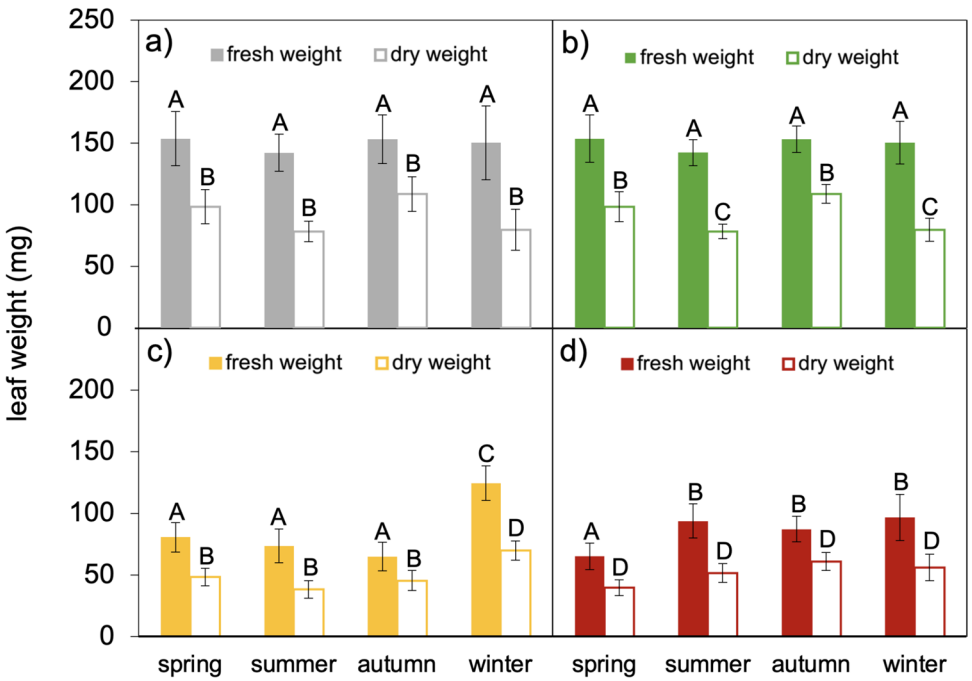


Fig. 2 Fresh and dry weights over one growth cycle: control (**a**), fertilized (**b**), inoculated (**c**) and fertilized + inoculated (**d**). The data are presented as the means ± SEs of n = 90 leaves per group. The data were analysed by two-way ANOVA with Tukey's HSD post hoc test. Homogeneity of variance was analysed by Bartlett's test, and normality was analysed by the Shapiro–Wilk test. The same letters indicate no significant differences between the results ($p \leq 0.05$) within each treatment group

both the different treatments and the various harvest times had significant effects on the leaf perimeter of the plants in winter. In particular, the combined treatment of inoculation and fertilization resulted in a significant ($p = 0.035$) difference from fertilization alone in winter, indicating seasonal influences on plant growth (Table 2).

When the mean leaf fresh weights were compared across seasons (data not shown), FERT presented higher values (149.9 ± 5.26 mg × leaf^{-1}) than CONT (147.6 ± 29.96 mg × leaf^{-1}). In contrast, INOC and FERT+INOC resulted in significantly reduced weights (85.9 ± 26.54 and 85.7 ± 14.33 mg × leaf^{-1}, respectively). As shown in Fig. 2, compared with CONT, fertilization was associated with more uniform leaf development, as evidenced by the reduced variance in fresh weight. Inoculation, either alone or in combination with fertilization, tended to produce lighter leaves. The combination of inoculation and fertilization did not result in significant differences in leaf fresh weight compared with that of inoculated plants, which indicated that the predominant effect was caused by inoculation.

Significant differences in the mean values of dry weight (mg) of leaves were observed between the FERT and the inoculated groups (Fig. 2), confirming the significant influence of inoculation. No significant difference was noted between FERT+INOC and INOC.

The interannual average drying ratio remained constant at 0.61 ± 0.01 across all the groups and was unaffected by the treatments but varied with the harvest timing. The highest drying ratios were measured in autumn (0.71 ± 0.01 for CONT and FERT; 0.70 ± 0.01 for INOC and FERT+INOC),

with the lowest values recorded in winter for CONT and FERT and in summer for INOC and FERT+INOC (ranging from 0.52 ± 0.01 to 0.55 ± 0.03).

The leaf area-to-perimeter ratio, or the leaf index (LI), serves as a measure of leaf shape and efficiency. FERT and CONT presented interannual average LIs of 0.51 ± 0.1 and 0.51 ± 0.11, respectively, in contrast to INOC (0.44 ± 0.11) and FERT+INOC (0.45 ± 0.08). The broadest leaves were formed in spring by FERT (0.81 ± 0.30) and CONT (0.64 ± 0.10), which then significantly narrowed, reaching their minimum in summer. The narrowest leaves were observed in autumn for both inoculated groups, with similar values (0.33 ± 0.07 for INOC and 0.34 ± 0.07 for FERT+INOC). A pattern of narrowing from spring to autumn, followed by a widening (not significant) in late winter, was observed across all groups, with summer leaves tending toward smaller sizes in all groups except for FERT+INOC, which presented the highest ratio (0.54 ± 0.086) in this season (Fig. 3).

The specific leaf area (SLA), a critical measure of leaf efficiency indicating the surface area of a leaf relative to its mass, tended to reach peak values in all groups during spring, as represented in Fig. 3. INOC and FERT+INOC displayed mean interannual SLAs of 10.70 ± 1.87 and 9.28 ± 1.59 m^2/kg, respectively, which were higher than those of FERT (6.96 ± 1.12 m^2/kg) and CONT (6.83 ± 0.74 m^2/kg), indicating thinner leaves with a greater surface area relative to their mass, although these differences were not statistically significant when considering 95% confidence intervals. The interannual fluctuation in the SLA was pronounced within the groups, except for the control group, which showed a nearly linear decrease from spring (8.75 ± 0.70 m^2/kg) to winter (5.12 ± 0.84 m^2/kg). Further, this pattern partly resembled the pattern of the LI. When the different samples were compared, INOC presented a similar SLA trajectory to that of the control group, with a nearly linear decrease in SLAs over the year. The two inoculated groups achieved the highest SLAs (16.37 ± 4.16 m^2/kg for INOC and 16.32 ± 2.61 m^2/kg for FERT+INOC), with the lowest values measured in winter for INOC and CONT and in autumn for FER+INOC, whereas FERT presented the lowest value in summer (4.78 ± 2.17 m^2/kg). The SLA development of FERT paralleled that of FERT+INOC, with maximum values in spring (11.01 ± 2.71 m^2/kg) and minimum values in summer and autumn (4.78 ± 1.25 m^2/kg and 4.87 ± 1.73 m^2/kg, respectively), indicating the influence of fertilization on the leaf pattern.

Since the SLA data were not normally distributed except for the control group, the Kruskal–Wallis test was used to assess overall differences between treatment groups and revealed a statistically significant effect of treatment on

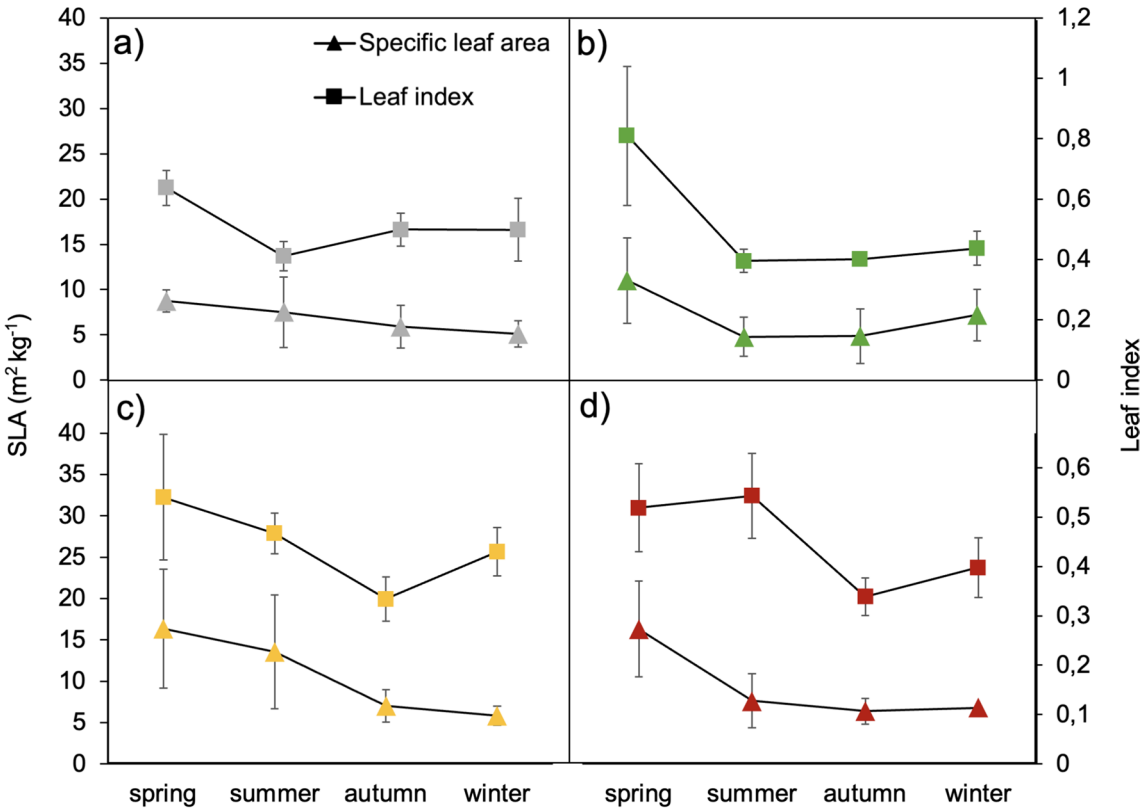


Fig. 3 Comparison of the specific leaf area (SLA) and leaf index (leaf area/leaf perimeter ratio): control (**a**), fertilized (**b**), inoculated (**c**) and fertilized + inoculated (**d**). The data are presented as the means ± SEs of n = 90 leaves per group

SLA. Pairwise comparisons conducted with the Mann–Whitney U test indicated that INOC differed significantly from both CONT and FERT. When SLA values were compared across harvest times, significant seasonal differences were also observed, mainly reflecting higher values in spring compared with later seasons.

Flowering

In the first year of cultivation within the controlled pot environment, the experimental plants presented a mean floral yield of less than two inflorescences per individual (Fig. 4A). In contrast, the subsequent year experienced substantial increases, with the mean floral yield approaching nearly twelve inflorescences per plant (Fig. 4B).

The initial flowering phase was observed uniformly across all treatment groups around mid-March, irrespective of the experimental conditions imposed (Supplementary Figure S1). The peak daily floral yield was observed in the temporal window extending from early to mid-April. Despite the homogeneity in daily floral patterns across the various groups, a discernible variance in the aggregate floral yield was substantiated after the application of the Mann–Whitney U test, which was further adjusted by the Bonferroni correction (Fig. 4). CONT had the greatest floral yield, with 2.84 ± 0.41 inflorescences per plant, followed by INOC, with a yield of 2.12 ± 0.409 inflorescences. FERT and FERT+INOC presented comparable yields, with yields of 1.24 ± 0.25 and 1.34 ± 0.28 inflorescences per plant, respectively. The interval spanning from early May to late June was characterized by a residual floral yield, a period typically associated with the blooming of lateral shoots. Owing to the small size of the specimens, only a marginal fraction of the lateral shoots attained the blooming phase.

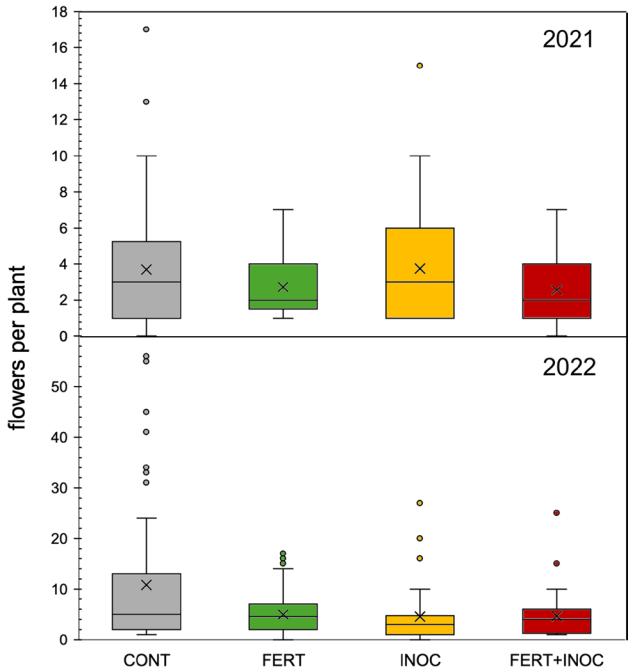


Fig. 4 Boxplots of cumulative flowering per treatment group for the first (2021) and second (2022) years. Since the homogeneity of the variances was not given, the Kruskal–Wallis test was used to assess differences between treatment groups. The Mann–Whitney U test was subsequently performed for each pairwise comparison of the flowering data with the subsequent Bonferroni correction. All the treatment groups differed significantly from each other. In the first flowering stage (2021), all the treatment groups even presented significant differences after the Bonferroni correction; however, in the second year, only FERT and CONT differed significantly from each other and from the other groups according to the corrected Mann–Whitney U test

In the second year of pot cultivation, the onset of flowering in the groups subjected to fertilization (FERT and FERT-INOC) advanced by an average of three weeks compared with that in the first year (Supplementary Figure S1). Conversely, CONT and INOC exhibited an onset of flowering that was only marginally earlier, by less than a week, than it was in the inaugural year, as delineated by the corresponding date of the previous year. An analysis of the cumulative floral yield also revealed statistically significant differences across treatment groups. Nonetheless, subsequent to the application of the Bonferroni correction, these differences were predominantly attributed to inoculation. CONT resulted in the highest mean floral yield per specimen (flowers/single plant), with a value of 16.53 ± 1.96, followed by FERT, with 14.08 ± 0.45. FERT+INOC and INOC resulted in significantly lower mean floral yields, with values of 9.21 ± 0.64 and 7.58 ± 1.05, respectively. FERT was observed to exert a regulatory effect on vegetative development, as evidenced by the reduced variability in floral yield among the fertilized specimens relative to CONT. Conversely, the daily floral yield did not differ significantly, although the blooming of lateral shoots was more pronounced in the second year.

Correlation analyses (Supplementary Figure S8) revealed a significant negative correlation between fertilization in the first year and both the cumulative and daily floral yields, whereas inoculation was negatively correlated solely with the cumulative floral yield. The floral yield data for the second year indicated that inoculation was negatively correlated with both the cumulative and daily floral yields, with a more pronounced correlation observed with the cumulative floral yield. Fertilization did not significantly correlate with the cumulative floral yield; however, a weak negative correlation, which was statistically significant, was observed with the daily floral yield.

Growth Parameters

The investigation of plant growth dynamics under various conditions revealed significant disparities in growth

uniformity. The observed hierarchy in terms of homogeneity was as follows: FERT > FERT+INOC > INOC > CONT. Specifically, FERT presented the most consistent growth patterns, characterized by elongated shoots devoid of lateral branching throughout the researched phase of cultivation. These plants also presented a compact yet expansive morphology. Conversely, CONT displayed a broad spectrum of growth forms and heights, with a marked proliferation of lateral branches (Supplementary Figure S2). Both INOC and FERT+INOC presented growth patterns akin to that of CONT, characterized by robust lateral shoot development. However, FERT+INOC exhibited marginally greater homogeneity than did INOC.

An aspect pertinent to the stress response was the quantification of nodes (leaf levels on a branch), with a lower stress level correlating with a greater leaf support capacity. The annual mean node count was highest in FERT and CONT, at 8.33 ± 2.02 and 8.14 ± 1.79, respectively. In contrast, INOC and FERT+INOC manifested significantly reduced node counts of 6.1 ± 1.29 and 5.76 ± 1.23, respectively.

No statistically significant differences were observed in maximum height growth (the tallest shoots of n = 33 individuals per treatment group with regular growth, Fig. 5a). CONT exhibited relatively uniform height growth, with an average plant height of 43.4 cm and extremities between 35 and 48 cm. FERT attained an average height of 46.0 cm, ranging from 35 to 53 cm. The INOC plants were marginally shorter than the CONT plants were, achieving an average height of 38.8 cm, with a maximum height of 46 cm and a minimum height of 32 cm. FERT+INOC displayed an average height of 39.3 cm, with a maximum height of 45 cm and a minimum height of 33 cm.

During the initial year, trunk perimeter measurements did not differ significantly across treatment groups (Fig. 5b). However, in the second year, both fertilized groups presented a markedly larger trunk perimeter, with fertilized plants achieving the greatest circumference of 32.57 ± 0.21 cm, resulting in the least variation among individuals. No significant differences in trunk perimeter were noted between the control and both inoculated groups.

Distinct variations in branch growth among the treatment groups were evident, with INOC impeding the growth of both the main and lateral branches, whereas FERT positively impacted plant height and growth velocity. FERT+INOC yielded intermediate results in terms of shoot growth and development pace. No significant differences were noted in lateral shoot growth between CONT and FERT, although the main shoots of fertilized plants exhibited significantly faster growth (Fig. 6).

Interestingly, the maximum height of the plants was not significantly affected by the different growth conditions of the shoots. This result is mainly because the inoculated plants developed their side shoots close to the terminal umbelliform cyme of the main shoot, whereas those of CONT and FERT developed their side shoots further down.

Plant mortality rates, as presented in Fig. 7, highlighted seasonal inflection points, suggesting the influence of environmental or external factors across all treatment groups. No plant deaths were recorded in spring, and no deaths were observed among the INOC and FERT+INOC groups during summer. However, mortality rates of 9% and 5% were observed for CONT and FERT, respectively, at the end of summer, with the majority of deaths occurring in autumn and winter. In the summer of the second year, FERT and CONT presented the highest survival rates, with values of 60% and 52%, respectively. In contrast, the survival rates of the INOC and FERT+INOC groups were only 28% and 40%, respectively.

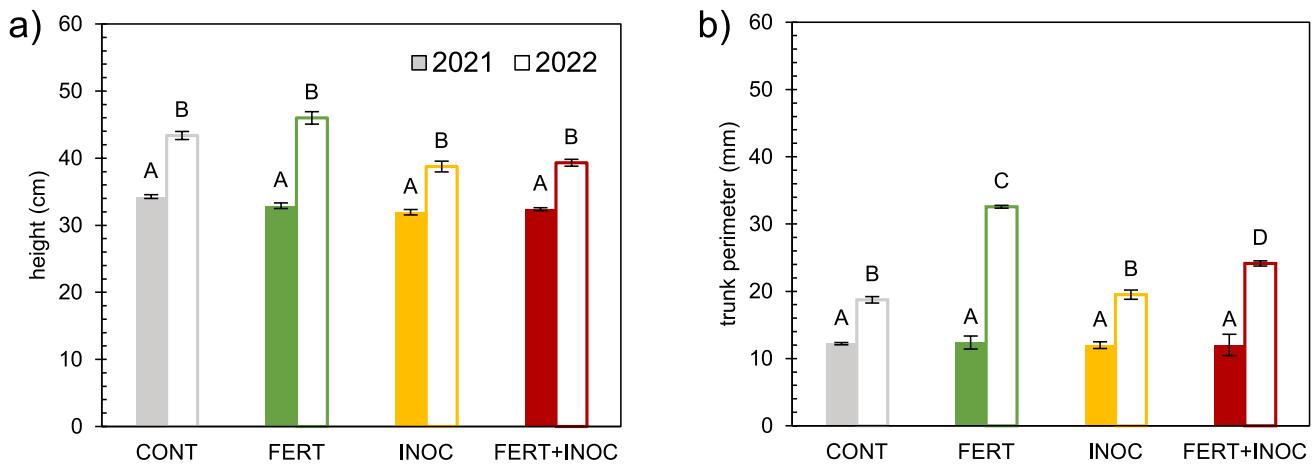


Fig. 5 Mean height of plants in the first and second years of growth in pots (**a**) and trunk perimeter at the base (**b**). The data are presented as the means ± SEs of n = 33 individuals per treatment group. The data were analysed by two-way ANOVA with Tukey's HSD post hoc test. Homogeneity of variance was analysed by Bartlett's test, and normality was analysed by the Shapiro–Wilk test. The same letters indicate no significant differences between the results ($p > 0.05$)

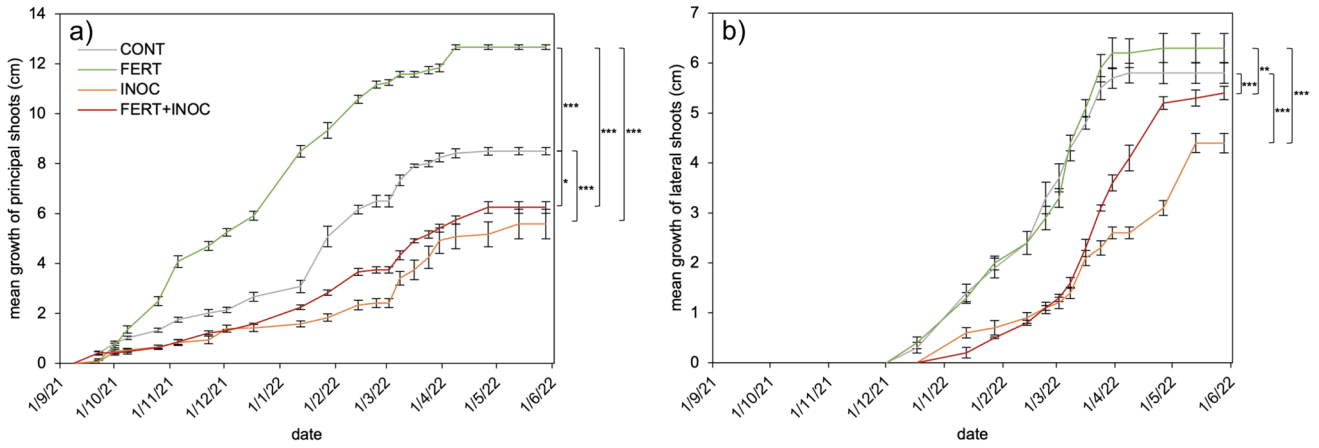


Fig. 6 Mean growth of the principal shoots (**a**) and lateral shoots (**b**) measured between the shoot apical meristem and first leaf node during the main growth season (n = 15 shoots per treatment group ± SE). The data were analysed by one-way ANOVA with Tukey's HSD post hoc test. Statistical significance is represented by *** for $p \leq 0.001$, ** for $0.001 < p \leq 0.01$, and * for $0.01 < p \leq 0.05$

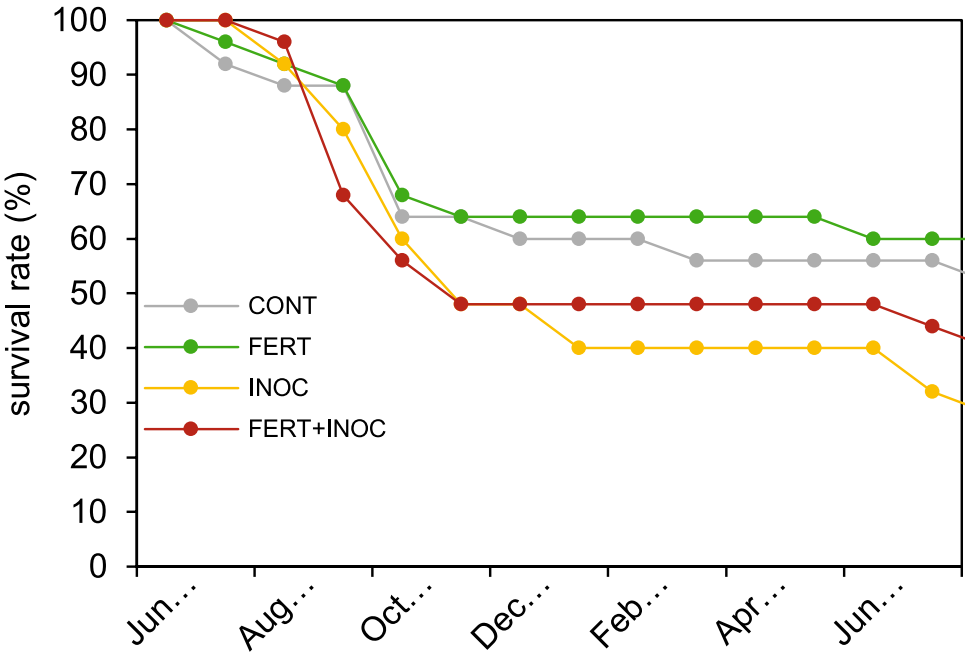


Fig. 7 Survival rates of all the treatment groups during pot culture from December 2020 to July 2022

Deceased plants were characterized by having 3 or fewer leaf nodes with living leaves, equating to a total of 6 or fewer leaves on each of all the shoots. Plants exhibiting signs of illness survived if they retained leaves on more than 3 leaf nodes.

The correlation analysis did not reveal a significant relationship between plant mortality and the total phenol content or antioxidant capacity. However, moderately to strongly significant correlations, both positive and negative, were observed with several of the analysed polyphenols. Notably, dehydrated tergallic C-glucoside, prodelphinidin B2-3'O-gallate and myricetin hexoside demonstrated strong and very significant correlations with plant survival rates. On the other hand, quercetin glucoside and ellagic acid 7-xiloside were significantly strongly correlated with plant death (Fig. 8).

Total Phenolic Content

The quantification of the TPC, as shown in Table 3, was conducted using the Folin–Ciocalteu assay. This analysis revealed a variation in the TPC across extracts, ranging from 3.72 to 38.85 mg GAE/g extract, with an aggregate mean value of 18.48 mg GAE/g extract observed in CONT. FERT presented phenolic concentrations ranging from 3.97 to 36.73 mg GAE/g extract, resulting in a mean value of 18.45 mg GAE/g extract. INOC presented phenolic concentrations ranging from 17.86 to 35.21 mg GAE/g

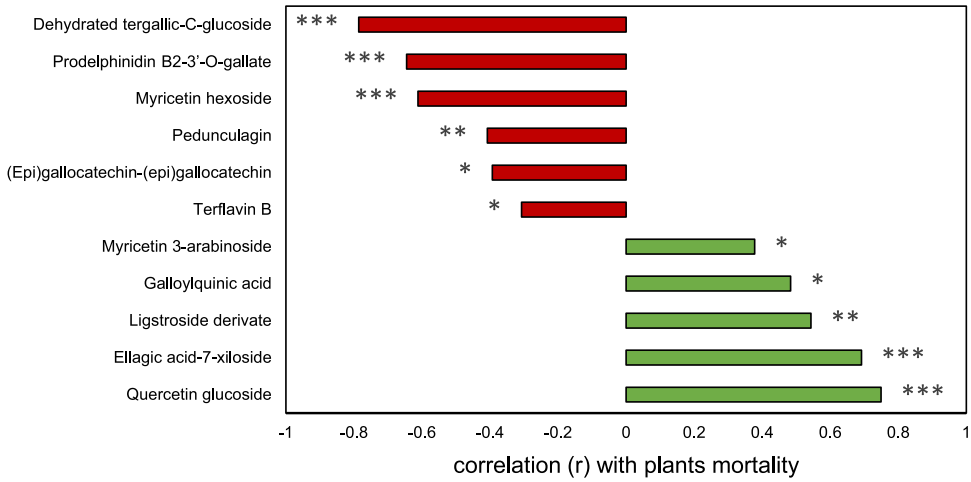


Fig. 8. Pearson's correlation coefficients (r) between plant mortality and the analyzed polyphenols during one vegetative cycle. Only statistically significant (**p** < 0.05) correlations are presented: *** for $p \leq 0.001$, ** for $0.001 < p \leq 0.01$, * for $0.01 < p \leq 0.05$. Positive values indicate that a measured polyphenol correlates with plant death; negative values correlate with the survival of the plants

Table 3 Results for the total phenolic content of *C. albidus* aqueous extracts from leaves harvested in four seasons determined using the Folin–Ciocalteu assay and presented in gallic acid equivalents (GAEs) per gram of plant extract (data are presented as the means ± SEs of n = 3 samples). The data were analysed by one-way ANOVA with Tukey's HSD post hoc test. Means in the same column followed by different letters are significantly different ($p < 0.05$)

	SPRING		SUMMER		AUTUMN		WINTER	
CONT	8.89	± 0.61 ^{a,b}	38.85	± 0.31 ^{a}	3.72	± 1.41 ^{a}	22.46	± 1.95 ^{a}
FERT	4.87	± 1.7 ^{a}	28.21	± 4.45 ^{a}	3.97	± 1.32 ^{a}	36.73	± 1.73 ^{b}
INOC	21.10	± 1.07 ^{a,b}	33.59	± 2.24 ^{a}	17.86	± 1.78 ^{b}	35.21	± 0.94 ^{b}
FERT+INOC	13.34	± 1.41 ^{b}	36.32	± 4.58 ^{a}	12.75	± 2.62 ^{b}	31.4	± 1.29 ^{b}

Table 4 Results of the TEAC assay of *C. albidus* extracts from leaves harvested in four seasons, presented as mmol equivalent Trolox/100 g extract (data are presented as the means ± SEs of n = 3 extracts). The data were analysed via one-way ANOVA with Tukey's HSD post hoc test. Means in the same column followed by different letters are significantly different ($p < 0.05$)

	SPRING		SUMMER		AUTUMN		WINTER	
CONT	9.92	± 0.23 ^{a}	8.42	± 0.8 ^{a}	3.93	± 0.72 ^{a}	7.09	± 0.16 ^{a}
FERT	6.34	± 1.16 ^{b}	8.44	± 0.08 ^{a}	4.05	± 0.54 ^{a}	7.84	± 0.05 ^{a}
INOC	8.4	± 0.94 ^{ab}	8.24	± 0.6 ^{a}	15.74	± 0.43 ^{b}	6.9	± 0.67 ^{a}
FERT+INOC	10.27	± 0.15 ^{a}	9.78	± 0.95 ^{a}	11.8	± 0.9 ^{c}	7.63	± 0.21 ^{a}

extract, with a calculated mean of 26.94 mg GAE/g extract. FERT+INOC presented phenolic levels ranging from 12.75 to 36.32 mg GAE/g extract, with an overall mean of 23.45 mg GAE/g extract. Notably, the peak phenolic concentration was identified in CONT during summer, whereas the lowest value was also observed in CONT but in autumn, at 3.72 mg GAE/g extract.

In general, all the treatments consistently yielded the highest TPC, particularly in the summer and, to a lesser extent, in the winter, which confirms the strong influence of high and low temperatures on polyphenol biosynthesis, as observed in other studies (Balasooriya et al. 2019; Zhou et al. 2018; Sharma et al. 2019). A comparative analysis across the four treatment groups revealed a descending order of average interannual TPC as follows: INOC > FERT+INOC > CONT > FERT, with CONT and FERT showing nearly equal concentrations.

The statistical analysis revealed significant differences in spring, autumn and winter among several different treatment groups. The most significant differences were found in autumn between the inoculated plants (INOC and FERT+INOC) and the other treatment groups. In the summer, no differences were detected between any of the groups, whereas in the winter, the differences were limited to CONT and the remaining plants. Interestingly, for all seasons, no significant differences were observed between INOC and FERT+INOC. Furthermore, no significant correlations with TPC were observed for any of the treatments.

Antioxidant Capacity

A TEAC assay was employed to evaluate the antioxidant potential of *C. albidus*, as described in Table 4, with a focus on the reactional variability across the various treatment groups throughout the designated harvest periods.

The total antioxidant activity exhibited variability across the treatment groups. For CONT, the activity ranged from 3.93 to 9.92 mmol TE/100 g extract, with an interannual

mean of 5.21 mmol TE/100 g extract. FERT displayed antioxidant activity ranging from 4.05 to 8.44 mmol TE/100 g extract, yielding an overall mean of 6.67 mmol TE/100 g extract. INOC manifested a total antioxidant capacity ranging from 6.90 to 15.74 mmol TE/100 g extract, with a mean value of 9.82 mmol TE/100 g extract. Furthermore, FERT+INOC exhibited antioxidant capacities between 7.63 and 11.8 mmol TE/100 g extract, with a mean value closely paralleling that of INOC at 9.87 mmol TE/100 g extract.

In general, the inoculated treatment groups, irrespective of the fertilization status, consistently yielded the highest TEAC values, particularly in autumn, indicating a temporal increase in antioxidant capacity attributed to inoculation toward winter. A comparative analysis of the interannual average antioxidant capacities revealed a descending order of mean antioxidant capacity as follows: FERT+INOC > INOC > CONT > FERT. FERT+INOC and INOC showed almost the same results, 39.48 and 39.28 mmol TE/100 g extract, respectively. INOC demonstrated a markedly superior antioxidant capacity in autumn, approximately quadrupling that of CONT. These results were significantly different from each other, with inoculation having the most significant effect on the antioxidant capacity ($p = 6.83 \times 10^{-5}$) and fertilization in combination with inoculation enhanced this effect ($p = 0.015$). However, significant differences were observed only in spring and autumn when the combined results of the same harvest times across all the treatment groups were considered. In contrast, no such differences were found between summer and winter or between individual treatment groups during those seasons. The most significant differences were observed in autumn between the inoculated plants (INOC and FERT+INOC) and those in the other treatment groups. Further analysis confirmed these observations, with the antioxidant capacity correlated with INOC ($r = 0.501$, $p = 0.0003$) and FERT+INOC ($r = 0.306$, $p = 0.03$). In spring, the differences were less pronounced and could be found only between FERT and FERT+INOC and between FERT and CONT.

Correlation Between the Antioxidant Capacity and TPC

The correlation between the TPC and the antioxidant capacity was examined to gain a deeper understanding of the relationship between these two parameters in different extracts.

The correlations between the TPC and antioxidant capacity were generally not significant across the harvest times. However, in spring, FERT+INOC showed a very strong correlation ($r = 0.99$, $p = 0.018$). With respect to the treatments only, a significant positive correlation was observed between the antioxidant capacity and TPC for FERT ($r = 0.663$, $p = 0.026$). When individual compounds were examined, predominantly weak and statistically nonsignificant correlations with antioxidant capacity were found (data not shown).

Extract Composition

The chemical composition of the extracts was evaluated by UHPLC–MS as described in the Methods section. Overall, depending on the harvest time, 30 major peaks were observed, representing 92% to 100% of the total UHPLC peak area. Among these peaks, which corresponded up to 16 compounds divided into several isomers, five flavonols (myrecitin 3-O-(2″-O-galloyl)-hexoside, myricetin hexoside, myricetin 3-arabinoside, quercetin glucoside, kaempferol diglycoside), two flavanols ((-)-(epi)gallocatechin-(epi)gallocatechin dimer, prodelphinidin B2-3'-O-gallate), five ellagitannins (dehydrated tergallic-C-glucoside, punicalagin, pedunculagin, terflavin B, ellagic acid-7-xiloside), three phenolic acids (coumaroylquinic acid, galloylquinic acid, caffeoyl hexose), and one secoiridoid glycoside (ligstroside derivate) were identified (Table 5). Extracts from all the treatment groups had an annual average of 21 peaks. Most peaks were observed in the extracts obtained in spring from FERT, with 30 detected compounds, followed by CONT, with 29. The fewest peaks were found in winter for INOC, with only 17 identified compounds. The relative areas of the peaks identified as isomers were summarized with the corresponding main compounds and are not shown individually Fig. 8.

Phytochemicals

The results of the relative distribution of the studied polyphenols obtained via UHPLC–MS are shown in Table 5 and Fig. 9. The compounds organized by substance class were significantly influenced by the harvest time, with the exception of ellagitannins. With respect to the treatments, among the substance classes analysed, significant differences between the treatment groups were observed only for ellagitannins ($p = 0.0082$).

Significant differences in concentration related to harvest time were observed for all individual compounds, except for myricetin 3-arabinoside (Table 5). Regarding the experimental treatments, no significant differences were found in the measured concentrations, with the sole exceptions of caffeoyl hexose and pedunculagin. Furthermore, a comparison of individual compounds across treatments throughout the vegetative cycle using the Kruskal–Wallis test with Bonferroni correction revealed no statistically significant differences.

Compound concentrations were analyzed by averaging all treatment groups for each season and, conversely, by

Table 5 Effects of the treatment (CONT, FERT, INOC, and FERT+INOC) and harvest time (spring, summer, autumn, and winter) on the composition of *C. albidus* aqueous extracts. UHPLC–MS retention times (Rt) of the 16 evaluated peaks, mass data (base ions in both positive and negative mode [M−H]+ and [M−H]−), main fragment ions (MS/MS), peak identification and relevance of compounds (range of relative area percentages at 280 nm). The relative area is represented by the mean value ± standard deviation of three replicates. The data were analysed by one-way ANOVA with Tukey's HSD post hoc test. Statistical significance is shown separately for each treatment group and harvest time. The same letters within each observation (treatment, harvest time) are not significantly different ($p \leq 0.05$)

Proposed Compound	[M-H]−(m/z)	MS/MS (m/z)	RT (min)	Literature	Relative area depending on treatment (%)				Relative area depending on harvest time (%)			
					CONT	FERT	INOC	FERT+INOC	SPRING	SUMMER	AUTUMN	WINTER
Pedunculagin	783	275, 301, 451, 481	10.1	Fischer et al. 2011)	2.98±2.14 ^{a}	2.65 ± 1.77 ^{bc}	3.32 ± 2.57 ^{ab}	3.59 ± 2.49 ^{c}	2.79 ± 1.83 ^{a}	5.23 ± 1.40 ^{b}	2.02 ± 1.05 ^{a}	2.22 ± 2.43 ^{a}
(-)-(Epi)gallocatechin-(epi)gallocatechin dimer	609	423, 441	10.6	Barrajón-Catalán et al. 2011; Santagati et al. 2008)	2.69±1.75 ^{a}	3.06 ± 2.53 ^{a}	5.04 ± 4.40 ^{a}	3.26 ± 1.57 ^{a}	1.75 ± 1.97 ^{a}	5.99 ± 2.18 ^{b}	3.08 ± 0.53 ^{a}	1.68 ± 1.47 ^{a}
Prodelphinidin B2-3'-O-gallate	761	423, 609	10,6	Tomás-Menor et al. 2015)	5.94±8.0 ^{a}	6.31 ± 5.74 ^{a}	3.57 ± 2.35 ^{a}	6.44 ± 8.80 ^{a}	13.67 ± 5.17 ^{a}	4.68 ± 3.75 ^{b}	1.37 ± 0.35 ^{bc}	1.71 ± 0.76 ^{bc}
Coumaroylquinic acid	337	161	10,7	Clifford et al. 2003)	11.71±7.56 ^{a}	10.51 ± 5.52 ^{a}	11.53 ± 10.03 ^{a}	8.37 ± 3.92 ^{a}	8.13 ± 2.15 ^{a}	6.33 ± 4.38 ^{ab}	17.92 ± 6.40 ^{c}	5.59 ± 2.21 ^{ab}
Terflavin B	783	451, 631	11,7	Salih et al. 2021; Singh et al. 2016)	1.77±1.27 ^{a}	2.11 ± 1.94 ^{a}	2.32 ± 2.01 ^{a}	3.57 ± 3.15 ^{a}	3.63 ± 2.73 ^{a}	1.16 ± 2.11 ^{b}	1.94 ± 0.45 ^{ab}	1.72 ± 0.71 ^{ab}
Punicalagin	1083	301, 601, 781	14.2	Barrajón-Catalán et al. 2011; Barros et al. 2013; Maggi et al. 2016; Moreira et al. 2017)	2.66±2.64 ^{a}	2.56 ± 2.19 ^{a}	3.13 ± 2.91 ^{a}	0.85 ± 0.61 ^{a}	4.38 ± 2.78 ^{a}	0.00 ± 0.00 ^{b}	1.01 ± 0.80 ^{b}	1.39 ± 0.44 ^{b}
Galloylquinic acid	343	151, 169, 191	14,5	Sannomiya et al. 2005)	3.65±3.24 ^{a}	6.44 ± 3.01 ^{a}	7.34 ± 4.62 ^{a}	6.29 ± 3.87 ^{a}	3.07 ± 2.02 ^{a}	1.83 ± 1.96 ^{ab}	11.65 ± 9.16 ^{c}	7.46 ± 2.81 ^{ac}
Cafeoyl-hexose	341	179	14,6	Maatta et al. 2001)	6.39±2.82 ^{a}	6.09 ± 2.92 ^{ab}	8.24 ± 3.53 ^{b}	6.92 ± 3.25 ^{ab}	7.91 ± 2.26 ^{abc}	6.03 ± 2.26 ^{b}	10.40 ± 3.71 ^{c}	6.14 ±3.09 ^{abd}
Ligstroside derivate	453	151, 169, 313	14,9	Cardoso et al. 2011)	5.39±2.49 ^{a}	6.42 ± 1.52 ^{a}	5.13 ± 3.21 ^{a}	6.01 ± 2.17 ^{a}	5.70 ± 2.02 ^{a}	3.51 ± 2.19 ^{a}	6.23 ± 1.63 ^{ab}	9.22 ± 4.32 ^{b}
Putative Myricetin 3-O-(2″-O-galloyl)-hexoside or myricetin-galactoside-gallate	631	317, 479	17,1	Pereira et al. 2017; Saldanha et al. 2013)	3.22±0.62 ^{a}	4.41 ± 2.36 ^{a}	3.25 ± 1.83 ^{a}	3.46 ± 2.37 ^{a}	2.70 ± 3.37 ^{a}	4.42 ± 0.95 ^{b}	2.82 ± 0.93 ^{c}	3.20 ± 1.71 ^{d}
Myricetin hexoside	479	179, 271, 316	17,7	Maatta et al. 2001)	11.28±6.79 ^{a}	9.39 ± 6.03 ^{a}	10.21 ± 3.90 ^{a}	8.44 ± 3.60 ^{a}	11.16 ± 2.13^{a}	15.91 ± 3.74 ^{b}	8.83 ± 4.84 ^{ac}	5.85 ± 3.25 ^{c}
Dehydrated tergallic-C-glucoside	615	301	18,3	García-Beneytez et al. 2003)	4.52±3.96 ^{a}	5.14 ± 2.46 ^{a}	4.38 ± 3.21 ^{a}	6.12 ± 4.00 ^{a}	7.19 ± 1.97 ^{a}	8.46 ± 1.60 ^{a}	3.82 ± 2.88 ^{b}	2.88 ± 0.90^{b}
Myricetin 3-arabinoside	449	179, 271, 316	19.2	Tomás-Menor et al. 2015)	7.77±1.48 ^{a}	10.03 ± 5.90 ^{a}	9.53 ± 2.93 ^{a}	11.87 ± 3.25 ^{a}	8.03 ± 2.76 ^{a}	9.26 ± 2.39 ^{a}	10.48 ± 5.46 ^{a}	13.26 ± 7.76 ^{a}

Table 5 (continued)

Proposed Compound	[M-H]−(m/z)	MS/MS (m/z)	RT (min)	Literature	Relative area depending on treatment (%)				Relative area depending on harvest time (%)			
					CONT	FERT	INOC	FERT+INOC	SPRING	SUMMER	AUTUMN	WINTER
Quercetin glucoside	463	151, 301	21,4	Tomás-Menor et al. 2015)	8.79±7.22 [a]	14.39 ± 11.86 [a]	12.49 ± 7.39 [a]	15.15 ± 9.02 [a]	5.55 ± 3.61 [a]	8.59 ± 5.59 [a]	8.97 ± 2.63 [a]	22.49 ± 6.72 [b]
Ellagic acid-7-xiloside	433	301	26.6	Soong and Barlow 2005)	12.81±6.66 [a]	9.69 ± 3.63 [a]	14.81 ± 8.99 [a]	12.23 ± 3.98 [a]	7.07 ± 2.11 [a]	9.33 ± 1.72 [a]	14.89 ± 5.83 [b]	18.23 ± 7.02 [b]
Kaempferol diglycoside	593	285, 447	37,1	Tomás-Menor et al. 2015)	6.94±2.83 [a]	6.45 ± 3.34 [a]	8.27 ± 3.09 [a]	6.79 ± 1.05 [a]	7.29 ± 2.47 [a]	9.26 ± 1.46 [ab]	4.65 ± 1.75 [c]	7.13 ± 3.46 [ac]

averaging all harvest times for each individual treatment group (Table 5) to provide a detailed evaluation of both factors. Boxplots illustrating the cumulative concentrations of all studied compounds throughout the vegetative cycle across different treatments are presented in Supplementary Figures S3 to S7.

Flavan-3-ols

The seasonal average concentrations of flavan-3-ols (represented by blue bars in Fig. 9) in all the treatment groups were highest in spring. Specifically, prodelphinidin B2-3'-O-gallate showed a significant peak in spring, whereas (-)-(epi) gallocatechin-(epi)gallocatechin was either present at low or undetectable concentrations during this time but reached its maximum in summer (peak 2). By summer, the concentration of prodelphinidin B2-3'-O-gallate decreased significantly, continuing to decrease through autumn, with a slight increase observed in winter. In contrast, (-)-(epi)gallocatechin-(epi)gallocatechin displayed less variation throughout the year, peaking in summer, slightly decreasing in autumn, and maintaining similar concentrations in both spring and winter.

The highest average concentrations of prodelphinidin B2-3'-O-gallate across all harvest periods were observed under fertilized conditions (FERT and FERT+INOC), with comparable values for both treatment groups, while the lowest levels were observed for the INOC treatment group. For (-)-(epi)gallocatechin-(epi)gallocatechin, the highest average concentration was observed for INOC, and the lowest was observed for CONT. However, none of these differences were statistically significant.

Interestingly, significant ($p < 0.05$) differences were observed between individual treatments and harvest periods for flavan-3-ols. For example, no (-)-(epi)gallocatechin-(epi)gallocatechin was detected in the CONT and INOC treatment groups in spring, and it was also absent from the INOC treatment group in winter, whereas prodelphinidin B2-3'-O-gallate was absent in the FERT+INOC treatment group during summer.

Throughout the year, the correlation between the concentrations of the two flavan-3-ols was negligible. However, a strong negative correlation was observed between the harvest period and the concentration of prodelphinidin B2-3'-O-gallate ($r = -0.990$, $p = 1.28 \times 10^{-3}$). As the year progressed from spring to winter, the concentration of this compound decreased significantly, regardless of the treatment applied.

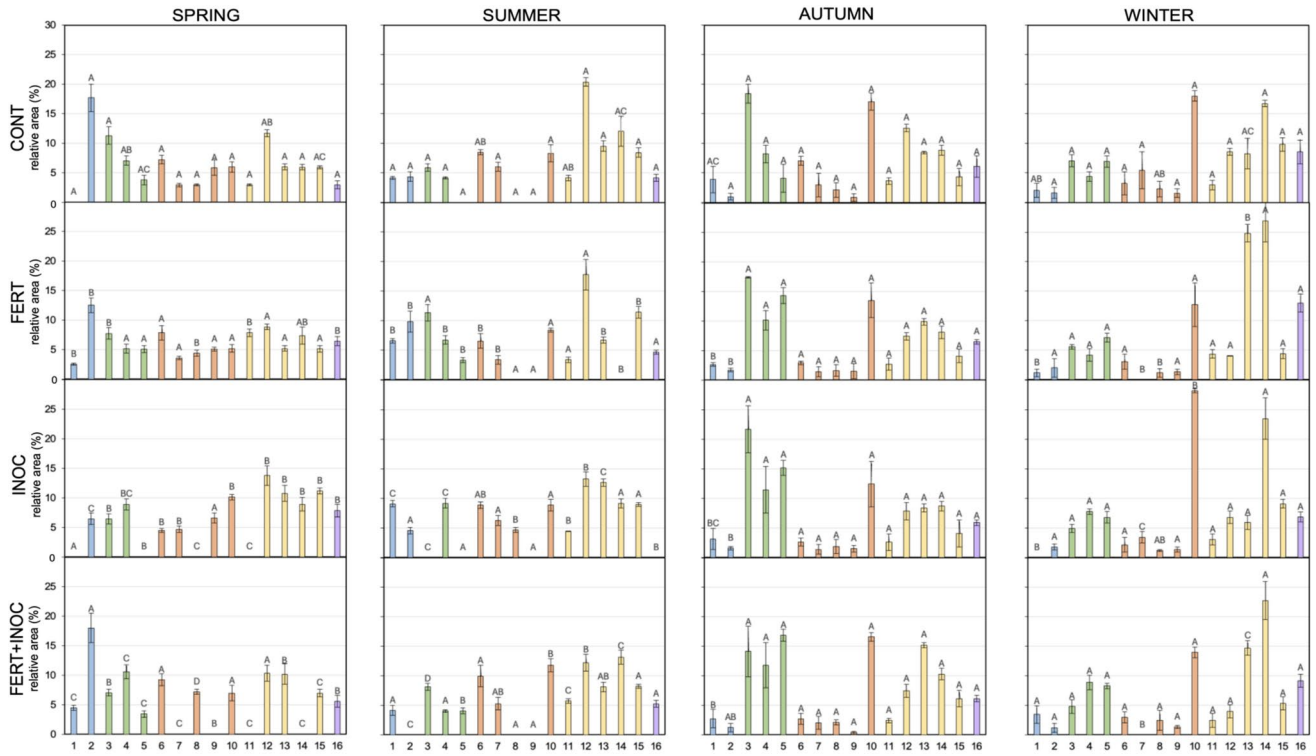


Fig. 9 Seasonal profiles of the relative distributions of the studied polyphenols extracted with water according to the relative area of each UHPLC–MS profile. Blue: flavan-3-ols; green: phenolic acids; brown: ellagitannins; yellow: flavonols; purple: secoiridoid glycosides. Compounds: 1. (-)-(epi)gallocatechin-(epi)gallocatechin dimer, 2. prodelphinidin B2-3'-O-gallate, 3. coumaroylquinic acid, 4. caffeoyl hexose, 5. galloylquinic acid, 6. dehydrated tergallic-C-glucoside, 7. pedunculagin, 8. terflavin B, 9. punicalagin, 10. ellagic acid-7-xiloside, 11. myrecitin-3-O-(2″-O-galloyl)-hexoside, 12. myricetin hexoside, 13. myricetin 3-arabinoside, 14. quercetin glucoside, 15. kaempferol diglycoside, 16. ligstroside derivative. The relative area is represented by the mean value ± standard deviation of three replicates. The data were analysed by one-way ANOVA with Tukey's HSD post hoc test. Statistical significance is shown between the same compounds for each harvest time. The same letters within each observation (subplot) are not significantly different ($p \leq 0.05$)

Phenolic Acids

Except for caffeoyl hexose (peak 8), the analysed phenolic acids (green bars, Fig. 9) exhibited significant seasonal fluctuations ($p < 0.0001$) characterized by a pronounced maximum in autumn and a significant minimum in winter.

When all the treatment groups were averaged across seasons, the analysed phenolic acids exhibited several notable patterns. For coumaroylquinic acid (peak 4), no clear yearly trend emerged. Its concentration slightly decreased from spring to summer, increased significantly from summer to autumn and then decreased sharply to its lowest average concentration in winter. The concentration of galloylquinic acid (peak 7) decreased during the seasons with longer daylight (spring to summer) but increased significantly in the seasons with shorter daylight, peaking in autumn before decreasing again in late winter. Caffeoyl hexose concentrations were more stable throughout the year, with a significant peak in autumn.

The harvest time was strongly positively correlated with the concentrations of coumaroylquinic acid (r = 0.898, p = 0.038) and galloylquinic acid (r = 0.988, $p = 1.58 \times 10^{-3}$) but strongly negatively correlated with the concentration of dehydrated tergallic C-glucoside (r = -0.949, p = 0.013).

When the average concentration measured in all harvest periods for each treatment group was considered, only caffeoyl hexose was significantly different (p = 0.01) between the INOC treatment and CONT groups. However, no significant differences were observed between INOC and FERT+INOC or between INOC and FERT. The concentrations of other phenolic acids did not differ significantly across the treatment groups.

Significant differences were observed between the treatments and harvest periods for the concentrations of some phenolic acids. Compared with the other treatments, coumaroylquinic acid presented significantly higher concentrations in CONT in spring, although the differences in other seasons were not significant, except for INOC in summer, where coumaroylquinic acid was not detected. The concentration of caffeoyl hexose was significantly different between the treatment groups in spring and summer but not in autumn and winter, with both the INOC and FERT+INOC

treatment groups presenting increased concentrations of this compound, except for FERT+INOC in summer.

Correlation analyses between individual phenolic acids revealed a significant positive relationship between the concentrations of caffeoyl hexose and coumaroylquinic acid (r = 0.459, $p = 2.89 \times 10^{-3}$). While FERT and FERT+INOC showed no significant correlations with substance concentrations (with all correlations being either close to zero or weakly negative), a significant positive correlation was observed between INOC and the caffeoyl hexose concentration (r = 0.397, $p = 0.0084$).

Ellagitanins

In general, the concentrations of ellagitannins (brown bars in Fig. 9) increased over the course of the vegetative cycle. The average concentrations in all treatment groups per season reached their maximum values in late winter, primarily due to an increase in the ellagic acid-7-xyloside concentration (peak 15). In contrast, the lowest values were observed in summer, mainly because terflavin B (peak 5) was not detected in all the treatment groups except for INOC. The level of dehydrated tergallic-C-glucoside (peak 12) exhibited an increasing trend through spring and summer but decreased significantly in autumn, reaching its lowest concentration in winter. The concentration of pedunculagin (peak 1) fluctuated throughout the year, with no consistent trend, although higher concentrations were generally observed during the warmer seasons, peaking significantly in summer. Its lowest concentrations appeared sporadically, especially in autumn. The concentrations of punicalagin (peak 6) exhibited pronounced seasonal fluctuations, with its highest levels typically observed in spring. Its concentrations decreased significantly through autumn and winter and were not detected during summer, indicating a clear seasonal pattern in its biosynthesis. Terflavin B exhibited a developmental pattern comparable to that of punicalagin, characterized by peak concentrations in spring followed by a significant decline in summer, suggesting a robust response to environmental or physiological shifts. Conversely, ellagic acid-7-xyloside concentrations showed a progressive increase throughout the year, with higher levels recorded during the colder months. Specifically, levels reached a minimum in spring, rose gradually through summer, and peaked significantly in autumn before reaching their maximum values in winter.

In terms of treatment, significant differences were found in the seasonal accumulation of ellagitannins. However, these differences were primarily due to pedunculagin, which responded significantly to fertilization at relatively low concentrations. The highest values were recorded in INOC, followed by CONT. The concentrations of other ellagitannins did not differ significantly among the treatment groups. Significant differences between individual treatment groups and harvest times were also observed for the concentrations of ellagitannins. Pedunculagin was not detected in FERT+INOC during spring and winter or in FERT during winter. Terflavin B was absent from INOC in spring but present in summer across all the treatment groups except INOC. Punicalagin was not detected in FERT+INOC in spring or in any treatment group during summer. Ellagic acid-7-xyloside was present across all the treatment groups, with the highest concentration recorded in INOC during winter, which was five times greater than the value measured in FERT during spring. All the measured ellagitannins exhibited significant, strong correlations with the harvest time. The correlations were strongly negative for dehydrated tergallic C-glucoside, pedunculagin, punicalagin, and terflavin B, with very significant values approaching 1. However, ellagic acid-7-xyloside was positively correlated with the harvest time. Moderate positive correlations were detected between terflavin B and punicalagin concentrations (r = 0.697, $p = 2.18 \times 10^{-4}$) and between punicalagin and pedunculagin concentrations (r = 0.603, $p = 6.23 \times 10^{-3}$) during the vegetative cycle. Negative correlations were observed between ellagic acid-7-xyloside and punicalagin concentrations (r = -0.599, $p = 2.8518 \times 10^{-4}$), as well as between ellagic acid-7-xyloside and terflavin B concentrations (r = -0.598, $p = 6.15 \times 10^{-4}$), indicating a moderate inverse relationship. No significant correlations were observed for the other compounds.

Flavonols

The quantified flavonols (yellow bars, Fig. 9) generally exhibited significant seasonal variation, with primary peaks occurring in summer and secondary, less pronounced increases in winter. However, quercetin glucoside (peak 14) and myricetin arabinoside (peak 13) were present at the highest concentrations in winter. The concentrations of most of the compounds did not differ significantly between spring and autumn.The concentration of myricetin-3-O-(2″-O-galloyl)-hexoside (peak 10) displayed a relatively stable pattern, starting with a lower level in spring and gradually decreasing throughout the year. The concentration of myricetin hexoside (peak 11) exhibited significant fluctuations, with concentrations increasing during the warmer months and peaking in summer, followed by the lowest level in winter. The concentration of myricetin 3-arabinoside (peak 13) also varied, with an increasing trend toward the end of the year, the lowest concentration occurring in spring and the maximum concentration occurring in winter. Similarly, quercetin glucoside concentrations displayed pronounced fluctuations, with the highest concentration occurring in

winter and the lowest occurring in spring. Kaempferol diglycoside concentrations (peak 16) were initially relatively low in spring, increased to a peak in early summer, and then declined until autumn. This decline stabilized somewhat as winter approached, with a slight increase in concentration.

In terms of the seasonal averages of the individual treatment groups, the highest flavonol concentrations were observed in FERT+INOC, followed by INOC, with lower levels detected in FERT and CONT, with the latter presenting the lowest levels. However, no statistically significant differences were observed between the treatment groups for any of the individual flavonols analysed.

The analysis of individual treatment groups within each season revealed statistically significant differences in the concentrations of all flavonols in spring and summer, whereas in winter, such differences were only significant for myricetin arabinoside.

Regarding seasonal influence, only two compounds showed significant correlations with harvest time: myricetin 3-arabinoside exhibited a strong positive correlation ($r = 0.909$, $p = 0.03$), whereas myricetin-3-O-(2″-O-galloyl)-hexoside was significantly negatively correlated ($r = -0.964$, $p = 8.19 \times 10^{-3}$). The remaining flavonols showed no significant associations, suggesting that their concentrations are either independent of seasonal shifts or governed by other predominant factors.

Additionally, the concentrations of most flavonols showed weak or nonsignificant correlations with each other, except for myricetin hexoside, which was positively correlated with kaempferol diglycoside ($r = 0.517$, $p = 2.31 \times 10^{-4}$) and negatively correlated with quercetin glucoside ($r = -0.440$, $p = 3.51 \times 10^{-3}$) and myricetin arabinoside ($r = -0.306$, $p = 0.04$). These correlations suggest that these compounds may be involved in compensatory biosynthetic pathways.

Secoiridoid Glycosides

The only secoiridoid glycoside studied (purple bars in Fig. 9) was a ligstroside derivative (peak 9). The analysis of this compound revealed distinct seasonal patterns, with a general increasing trend throughout the year and a peak in winter. Significant differences were observed in winter compared with spring and summer.

Compared with CONT, fertilization and inoculation (FERT, INOC, and FERT+INOC) resulted in significantly higher average levels of the ligstroside derivative in spring. However, no significant differences were observed between treatment groups in the other seasons, except for INOC in summer, where the ligstroside derivative was not detected.

Effects of the Treatments and Harvest Timing

The Bartlett tests for homogeneity of variance revealed significant results, indicating unequal variance between treatment groups. This result suggests that certain treatments may affect some compounds in ways that cannot be identified through comparisons of mean values alone but may instead be due to individual plant responses or specific conditions. Consequently, treatment effects cannot be fully understood without considering seasonal influences.

Box plots (Figures S-3 to S-7 in the Supplementary Information) illustrate the distribution of substance concentrations across treatment groups. Some overlap between the groups was evident, with the INOC group showing higher medians and a broader interquartile range, indicating both higher concentrations and greater variability. FERT had a distribution similar to that of CONT, with a slight tendency toward higher concentrations, although the variability between these groups appeared similar. Compared with CONT, INOC presented a greater mean and standard error. The means for the fertilized and nonfertilized groups were comparable, with a slight increase in the standard error for FERT.

Overall, fertilization seems to have a minimal effect on both the growth parameters and the phytochemical composition of the leaves. Significant differences between treatment groups throughout the vegetative cycle were observed only for pedunculagin and caffeoyl hexose concentrations (Table 3). All other compounds exhibited only minor deviations in annual average concentrations. Inoculation led to greater fluctuations in the values, although these fluctuations were not statistically significant, with the exception of the caffeoyl hexose concentrations.

In Mediterranean shrubs such as *C. albidus*, phenolic compounds and antioxidant capacity are closely linked to stress physiology, redox regulation, and defence strategies (Zouhri et al. 2024; Kielar et al. 2025). Therefore, the increased total phenolic content and antioxidant capacity observed in inoculated plants, particularly during autumn, may be interpreted as indirect indicators of altered physiological status rather than direct consequences of enhanced nutrient uptake. This response is consistent with a trade-off between growth and secondary metabolism, frequently reported under biotic or abiotic stress conditions.

Most compounds presented significant differences in variance between treatment groups at specific harvest times (Fig. 9). Some compounds were more sensitive to particular treatments at certain times of the year, as indicated by greater variability in their concentrations. Significant differences were most apparent in spring and summer, whereas differences in autumn and winter were less pronounced or absent. For example, the concentrations of flavan-3-ols

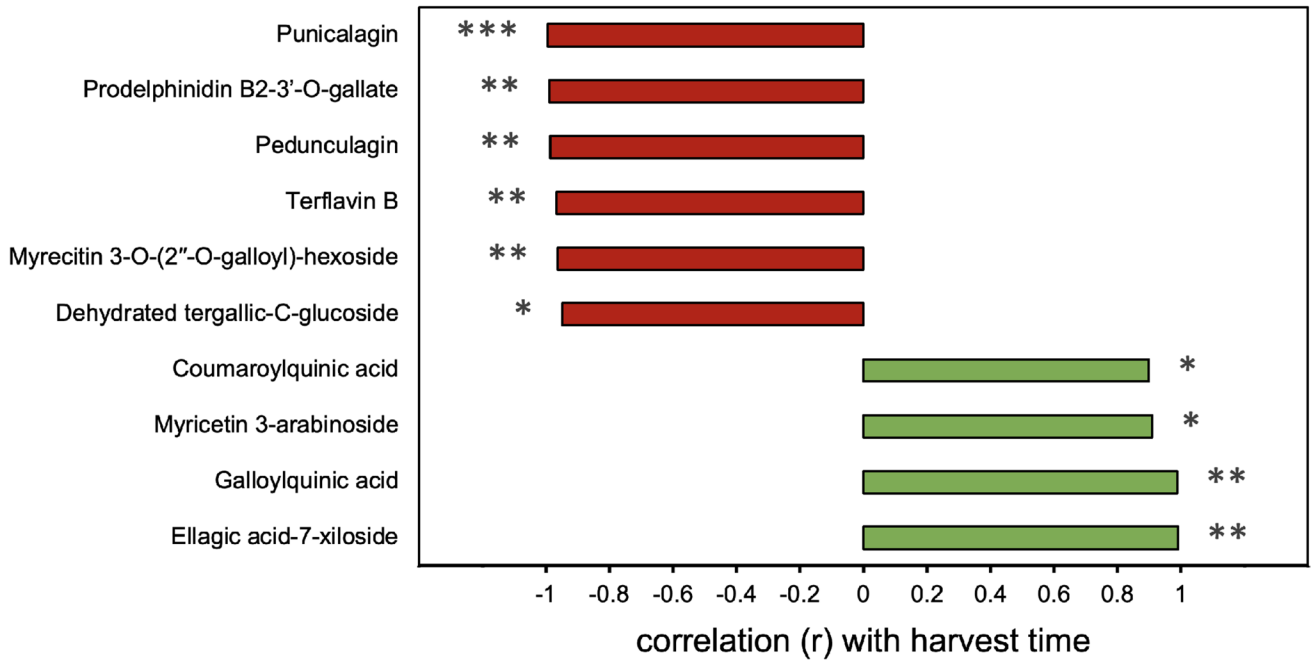


Fig. 10 Pearson's correlation coefficients (r) between single compounds and the harvest time during one vegetative cycle. Only statistically significant ($p < 0.05$) correlations are presented: *** for $p \leq 0.001$, ** for $0.001 < p \leq 0.01$, * for $0.01 < p \leq 0.05$. Positive values indicate that the concentration of a measured polyphenol correlates with the end of the vegetative cycle; negative values correlate with earlier harvest times

Table 6 Parameters evaluated for determining the harvest time included the mean leaf dry weight (mg per leaf), antioxidant capacity (TROLOX equivalents per 100 g extract, TEAC) and total phenol content (gallic acid equivalent per gram extract (TPC)) independently of the treatment from leaves harvested in four seasons. The data were analysed by one-way ANOVA with Tukey's HSD post hoc test. Within each observation, the letters indicate a significant difference ($p < 0.05$) compared with spring, summer, autumn and winter (data are presented as the means ± SEs of n = 90 leaves for leaf dry weight and n = 100 each for TEAC and TPC analyses)

	Mean leaf dry weight (mg)	TEAC (mmol equivalent Trolox/100 g extract)	TPC (gallic acid equivalent/g)
Spring	71.37 ± 8.68 [a]	8.73 ± 0.57 [a]	8.52 ± 1.36 [ac]
Summer	58.2 ± 4.83 [b]	8.72 ± 0.35 [a]	27.09 ± 4.84 [b]
Autumn	77.5 ± 8.00 [a]	8.88 ± 1.56 [a]	9.58 ± 1.98 [c]
Winter	76.8 ± 5.74 [a]	7.18 ± 0.24 [a]	31.45 ± 1.79 [bd]

(epi)gallocatechin-(epi)gallocatechin and prodelphinidin B2-3'O-gallate differed significantly across all the treatment groups within each season, with only (epi)gallocatechin-(epi)gallocatechin significantly influenced by inoculation throughout the entire vegetative cycle. Terflavin B and myricetin 3-O (2''-O-galloyl)-hexoside concentrations followed a similar pattern, with inoculation significantly affecting the concentrations of these compounds in spring and summer. In contrast, ellagic acid 7-xyloside and myricetin 3-arabinoside concentrations were significantly affected by fertilization throughout the vegetative cycle, except in autumn.

In general, the harvest timing had the most substantial effect on phytochemical parameters (Table 3). Strong, significant correlations were identified for 10 of the 16 analysed phytochemicals. The contents of six compounds decreased throughout the vegetative cycle, whereas the contents of myricetin arabinoside, galloylquinic acid, coumaroylquinic acid, and ellagic acid 7-xyloside increased significantly over this period (Fig. 10). However, no significant correlation was found between the harvest timing and total phenol content or antioxidant capacity, possibly because of opposing trends in the concentrations of individual compounds.

The potential harvest time were assessed by evaluating three key variables: the mean leaf dry weight and the biochemical components TEAC and TPC (Table 6). Significant differences in harvest timing, independent of treatment, were observed only for leaf dry weight in summer and TPC between all seasons, except spring and autumn and summer and winter, whereas TEAC did not show significant differences. These findings suggest that the harvest timing could significantly impact production quality and yield.

Given the significant peak in TPC and the lack of variation in annual TEAC values, late winter appears to be the optimal harvest time, which is further supported by the high mean leaf dry weight during this period.

Discussion

Vegetative Observations

Seasonal variation emerged as the dominant driver of vegetative performance in *C. albidus*, with spring promoting maximal growth across all treatments and significantly shaping leaf area, LI, and SLA, largely independent of fertilization, inoculation, or water availability. Although soil moisture was maintained at field capacity throughout the experiment, plants nevertheless exhibited physiological responses indicative of environmental constraint, suggesting that factors such as temperature, photoperiod or cultivation conditions contributed to seasonal growth modulation. The environmental conditions are listed in the supplementary information and have been considered along the discussion.These findings underscore the strong influence of seasonal cues on leaf development in *Cistus* species and are consistent with previous observations highlighting the complex interaction between environmental drivers and plant physiological traits (Catoni et al. 2012).

Although arbuscular mycorrhizal inoculation is commonly associated with enhanced plant growth and nutrient acquisition, inoculation with the selected *Rhizophagus* and *Glomus* species did not confer growth benefits to *C. albidus* under the experimental conditions applied. Inoculated plants presented significantly reduced leaf area and leaf biomass, together with increased inter-individual variability in leaf area, indicating a growth penalty rather than a growth-promoting response. Inoculated plants also exhibited higher SLA values, indicating a shift toward thinner leaves and altered leaf structural traits. Inoculation was further associated with less uniform vegetative growth and reduced node numbers.

Because mycorrhizal colonization was not directly assessed, these effects should not be interpreted as evidence of a failed symbiosis but rather as plant responses to the inoculation treatment itself, potentially reflecting stress-related physiological adjustments, altered carbon allocation, or rhizosphere-mediated interactions. Such responses may be amplified under pot culture conditions, where limited substrate volume and nutrient competition between host plants and fungal partners can constrain plant performance.

Previous studies have reported successful AMF associations in *Cistus* species, including *C. albidus*, resulting in improved growth and stress tolerance (Ortuño et al. 2018). However, these outcomes appear to be highly dependent on plant–fungus specificity and environmental context. Notably, the AMF taxa reported to be particularly effective under Mediterranean conditions were not included in the inoculum used here, which may partly explain the observed growth inhibition. Moreover, the absence of water limitation may have reduced the potential benefits of AMF inoculation, as positive responses are often more pronounced under drought stress (Rubin et al. 2017; Chandra et al. 2019).

Inoculated plants exhibited lower flower production in the second year, indicating a shift in resource allocation away from reproductive output. Flowering onset was uniform across treatment groups and primarily driven by environmental cues such as photoperiod and temperature rather than by the applied treatments. This observation aligns with the literature, which confirms that plants may rely on specific environmental signals to initiate flowering (Amasino 2010; He et al. 2020). The low initial flowering yield likely resulted from plants allocating energy to root and vegetative growth rather than reproduction. A significant increase in flowering in the second year, particularly in the fertilized groups, demonstrated improved reproductive stability and earlier flowering rather than increased absolute floral output.

Differences in total flowering yield among the treatment groups highlight the distinct impacts of fertilization and inoculation on reproductive output and suggest that inoculated plants might allocate more energy toward root growth because of improved nutrient uptake. Inoculated plants exhibited reduced reproductive output, which may reflect a shift toward belowground or vegetative investment under altered physiological conditions rather than enhanced reproductive performance. Fertilized plants, by contrast, displayed more uniform growth and elongated shoot architecture, whereas control plants showed increased lateral branching. These contrasting growth forms are consistent with the optimal partitioning theory, whereby plants adjust biomass allocation in response to resource availability (Kobe et al. 2010). The absence of significant differences in plant height between treatments highlights a complex interplay between genetic and environmental factors and resource allocation strategies (Obeso 2002).

Survival rates and correlations among plant mortality rates and leaf node counts revealed critical thresholds for *C. albidus* survival under the experimental conditions. Mortality peaked between late winter and early spring and was more pronounced between late summer and autumn, which is consistent with seasonal changes in the ABA concentration. This finding is consistent with observations from previous studies, where leaf samples from winter plants presented low ABA concentrations, which increased in spring and summer (López-Carbonell et al. 2009). The comparatively higher survival rates in the treated groups despite the physiological stress responses suggest a moderating effect of fertilization on stress tolerance.

Oxidative stress caused by reactive oxygen and nitrogen species (ROS/RNS), is a central component of plant stress physiology, and plant-derived antioxidants, particularly polyphenols, are known to mitigate biotic and abiotic

stress effects (Sharma et al. 2019; Harman 1956; Šamec et al. 2021). Importantly, several phenolic compounds in this study appeared to act as markers of severe stress rather than indicators of protection (Close and McArthur 2002). Compounds such as ellagic acid 7-xyloside and galloylquinic acid were positively correlated with plant mortality, indicating that elevated concentrations may reflect substantial physiological stress. Although ellagic acid derivatives and galloylquinic compounds exhibit antioxidant properties (Khan et al. 2017; Abu El-Soud et al. 2013; Baratto et al. 2003), derivatization can reduce their radical-scavenging capacity (Lesjak et al. 2018). Consequently, high concentrations of these phenolics may signal stress levels approaching the tolerance limits of the plant rather than effective protection.

In contrast, dehydrated tergallic-C-glucoside and prodelphinidin B2-3′-O-gallate showed strong negative correlations with plant mortality, suggesting an association with healthier physiological states. Flavan-3-ols such as prodelphinidin B2-3′-O-gallate possess high antioxidant potential due to their multiple hydroxyl groups and may contribute to improved stress buffering. The lower mortality observed in fertilized plants may therefore reflect an enhanced capacity to cope with seasonal environmental constraints, potentially supported by higher flavan-3-ol levels. These findings indicate that promoting the biosynthesis of specific polyphenolic profiles through agronomic practices could enhance plant resilience, while excessive accumulation of other phenolics may serve as early indicators of critical stress.

The combined growth limitation, altered allocation patterns, and metabolite-level responses observed under inoculation are more consistent with stress-associated signalling effects than with a growth-promoting functional mycorrhizal symbiosis under the conditions tested (Pozo and Azcón-Aguilar 2007).

Phenolics and Antioxidant Capacity

The antioxidant capacity of *C. albidus* extracts, as assessed through the TEAC assay, exhibited a clear pattern of seasonal adaptation driven by the plant's need to cope with environmental stress (Jubany-Mari et al. 2008). Agricultural treatments such as inoculation and fertilization also influenced the antioxidant capacity, with inoculation, irrespective of fertilization, resulting in the highest mean antioxidant capacity. Seasonal variations affected antioxidant capacity, with inoculated groups showing the highest values toward the end of the year, particularly in autumn. Inoculation was associated with increased concentrations of selected phenolic compounds and higher antioxidant capacity, consistent with the activation of stress- and signalling-related metabolic pathways.

Under the non-drought conditions of the experiment, the increased antioxidant capacity observed in inoculated plants relative to controls suggests that AMF inoculation contributed to antioxidant responses independent of water limitation. Increases in secondary metabolite production following microbial inoculation and/or nutrient amendments have been reported in other plant species (Parada et al. 2019).

Overall, these results indicate that water availability played a subordinate role in modulating antioxidant capacity in *C. albidus* under the present conditions, consistent with observations that reduced rainfall does not necessarily affect phenolic content (Moreira et al. 2020). Because soil moisture was maintained at field capacity throughout the experiment, variation in antioxidant capacity is more consistent with seasonal cues and treatment effects than with differences in water availability.

Secondary metabolites contribute not only to redox buffering but also to stress-related signalling processes in plants (Bekheet 2011), and the lower antioxidant capacity observed in control plants may reflect reduced environmental stimulation under controlled cultivation conditions compared with naturally exposed populations, as suggested by previous studies (Lukas et al. 2021). Notably, significant seasonal differences in antioxidant capacity were not always mirrored by changes in total phenolic content (TPC), indicating that nonphenolic antioxidants and matrix effects contributed substantially to overall antioxidant activity.

Interactions among phenolic compounds and other constituents within the plant matrix can modulate antioxidant capacity through synergistic or antagonistic effects (Giordano and Lionetto 2023; Freeman et al. 2010). Furthermore environmental factors such as light, temperature and nutrient availability influence the biosynthesis of redox-active compounds. This complexity likely explains why a significant positive correlation between TPC and antioxidant capacity was observed only in fertilized plants.

Seasonal differences in TPC highlight the influence of environmental and developmental factors on phenolic biosynthesis, consistent with reports in other medicinal and crop species (Ahanger et al. 2014; Hashem et al. 2018; Semenyutina et al. 2022; Marchioretto et al. 2007; Oyungerel et al. 2017). Correlation analysis revealed that fertilization promoted a tighter coupling between phenolic content and antioxidant capacity (Onofrei et al. 2017; Azaizeh et al. 2005), whereas the absence of such a relationship in the combined treatment suggests antagonistic or compensatory effects among antioxidant components. Similar decoupling between phenolic content and antioxidant activity has been reported in rapeseed (Amarowicz et al. 2024), underscoring the multifactorial nature of antioxidant regulation in plants.

Phytochemicals

Inoculated plants exhibited elevated concentrations and increased inter-individual variability across several phytochemical classes, indicating that secondary metabolism in *C. albidus* is not uniformly modulated by cultivation practices but reorganised in a selective and context-dependent manner. Rather than responding through generalized increases or decreases in metabolite abundance, plants displayed coordinated, class- and compound-specific shifts, reflecting differential regulatory control across biosynthetic pathways (Pichersky and Lewinsohn 2011).

Across the phytochemical profile, a clear hierarchy of responsiveness emerged. Flavan-3-ols and flavonols showed selective and context-dependent responsiveness to microbial inoculation and seasonal progression, whereas phenolic acids, ellagitannins, and the analysed secoiridoid glycoside showed comparatively limited treatment sensitivity and were shaped predominantly by harvest time and developmental stage. This pattern indicates that not all secondary metabolites contribute equally to short-term physiological adjustment, but occupy distinct positions within a hierarchically organised regulatory system (Walker et al. 2022).

Flavan-3-ols and flavonols represented the most metabolically plastic pools among the classes analysed. Their pronounced responsiveness to inoculation and season is consistent with their established roles in redox buffering, stress perception, and signalling-related modulation of plant physiology (Agati et al. 2012, 2020; Mittler et al. 2022). In addition to antioxidant functions, flavonoids are known to interact with phytohormone signalling networks, including the modulation of auxin transport and developmental patterning (Brown et al. 2001; Brunetti et al. 2018; Peer and Murphy 2007). In this context, the inoculation-associated shifts observed here are more plausibly interpreted as signalling-dominant responses to altered biotic context than as uniform enhancements of nutritional performance, in line with evidence that flavonoids participate in root–rhizosphere communication and plant–microbe interactions (Hassan and Mathesius 2012; Kessler and Kalske 2018).

Within these responsive classes, compound concentrations varied markedly across harvest times, underscoring strong modulation by seasonal and developmental factors. The progressive decline of prodelphinidin B2-3′-O-gallate over the vegetative cycle is consistent with a temporal reallocation of flavan-3-ol pools during plant development, likely reflecting a shift from active vegetative growth toward reproductive investment and associated carbon reallocation (Dixon and Paiva 1995; Neilson et al. 2013). Divergent trajectories of individual flavonols (e.g. quercetin- versus myricetin-derived compounds) further indicate that the flavonoid pool is not governed by a single "stress intensity" axis but by compound-specific regulation integrating developmental stage, carbon status, and signalling function (Agati et al. 2020; Brunetti et al. 2018).

In contrast, phenolic acids showed pronounced temporal dynamics but comparatively limited responsiveness to treatments, suggesting a stronger coupling to developmental programmes and baseline environmental regulation than to short-term perturbation (Ma et al. 2016). The selective sensitivity of specific phenolic acids to inoculation, such as caffeoyl-hexose-related responses, likely reflects targeted activation of discrete branches within the phenylpropanoid network, superimposed on a seasonally constrained metabolic background. This behaviour aligns with the broader principle that specialised metabolites mediate ecological interactions while remaining embedded within higher-order regulatory constraints (Kessler and Kalske 2018; Erb and Kliebenstein 2020).

Ellagitannins exhibited strong harvest-time structuring, with compound-specific accumulation and depletion patterns over the course of development. Similar seasonal dynamics of individual hydrolysable tannins have been reported in other woody species, where these compounds are interpreted as developmentally regulated components of constitutive defence and storage–turnover systems (Grundhöfer et al. 2001; Salminen et al. 2004). Such behaviour supports the view that ellagitannins primarily reflect long-term physiological state and defensive architecture rather than functioning as rapidly inducible front-line responses to transient environmental stress.

Finally, the relative insensitivity of the analysed secoiridoid glycoside to fertilisation and inoculation, combined with pronounced seasonal regulation, supports the existence of metabolite subsets that are predominantly governed by intrinsic developmental programmes and long-term environmental integration rather than by short-term agronomic inputs (Alagna et al. 2012; Gómez-Rico et al. 2009). Within this framework, these compounds may serve as stable markers of developmental timing against which more plastic, signalling-responsive metabolites fluctuate (Erb and Kliebenstein 2020; Devireddy et al. 2021).

These findings indicate that the phytochemical profile of *C. albidus* responds to cultivation practices not through uniform amplification of secondary metabolism, but through a selective and hierarchical reconfiguration of metabolite classes and individual compounds. Metabolically plastic phenolic pools, particularly flavan-3-ols and flavonols, encode short-term information about biotic context and seasonal conditions, whereas phenolic acids, ellagitannins, and the analysed secoiridoid glycoside primarily reflect developmental state and long-term environmental integration. This structured organisation of the phytochemical matrix provides a mechanistic framework for understanding how

cultivation practices reshape medicinally relevant profiles by recalibrating growth–defence–signalling trade-offs rather than by maximising compound abundance per se (Mittler et al. 2022; Kessler and Kalske 2018; Erb and Kliebenstein 2020). Here, the term "phytochemical matrix" refers to the integrated composition, relative abundance, and temporal organisation of specialised metabolites, rather than to isolated compound concentrations. This hierarchical pattern of metabolite responsiveness raises the question of how primary metabolic constraints shape secondary metabolic allocation across development and environmental context.

Primary and Secondary Metabolism

The differential and class-specific responsiveness observed across the phytochemical profile suggests a structured organisation of secondary metabolism. Not all secondary metabolites appear to serve equivalent roles in short-term physiological adjustment, with certain compound classes acting as metabolically plastic response elements and others reflecting more conserved, developmentally programmed functions. The class- and compound-specific phytochemical patterns observed in *C. albidus* can be mechanistically interpreted through the dynamic coupling between primary and secondary metabolism. Rather than representing independent metabolic layers, these systems form an integrated regulatory network in which resource availability, developmental stage, and environmental signalling jointly shape the allocation of carbon, nitrogen, and reducing power (Erb and Kliebenstein 2020; Hartmann 2007).

Classical frameworks such as the carbon–nutrient balance hypothesis and the protein competition model provide a useful conceptual basis for understanding this integration. Both models emphasize that secondary metabolism is constrained by the demands of primary metabolism and that shifts in growth, development, or nutrient status inevitably alter the availability of precursors, cofactors, and energy for secondary biosynthetic pathways (Dixon and Paiva 1995; Herms and Mattson 1992; Bryant et al. 1983). In this context, variation in secondary metabolite profiles does not primarily reflect isolated up- or down-regulation of individual pathways, but rather the outcome of competitive allocation decisions within a finite metabolic budget (Neilson et al. 2013).

Importantly, the present data indicate that not all classes of secondary metabolites occupy equivalent positions within this allocation hierarchy. Flavan-3-ols and flavonols represent comparatively metabolically plastic pools that are closely coupled to primary metabolic fluxes and cellular redox status. Their biosynthesis relies heavily on carbon-rich intermediates of the phenylpropanoid pathway and is therefore particularly sensitive to changes in carbon availability, nitrogen limitation, and developmental transitions (Agati et al. 2020; Dixon and Paiva 1995). As a consequence, these compounds respond readily to short-term physiological perturbations, including microbial signalling and seasonal shifts, and function as flexible modulators of stress perception, signalling, and growth–defence trade-offs (Erb and Kliebenstein 2020). In contrast, phenolic acids, ellagitannins, and secoiridoid glycosides occupy more constrained positions within the metabolic network. Although they share upstream precursors with more plastic phenolic classes, their downstream biosynthesis, modification, compartmentalisation, and turnover are more tightly embedded in developmental programs and long-term environmental integration (Salminen et al. 2004; Hartmann 2007). These compounds tend to accumulate within structurally or functionally stabilised pools, often associated with constitutive defence, storage, or long-horizon adaptive strategies. As a result, their concentrations are shaped predominantly by ontogeny and seasonal progression rather than by transient agronomic or biotic inputs (Herms and Mattson 1992).

This differential positioning within the primary–secondary metabolic interface explains why cultivation practices in the present study led to selective reconfiguration of the phytochemical matrix rather than uniform shifts across all metabolite classes. Fertilization primarily influenced overall growth performance and resource availability, thereby indirectly stabilising metabolically demanding secondary pathways. In contrast, microbial inoculation is consistent with acting as a signalling perturbation that preferentially engaged metabolically plastic phenolic pools, triggering shifts in compounds involved in redox regulation and defence signalling without producing proportional benefits for growth or reproduction (Neilson et al. 2013; Erb and Kliebenstein 2020).

Developmental phase further modulated these interactions by redefining metabolic priorities over time. During early and mid-vegetative stages, investment in carbon-intensive, signal-active secondary metabolites is compatible with growth demands and defensive needs. As plants transition toward reproductive development, however, primary metabolism increasingly prioritises resource allocation to reproductive structures, leading to a down-regulation or redistribution of metabolically expensive secondary pools (Herms and Mattson 1992; Bryant et al. 1983). More conserved metabolite classes, whose functions are aligned with long-term defence architecture or developmental state, remain comparatively stable across this transition (Salminen et al. 2004).

Together, these observations support a view of secondary metabolism as a hierarchically organised extension of primary metabolism rather than as an independent response layer. Within this framework, phytochemical profiles

emerge from the dynamic balance between metabolically plastic compounds that mediate short-term signalling and stress calibration, and more conserved compounds that encode developmental state and long-term environmental integration. Such organisation provides a coherent explanation for the coexistence of growth limitation, enhanced stress signalling, and selective metabolite accumulation observed under different cultivation regimes in *C. albidus* (Erb and Kliebenstein 2020).

Limitations

A limitation of this study is the absence of direct measurements of arbuscular mycorrhizal colonisation and molecular stress markers. The experimental design focused on seasonal-scale phenotypic and metabolic integration under controlled cultivation rather than on mechanistic resolution of early symbiotic establishment or host stress signalling. While this limits causal inference regarding functional mycorrhizal mutualism, the consistent growth suppression, increased phenolic investment, and altered survival patterns observed in inoculated plants support interpretation at the level of whole-plant regulatory responses. Future studies integrating root colonisation assessments and stress and redox-related biomarkers would help disentangle functional symbiosis from indirect microbial signalling effects.

Conclusions

This study demonstrates that growth performance, phenolic composition, and antioxidant capacity in *C. albidus* are shaped by a tightly interlinked interplay between seasonal cues, developmental stage, and cultivation practices. Seasonal progression emerged as the dominant driver of vegetative growth and phenolic dynamics, overriding direct effects of fertilization, inoculation or water availability. In contrast to expectations of growth promotion, arbuscular mycorrhizal inoculation primarily induced growth limitation, altered allocation patterns, and selective modulation of secondary metabolism, consistent with stress-associated signalling rather than with a uniformly beneficial symbiotic interaction under the tested conditions.

At the phytochemical level, responses were highly structured and class-specific. Rather than uniform amplification of secondary metabolism, cultivation practices led to a selective and hierarchical reconfiguration of the phytochemical profile. Metabolically plastic phenolic pools, particularly flavan-3-ols and flavonols, responded sensitively to microbial and seasonal cues, whereas phenolic acids, ellagitannins, and the analysed secoiridoid glycoside were predominantly governed by developmental programmes and long-term environmental integration. Antioxidant capacity followed seasonal dynamics that were not fully explained by total phenolic content alone, highlighting the importance of matrix effects, nonphenolic antioxidants, and compound interactions in determining functional bioactivity.

These findings support a view of plant secondary metabolism as an integrative regulatory layer rather than a simple output of stress intensity or agronomic input. The phytochemical profile of *C. albidus* reflects coordinated allocation decisions that balance growth, defence, and signalling across developmental time and environmental context. Such organisation provides a conceptual framework for understanding how cultivation conditions reshape biologically relevant plant traits not by maximising compound abundance, but by recalibrating the qualitative and temporal structure of the phytochemical matrix. This perspective has implications for the cultivation, interpretation, and application of medicinal plants, where biological effects are likely to emerge from structured metabolic configurations rather than from isolated compounds alone.

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Data Availability Data will be made available on request.

Declarations

Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have

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