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Effects of water limitation on the production of key secondary metabolites with medicinal properties in *Plantago lanceolata* and *Tanacetum parthenium*

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ABSTRACT

Plant secondary metabolites play vital roles in growth, defence, and human pharmacological applications, with medicinal plants historically used to treat diseases. However, climate-change-induced drought may threaten medicinal plants' growth and metabolite production. This study examines drought effects on two medicinal plants common in the UK: *Plantago lanceolata* and *Tanacetum parthenium*. Under glasshouse conditions, we compared growth, chlorophyll content, and photosystem II efficiency (Fv/Fm) in well-watered vs. low-watered plants. High-performance liquid chromatography (HPLC) measured the concentration of parthenolide in *T. parthenium* and verbasco-side in *P. lanceolata* leaves, with both compounds being the two most abundant with medicinal relevance. Results showed significantly reduced growth for both species under drought, though photosynthetic performance remained unaffected. Parthenolide content in *T. parthenium* decreased with low water, while verbasco-side in *P. lanceolata* showed no significant change. This study highlights drought's impact on medicinal plants' growth and metabolite production, emphasizing the need for further research on environmental factors affecting secondary metabolites under climate change.

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KEYWORDS

Drought; medicinal plants; parthenolide; verbasco-side

Introduction

Medicinal plants have long been an important component of health care in human populations, with written records of the preparation of drugs from plants found on clay slabs dating back to over 5000 years ago (Hassan 2015) and archaeological remains dating back over 70,000 years (Wadley et al. 2011). Of the approximately 308,000 vascular plant species which have been described and accepted globally to date (Christenhusz and Byng 2016), over 50,000 are known to be used (historically or currently or both) for medicinal purposes (Chen et al. 2016). Plants produce a diverse array of secondary metabolites that play different roles in plant growth and development, including regulating flowering and defence against pathogens and herbivores (Teoh 2015; Erb and Kliebenstein 2020). These secondary metabolites have a variety of applications for humans, for example, as food additives, agrochemicals, biopesticides (Jamwal et al. 2018), and pharmaceuticals (Tungmunnithum et al. 2018). The value of plants as sources of medicine has been supported historically

through the production of important plant-derived drugs such as artemisinin found in *Artemisia annua* L. and used in the treatment of malaria (Qamar et al. 2024), paclitaxel extracted from *Taxus brevifolia* and widely used in chemotherapy (Weaver 2014), or galanthamine obtained from the family Amaryllidaceae and used in the treatment of Alzheimer's disease (Sramek et al. 2000). The contribution of plants to the treatment and prevention of disease is still enormous today, with 11% of the 252 drugs the World Health Organization (WHO) considers 'essential' and 'basic' being exclusively derived from plants (Veeresham 2012). It is generally agreed that plants still hold great potential for the discovery and development of new drugs (Miller 2011; Sezer et al. 2024), and despite sustained research activity in the field, further work is required to improve our understanding of the impact of climate change on medicinal plants (Applequist et al. 2020).

Climate plays a primary role in defining the distribution of species along the latitudinal and elevational gradients of our planet (Stevens 1989; Stevens

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1992; Rahbek 2005; Lenoir et al. 2020). Changes in climatic conditions affect plant species, which need to move or adapt to the novel growth conditions to avoid perishing (Araújo and Pearson 2005; Parmesan 2006). Climate change in ecosystems, therefore, may lead to changes in plant species distribution and composition, including species invasions and extinctions, but may also affect species physiology, phenology, and interaction with other organisms and their environment (Bellard et al. 2012; Fontúrbel et al. 2021; Kumari et al. 2024). Overall, climate change is considered a major threat to biodiversity and ecosystem functioning. In the context of medicinal plants, climate change may not only affect species distribution (and survival) but may also affect plant growth and the synthesis and production of plant secondary metabolites with medicinal properties (Sun et al. 2023; Alum 2024). Climate change can affect plant morphology, including changes in the root-to-shoot ratio (Eziz et al. 2017), specific root length (Nicotra et al. 2010), leaf area, foliage distribution (Gratani 2014), and the overall plant biomass (Misra and Srivastava 2000), which can also affect the quantity of desirable metabolites harvested from plants (Applequist et al. 2020). Abiotic stresses associated with climate change, such as changes in temperature and water availability, can affect the secondary metabolite content of medicinal plants (Gairola et al. 2010; Applequist et al. 2020; Sun et al. 2023; Alum 2024), which in turn may affect the medicinal properties and safety in therapeutic use – e.g., posing a health risk if stress results in an unexpected increase in the concentration of secondary metabolites that can become toxic at higher concentrations (Applequist et al. 2020). Prior work has found that drought tends to increase the content of secondary metabolites in plants, particularly of those involved in drought protection response (Yang et al. 2018), and also tends to increase overall metabolite content specifically in medicinal plants (Selmar and Kleinwächter 2013). However, the effect of environmental stress on plant secondary metabolites appears variable and has been found to depend on the type of stress and the identity of the chemicals considered (Yang et al. 2018). For example, baicalin content, a flavonoid with therapeutic uses extracted from *Scutellaria baicalensis* Georgi, was found to increase under mild to moderate drought stress conditions but to decrease under severe stress conditions (Cheng et al. 2018). Similarly, glycyrrhizin content, a triterpenoid

saponin with therapeutic properties extracted from *Glycyrrhiza glabra* L., decreases under conditions of excessive drought compared to mild and moderate drought (Hosseini et al. 2018). Moreover, in *Mentha piperita* L. and *Catharanthus roseus* (L.) G. Don, the concentration of some secondary metabolites such as total phenols, flavonoids, and saponins decreases whilst the concentration of others such as tannins, terpenoids, and alkaloids increases when exposed to the same level of drought stress (Alhaithloul et al. 2019). These studies highlight the varying responses of secondary metabolites in medicinal plants when exposed to varying levels of drought stress. Further research is, therefore, required in order to extend our knowledge on the effects of climate change, in particular water deprivation, to other medicinal plant species and their secondary metabolites.

Here, we aimed to investigate the effects of water availability on the growth and performance of two herbs with known medicinal properties, i.e., *Tanacetum parthenium* L. (Feverfew) and *Plantago lanceolata* L. (Ribwort Plantain). We further quantified changes in two key secondary metabolites with known medicinal properties, parthenolide in *T. parthenium* and verbascoside in *P. lanceolata*, in response to the different water regimes. Verbascoside and parthenolide were chosen as they are the most abundant secondary metabolites with known therapeutic properties in our study species (Bahadori et al. 2020; Kashkooe et al. 2024).

Materials and methods

Study species

Two species with medicinal uses that can be found widespread across Northern Europe were used in this study: *Plantago lanceolata* (Ribwort Plantain) and *Tanacetum parthenium* (Feverfew). Both species have well-established therapeutic uses (Pareek et al. 2011; Abate et al. 2022).

T. parthenium is a member of the Asteraceae (Compositae) family. Its common name – Feverfew – reflects its long-term use as a medicinal plant, as it stems from ‘febrifuge’, meaning a medicine to ‘reduce fever’ (Kashkooe et al. 2024). Parthenolide is the primary active phytochemical found in *T. parthenium* and is one of the many sesquiterpene lactones this species is known to produce (Kashkooe et al. 2024). Among

other applications, this metabolite has been studied with regards to its use for the treatment of acute myelogenous leukemia (Guzman et al. 2005), reducing atherosclerotic lesions (López-Franco et al. 2006), and its anti-inflammatory properties (Kwok et al. 2001). *T. parthenium* is mainly used in the treatment and prevention of migraines (Kashkooe et al. 2024).

P. lanceolata belongs to the family Plantaginaceae and has been historically used as a food source and traditional medicine (Bahadori et al. 2020). Its medicinal purposes include the treatment of wounds and inflammation (Abate et al. 2022) and as an antimicrobial agent (Ferrazzano et al. 2015). The active phytochemicals present in *P. lanceolata* include verbascoside – a phenylethanoid glycoside (Alipieva et al. 2014) – and iridoid glycosides aucubin and catalpol (Abate et al. 2022). Studies have investigated several applications of verbascoside, including in the treatment of oxidative-stress-related neurodegenerative diseases (Esposito et al. 2010), inflammatory diseases (Pesce et al. 2015), and *Staphylococcus aureus* infections (Yang et al. 2021). Aucubin and catalpol have also been found to improve the prognosis of Parkinson's disease and Alzheimer's disease (Yang et al. 2022), offer neuroprotection in primary diabetic encephalopathy (Xue et al. 2008), and alleviate diabetic complications (Bai et al. 2019).

Germination and experimental set up

P. lanceolata and *T. parthenium* plants were grown from seeds (King's seeds, Colchester, UK) under glasshouse conditions with natural lighting and temperature ranging from 16°C to 29°C, in the Talybont Greenhouses plant growth facilities, as part of our Plant Growth Technology Hub (School of Biosciences, Cardiff University). Seeds were sown on the 21st April 2022 into germination trays filled with a mix of John Innes n° 2, BrettTM horticultural sand, and perlite (5:5:1). Three weeks after sowing, on the 13th May 2022, 60 seedlings per species were transplanted into individual 8 cm round pots filled with the same mix of compost, sand, and perlite and kept in the same glasshouse. Soil pH (measured with a pH tester HI98103, Hanna Instruments) was 7.6 (\pm 0.1), which is within the pH range adequate for both species (Fitter and Peat 1994).

Following transplanting, these 60 seedlings per species were allowed to establish and on the 6th of June

2022 were then randomly allocated to two different levels of water availability. Well-watered (WW) plants ($n = 30$ for *P. lanceolata* and $n = 30$ for *T. parthenium*) were watered to field capacity every other day. The soil of plants in the low-watered (LW) treatment ($n = 30$ for *P. lanceolata* and $n = 30$ for *T. parthenium*) was allowed to dry between watering, with plants showing signs of wilting before re-watering to field capacity (at 5–6 days of water deprivation). LW plants showed signs of wilting that fitted the description of stage 2 of observable wilt, with visible wilted leaves and dropping stems, as described by Jackson and Fonteno (2017). To monitor soil water content and determine the extent of water restriction, we used the gravimetric method by weighing all LW pots and a subsample of HW pots before and after watering at field capacity. The difference in pot mass before and after watering reflects the change in soil water content, with LW pots showing an average decrease of 19.7% in mass prior to re-watering at field capacity and HW pots showing an average decrease of 8.2% in mass prior to re-watering at field capacity. This indicates that LW pots experienced a water restriction of approximately 58% compared to HW plants. Plants were harvested on the 28th of June 2022, 22 days after water treatments started (46 days after transplantation), during which the LW plants underwent wilting four times.

Chlorophyll content and photosynthetic performance

Chlorophyll content was estimated prior to harvest on the 28th June 2022, by measuring the first to third youngest fully expanded leaves from the shoot apex of each plant ($n = 60$ (LW = 30, WW = 30) for *P. lanceolata* and $n = 30$ (LW = 30, WW = 30) for *T. parthenium*) using a handheld chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan), which calculates an index based on absorbance at 650 and 940 nm. SPAD values are positively correlated with the chlorophyll content of leaves (Richardson et al. 2002).

The maximum quantum yield of PSII, Fv/Fm, was also measured on the 28th June, 2022, by measuring the two youngest fully expanded leaves from the shoot apex of each plant ($n = 60$ (LW = 30, WW = 30) for *P. lanceolata* and $n = 60$ (LW = 30, WW = 30) for *T. parthenium*) using a MINI-PAM II fluorometer (Walz, Germany), which measures chlorophyll fluorescence. DLC-8 dark leaf clips were attached to these leaves

to ensure a 20-min dark-adaptation period, which is required for Fv/Fm measurements, before taking readings. Fv/Fm is a non-invasive measurement of plant photosynthetic performance, with values lower than 0.7 indicating stress and/or photoinhibition (Maxwell and Johnson 2000).

Plant growth and metabolite analysis

On the 28th June 2022, plants were harvested, and their biomass was separated into aboveground and belowground parts. Roots were separated from the soil by washing through a 2 mm² sieve from which roots were retrieved. Above- and belowground dry mass were obtained after drying in an oven at 50°C for 72 h (to constant mass) followed by weighing using an analytical balance (ME204, Mettler-Toledo, Switzerland, accuracy 0.0001 g).

Extraction of parthenolide and verbascoside from the aboveground dry mass was adapted from Hakimian and Maziah (2009). The aboveground dry mass of each plant ($n = 60$ (LW = 30, WW = 30) for *P. lanceolata* and $n = 60$ (LW = 30, WW = 30) for *T. parthenium*) was ground to a fine powder, and a sample of 1 g was mixed with 10 mL of methanol (99.9%) for 30 min, agitating it every 10 min. Extracts were then filtered using Whatman No 2 filter paper, and the methanol was evaporated under a flow of nitrogen until 1 mL of extract remained. Extracts were then centrifuged at 13,000 rpm for 5 min, and the supernatant was resuspended in methanol (99.9%) in a ratio of 9:1 (methanol: supernatant) prior to analysis using high-performance liquid chromatography (HPLC). HPLC analyses were carried out using a Waters[®] Autopurification[™] HPLC system using standard protocol and conditions, as described in Webster et al. (2020), with an injection volume of 20 μ L. A standard solution of parthenolide (10 mg/ml concentration) was prepared by dissolving 3.6 mg of parthenolide standard (CAS 20554-84-1 – Calbiochem, $\geq 97\%$ HPLC, Sigma-Aldrich) in 360 μ L of HPLC-grade methanol (99.9%, Thermo Fisher Scientific) (as described in Mahood et al., 2022). A standard solution of verbascoside was prepared in the same manner using verbascoside standard (CAS 61276-17-3, 99.21%, APExBIO). The mobile phases consisted of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). A solvent gradient was used to achieve separation, beginning with

95% of solvent A and 5% of solvent B and ending with 5% of solvent A and 95% of solvent B after 15 minutes. The peak retention time for parthenolide was between 6.68 and 6.74 min. For verbascoside, the peak retention time was between 4.28 and 4.29 min. Parthenolide and verbascoside peak heights were found using MassLynx V4.1 and V4.2 software (Waters[®]) from chromatograms filtered by wavelengths of 214 and 330 nm, respectively, and peak areas were calculated.

Data analysis

All statistical testing was carried out using R version 4.1.1 (R Core Team 2022). The effects of watering treatment on aboveground, belowground, and total dry mass, root:shoot ratio, chlorophyll content, Fv/Fm, and parthenolide and verbascoside content were determined by means of a one-way analysis of variance (ANOVA), using the 'lm' function in R with water availability (WW, LW) as a fixed factor, separately for each plant species. *P*-values were obtained by calling the Anova function in the 'car' package, using sum of squares type 'III'. Models were assessed visually for assumption of normality and homoscedasticity, and variables were transformed to meet model assumptions. Chlorophyll content and root:shoot ratio of *T. parthenium* were transformed to the power of two. Aboveground dry mass and parthenolide content of *T. parthenium* and the belowground dry mass, root:shoot ratio, and verbascoside content of *P. lanceolata* were log₁₀-transformed. The chlorophyll content of *P. lanceolata* was square root-transformed, and the reciprocal of the square root transformation was applied to the aboveground dry mass of *P. lanceolata*.

Results

Tanacetum parthenium

The aboveground, belowground, and total dry mass of *T. parthenium* plants were significantly reduced in response to water limitation (Figure 1a, b, and c, respectively). LW plants accumulated, on average, 38.7% less total dry mass than control plants (Figure 1c); however, biomass allocation was not significantly affected by water availability as the root:shoot ratio was similar in WW and LW plants (Figure 1d). Similarly, the chlorophyll content and the maximum quantum yield of PSII (Fv/Fm) were also not significantly

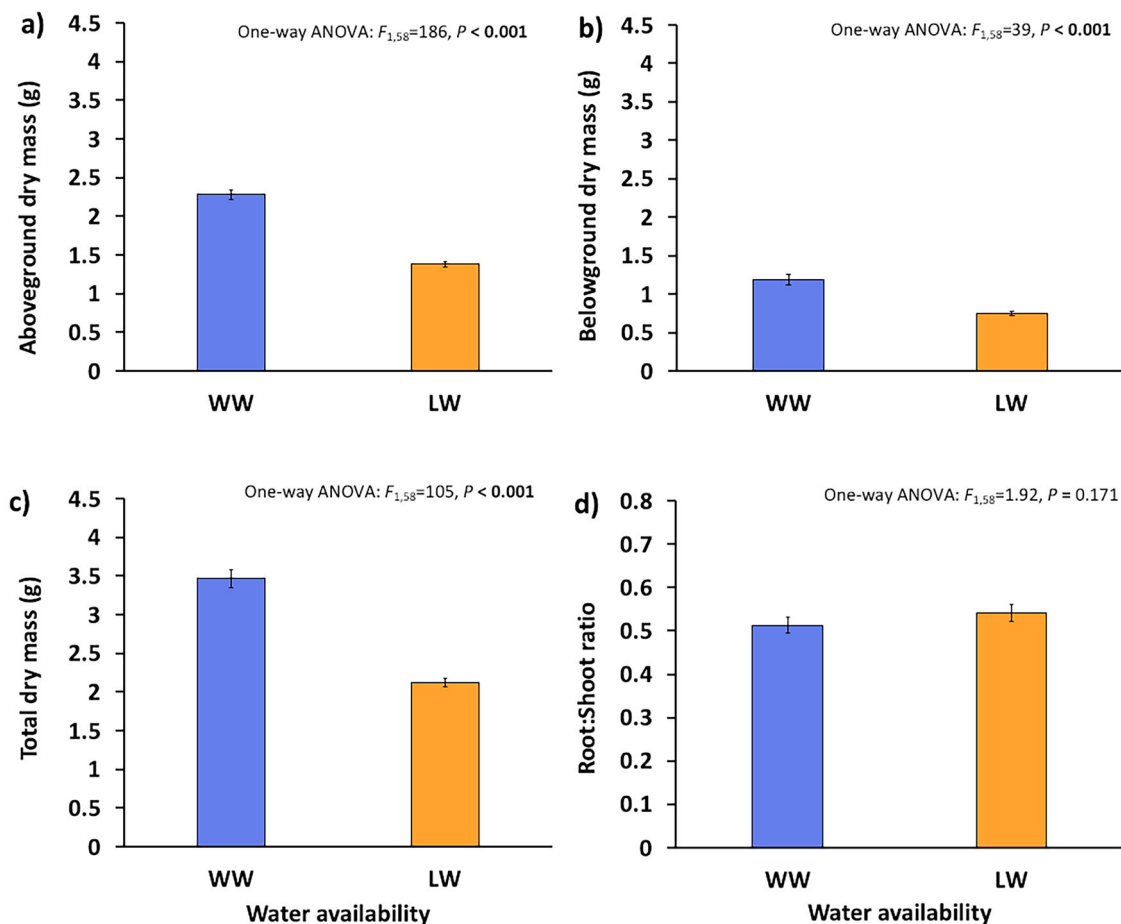


Figure 1. Dry mass measurements of *T. parthenium*: (a) aboveground dry mass, (b) belowground dry mass, (c) total dry mass, and (d) root-to-shoot ratio. Bars represent mean values ($n = 30$), and error bars indicate standard error. One-way ANOVA output is also shown; P -values < 0.05 are highlighted in bold.

affected by the water availability (Figure 2a,c, respectively). Fv/Fm was on average 0.835, indicating that the plants were not affected by water limitation.

At the time of harvest, LW plants had significantly lower parthenolide content than WW plants (Figure 3a).

Plantago lanceolata

Similarly to *T. parthenium*, *P. lanceolata* plants accumulated less aboveground, belowground, and total dry mass under restricted water conditions (Figure 4a, b, and c, respectively), and the root:shoot ratio was not statistically affected by water availability (Figure 4d). However, the chlorophyll content was significantly greater in LW than in WW plants (Figure 2b), and again, no differences were found in the maximum quantum yield of PSII (Fv/Fm) in response to the changing water availability (Figure 2d).

In contrast to the response of parthenolide in *T. parthenium* plants, at the time of harvest, no differences were found in the verbascoside content (mg/g dry leaf matter) in *P. lanceolata* plants in response to different water availability (Figure 3b).

Discussion

More intense and more abrupt droughts are becoming increasingly common globally as a result of climate change (Mukherjee et al. 2018). These changes are affecting not only the survival and distribution of medicinal plants but also the production of their secondary metabolites (Applequist et al. 2020). In this study, we investigated the growth responses of two commonly used plants with medicinal properties, *T. parthenium* and *P. lanceolata*, and measured the accumulation of two compounds with well-established

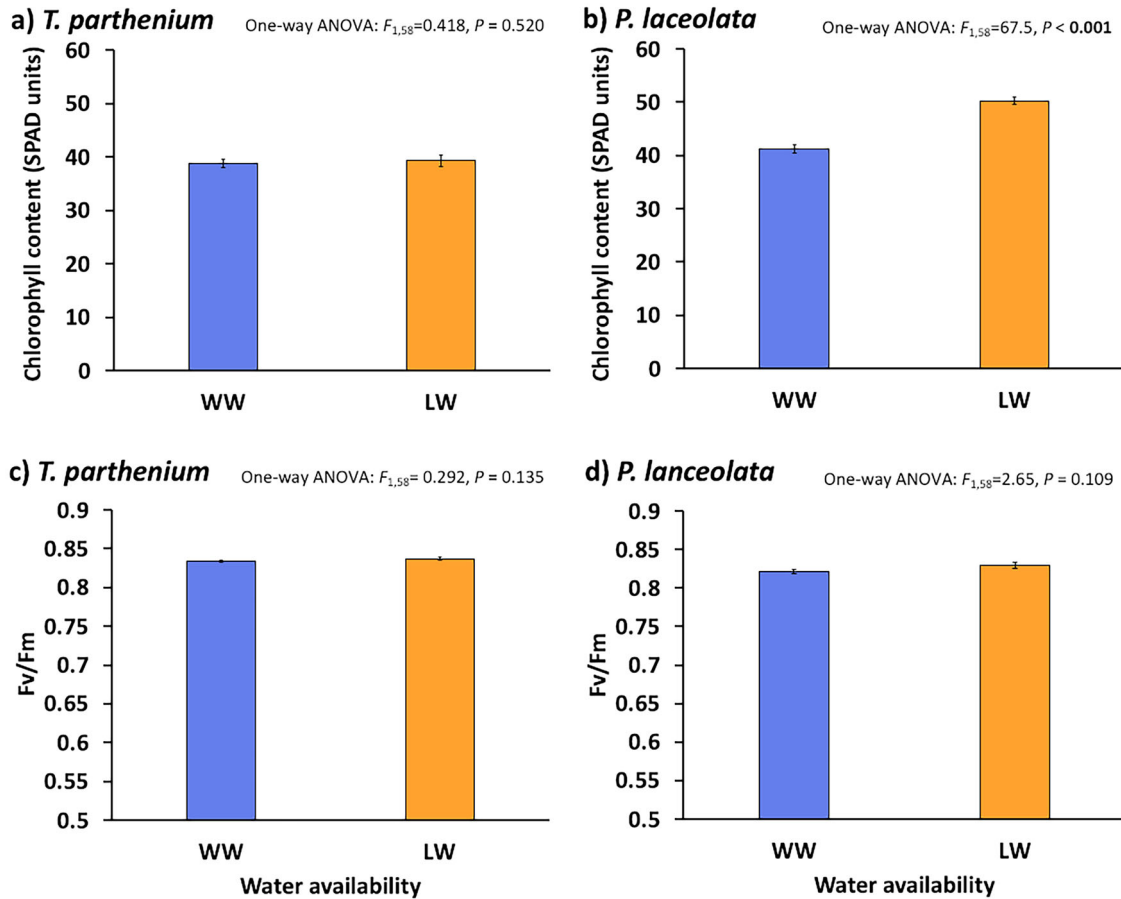


Figure 2. Chlorophyll content (SPAD units) for *T. parthenium* (a) and for *P. lanceolata* (b) and Fv/Fm, maximum quantum yield of PSII, for *T. parthenium* (c) and for *P. lanceolata* (d). Bars represent mean values ($n = 30$). Error bars indicate standard error. One-way ANOVA output is also shown; P -values < 0.05 are highlighted in bold.

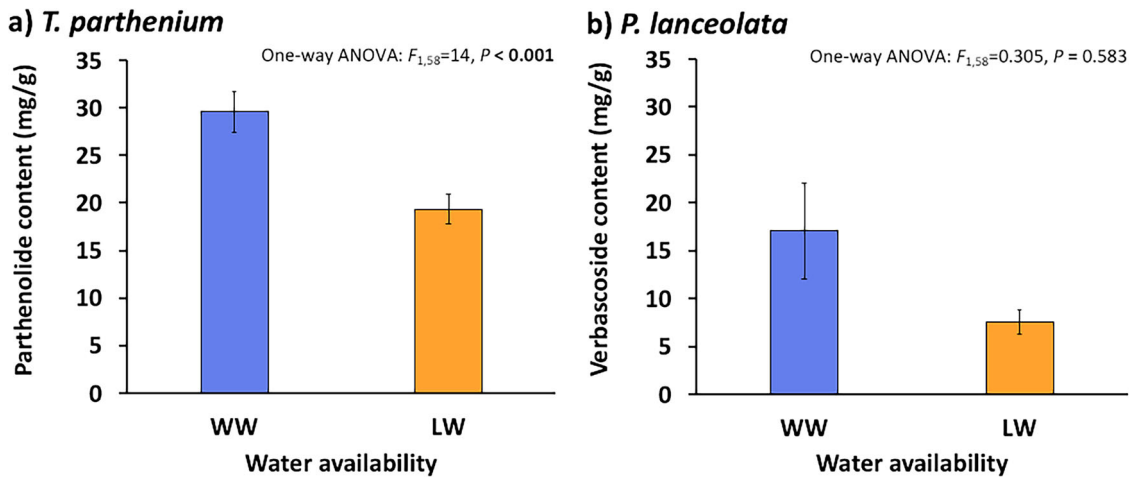


Figure 3. (a) Concentration of parthenolide (mg/g of dried leaves) in *T. parthenium* and (b) concentration of verbascoside (mg/g of dried leaves) in *P. lanceolata* under different water availability levels. Bars indicate mean values ($n = 30$). Error bars indicate standard error. One-way ANOVA output is also shown; P -values < 0.05 are highlighted in bold.

medicinal properties. By reducing water availability/frequency in our experiment, we expected to see an increase in parthenolide and verbascoside content

in *T. parthenium* and in *P. lanceolata*. Even though our water limitation treatment reduced plant growth in both species, the production of the secondary

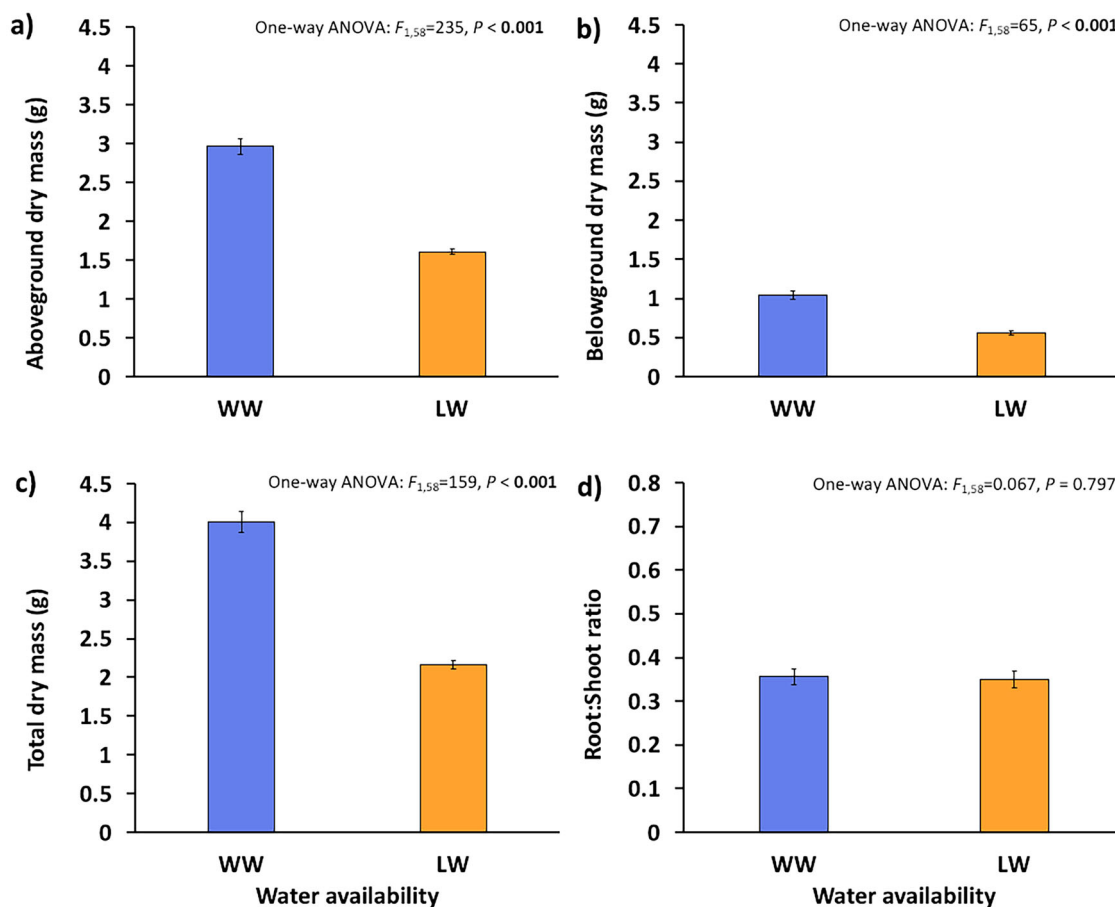


Figure 4. Dry mass measurements of *P. lanceolata*: (a) aboveground dry mass, (b) belowground dry mass, (c) total dry mass, and (d) root-to-shoot ratio. Bars represent mean values ($n = 30$), and error bars indicate standard error. One-way ANOVA output is also shown; P -values < 0.05 are highlighted in bold.

metabolites investigated was only negatively affected in *T. parthenium*, whilst verbascoside content was not affected in *P. lanceolata*.

It is well known that environmental stress affects the quantity and composition of metabolites in medicinal plants (Zehra et al. 2019), and an increase in the overall content of secondary metabolites has been found in response to drought stress in many medicinal plant species (Selmar and Kleinwächter 2013; Shil and Dewanjee 2022; Ghasemi et al. 2023). However, it appears that not all secondary metabolites are affected equally in response to drought stress. In some cases, particularly for active phytochemicals such as alkaloids, tannins, and terpenoids, drought stress increases their content, whilst content is reduced for phenols, flavonoids, and saponins (Alhaithloul et al. 2019; Shil and Dewanjee 2022). Varying responses can occur even within the same group of metabolites, for example, within terpenoids, it has been found that drought-treated plants of the same species showed an increase

in overall monoterpene synthesis and decline in total sesquiterpene synthesis (Chandrasekaran et al. 2022). Sesquiterpene lactones – a subgroup of terpenoids to which parthenolide belongs – have been found to decrease in other species, such as artemisin found in *Artemisia annua*, in response to increasing drought stress (Yadav et al. 2014). Previous work on *T. parthenium* has found reduced levels of parthenolide in plants which have experienced drought stress but an increase in total phenolic content (Fonseca et al. 2006; Fonseca et al. 2008). Not much is known regarding the changes in verbascoside – a phenylethanoid glycoside – in *P. lanceolata* in response to water stress, and the lack of response here may be in part explained by the resilience of this species to the drought intensity imposed in our experiments (not severe, as indicated by the physiological parameters investigated), with some studies suggesting that it is moderately drought-tolerant (Morales et al. 2021; Miszalski et al. 2023). However, it has also been found that prolonged

drought causes contrasting changes in the levels of different secondary metabolites of *P. lanceolata*, with some being enhanced and others reduced (Orians et al. 2019).

Fonseca et al. (2008) point out that trade-offs may occur between the contents of different metabolites in response to changing environments, with increases in certain metabolites occurring at the expense of decreasing the content of other metabolites. This highlights the complexity surrounding the production of secondary metabolites in plants (Li et al. 2020), which is influenced not only by the plant genetics (Li et al. 2020; Qaderi et al. 2023) but also by environmental conditions such as water availability, temperature, light, and soil nutrients (Gobbo-Neto and Lopes 2007) and any changes herein. Furthermore, differences in the intensity and duration of the imposed stress may lead to different responses in the development and production of secondary metabolites in medicinal plant species (Qaderi et al. 2023).

As expected, the aboveground, belowground, and total dry mass of *P. lanceolata* and *T. parthenium* were significantly reduced in plants that experienced periods of water deficit. However, the root-to-shoot ratios were not affected by the changing water regimes in either of the study species. Increased root:shoot ratio under water stress has been widely reported across many different plant species, and it is considered a drought avoidance strategy whereby growing plants allocate their resources more heavily to the root systems to increase water uptake capacity at the expense of developing aboveground organs (e.g., Makbul et al. 2011; Eziz et al. 2017). However, equally, decreased root:shoot ratios have been reported across different plant species (Kou et al. 2022). It has been suggested that in some cases plants develop a survival strategy when experiencing periods of drought, allowing part of the roots to die rather than investing more on them and reinvesting in new root development only when favourable conditions return (Whitmore and Whalley 2009; Kou et al. 2022). Such variation in root allocation strategies may occur in response to the intensity and duration of drought stress, along with the identity of the plant species (Xu et al. 2015).

To fully understand the mechanisms responsible for the changes in secondary metabolites because of drought, a more detailed molecular and genetic study should have been performed, in addition to measuring the primary metabolism (i.e., photosynthetic capacity)

of the plants. Moreover, it would be interesting to consider how potential acclimation to water stress (see Charng et al. 2023 and references there) may have affected secondary compound concentrations in our study species. Unfortunately, this was not possible in our study. Nevertheless, we measured chlorophyll content and Fv/Fm as proxies of the physiological status of the plants. Chlorophyll content was significantly higher in low-watered plants of *P. lanceolata* than in those that were well-watered, but no differences were found between the treatments in *T. parthenium*. The effect of drought on chlorophyll content has been found to vary depending on plant genotype and environmental conditions (including frequency and duration of drought) (Junaid et al. 2023). It is generally accepted that drought stress causes a reduction in plant chlorophyll content, likely due to damage to the thylakoid membranes within chloroplasts as a result of water deficit (Monteoliva et al. 2021). However, some research has also found that the level of chlorophyll in leaves may increase in water-stressed plants (Ramírez et al. 2014) – similar to our findings for *P. lanceolata*. It has been suggested that different causes, including a loss of turgor or a reduction in leaf growth caused by drought, could lead to higher chlorophyll concentration (Rolando et al. 2015), which would explain the high SPAD readings measured in *P. lanceolata* in response to lack of water. The lack of a significant reduction in chlorophyll content under reduced water availability could partly explain the maintenance of the maximum quantum efficiency of photosystem II (Fv/Fm) – with similar values in low- and well-watered plants – as any potential damage to the photosynthetic machinery as a result of stress would typically impair the energy capture and transfer processes within the photosystem, leading to a decline in Fv/Fm values (Murchie and Lawson 2013).

Conclusions

Compared to agricultural crops, there is still limited knowledge of the effects of drought and drought tolerance in medicinal plants (Bistgani et al. 2024). Overall, this study has shown that the growth of *T. parthenium* and *P. lanceolata* is negatively affected by drought. Moreover, we found variable effects of changing watering conditions on plant performance and metabolite production in these two species, with parthenolide content decreasing, whilst verbascoside was not

affected in response to water deficit. Our findings highlight the potential effects that climate change and drought are having on medicinal plant populations and the production of pharmaceutical compounds.

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Disclosure statement

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Author contributions

EG & JSV: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. JSV & BdG: Supervision, Resources, Writing – review & editing. SV: Resources, Writing – review & editing. All authors read and approved the final manuscript before submitting it for publication.

Data availability statement

The data that support the findings of this study are available at <https://doi.org/10.17035/cardiff.27691971.v1>

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