

Relationship between root traits and arbuscular mycorrhizal fungi in three species of weeds with different synanthropy index

Relación entre las características de las raíces y los hongos micorrízicos arbusculares en tres especies de malezas con diferente índice de sinantropía

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RESUMEN

Antecedentes: Las estrategias de búsqueda de nutrientes entre raíces delgadas y gruesas, incluyendo la simbiosis micorrízica son costosas. Estas estrategias pueden variar con el índice de sinantropía (grado en que una especie se asocia con hábitats antropogénicos), maximizando los beneficios para adquirir recursos.

Objetivos: Cuantificar la colonización micorrízica arbuscular en especies del género *Melampodium* con diferente índice de sinantropía, y determinar las correlaciones entre la colonización micorrízica y las características arquitectónicas y morfológicas de las raíces.

Métodos: Se colectaron raíces de *Melampodium divaricatum*, *M. perfoliatum* y *M. tepicense*, con índices de sinantropía de mayor a menor, respectivamente. Se cuantificó la tasa de ramificación de raíces (RBR), longitud de raíces (TRL), diámetro de raíces de primer orden (FOR), densidad del tejido radicular (RTD), longitud de raíz específica (SRL), colonización micorrízica, y se realizaron análisis químicos del suelo.

Resultados y conclusiones: *Melampodium tepicense* tuvo los menores valores de FOR, los mayores de SRL, y menor colonización micorrízica. *Melampodium divaricatum* y *M. perfoliatum* tuvieron valores opuestos. Adicionalmente, *M. divaricatum* y *M. perfoliatum* tuvieron valores altos de TRL, RTD y RBR, sugiriendo que ambas estrategias, micorrizas y raíces finas, son usadas para adquirir recursos, independientemente de sus relaciones filogenéticas y nutrientes del suelo.

Palabras clave: características arquitectónicas de las raíces, características morfológicas de las raíces, Nayarit, malezoide

ABSTRACT

Background: Nutrient foraging strategies between thin and thick roots, including mycorrhizal symbionts are resource-costly, and therefore a trade-off could exist. These strategies can vary with the synanthropy index (degree to which a species associates with anthropogenic habitats), thus maximizing the benefits for the acquisition of soil resources.

Objectives: To quantify the arbuscular mycorrhizal colonization of *Melampodium* species with different synanthropy index, and to determine the correlations between arbuscular mycorrhizal colonization and the architectural and morphological root traits.

Methods: Roots of *Melampodium divaricatum*, *M. perfoliatum* and *M. tepicense*, with highest to lowest synanthropy index, respectively were collected. The root branching rate (RBR), total root length (TRL), diameter of first-order root, (FOR), root tissue density (RTD), specific root length (SRL), and arbuscular mycorrhizal colonization were quantified. Additionally, soil chemistry analyses were done.

Results and conclusion: *Melampodium tepicense* had lowest FOR, highest SRL and lowest arbuscular mycorrhizal colonization, whereas *M. divaricatum* and *M. perfoliatum* had the opposite values. Additionally, *M. divaricatum* and *M. perfoliatum* had higher TRL, RTD, and RBR, suggesting that both strategies, arbuscular mycorrhiza and fine roots, are used for acquisition of nutrients, independently of their phylogenetic relationship and soil nutrients.

Keywords: architectural root traits, morphological root traits, Nayarit, weediness

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INTRODUCTION

The plants have evolved a suite of strategies for acquiring nutrients from the soil and to respond to changes in their availability in time and space. These strategies include architectural and morphological root traits, and also biotic root interactions with fungi and bacteria (Bardgett *et al.*, 2014; Kong *et al.*, 2017). Architectural traits determine the spatial configuration of root system of an individual plant and include root branching rate (Bardgett *et al.*, 2014). Whereas morphological traits are features on individual roots such as total root length, specific root length, root tissue density, and diameter of first-order roots (Bardgett *et al.*, 2014). It has been suggested that plant species with thin and thick-roots represent two strategies for foraging soil mineral nutrients, but they are resource-costly (Eissenstat, 1992; Comas *et al.*, 2014; Kong *et al.*, 2014). Therefore, many plants do not invest resources in multiple nutrient-acquisition mechanisms, because there is a trade-off between one strategy at the expense of another (Chen *et al.*, 2016). Recent evidence also shows that thin and thick-roots interact in different ways with arbuscular mycorrhizal (AM) fungi. For instance, thick root systems are not very efficient at nutrient foraging and they invest instead in mycorrhizal symbionts to meet their nutrient demands (Kong *et al.*, 2016; Lugli *et al.*, 2020). Additionally, thick-roots are more colonized by AM fungi because their cortex provides greater space for mycorrhizal colonization (Kong *et al.*, 2017). Arbuscular mycorrhizal fungi belonging to subphylum Glomeromycotina (Spatafora *et al.*, 2016) are an ancient lineage (Strullu-Derrien *et al.*, 2018) of obligate biotrophs that colonize the roots of most terrestrial plant species, including cultivated plants, around 80 % of plant species and 92 % of plant families (Wang and Qui, 2006). Because of its important ecological and agricultural implications, the impact of this symbiosis has been studied, not only, on the host plant's growth and development, but also on soil adherence, soil stability, mineral redistribution, water retention and restoration of disturbed ecosystems (Álvarez-Sánchez and Peña, 2009; Gianinazzi *et al.*, 2010; Cuenca, 2015; Pickles and Simard, 2017; Ji *et al.*, 2019; Li *et al.*, 2020). The relationship between AM fungi and the majority of land plants is often of a bidirectional nature, i.e., the fungi procure inaccessible minerals and water (Smith and Read, 2008) to the host plant and in return they

receive up to 20 % of photosynthates produced by the host plant (Jakobsen and Rosendahl, 1990; Drigo *et al.*, 2010). Due to the improved nutrient acquisition, mycorrhizal plants obtain other benefits; for instance, they are better at tolerating stresses, both biotic, e.g. herbivores, pathogens (Sharma *et al.*, 2017) and abiotic drought, heavy metals, salinity (Miransari, 2010; Latif *et al.*, 2016).

It is possible that root traits and AM symbionts play an important role in the success of plants to grow in several environments, including habitats strongly modified by humans such as cultivated land. Plant species from the same genus but that differ in their degree to which they associate with anthropogenic habitats (synanthropy index or weediness), are suitable to evaluate if there are differences in their root traits (thin vs thick) and if these types of roots differ in their association with AM fungi. Therefore, in the present study, the mycorrhizal colonization by AM fungi, and both architectural (root branching rate) and morphological (total root length, diameter of first-order root, root tissue density, and specific root length) traits were quantified in three weed species from the same genus (*Melampodium*) but differing in their synanthropy index (weediness).

Three *Melampodium* species were studied because their phylogenetic relationships are known (Stuessy *et al.*, 2011), their synanthropy indexes were previously estimated (Hanan-A *et al.*, 2016), and also can coexist in the same habitats (Hanan-A *et al.*, 2016). The studied species are: (i) *Melampodium divaricatum* (Rich.) DC. is widespread in subtropical habitats from Mexico to Central America, Colombia, and Brazil. It has been introduced in Cuba, Burma, Puerto Rico and the Virgin Islands. This species can be found in disturbed sites, it blooms all year-round, and it has the highest synanthropy index (2.06). (ii) *M. tepicense* B.L. Rob. has distribution in cloud pine-oak and tropical dry forests in the states of Nayarit, Jalisco, Colima and Michoacán, it blooms from August to February, and it has the lowest synanthropy index (1.67). Additionally, *M. divaricatum* and *M. tepicense* (the weediest and least weedy, respectively) differ in their life history traits (Hanan-Alipi *et al.*, 2021). For instance, *M. divaricatum* has a fast germination, it produces flowers earlier, and also has a more sustained growth rate than *M. tepicense*. Although *M. divaricatum* produces more biomass, the majority is allocated to stems, leaves and flowers compared with *M. tepicense* which

allocates more biomass to roots (Hanan-Alipi *et al.*, 2021). These life history traits could explain the success of *M. divaricatum* to colonize different habitats including anthropogenic habitats. (iii) *M. perfoliatum* (Cav.) Kunth is found in tropical dry and pine-oak forests in Mexico, Guatemala, and Costa Rica, and it was introduced in Cuba and southern California. It blooms all year-round, and it has an intermediate synanthropy index (2.0) between *M. divaricatum* and *M. tepicense* (Hanan-A *et al.*, 2016).

The specific aims of this study were: (i) to quantify the arbuscular mycorrhizal colonization of *Melampodium* species with different synanthropy index, (ii) to determine the correlations between AM colonization and the architectural and morphological root traits. Given that mycorrhizal plants often obtain benefits such as greater amount of mineral nutrients, water and buffering to biotic and abiotic stresses, a range from highest to lowest AM colonization proportional to that of synanthropy index (*M. divaricatum* - *M. perfoliatum* - *M. tepicense*) was predicted. Additionally, it was expected that AM colonization would correlate positively with diameter of first-order root and root tissue density, but negatively with total root length, specific root length and root branching rate in agreement with the root economic spectrum (Kong *et al.*, 2017), i.e., maximizing the benefits and minimizing costs for soil resource acquisition.

MATERIALS AND METHODS

Study site

Roots from *Melampodium* species and soil sampling were carried out along a stretch of the highway San Blas-Tepic (Federal highway Mexico 76), heading northeast, past the locality of Jalcoctán, in the municipality of San Blas, in the state of Nayarit, Mexico, where individuals of the three species have been previously located. We sampled the roots and the soil in three sites (Table 1) along a stretch of the highway, which measures a total of ~8.6 km, and the distance between two sampled points (sites) was of 2 km and 4.6 km approximately.

The vegetation along the sampling sites is tropical sub-deciduous (semi-deciduous) forest, however, there is high fragmentation because of extensive crops such as mango, coffee, avocado and banana. The climate is warm sub-humid with rainfalls in summer (INEGI, 2017), the minimum average precipitation (10 mm) and temperature (10.5 °C) occur in May and January respectively, while the maximum average precipitation (943.5 mm) and temperature (39.8 °C) occur in August and October, respectively (Servicio Meteorológico Nacional, 2020).

Soil chemistry

In October 2016, we collected soil samples from all sites. The soil samples were taken using a metal core

Table 1. Localization, number of plant species sampled per site, and soil chemistry parameters in the three sites

	Sites		
	1	2	3
Coordinates	21° 31' 44.4" N, 105° 02' 09.3" W	21° 29' 46.6" N, 105° 04' 59.0" W	21° 31' 14.2" N, 105° 02' 59.5" W
Sampled plants	25 <i>M. tepicense</i> , 7 <i>M. divaricatum</i>	18 <i>M. divaricatum</i> , 14 <i>M. perfoliatum</i>	11 <i>M. perfoliatum</i>
MO (%)	8.76	3.11	4.52
N (kg ha ⁻¹)	258	85	141
P (mg kg ⁻¹)	115.3	225.7	49.6
K (Cmol kg ⁻¹)	0.7	0.87	1.57
Ca (Cmol Kg ⁻¹)	15.7	3.31	11.81
Mg (Cmol Kg ⁻¹)	7.16	2.85	4.91
pH	6.03	5.94	6.45

MO = organic matter, N = total nitrogen, P = total phosphorus, K = potassium, Ca = calcium, Mg = magnesium.

with 2.5 cm of diameter and 10 cm from the top. Three samples from each site were collected and homogenized them before chemical analyses were done. The following chemical soil parameters were determined according to the Mexican Official Standard NOM-021-PROY-NOM-021-RECNAT-2000 (DOF, 2000): soil pH in water (1:2), organic matter (OM %) by Walkey-Black, total nitrogen (N kg ha⁻¹) by Kjeldahl, total phosphorus (mg kg⁻¹) and potassium (K Cmol kg⁻¹) by flammometry, calcium (Ca Cmol kg⁻¹) and magnesium (Mg Cmol kg⁻¹) by spectrophotometry (Table 1). All analyses were carried out in the "Laboratorio de Análisis de Suelo, Agua y Plantas" of the "Unidad Académica de Agricultura", Autonomous University of Nayarit, Mexico.

Root trait measurements

For each weed species, also in October 2016, roots from a total of 25 flowering individuals (with the aim of distinguishing between species) per species were sampled. Some species sampled coexisted in two sites (Table 1). The soil was carefully excavated 0-20 cm deep. Once that part of root system was exposed, root branches (including first and second-order roots) were cut from the main roots and transported within a few hours to the laboratory in plastic bags with soil. In the case of *M. tepicense*, all individual plants as a whole because of their small size were collected, in order to have enough root for root traits measurements and AM colonization. In the laboratory, the soil was removed and the roots were washed with enough tap water. Then, a subsample of each plant's roots was studied, including first and second-order roots, which were photographed alongside a 20 cm ruler to serve as a reference scale. Subsequently, these subsamples were dried in an oven at 60°C over a period of three days and then weighed on an analytical balance to determine the root's dry weight. The images were analyzed utilizing a plugin for the image processing program ImageJ (Rasband, 2016) called SmartRoot (Lobet *et al.*, 2011). Utilizing SmartRoot it was possible to estimate total root length (TRL, cm), volume (cm³), and diameter of first-order roots (FOR, cm). These parameters were utilized to then calculate root tissue density (RTD), specific root length (SRL) and root branching rate (RBR). Root tissue density (g cm⁻³) was calculated by dividing the root dry weight by the volume. Specific root length (m g⁻¹) was calculated by dividing the

total length of the root by the root's dry weight of the subsample. The root branching rate was calculated by dividing the number of first-order roots by the number of second-order roots. In addition, the basal diameter of stem (BDS) from all sampled plants was measured before collecting their roots. Under field conditions the size of flowering plants is heterogeneous, therefore, the BDS can be used as covariable.

Arbuscular mycorrhizal measurements

In the field, a subsample of fine roots from all sampled individuals was taken and stored in ethanol and distilled water (1:1, respectively). Roots were processed according to the method of Koske and Gemma (1989) and stained with trypan blue (0.05 %). The colonization of roots was quantified by AM fungal structures (hyphae, vesicles and arbuscules) in 15 root segments of approximately 15 mm long per plant, and they were fixed in polyvinyl-lacto-glycerol (PVLG). Each root fragment was scanned at three equally spaced points under a light microscope (ZEISS Primo Star) at 100x total magnification, using the cross-hair intersection method (McGonigle *et al.*, 1990). This method consists of scanning the presence or absence of fungal structures across the equator of each field of view (45 fields of view) under the microscope. The AM hyphae were identified as smooth and coenocytic hyphae (aseptate) stained blue by trypan blue. To estimate the percentage of hyphae, vesicles and arbuscules, positive counts were summed and weighted by the total number of fields of view analyzed. Given that the field roots have different thickness it is difficult to properly observe the presence or absence of fungal structures, therefore, these roots were not taken into consideration when calculating percentages. Therefore, the number of fields of view varied from 16 to 45.

Statistical analyses

All statistical tests were done using the R statistical programming language (R Core Team, 2020). Before running the tests, we performed a graphic data exploration to check for outliers, homogeneity of variance, and normality, and to evaluate the best potential models with which to analyze each type of response variable (Zuur *et al.*, 2010). For all the models, we verified whether the residuals were normally distributed, the variances were homogeneous, and when it was necessary the variables were transformed.

To test for differences in the BDS between species, we used one-way ANOVA. To meet the model's assumptions, the Neperian logarithmic transformation was applied to the BDS. To test whether the variables TRL, RTD, SRL, FOR, RBR and the colonization by hyphae differed between species, ANCOVA was used; the BDS was used as a covariable. To meet the model's assumptions, the SRL and RBR were transformed with Neperian logarithm. The square root transformation was applied to TRL, RTD, FOR, and the percentage of hyphae.

Vesicles and arbuscules were analyzed as binary variables (presence/absence) because several individuals did not show these fungal structures. For these binary variables, generalized linear models (GLM) with a quasibinomial error structure and the logit link function were used. For both ANCOVA and GLM, when there were significant differences between species these were explored using a *posteriori* contrast based on *t*-tests (Warnes *et al.*, 2018).

Spearman correlation coefficients were employed to determine the relationship between AM colonization by hyphae and root traits (TRL, RTD, SRL, FOR and RBR), first for all the species together and then for each one of them. The correlations were calculated separately for each root variable and the hypothesis of "true rho is not equal to zero" was used. Given that vesicles and arbuscules were binary variables, these data were not used in the Spearman correlation.

RESULTS

Root trait measurements

The weed species differed significantly in the BDS ($F_{2,72} = 33.38$, $P < 0.001$), *Melampodium divaricatum* and

M. perfoliatum had the higher BDS (mean \pm standard error: 1.472 ± 0.137 cm and 2.116 ± 0.313 cm, respectively), and differed significantly with *M. tepicense* (0.524 ± 0.026 cm).

For all root traits there were statistically significant differences between the weed species (Table 2). *Melampodium divaricatum* was the weed species with the highest values of RTD (0.350 ± 0.018 g cm³), compared with *M. perfoliatum* (0.291 ± 0.028 g cm³) and *M. tepicense* (0.238 ± 0.056 g cm³), which did not differ. TRL, FOR, and RBR showed significant differences between species in agreement with a *posteriori* contrast, the values matched with the synanthropy index from highest to lowest: *M. divaricatum* (TRL: 84.180 ± 7.429 cm, FOR: 1.955 ± 0.157 cm, RBR: 30.304 ± 2.625), *M. perfoliatum* (TRL: 69.030 ± 10.799 cm, FOR: 1.693 ± 0.292 cm, RBR: 19.790 ± 3.825), and *M. tepicense* (TRL: 22.198 ± 2.332 cm, FOR: 0.267 ± 0.030 cm, RBR: 7.877 ± 1.832). But in contrast, *M. divaricatum* was one of the weed species with low SRL (151.117 ± 78.428 m g⁻¹), and there was no significant difference with *M. perfoliatum* (64.213 ± 31.328 m g⁻¹), while *M. tepicense* had the highest SRL (922.273 ± 191.996 m g⁻¹).

Except for RTD and SRL, root traits were found to be related with the covariable (Table 2). In general, larger plants developed higher TRL, FOR and RBR (Figure 1a, b and c), but *M. divaricatum* with the intermediate BDS and highest synanthropy index had the greatest values of TRL, FOR and RBR compared with *M. perfoliatum* and *M. tepicense*. *Melampodium tepicense* had the lowest values of TRL, FOR and RBR (Figure 1a, b and c), and differed significantly with the other two weed species. *M. tepicense* is the weed with the lowest size (BDS), and also lowest synanthropy index. While *M. perfoliatum* had intermediate values of TRL, FOR and

Table 2. Summary of statistical results of ANCOVA for root traits and mycorrhizal colonization by hyphae, BDS (basal diameter of stem) was used as covariate

Source of variation	df	TRL (cm)		RTD (g cm ³)		SRL (m g ⁻¹)		FOR (cm)		RBR		Hyphae %	
		F	P	F	P	F	P	F	P	F	P	F	P
Species	2	44.875	<0.001	5.709	0.005	19.669	<0.001	79.817	<0.001	34.38	<0.001	9.734	<0.001
BDS	1	58.619	<0.001	0.204	0.652	3.863	0.053	57.663	<0.001	5.166	0.026	2.258	0.137
Species:BDS	2	0.022	0.977	2.094	0.130	1.007	0.370	0.093	0.911	0.892	0.414	1.949	0.150
Residuals	69												

TRL = total root length, RTD = root tissue density, SRL = specific root length, FOR = diameter of first-order root, RBR = root branching rate. Significant values are shown in bold.

RBR, it is the weed with the greatest size but it has an intermediate synanthropy index, and it differed significantly of *M. divaricatum* and *M. tepicense* (Figure 1a, b and c). Finally, we did not find significant differences in the interaction species:covariable (Table 2).

Arbuscular mycorrhizal measurements

The arbuscular mycorrhizal colonization showed significant differences between weed species (Table 2). The percentage of hyphae was higher in *M. divaricatum* and *M. perfoliatum*, and there were no differ-

ences between them, while *M. tepicense* had the lowest percentage of hyphae (Figure 2a). The same pattern was observed in the vesicles (Deviance₂ = 13.944, $P = 0.001$, Figure 2b) and arbuscules (Deviance₂ = 12.657, $P = 0.002$, Figure 2c).

Only for the vesicles the covariable was significant (Deviance₁ = 4.482, $P = 0.042$), *M. tepicense* showed the lowest colonization by the vesicles and it was also the species with the lowest BDS. We did not find significant differences in the interaction of the species with the covariable explaining the AM colonization by hyphae

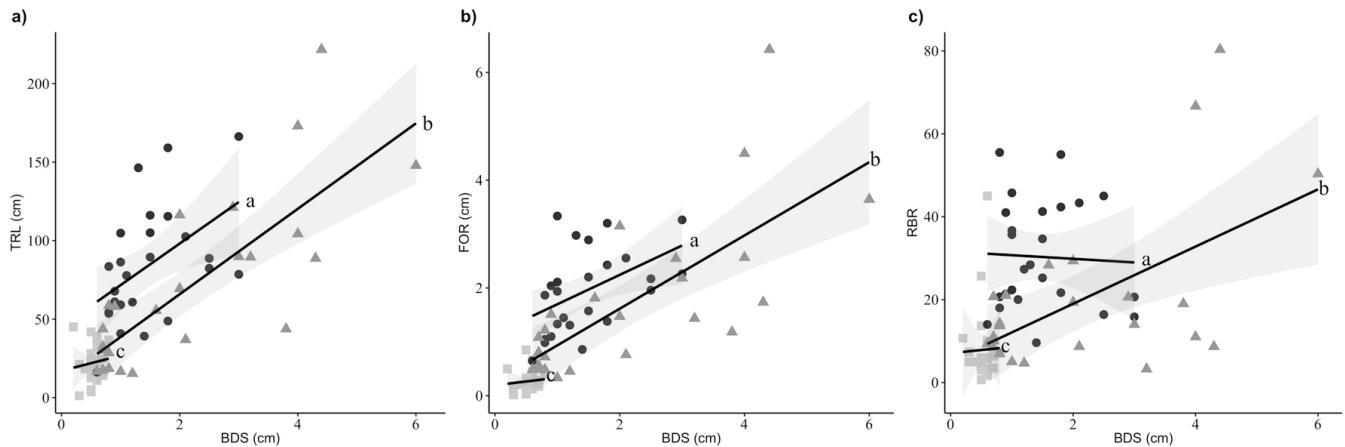


Figure 1. Interactive effects of basal diameter of stem (BDS, covariate). a: Total root length (TRL). b: Diameter of first-order root (FOR). c: Root branching rate (RBR) for *Melampodium divaricatum* (black circles), *M. perfoliatum* (grey triangles), and *M. tepicense* (grey squares). The lines represent the regression lines with the respective confidence intervals (shaded). Different letters beside the lines indicate statistically significant differences between the weed species according to a *posteriori* contrast $P < 0.05$. Data are given without transformation.

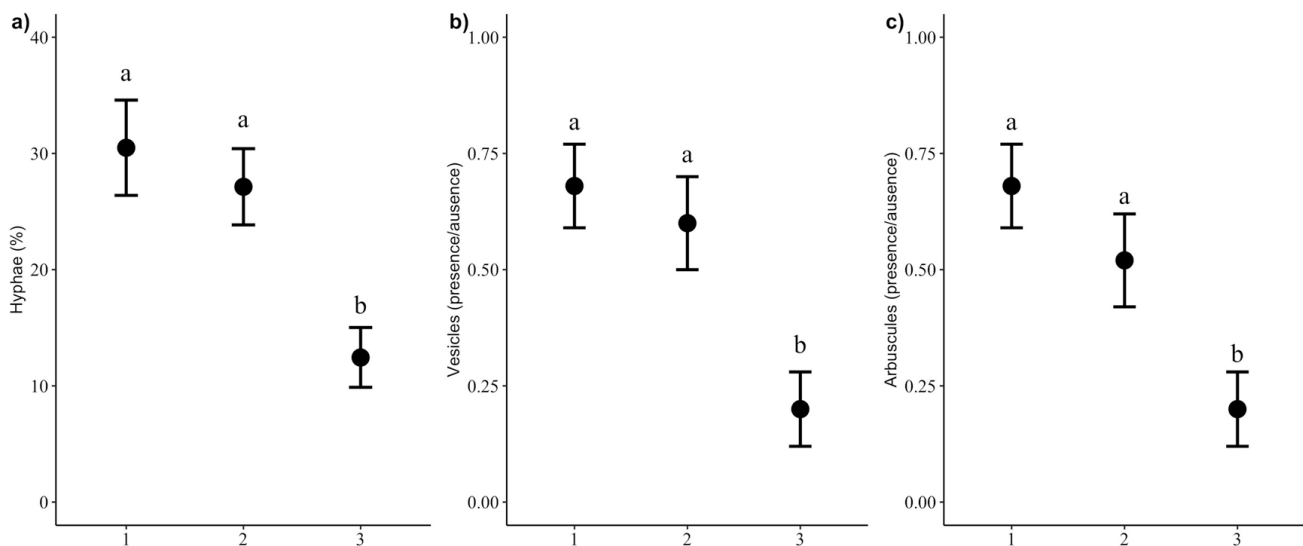


Figure 2. a: Percentage of root colonized by hyphae. b: Presence/absence of vesicles. c: Presence/absence of arbuscules in the weed species. The numbers in the axis y indicate the species: 1 = *Melampodium divaricatum*, 2 = *M. perfoliatum*, and 3 = *M. tepicense*. Means \pm standard errors are given without data transformation by hyphae. Different letters indicate significant differences between species according to a *posteriori* contrast $P < 0.05$.

(Table 2), vesicles (Deviance₂ = 0.579, $P = 0.766$) and arbuscules (Deviance₂ = 1.966, $P = 0.405$).

Spearman correlation analyses showed, considering the three species, a positive correlation between mycorrhizal colonization by hyphae, and TRL, FOR and RBR (Figure 3a, d, e). Yet, there was no correlation between mycorrhizal colonization by hyphae and RTD (Figure 3b), and SRL (Figure 3c). But when the weed species were analyzed separately there were no correlations between mycorrhizal colonization by hyphae and the root traits (Table 3).

DISCUSSION

In this study, we observed mycorrhizal colonization by hyphae, vesicles and arbuscules in the three weeds, our finding is consistent with previous studies, which showed mycorrhizal colonization in weeds (Baumgartner et al., 2005; Vatovec et al., 2005; Daisog et al.,

2012; Li et al., 2016). The arbuscules are the specific fungal structures that perform the exchange of nutrients between fungi and plants, therefore, this could indicate a functional symbiosis in the three species. However, the weed species could obtain different benefits from their mycorrhizal symbionts in agreement with their synanthropy index (i.e., the degree to which a species associates with anthropogenic habitats) and their root traits.

Melampodium tepicense is the weed with the lowest synanthropy index and it also had the lowest arbuscular mycorrhizal colonization. Lower AM colonization in this species was consistent with its smallest FOR and greatest SRL, compared with *M. divaricatum* and *M. perfoliatum*. Our findings agree with other studies showing the same pattern (Comas et al., 2014; Vega-Frutis et al., 2015; Kong et al., 2016, Wen et al., 2019). But in contrast, it has been suggested that species with high SRL produce a higher TRL and RBR (Liu et al., 2015;

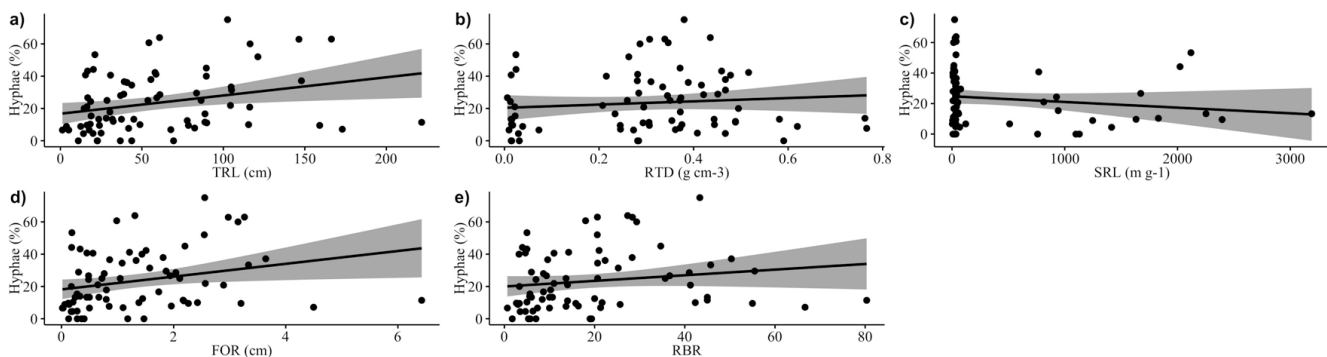


Figure 3. Relationships between mycorrhizal colonization by hyphae and root traits for the three species. Scatterplots show the relationship between mycorrhizal colonization and a: Total root length (TRL). b: Root tissue density (RTD). c: Specific root length (SRL). d: Diameter of first-order root (FOR). e: Root branching rate (RBR). Data are given without transformation.

Table 3. Spearman correlation coefficients between arbuscular mycorrhizal colonization by hyphae and root traits

Variables	Hyphae (%)							
	All species		<i>M. divaricatum</i>		<i>M. perfoliatum</i>		<i>M. tepicense</i>	
	rho	P	rho	P	rho	P	rho	P
TRL (cm)	0.329	0.004	0.049	0.816	-0.069	0.742	0.226	0.276
FOR (cm)	0.359	0.002	0.037	0.859	-0.096	0.646	0.116	0.580
RTD (g cm ⁻³)	0.139	0.235	0.090	0.667	0.152	0.467	0.065	0.755
SRL (m g ⁻¹)	0.123	0.293	0.145	0.487	0.105	0.617	0.198	0.341
RBR	0.271	0.019	0.061	0.774	-0.040	0.846	0.076	0.716

TRL = total root length, FOR = diameter of first-order roots, RTD = root tissue density, SRL = specific root length, RBR = root branching rate. Significant values are shown in bold.

Kramer-Walter *et al.*, 2016). However, *M. tepicense* had the lowest values of these root parameters. A previous experiment, under common-garden, showed that this weed produced lower total dry biomass, but used a relatively larger part of its biomass for roots than *M. divaricatum* (Hanan-Alipi *et al.*, 2021). These findings suggest that roots of *M. tepicense* could have phenotypic plasticity and can respond to soil conditions (field vs experiment). Additionally, *M. tepicense* with a lower RTD, and highest SRL may increase the volume of soil explored and enable faster acquisition of mineral nutrients, hence, this could explain our results (Comas and Eissenstat, 2004; Lugli *et al.*, 2020).

Melampodium divaricatum and *M. perfoliatum* had the greater values of the FOR. Thick-root species have higher construction cost per unit root length, i.e., low SRL, as it was observed. Also, species with thick-root allocate more carbon to AM fungi to increase absorptive surfaces by trusting more on finely structural fungal hyphae, thus, often show lower values of TRL, RBR and RTD, but greater root lifespan (Liu *et al.*, 2015; Kramer-Walter *et al.*, 2016). Our findings are consistent with several studies (Liu *et al.*, 2015; Chen *et al.*, 2016) showing the same pattern, but opposite with the greater values observed for TRL, RBR and RTD. It is probable that *M. divaricatum* and *M. perfoliatum* would benefit by investing in both strategies at the same time, i.e., smaller diameter of hyphae could efficiently explore large soil volumes and the roots could obtain mineral nutrients around (Lugli *et al.*, 2020). Species that have higher RBR may be able to proliferate into localized patches of nutrients in the soil (Kramer-Walter *et al.*, 2016). A previous experiment showed that *M. divaricatum* had a fast germination and sustained growth rate than *M. tepicense* (Hanan-Alipi *et al.*, 2021), these life history traits along with the greater mycorrhizal colonization, RBR and TRL observed in this study might be key to explain the success of this species to colonize numerous habitats compared with *M. tepicense*. Opposite to our hypothesis, RTD was also significantly highest in *M. divaricatum*. It has been suggested that the plants could have high RTD and slow SRL, as in *M. divaricatum*, especially in soils where nutrients are not available. However, some studies have shown a relation between RTD and SRL (Kong *et al.*, 2014; Kramer-Walter *et al.*, 2016). In our study, *M. tepicense*, which had highest SRL and lower RTD seems to benefit in terms of nutrient acquisition as it was previously mentioned.

We also hypothesized that AM colonization would correlate positively with FOR and RTD, but negatively with TRL, SRL and RBR. However, our hypothesis was partially supported. Specifically, AM colonization by hyphae showed a positive correlation with FOR, thick-root species are generally more colonized by AM fungi, because their cortices are thicker and can provide greater space for intraradical fungal structures, and hence a higher absorption rate (Guo *et al.*, 2008; Bardgett *et al.*, 2014; Kong *et al.*, 2014; Liu *et al.*, 2015). This finding is consistent with several investigations, including plant species with different life histories and phylogenetic relationships. For instance, Liu *et al.* (2015) evaluated the root traits of 14 subtropical tree species, Kong *et al.* (2014) in 96 subtropical plant species, and Vega-Frutis *et al.* (2015) in 15 rainforest tree and palm species. RTD is considered as an important predictor of plant strategies (Birouste *et al.*, 2013; Kramer-Walter *et al.*, 2016) and has been associated with slow-growing species, although it is variable among woody species differing in moisture availability (Comas and Eissenstat, 2004). Also, RTD has been associated with AM fungi, although we observed the highest mycorrhizal colonization and RTD in *M. divaricatum*, we did not find a correlation between these variables, as we hypothesized. Liu *et al.* (2015) also did not find a correlation, they evaluated 14 subtropical tree species in China, and Lugli *et al.* (2020) found the same pattern in Central Amazonia using roots from 32 plots with similar soil and vegetation.

Regarding TRL, SRL and RBR, some studies have shown that species with higher AM colonization show a negative correlation with these variables. In this study, there was no correlation between AM fungi and SRL, this is consistent with Liu *et al.* (2015) and Maherali (2014). We observed a positive correlation between AM fungi and both TRL and BRB, these findings were opposite to our initial hypothesis. Other reports have demonstrated that mycorrhizal plants often have thinner roots and greater branching intensity (Comas *et al.*, 2014), therefore suggesting that the plants could use both foraging strategies, i.e., thin roots with more branching and mycorrhizas for the acquisition of mineral soil nutrients, thus maximizing the benefits and minimizing costs for soil resource acquisition (Bardgett *et al.*, 2014; Liu *et al.*, 2015). Additionally, TRL and RBR are key traits in determining root plastic response to nutrient patches, hence they may have high values in soils with low fer-

tility (Kong *et al.*, 2014). Consequently, weed species could be more competitive in environments where nutrients are heterogeneous in space and time (Chen *et al.*, 2016) such as anthropogenic habitats.

It is important to mention that we did not find any correlation between AM colonization by hyphae and root traits when the species were analyzed separately. In a meta-analysis used to evaluate the relationships between root architectural and mycorrhizal growth response, Maherali (2014) did not detect any relationship between the variables, probably because the plants have the ability to respond plastically to fungal colonization. Therefore, studies comparing inoculated and non-inoculated plants are necessary to test whether root traits *per se* display phenotypic plasticity to colonization by AM fungi (Comas *et al.*, 2014; Maherali, 2014).

The weed plants were collected in three sites, but in the first site coexisted *M. tepicense* and *M. divaricatum*, i.e., the species with lowest and highest weediness and also, they are the species which are closer phylogenetically. These species had access to the same pool of nutrients, but they showed different strategies to explore soil mineral nutrients independently of their phylogenetic relationship, i.e., the weeds have different foraging strategies, probably via natural selection (Comas *et al.*, 2014) given that there was no phylogenetic signal ("tendency of related species to resemble each other more than species drawn at random from the same tree", Münkemüller *et al.*, 2012). Although the amount of nitrogen and phosphorus (the phosphorus is used in an array of plant processes such as photosynthesis and respiration) were high in the sites in agreement with the Mexican Official Standard (DOF, 2000), we did not quantify the amount available to the plants, but the total amount. In the second site *M. divaricatum* and *M. perfoliatum* coexisted, these species have similar synanthropy index and there were no significant differences in AM colonization and SRL between them. Regarding the TRL, RTD, FOR and RBR *M. perfoliatum* showed values more closer to *M. divaricatum* than *M. tepicense* and this is consistent with their synanthropy index.

Finally, roots perform multiple pivotal functions in plants and the ecosystem functions. For instance, absorption of both mineral nutrients and water from the soil, physical anchoring, resource storage, and vegetative reproduction. Additionally, they are the main point of interface with soil symbiotic organisms. However,

the role of root traits is not well understood compared with leaf and stem traits (Kramer-Walter *et al.*, 2016). Future studies may incorporate more weed species to understand how root traits, AM fungi and soil conditions mediate nutrient-acquisition mechanisms.

CONCLUSIONS

The three *Melampodium* species showed AM colonization, and the weediest (*M. divaricatum*) and least weedy (*M. tepicense*) species had the highest and lowest AM colonization, respectively. These species differed also in their morphological and architectural root traits, suggesting that they have different foraging strategies even though phylogenetically they are closer, whereas *M. perfoliatum* and *M. divaricatum* had similar values regarding AM colonization and root traits, and also a similar synanthropy index. Our findings suggest that proximate causes are operating.

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