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Arbuscular mycorrhizal fungi prevent the negative effect of drought and modulate the growth-defence trade-off in tomato plants

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Abstract

Introduction: A wide range of arbuscular mycorrhizal fungi (AMF) can be applied to agricultural soils as biofertilizers for increasing crop growth and yield. Current research also shows that AMF can stimulate plant defences against a range of herbivores and pathogens. However, to date, the efficient use of AMF in agriculture is largely impaired by our inability to predict the performance of different AMF-plant complexes in variable environments. For instance, AMFs by increasing plant foraging capacity might alleviate allocation constraints in relation to growth versus defences. However, whether this effect occurs might depend on the in situ conditions. The main goal of this study was to investigate the context-dependency of the ability of AMF to modulate plant growth and resistance against herbivores under variable soil water availability.

Materials and Methods: To address our goal, we performed a greenhouse experiment for measuring the effect of different AMF inocula (*Funneliformis mosseae*, *Rhizophagus irregularis*, or both) on tomato plants (*Solanum lycopersicum*) growth and defences against an insect herbivore under two conditions: a normal watering regime or drought conditions. We measured the functional, physiological and chemical traits of the plants.

Results: We found that AMF presence generally decreased plant growth, but increased chemical defences and resistance against generalist caterpillars. Such growth-defence trade-off was nonetheless dependent on the identity of the mycorrhizal inoculum and on soil water content. Under drought, inoculated tomato plants lowered their investment to defence and noninoculated plants lowered their growth.

Conclusion: This study highlights the influence of abiotic factors and fungal identity on plant–AMF–herbivore interactions. In a broader sense, our results point to the necessity of finding AMF species that have reduced context-dependency to climatic factors, for more widespread use in organic agriculture.

KEYWORDS

biological control, chemical defence, climate change, glycoalkaloids, hydric stress, plant-microbe-insect interactions

1 | INTRODUCTION

Being at the base of the food chain, plants are constantly under herbivore attack. In response, plants have evolved various defence strategies, including the production of physical structures, such as spines or trichomes, the production of toxic chemicals (also known as plant secondary metabolites) or the reallocation of nutrients in their tissues (Walters, 2010), among others. Because the production of defences is energetically costly (Cipollini & Redman, 1999; Gershenzon & Dudareva, 2007), both wild and cultivated plants continuously face the dilemma of how to allocate their energy to growth, reproduction or defences (Herms & Mattson, 2015). Specifically, plants, throughout their lifetime have to accommodate their resources within multiple axes of trade-offs, such as choosing to allocate energy to produce new tissues (plant growth) or to produce chemical defences (Herms & Mattson, 2015). The strength of these trade-offs is largely dictated by resource availability (Coley et al., 1985; Defossez et al., 2018; Rasmann et al., 2011). For example, when nutrients are limited, plants tend to grow slower to divert energy to defences since even small tissue losses can be fatal to the plant (Karban, 2011).

To alleviate resource constraints, plants can associate with a myriad of rhizosphere- or root-associated microbes (fungi and bacteria). In exchange for root exudates or photosynthetic products, soil microbes provide plants with increased mineral and water uptake (Schnitzer et al., 2011). Association with soil microbes can enhance plant growth, especially when nutrients are limited, or when facing other environmental stresses, such as drought, heat, salinity, flooding or heavy metals (Grover et al., 2011; Kumar & Verma, 2018; Singh et al., 2018; Vimal et al., 2017). In addition, soil microbes can stimulate constitutive resistance and induced defence responses to above- and belowground plant consumers, including herbivores and pathogens (Formenti & Rasmann, 2019; Guerrieri et al., 2004; Lugtenberg & Kamilova, 2009; Martinez-Medina et al., 2016; Van Wees et al., 2008). Therefore, the association with beneficial microbes alleviates water and nutrient resources deficits in the plants, thereby mitigating the growth-defence trade-off (Dias et al., 2017; Weyens et al., 2009; Yang et al., 2009).

Within the diversity of soil-inhabiting microbes, the group of arbuscular mycorrhizal fungi (AMF; Glomeromycota) can associate with many plants in practically all terrestrial ecosystems (Smith & Read, 2010). AMF play several key roles in plant health, by enhancing plant growth and reproduction (Bennett & Bever, 2007; Koide, 1991, 2010; Wilson & Hartnett, 1997) as well as by mediating plant–herbivore interactions (Gange, 2001; Hartley & Gange, 2009; Papantoniou et al., 2021; Pozo et al., 2009). Although being common and widespread, AMF-mediated stress tolerance in plants is highly dependent on the nature of the interaction. This includes both the genetic identity of the interacting partners, and the context (e.g., soil quality, or environmental conditions) in which the interaction is taking place (Davitt et al., 2011; Zytynska, 2021). While the application of AMF in itself is a highly promising alternative to synthetic fertilizers and pesticides (Gosling et al., 2006), its context-dependency requires further research to understand how it affects the outcome of AMF–plant interactions (Hoeksema et al., 2010; Simard & Durall, 2004). Because several microbe-based products already available in the market display variable degrees of efficacy (Hart et al., 2018; Holland et al., 2018; Kokkoris et al., 2019), understanding their efficacy across variable environmental conditions is of prime importance (Hartman & Tringe, 2019; Lenoir et al., 2016; Teotia et al., 2017; Yadav et al., 2017). Experimentally assessing the context-dependency of plant–microbe interactions in terms of plant growth-defence trade-offs may help to understand the high variability of microbial-based fertilizer effects and spur their commercial utilization (Bulgari et al., 2019; Lee Díaz et al., 2021).

One of the major challenges crop producers have to face nowadays is the increase in drought events due to climate change (Li et al., 2009; Olesen et al., 2011; Porter & Semenov, 2005). Soil microbes have been shown to alleviate drought stress in several systems (Kim et al., 2012). For instance, the AMF species *Funneliformis mossae* and *Rhizophagus irregularis* were shown to alleviate drought stress in tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) plants (Latef et al., 2016; Yooyongwech et al., 2016). So far, most studies mainly explored the effect of single AMF strains on plants. Nonetheless, natural soils as well as commercial products contain more complex mixtures of AMF (Brígido et al., 2017). Likely because of this soil biological complexity, studies on the effects of AMF on plant health and growth across environments have reached diverging conclusions (Bennett & Bever, 2007; Bennett et al., 2018; Maherali & Klironomos, 2007). The microbial consortium approach (i.e., inoculating more than one species at a time) is an additional axis to study fungal–plant–insect interactions (Aguilera et al., 2022). In the context of crop production and product development, it is also important to study how mixtures of soil microbes perform under abiotic stress, such as drought.

To address these gaps, we explored how combinations of different AMF species can facilitate plant growth and defences under drought conditions. For this, we focused on the domesticated tomato *Solanum lycopersicum* 'MoneyMaker' as the model system for tomato crop production. Specifically, we asked: (1) Are AMFs generally beneficial for tomato plants under abiotic stress? We predicted that mycorrhized plants would produce more biomass, have higher levels of chemical defences and would be more resistant against insect herbivores under drought conditions than nonmycorrhized plants. (2) Is the combination of two AMF strains better than single-strain inoculations for protecting tomato plants against herbivory under drought stress? We predicted that the double-strain inoculation would increase plant growth defences and resistance more than the single-strain inoculations. Finally, we asked (3) how does AMF inoculation affect the growth-defence trade-offs plants may face? We predicted that AMF should alleviate potential growth-defence trade-offs because they can increase resource acquisition in plants. Our study aims to a better understanding of the context-dependency in plant–microbe–herbivore interactions, and in turn clarify the influence of abiotic factors while using AMF-based fertilizers and biostimulants for tomato production.

2 | MATERIAL AND METHODS

2.1 | Biological material

To address our questions, we cultivated seedlings of tomato plants (*Solanum lycopersicum*, cultivar 'MoneyMaker', SativaRheinau, Switzerland), which were inoculated with different species of AMF under different watering regimes. The AMF treatment consisted of single inoculations of the AMF *Rizophagus irregularis* (formerly *Glomus intraradices*) and *Funneliformis mosseae* (Glomeraceae), as well as their combinations. The two AMF species originated from the Swiss collection of AMF (Agroscope) and reproduced in sandy soil with *Plantago lanceolata* as nursery plant species (Wagg et al., 2011).

2.2 | Experimental design

For germination, tomato seeds were first surface-sterilized in a water-sodium hypochlorite (5% NaClO) solution for 10 min, rinsed thoroughly with deionized water and then germinated on autoclaved potting soil (Ricoter). The seedlings were maintained in the greenhouse with natural daylight at 24°C/18°C day/night until transplantation into inoculated soil 5 weeks after germination. For each treatment group, 11 individual seedlings ($n = 11$) were transferred to 3 L plastic pots containing a sterile mixture of potting soil, wood fibre and sand (2:2:1). At transplantation, inoculated plants received the mycorrhizal treatment consisting of adding 5% (v:v) of the AMF substrate inoculum to a sterile soil mixture. The control plants received the same amount of autoclaved AMF substrate. In addition, all plants received a 5 ml aliquot of a filtrate (<20 µm) of the inoculum substrate to homogenize the microbial population across all treatments. Plants did not receive any supplementary fertilization. Right after transplantation and inoculation, the watering treatment started, and the insect bioassay experiment was performed 9 weeks after and 1 week before harvesting the plants. To test for the interactive effect of drought and different AMF inoculations, we performed a full-factorial randomized designed with two main factors: (1) AMF treatments, with four levels (*R. irregularis* inoculation (R), *F. mossae* inoculation (F), both AMF species together (RF), and a control (C) without AMF); and (2) watering treatments, with two levels (normal watering regime and drought; 11 replicates per treatment, 88 plants in total). The watering treatments were imposed by either watering to maintain field capacity for the normal watering regime, or not watering until reaching the wilting point for the drought regime.

2.3 | Measurements of functional traits and samples collection

At the end of the experiment, the following functional traits related to plant growth and biomass accumulation were measured on all replicates: (1) Plant height (mm), measured as the main stem length, from the cotyledon insertion point to the terminal apex. (2) Photosynthetic activity of the leaf, measured 6 weeks after inoculation with a chlorophyllometer (SPAD, Konica Minolta, Inc.). Measurements were performed on the terminal leaflet of the last five fully developed leaves for each plant and the mean value per plant was used for statistical analyses. (3) Specific leaf area (SLA $cm^2.mg^{-1}$), which was calculated by dividing the dry weight by the area of a fully expanded leaf from the middle part of the plant. Leaf area was determined using the public domain software ImageJ (Schneider et al., 2012). (4) Carbon to nitrogen ratio (CN), measured with an organic elemental analyser (Flash2000, Thermo Scientific). Finally, we measured the fresh biomass of the plants (5) aboveground (leaves and stem) and (6) belowground (roots). Fresh roots were collected, frozen and stored for AMF colonization rate determination, leaf samples were collected and fast frozen in liquid nitrogen for metabolomic analyses.

2.4 | Insect resistance bioassay

Nine weeks after AMF inoculation and the start of the watering regimes, one fully expanded terminal leaf was collected per plant and placed in a 250 ml plastic box with the petiole maintained humidified and wrapped in wet tissue. Each box ($n = 11$ per treatment; 88 in total) received five caterpillars (2nd instar) of the highly polyphagous Egyptian cotton leafworm (*Spodoptera littoralis*, Lepidoptera, Noctuidae) for a total weight of 55 mg (±10%). Caterpillars' eggs provided by the Farce laboratory of the University of Neuchâtel were previously fed on tomato leaves and grown up to 2nd instar. The larvae were left in the boxes with the leaves for 7 days at 22°C with a 12 h photoperiod. Finally, the fresh biomasses of the five caterpillars were averaged to obtain a larval weight gain value for each plant replicate for all treatments (440 caterpillars in total).

2.5 | Determination of mycorrhizal inoculation

Mycorrhizal inoculation was determined after carefully cleaning the roots under tap water. The roots were washed in KOH (10%) for 30 min at 90°C and rinsed with water acidified with acetic acid (1%). The fungal structures present in the roots were stained with a staining solution (5% blue ink dissolved in 5% acetic acid) following the method described in Vierheilig et al. (2005). The mycorrhizal colonization rate was calculated using the gridline intersection method (Giovannetti & Mosse, 1980) under a stereo microscope at 100X (Leica MGD33). We calculated the colonization rate as the percentage of root length colonized by the AMF, considering hyphae, arbuscules and vesicles.

2.6 | Plant secondary metabolites

A targeted metabolomics approach was used to assess the effect of AMF, drought and herbivory on major defence metabolites in tomato plants. These included the two glycoalkaloids, tomatine and

dehydrotomatine, and the flavonoid, quercetin-3-O-rutinoside, also known as rutin. Leaf samples of each plant replicate were extracted following a procedure derived from De Vos et al. (2012). Briefly, freeze-dried leaf material was ground to powder and 20 mg of each sample was double extracted with an extraction solution, MeOH HPLC grade/Acetate buffer pH 4.8 (75:25, v/v %) in 2 ml reaction tubes holding two glass beads by shaking in a TissueLyser (Qiagen) at 30 Hz for 5 min, followed by 10 min centrifugation at 15,000 rpm at 4°C. Clear supernatants were combined and stored at −20°C until further processing. Next, the diluted crude extracts (1:50) were analysed with an UltiMate™ 3000 Standard Ultrahigh-Pressure Liquid Chromatography system (UHPLC, Thermo Scientific) equipped with an Acclaim® Rapid Separation Liquid Chromatography (RSLC) 120 column (150 × 2.1 mm, particle size 2.2 µm, ThermoFisher Scientific) using the following gradient at a flow rate of 0.4 ml/min: 0–2 min isocratic 95% A (water/formic acid 99.95/0.05 (v/v %)), 5% B (acetonitrile/formic acid 99.95/0.05 (v/v %)); 2–15 min, linear from 5% to 40% B; 15–20 min, linear from 40% to 95% B; 20–22 min, isocratic 95% B; 22–25 min, linear from 95% to 5% B; 25–30 min, isocratic 5% B. The eluted compounds were detected from m/z 90 to 1600 at a spectra rate of 3 Hz, using an ESI-UHR-QToF-MS (maXis impact quadrupole time-of-flight mass spectrometer, Bruker Daltonics) equipped with an Apollo II electrospray ion source applying the following instrument settings in positive ion mode: scan range 90–1600 m/z; acquisition rate 3 Hz; end plate offset 500 V; capillary voltage 3500 V; nebulizer pressure 2 bar, dry gas 10 L min−1, dry temperature 220°C. Mass calibration was performed using sodium formate clusters (10 mM solution of NaOH in 50/50 (v/v %) isopropanol water containing 0.2% formic acid.

Finally, for the data processing, the chromatograms from the raw QToF-MS Bruker data files were baseline-corrected, deconvoluted and aligned using mzMine 2.53. Assigning m/z groups to features was performed on mzMine 2.53 (Pluskal et al., 2010) and R4.0.5. R Core Team (2021) using the packages 'CAMERA' (Kuhl et al., 2012) and 'xcms' (Smith et al., 2006). We used the unique detected molecular feature to identify the compounds rutin, alpha-tomatine and dehydro-tomatine based on their fragmentation spectra at low energy under electrospray ionisation (ESI) in a positive mode (Supporting Information: Figure S1). For analyses, we used the relative amounts of the molecules based on the peak area of the cumulated fragmented ions.

3 | STATISTICAL ANALYSIS

Analyses were conducted using the software R4.0.5 (R Core Team, 2021). We performed the following analyses to address the questions posed in the introduction.

(1) To assess the effect of drought (two levels) and AM fungal inoculation treatments (four levels) on plant growth and defences, the nine plant traits (plant size, leaf chlorophyll index (SPAD), SLA, CN), aboveground biomass, belowground biomass, insect weight gain, alpha-tomatine, dehydrotomatine and rutin) were compared across all treatment combinations using a two-way multivariate analysis of variance (MANOVA, implemented with Wilks' lambda test) followed by univariate analyses of variance (ANOVAs) to test for individual main effects. Similarly, the effect of drought and AMF on larval weight gain were assessed with two-way ANOVAs. Chemical traits and larval weight gain were log-transformed before analyses to comply with the requirement of homoscedasticity of variance.

(2) To assess the effect of different inoculations on plant growth, defence and responses to the drought we calculated the effect size within each AMF treatment. Effect sizes were calculated using Cohen's *d* metric (Cohen, 2013), as estimated with the 'effsize' package (Torchiano, 2020). In our case, a positive effect size indicates that the plants, within each treatment, performed worst under drought conditions than under the normal watering regime. To summarize plant growth responses within one variable, all plant functional traits measured (as described above) were ordinated using a principal component analysis (PCA) calculated with the 'ade4' package (Dray & Dufour, 2007), and the first axis of the PCA was used for calculating effect sizes. We then calculated effect sizes on alpha-tomatine (known for defence properties), and on the larval biomass weight gain as proxies for plant chemical defences, and plant insect resistance, respectively.

(3) To assess the effect of AMFs and drought on the plant growth-defence trade-off, we built an interaction plot using the first axis of the coinertia analysis between the seven growth-related traits and the three chemical defence traits. When significant, the coinertia analysis (*coin* function in the 'vegan' package [Oksanen et al., 2020]) indicates a significant co-structuration (i.e., correlation) between the plant growth traits and the chemical defence matrices. This multi-trait trade-off was visualised using nonmetric multidimensional scaling (NMDS) implemented in the 'vegan' package. The AMF × drought treatment effect was tested using permutational multivariate ANOVA (PERMANOVA, using the *adonis* function in the 'vegan' package). The euclidean metric was used to calculate dissimilarity among samples for both the NMDS and PERMANOVA, although results were robust to other distance metrics. Finally, larval weight gain was fitted on the NMDS ordination using the function *envfit* in the vegan package.

4 | RESULTS

4.1 | Are AMFs beneficial for tomato plants under drought stress?

4.1.1 | Effects of drought on AMF colonization

AMF colonization was dependent on the species of AMF and their interaction with drought (D) (see significant AMF and D × AMF interaction effects in Table 1). Under drought conditions, all AMF species showed homogenous colonization rates of 3.7%, 4.8% and

4.2%, respectively for F (*F. mossae*), R (*R. irregularis*) and RF. Under the normal watering regime, *R. irregularis* had root colonization of 8.3% compared with only 2.6% of the root length for *F. mossae* (Table 1, Supporting Information: Figure S2). When both species were present, the colonization rate was the lowest, about 0.6% of the root length (Table 1, Supporting Information: Figure S2).

4.1.2 | Effects of AMF and drought on plant functional traits

We found strong main and interactive effects of drought and AMF treatments on all plant traits (MANOVA results for drought treatment; $F_{8,72} = 1479$, $p < 0.001$, AMF treatment effect; $F_{24,222} = 9.14$, $p < 0.001$, and drought × AMF interaction; $F_{24,222} = 4.06$, $p < 0.001$). Overall for both well-watered and drought-stressed plants, AMF-treated plants were 23% smaller and 47% lighter than control (nonmycorrhized) plants (Table 1, Figure 1). We also found an interactive effect between AMF and drought treatments across most traits studied (Figure 1, Table 1). In nonmycorrhized plants, drought decreased plant size by 26% (Figure 1a) and aboveground biomass by 34% (Figure 1b). Drought also increased belowground biomass by 76% (Figure 1c), the SLA by 28% (Figure 1e) and the C/N by 30% (Figure 1f). Mycorrhized plants were less strongly affected by the drought treatment. After inoculation by *R. irregularis*, drought decreased plant size by 23%, and the production of aboveground biomass only by 6%, the SLA increased by 8%, while belowground biomass and C/N were not different from that of well-watered plants (Figure 1). After inoculation with *F. mossae*, drought reduced plant size by 11%, the production of aboveground biomass by 7.6%, while belowground biomass increased by 9.4%, SLA by 21% and C/N by 12% compared with normal watering (Figure 1). After inoculation with the two AMF species mix, drought decreased plant size by 19.6%, the production of aboveground biomass by 22%, while belowground biomass increased by 31.6%, SLA by 2.5% and C/N by 39% (Figure 1). The chlorophyll content estimated by the SPAD measurement increased under drought for the control and the *R. irregularis* treatment, while it was less significantly affected for *F. mossae* and the double inoculation treatment.

When analysing all traits together, we observed positive and negative trait correlations. Specifically, plants with higher C/N and SLA values, mostly encompassing mycorrhized plants, attained a lower biomass and plant height (Figure 2a). In contrast, nonmycorrhized plants were bigger and heavier than AMF plants (Figure 2a). Therefore, we observed a shift in resource use. This can be seen when moving along the first axis of the PCA, which goes from a syndrome of plants investing more energy in biomass allocation and chlorophyll activity (the negative values) to a syndrome of high carbon storage and thicker leaves (positive values) mediated by the absence or presence of AMF on tomato roots (Figure 2a). This shift in carbon allocation strategies was also modulated by watering regimes. Particularly, we observed that nonmycorrhized drought-stressed plants invest more into root growth (Figure 1c) and have the lowest C/N in the leaves (Figure 1f). Similar trends in chlorophyll index (SPAD: Figure 1d) between *R. irregularis* and noninoculated control plants explain their proximity to the drought effect size (Figure 2c).

4.1.3 | Effects of AMF and drought on tomato chemical defences

We found a general positive effect of AMFs on the constitutive concentration of the three major secondary metabolites present in the tomato leaves (Figure 1g,h, Table 1). Overall, AMF treatments significantly increased the leaf concentrations of the two glycoalkaloids, alpha-tomatine and dehydrotomatine, and the flavonoid compound rutin (Table 1; AMF effects, $p < 0.001$). The concentrations of alpha-tomatine, dehydrotomatine and rutin were higher in the presence of AMF for both watering regimes. Compared with control plants, the concentration in alpha-tomatine increased by 37% in the AMF treatments, on average for both watering regimes together. Within well-watered plants, tomatine concentrations in the leaves of inoculated plants were 51.8% (F), 70.7% (R) and 86.8% (RF) higher than in control plants (Figure 1g). For the flavonoid, when exposed to a drought regime, leaves of AMF inoculated plants contain 18.9% (R), 17.8% (F) and 24.7% (RF) more rutin than nonmycorrhized plants (Figure 1i).

The effect of mycorrhiza on tomatine levels in the leaves was affected by its interaction with the watering regime (Table 1; D × AMF interaction effect, $p = 0.02$). In control plants, leaves of drought-stressed plants had 25.6% higher levels of tomatine, whereas in mycorrhized plants, the levels of tomatine were higher in well-watered plants (Figure 1g). Rutin and dehydrotomatine did not show interactive responses to drought and mycorrhization. The concentration of these three molecules in the leaves was not significantly affected by drought (Table 1; D effects $p > 0.05$), thus the concentrations show similar patterns in the two hydric regimes.

4.2 | How do AMF species and their combined inoculation differentially affect plants under drought stress?

4.2.1 | Effects of AMF and drought on plant growth

Within AMF treatments, we also observed that aboveground and belowground biomass are less affected by drought (Figure 1b) than noninoculated plants. To identify the underlying trait correlations, we performed a PCA. Overall, the PCA ordination showed positive and negative traits' correlations. Specifically, lower CN and SLA values were associated with higher root and leaf biomass, plant height and chlorophyll content (Figure 2a). Nonmycorrhized plants were bigger and heavier than AMF plants (Figure 2b). On the contrary, with AMF,

TABLE 1 Two-way interaction ANOVA table for measuring the effect of drought (two levels) and AMF treatments (four levels) on plant growth and chemical defence traits

Response variable	Treatment	Df	SumSq	*F* value	*p* value	Significance
AMF colonization	Drought (D)	1	4.4	1.17	0.28	
	AMF	3	462.2	40.56	<0.001	***
	D × AMF	3	143.3	12.57	<0.001	***
	Residuals	79	300.1			
Plant height	Drought (D)	1	705,990	37.10	<0.001	***
	AMF	3	874,752	15.32	<0.001	***
	D × AMF	3	123,335	2.16	0.1	
	Residuals	79	1,503,301			
SPAD	Drought (D)	1	54.9	7.50	0.01	**
	AMF	3	652.1	29.71	<0.001	***
	D × AMF	3	152.7	6.96	<0.001	***
	Residuals	79	578.1			
SLA	Drought (D)	1	0.0089	0.61	0.44	
	AMF	3	0.332	7.63	<0.001	***
	D × AMF	3	0.106	2.44	0.07	.
	Residuals	79	1.1461			
CN	Drought (D)	1	92.3	3.63	0.06	
	AMF	3	1700.5	22.33	<0.001	***
	D × AMF	3	1649.6	21.66	<0.001	***
	Residuals	79	2005.8			
AG biomass	Drought (D)	1	0.903	15.81	<0.001	***
	AMF	3	7.814	45.59	<0.001	***
	D × AMF	3	0.537	3.13	0.03	*
	Residuals	79	4.513			
BG biomass	Drought (D)	1	1.02E + 00	21.43	<0.001	***
	AMF	3	5.99E + 00	42.04	<0.001	***
	D × AMF	3	8.11E-01	5.7	0.001	**
	Residuals	79	3.75E + 00			
Alpha-tomatine	Drought (D)	1	7.62E + 11	3.65	0.06	.
	AMF	3	6.13E + 12	9.79	<0.001	***
	D × AMF	3	2.06E + 12	3.29	0.02	*
	Residuals	79	1.65E + 13			
Dehydrotomatine	Drought (D)	1	1.04E + 12	1.24	0.27	
	AMF	3	1.48E + 13	5.89	0.001	**
	D × AMF	3	4.47E + 12	1.78	0.16	
	Residuals	79	6.63E + 13			
Rutin	Drought (D)	1	1.01E + 11	0.34	0.56	
	AMF	3	6.99E + 12	7.73	<0.001	***

TABLE 1 (Continued)

Response variable	Treatment	Df	SumSq	*F* value	*p* value	Significance
	D × AMF	3	1.21E + 12	1.34	0.27	
	Residuals	79	2.38E + 13			

Abbreviations: AMF, arbuscular mycorrhizal fungi; CN, carbon to nitrogen ratio; SLA, specific leaf area; SPDA, leaf chlorophyll index.
Asterisks indicate significant effect.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

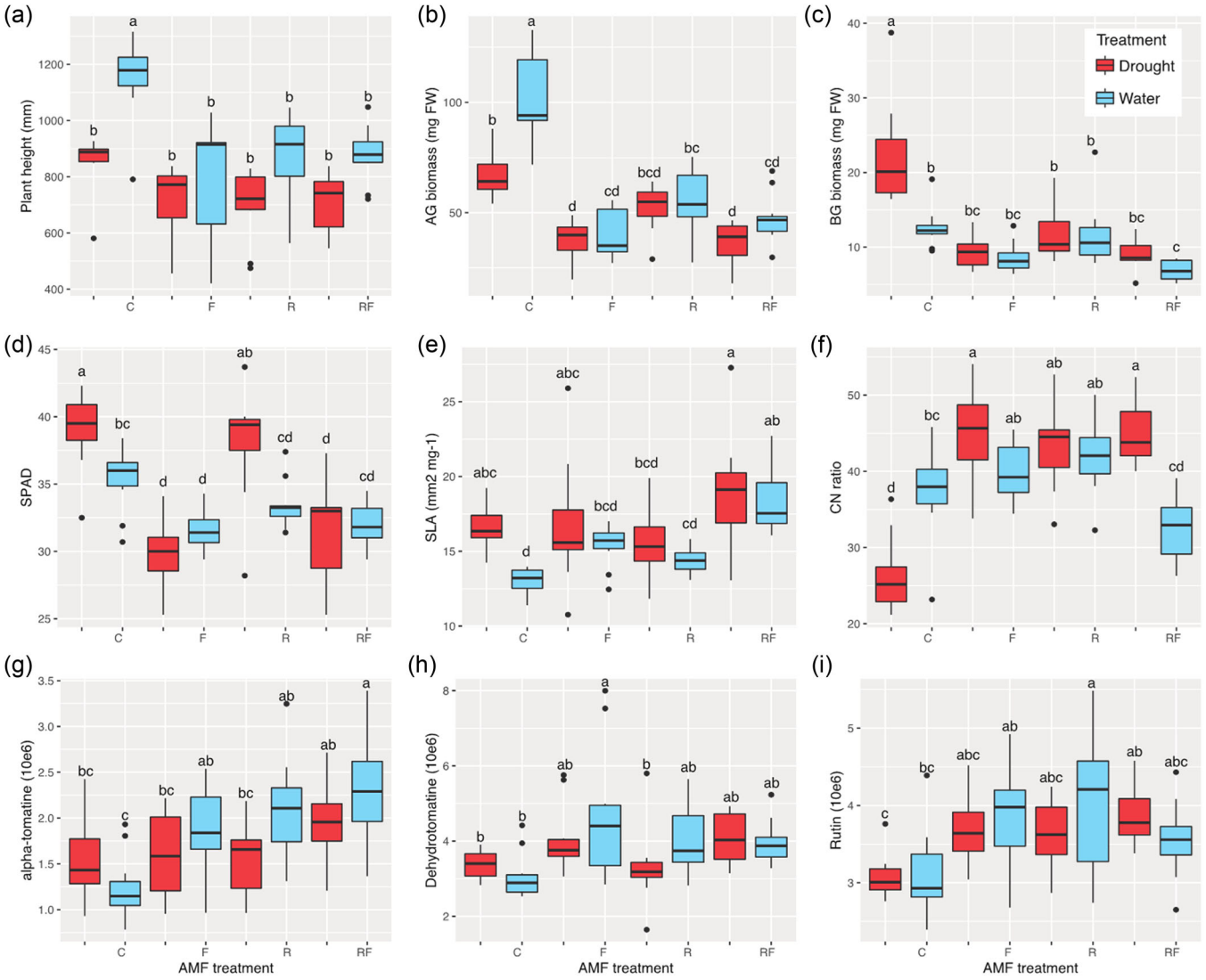

FIGURE 1 AMF and drought effects on plant growth and defences. Boxplots show for each AMF treatment (C: control; F: *Funneliformis mossae*; R: *Rhizophagus irregularis*; RF: *R. irregularis* + *F. mossae*) and each watering regime (blue: normal watering; red: drought condition) the raw data of the recorded variables: plant height (a); AG, aboveground biomass (b); BG, belowground biomass (c); SPAD, chlorophyll index (d); SLA, specific leaf area (e); carbon to nitrogen (C/N) ratio (f), and the relative chemical concentrations in the leaves before herbivory of alpha-tomatine (g); dehydrotomatine (h); rutin (i). Letters above boxplots indicate significant differences among AMF and drought treatments (Tukey HSD test, $p < 0.05$). Boxplots represent, from bottom to top, minimum, first quartile, median, third quartile and maximum, and dots represent the outliners.

tomato leaves had higher CN ratios and SLA values, indicating that they are thinner and contained 38% less nitrogen. Drought affected photosynthetic activity (SPAD, Figure 1d) for both noninoculated plants and inoculated with *R. irregularis*. This may explain why these treatments are projected closely to each other on the PCA1 (Figure 2c).

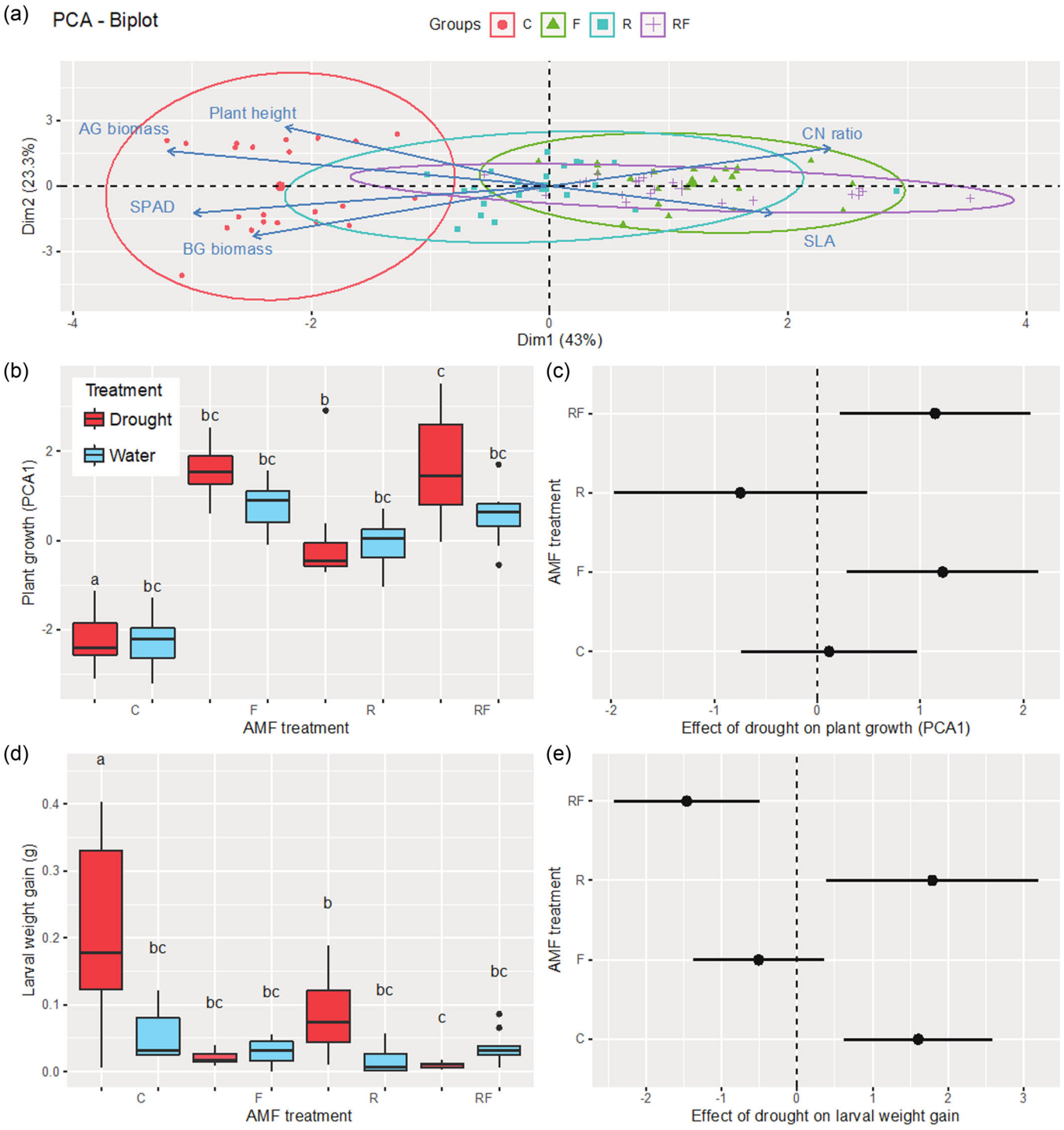

FIGURE 2 The effects of drought on plant growth responses with or without AMF. Shown are (a) principal component analysis (PCA) ordination plots for all plant growth-related traits (plant height, aboveground [AG] biomass, belowground [BG] biomass, chlorophyll content [SPAD], carbon-to-nitrogen [CN] ratio and specific leaf area [SLA]). Ellipses represent 95% confidence intervals around the different AMF treatments (C: control; F: *Funneliformis mossae*; R: *Rhizophagus irregularis*; RF: *R. irregularis* + *F. mossae*) (b) Average of the loadings of the first axis of the PCA (PCA1) as shown above, grouped by the drought treatment (blue: normal watering; red: drought condition), and by the different AMF treatments as described above in the (c) Cohens' *D* effect sizes between the normal watering and the drought condition across all AMF treatments using the loading of PCA1 (averages +/− 95% c.i.). (d) Average larval weight gain between plants under the two watering regimes and AMF treatments, and (e) Cohens' *D* effect sizes between the normal watering and the drought condition across all AMF treatments for larval weight gain (averages + /− 95% c.i.). Letters above boxplots indicate significant differences among AMF and drought treatments (Tukey HSD test, $p < 0.05$). Boxplots represent, from bottom to top, minimum, first quartile, median, third quartile and maximum, and dots represent the outliners.

4.2.2 | Effects of AMF and drought on insect performance

We found significant effects of AMF on *S. littoralis* caterpillar performance (Figure 2d; AMF effect; $F_{3,79} = 28.83$, $p < 0.001$). When fed on leaves of plants inoculated with AMF, caterpillar biomass decreased to 25% of that of caterpillars reared on leaves of nonmycorrhized tomato plants. This effect was strongly affected by drought (Figure 2d; drought effect; $F_{1,72} = 0.05$, $p < 0.001$) and the interaction between AMF and drought (Figure 2d,e; $F_{3,72} = 13.45$, $p < 0.001$). Under drought stress, all AMF treatments resulted in significantly smaller caterpillars, while under the normal watering regime, there were no significant differences in larval weight gain (Figure 2d). Drought also had a significant impact on herbivore performance (Table 1); overall, we found that *S. littoralis* larvae fed with leaves from well-watered plants grew 63% less than those fed with leaves of drought-stressed plants (Figure 2d). When comparing normal watering to drought stress for each AMF treatment, larval weight gain was 74% lower on well-watered control plants and 78% lower on well-watered *R. irregularis*-inoculated plants. Caterpillar biomass was generally low when fed on leaves from the mixed AMF inoculum (RF) and *F. mossae* for both well-watered and drought-stressed plants. The single inoculation with *F. mossae* did not affect significantly herbivore resistance across the two hydric regimes (Figure 2d,e).

4.3 | How do AMF inoculation and drought affect the growth-defence trade-off in tomato plants?

4.3.1 | Effects of AMF and drought on growth-defence trade-off

We found that control, nonmycorrhized plants invested more in biomass accumulation, while mycorrhized plants invested more in chemical defences (Figure 3, Supporting Information: Figure S3). AMF treatments clearly separated the plant phenotypes in the multivariate space (for the water treatment, Figure 4a, PERMANOVA with 999 permutations; $F_{3,42} = 5.71$, $p = 0.001$, and for the drought treatment, Figure 4b, $F_{3,43} = 4.40$, $p = 0.002$, Supporting Information: Figure S3). Specifically, we observed a negative correlation between aboveground biomass and the concentration of rutin in leaves (Supporting Information: Figure S4, $r = -0.38$, $p < 0.001$). Such intraspecific growth-defence trade-off was more pronounced when plants were well-watered, while it was alleviated (i.e., plants were more similar along the trade-off axis) when under reduced watering conditions (Figure 3). Finally, we observed that along this trade-off axis, AMF-treated plants, which invested more in defence, were also more resistant to *S. littoralis* feeding. However, this observable effect was only significant under drought conditions (correlation between larval weight gain and the growth-defence matrix under normal watering regimes; Figure 4a, $r = 0.3$, $p = 0.167$, and under drought conditions; Figure 4b, $r = 0.74$, $p = 0.001$).

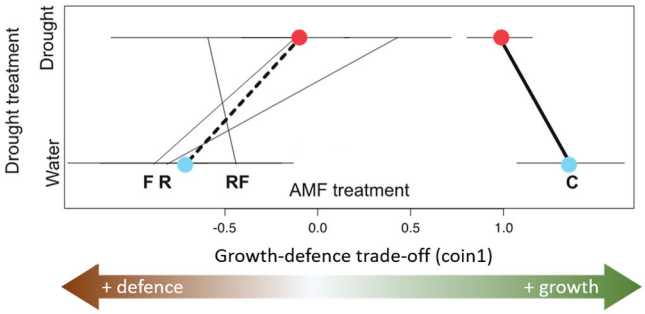

FIGURE 3 Interaction plot for the growth-defence trade-offs. The trade-off is based on the coinertia axis one calculated between the matrix of the seven growth traits and the three chemical defences (coinertia: $r = 0.188$, $p < 0.001$). Negative values of the coinertia axis are linked to a strategy of investing in chemical defences, while positive values are linked to a strategy of investing in biomass accumulation. Dots represent the average + /− 1 S.E. of each AMF treatment (C: control (bold plain line); F: *Funneliformis mossae*; R: *Rhizophagus irregularis*; RF: *R. irregularis* + *F. mossae* (bold dashed line, representing the average of the three AMF treatments)) across the two and each watering regime (blue: normal watering; red: drought condition).

5 | DISCUSSION

We explored plant–AMF–leaf herbivore interactions in domesticated tomato plants under drought stress. We found that drought generally decreased plant growth and resistance against generalist caterpillars of noninoculated plants in the timeframe of our study. The effect on growth and defence was context-dependent and related to the identity of the mycorrhizal inoculum and watering regimes. Interestingly, despite the fact that AMF-inoculated plants were mostly smaller, they had a lower reduction of height and biomass due to drought and could maintain a higher level of glycoalkaloids and rutin than nonmycorrhized plants. This indicates that the growth-defence trade-off was generally alleviated by the presence of AMFs in the tomato roots.

5.1 | AMF effects on plant growth and defences

Two major outcomes arose from our study; first, we show that AMF overall decreased plant growth but also increased defences and resistance against caterpillars. Second, we show that the effect of drought on plant functional traits was variable and interacted with the presence/absence or identity of the AMF inocula. That AMF reduce plant growth and biomass accumulation has been shown before, even though it may not be so commonly reported in the literature (Koltai & Kapulnik, 2010). Plausible reasons for this result are: (i) energy allocation trade-offs (Cavagnaro et al., 2021; Unger et al., 2016), (ii) costs of the mycorrhization exceeding the benefits of increased resource acquisition through the mycorrhizal network (Höpfner et al., 2015; Ngo et al., 2021; Sanchez-Bel et al., 2016) or (iii) physical constraints imposed by the potting system used (Lenoir et al., 2016; Wang et al., 2019). In our case, the best plausible

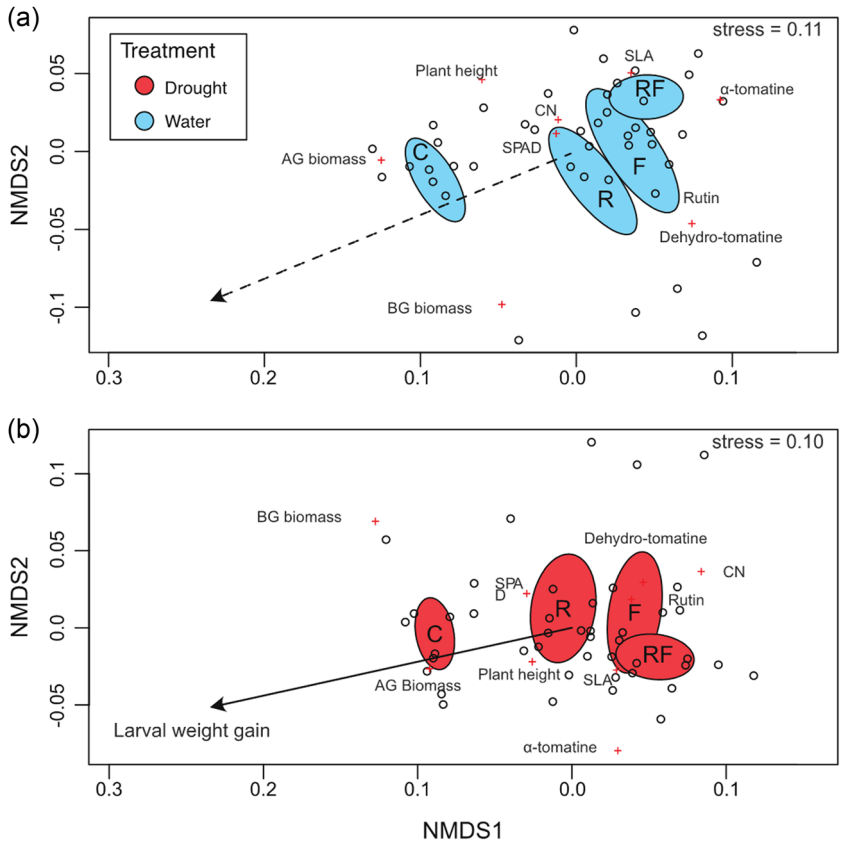

FIGURE 4 Nonmetric multidimensional scaling (NMDS) plot of the *Solanum lycopersicum* plant functional and chemical traits of ([a]: Water, for the normal watering regime; [b]: Drought, for hydric stress). Distance matrices were generated using the following variables: aboveground biomass, belowground biomass, plant height, specific leaf area (SLA), SPAD, carbon to nitrogen ratio of the leaves and concentrations in the leaves of the compounds: alpha-tomatine, dehydro-tomatine and rutin. The 95% confidence interval ellipses are represented based on the four different AMF treatments (Control, *F. mossae*, *R. irregularis*, *R.i. + F.m*). Stress values: $A = 0.11$; $B = 0.10$. For the water treatment, Figure 4a, PERMANOVA with 999 permutations; $F_{3,42} = 5.71$, $p = 0.001$, and for the drought treatment, Figure 4b, $F_{3,43} = 4.40$, $p = 0.002$). The arrows represent the plotted Bray-distance of the permanova of the Larval weight gain in the NMDS statistical space. The effect is significant for the drought treatment only (correlation between larval weight gain and the growth-defence matrix under normal watering regimes; Figure 4a, r = 0.3, $p = 0.167$, and under drought conditions; Figure 4b, $r = 0.74$, $p = 0.001$).

explanation is allocation trade-offs mediated by the AMFs. Indeed, when mycorrhized, plants grew less than nonmycorrhized plants but were more defended.

Second, we found that when the AMF inocula are mixed together, plant resistance to herbivory under drought conditions reached the highest level (Figure 2) and the mycorrhization is comparable to the other AMF treatments (Supporting Information: Figure S2). Under a normal watering regime, the two-species inoculum (RF) showed the lowest colonization rate, but comparable effects for herbivore performance to the single inoculation treatments (F and R). These results confirm our hypothesis that the response of the tomato plants to the beneficial fungi is both species- and environment-dependent (Barber et al., 2013). The growth of tomato plants was more impacted by drought when colonized by *R. irregularis*, while *F. mossae* and the mix of the two AMF species helped to alleviate the negative effects of drought. Accordingly, three-way interactions between fungi, plants and their enemies remain difficult to predict (Bennett et al., 2006), and even more so when in the presence of an additional factor, such as drought, a predominant abiotic factor impacting the growth of the inoculated plants (Bennett & Bever, 2007). Nevertheless, we demonstrated that tomato plants inoculated with AMF (*R. irregularis*, *F. mossae* or the mixture of both) increased the constitutive concentration of toxic alkaloids, independent of the watering regime. Previous work showed that the steroidal glycoalkaloids (including alpha-tomatine) produced by *S. lycopersicum* are detrimental to the growth and development of a wide range of insect herbivores, including generalist and specialist caterpillars (Friedman, 2002, 2004; Leite-Mondin et al., 2021; Papantoniou et al., 2021). Along the same lines, the increase of the flavonoid compound rutin (quercetin-3-O-rutinoside) in all AMF treatments, both under drought or normal watering regimes, indicates a clear influence of the mycorrhizal fungi on the chemical composition of tomato leaves (Figure 1j). Our findings thus corroborate the postulated effect of AMF on chemical defences, which is likely mediated by the triggering hormonal pathway related to chemical defence activation (Aseel et al., 2019; Delavaux et al., 2017; Minton et al., 2016).

5.2 | Effects of AMF inoculation and drought on the growth-defence trade-off in tomato plants

We showed complex biotic- and abiotic-mediated effects on tomato plants' growth-defence trade-offs. Under a normal watering regime, the noninoculated tomato plants invested more in growth, while AMF-treated plants contained higher concentrations of chemical defence compounds. More specifically, all AMF-inoculated treatments increased the production of alpha-tomatine, while noninoculated plants generally contained less alpha-tomatine and rutin in their leaves, compared with the plants with AMF. Interestingly, the magnitude of such trade-offs was more pronounced under normal watering conditions than under drought conditions. Under drought, inoculated tomato plants lowered their investment to defence and noninoculated plants lowered their shoot growth. This suggests that under good resource availability, in this case, water availability, mycorrhized plants are more plastic and are able to explore a wider phenotypical space, both in terms of growth and defences (Gratani, 2014; Toby Kiers et al., 2010). Or, said otherwise, environmental stress would tend to limit plant AMF-enhanced resistance to herbivores, suggesting that climate change can impact plant–herbivores–AMF interactions more strongly than when predicted from studies on bilateral interactions (Nicotra et al., 2010). Future research should aim to understand the physiological and metabolic bases of how AMFs mediate such growth-defence trade-offs (Meena et al., 2017). This would help to identify AMF strains that can be best used under elevated drought conditions (Grover et al., 2011). Interestingly, we also notice that all AMF-inoculated tomato plants presented a high constitutive concentration of both alpha-tomatine and rutin when the plants are well-watered, but also under 9 weeks of drought treatment. The enhancement of these glycoalkaloid and flavonoid compounds concentrations confirms the effect of AMF in triggering the constitutive chemical defence of the tomato plants under hydric physiological constraints. Whether this effect is mediated by physiological constraints, or whether this effect is the result of an adaptive response of the plants also needs further attention when developing tomato varieties that will be likely affected by intense drought events in the future (Basu et al., 2018).

6 | CONCLUSION

This study, combining functional, physiological and chemical variables aimed to bring new knowledge about the effect of AMF on tomato growth and defences under drought conditions. We showed that the watering regime during the culture cycle influences strongly how AMF interacts with the tomato plants. Particularly, while multiple microbe-based products are already available in the market, understanding their efficacy across the variation in climatic stresses is of prime importance. Our approach indicates that complex plant–microbe–insect–environment interactions have to be fully considered to obtain a real benefit in plant production. Our findings support that we need more data to fully understand the dependency of the AMF effects on eco-climatic factors for a precise projection of production systems with significant economic stakes. We therefore advocate measuring the effect of AMF under realistic climatic conditions and enriching these experiments with an extensive metabolomic investigation to identify further the molecular basis of abiotic and biotic stress attenuation induced by AMF colonization.

AUTHOR CONTRIBUTIONS

Dimitri Orine and Sergio Rasmann conceived the idea and designed the methodology. Dimitri Orine performed the experiment and collected the data with help of the Botanical Garden of Neuchâtel. Dimitri Orine, Fredd Vergara and Henriette Uthe carried out the chemical analyses. Dimitri Orine and Emmanuel Defossez did the metabolomic data processing. Dimitri Orine and Sergio Rasmann analysed the data. Dimitri Orine and Sergio Rasmann led the writing of the manuscript; Nicole M. van Dam edited the text; all authors reviewed the draft and gave final approval.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from OSF Repository at https://osf.io/dw7kq/(DOI 10.17605/osf. io/dw7kq).

ETHICS STATEMENT

The authors confirm that they have followed the ethical policies of the journal.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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