DROUGHT TOLERANCE OF ARTEMISIA TRIDENTATA IN RESPONSE TO HERBIVORY AND MYCORRHIZAL COLONIZATION

by

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The following individuals read and discussed the thesis submitted by student Mathew Geisler, and they evaluated the student's presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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ABSTRACT

Interactions with other organisms can affect a plant's ability to cope with drought. The re-establishment of *Artemisia tridentata*, a keystone species of the sagebrush steppe, is often limited by summer drought. This study investigated the effect of two biotic factors, herbivory and symbiosis with arbuscular mycorrhizal fungi (AMF), on the drought tolerance of *Artemisia tridentata* ssp. *wyomingensis* (Wyoming big sagebrush) seedlings. For this purpose, I conducted two separate but concurrent field experiments. The herbivory experiment had three treatments: seedlings without tree protectors and seedlings within Vexar or metal-mesh tree protectors. In the mycorrhizal experiment, all seedlings were within metal-mesh tree protectors, and the experiment had two treatments: without and with the addition of trap culture inoculum. The effects of the treatments were evaluated on seedling survival, leaf water potential, stomatal conductance, and inflorescence development. To better assess the impact of AMF colonization on drought tolerance, I also conducted greenhouse experiments. These experiments investigated whether AMF colonization affected the decline in stomatal conductance and photosynthesis induced by drought.

Herbivory damage, presumably caused by ground squirrels, mainly occurred in early spring, about five months after outplanting. Most seedlings recovered from this damage, but herbivory was associated with higher mortality during the subsequent summer, fall, and winter. Eighteen months after outplanting, the survival in plants within metal protectors was 12 and 39% higher than in those within plastic and no protectors,

respectively. In addition, the percentage of lived plants that developed inflorescences was approximately threefold higher in plants with protectors than those without them. The results of this experiment indicate that most plants that suffered herbivory did not die directly from this disturbance but from increased susceptibility to abiotic stresses, including drought.

The addition of trap culture inoculum increased AMF colonization by 23%, which represented a more than 100% rise over the levels measured in non-inoculated seedlings. However, this did not affect survival, which was above 90% for both treatments. The seedlings experienced water stress, as evidenced by water potentials ranging from -2 to -4 MPa and a decrease in stomatal conductance. Yet, despite the continued drought, the water potentials did not reach lethal levels and remained rather constant after midsummer. This response was partially mediated by reductions in stomatal conductance and plant hydraulic conductivity. The only response variable affected by inoculation was the percentage of plants developing inflorescence. These percentages were 45.4 and 59.0% for non-inoculated and inoculated seedlings, respectively ($p = 0.028$).

The greenhouse experiment showed that AMF colonization did not affect photosynthesis under well-watered conditions or instantaneous water use efficiency. In contrast, AMF colonization delayed the drought-induced decline in stomatal conductance and photosynthesis, or this decline occurred at a lower soil water content. These effects were not related to a lower water potential threshold for stomatal closure but rather to an increased ability to extract water from the potting substrate. The results are consistent with the notion that AMF colonization increases the drought tolerance of *A. tridentata* seedlings. However, the significance of the observed effect on increasing survival in

natural habitats remains to be tested under more extreme water stress conditions than those experienced by the plants in my field experiment.

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LIST OF ABBREVIATIONS

AMF	Arbuscular mycorrhizal fungi
DSE	Dark septate endophytes
Ψ_l	Leaf water potential
Ψ_w	Water potential
A	CO2 assimilation per unit leaf area
g_s	Stomatal Conductance
WUE	Water use efficiency
Tr	Transpiration per unit leaf area
ΦPSII	Photosystem II operating efficiency
rPC	Ratio of the pot weight to the weight at pot capacity
$rPC_{1/2}$	The ratio of the pot weight to the weight at pot capacity at which parameters reached one half their initial values
$t_{1/2}$	Time to reach one half the initial value for a given parameter
I	Initial value
P	Phosphorus
Zn	Zinc
l	litres
ml	Millilitres
MPa	Megapascal
PVC	Polyvinyl chloride
cm	Centimeters

mm	millimeters
µm	micrometers
m	meters
mmol	millimoles
µmol	micromoles
µg	micrograms
KOH	Potassium hydroxide
min	minutes
h	hours
s	seconds
d	days
°C	Degrees Celsius
WGA-HRP	Wheat germ agglutinin-horsradish peroxidase
ANOVA	Analysis of variance
RR	Response ratio
BLM	bureau of land management
Fig.	Figure

CHAPTER ONE: IMPACTS OF HERBIVORY AND MYCORRHIZAL COLONIZATION ON PLANT WATER STATUS AND SURVIVAL OF ARTEMISIA TRIDENTATA SEEDLINGS UNDER FIELD CONDITIONS

Introduction

Wyoming big sagebrush (*Artemesia tridentata subsp. wyomingensis*) is a keystone species of the sagebrush steppe. In this habitat, it is the most common shrub covering the landscape in stands surrounded by native perennial grasses, forbs, and biological soil crusts. Wyoming big sagebrush contributes to developing a heterogeneous landscape and provides forage and habitat for pygmy rabbits (*Brachylagus idahoensis*), pronghorn (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), songbirds, and greater sage grouse (*Centrocercus urophasianus*) (Aldridge and Boyce, 2007; Charley and West, 1977; Davies et al., 2007; Larrucea and Brussard, 2008). These organisms play vital environmental roles and add economic benefits. In 2011 alone, hunting retail sales earned Idaho $319,067,286; this included tags for pronghorn, deer, elk, and sage grouse ("Economics of Idaho's Hunting & Fishing," 2011; Whitney and Knopf, 1985).

Human use of the sagebrush steppe has left the landscape fragmented and degraded (Knick and Rotenberry, 1997). A long history of agricultural and grazing practices such as deliberate seeding of grasses for grazing and unchecked transportation of cattle have led to the introduction of non-native invasive annual grasses such as cheatgrass (*Bromus tectorum*) and medusahead *(Taeniatherum caput-medusae*) (Mack,

1981). These invasive annual grasses have changed the fire regime, increasing wildfire frequency (Knapp, 1996). This increase has led to a decline in the habitat occupied by sagebrush, which has prompted efforts to re-establish this species (Brooks et al., 2004; Baker, 2006; Dettweiler-Robinson et al., 2013).

Sagebrush includes various subspecies and cytotypes with differing root depths, growth rates, and climatic needs (Maier et al., 2001; Welch and Jacobson, 1988; Zaiats et al., 2020). Attempts to re-establish sagebrush have been extensive and costly with varied but generally low success rates (Baker, 2006; Brabec et al., 2015). The cause of this variability is not well understood. Low establishment following seeding or seedling outplanting may be related to the use of genotypes not well adapted to the local environment (Pellant et al., 2005). However, even when local genotypes are used, seedling recruitment can be very low due to climatic factors, particularly summer drought (Applestein et al., 2021; Brabec et al., 2015; Maier et al., 2001; Schlaepfer et al., 2014). The adverse effects of drought on seedling recruitment may be exacerbated by climate change and alterations in soil chemical and biological properties caused by fires and exotic plant invasions (Busby et al., 2013; Dierks et al., 2019; Williams et al., 2020).

The ability of plants to cope with drought is affected by interactions with other organisms (Barton and Shiels, 2020; Ortiz et al., 2015). Soil microorganisms such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizae (AM) can enhance drought tolerance (Marulanda et al., 2009; Ngumbi and Kloepper, 2016). In contrast, biotic stresses caused by pathogens or herbivory tend to reduce drought tolerance (Barton and Shiels, 2020; Gianoli and González-Teuber, 2005; Oliva et al., 2014). The extent to which soil microorganisms or biotic stresses limit *A. tridentata*

reestablishment is unclear (Davidson et al., 2016; Prado-Tarango et al., 2021; Takahashi and Huntly, 2010). However, during the summer, seedlings are often water-stressed to their limits of survival. This situation suggests that any biotic factor that somewhat affects responses to drought, such as root growth maintenance, solute accumulation, or antioxidant activity, would significantly impact survival.

As noted above, plants can enhance their growth and drought tolerance by forming symbiotic associations with AMF (Rodriguez et al., 2008). These fungi are obligate biotrophs that form arbuscules within the thalli of some bryophytes and roots of vascular plants. AMF are widespread in terrestrial ecosystems (Barbosa et al., 2017; Kivlin et al., 2011) and form associations with more than eighty percent of all land plants (Wang and Qiu, 2006a). Many studies have shown a mutualistic relationship between AMF and their host plants. Plants provide carbohydrates and lipids to the fungi (Keymer et al., 2017; Smith et al., 2009), and in exchange, AMF increase the uptake of minerals and nutrients, an effect that is particularly important for nutrients with low mobility in the soil, such as P and Zn (Smith and Smith, 2011). Although less studied, other beneficial effects of AMF on plants have been reported. AMF increased drought tolerance in several species, including corn (*Zea mays*), cowpea (*Vigna unguiculata*), and citrus (*Citrus sp. and Poncirus sp.*) (Begum et al., 2019; Oyewole et al., 2017; Wu et al., 2013). Researchers have also found that plants that form symbioses with AMF native to areas that experience high heat, salinity, or pathogen loads are better suited to deal with these stresses (Rodriguez et al., 2008).

Notwithstanding the benefits of the plant-AMF symbiosis, this symbiosis is not always mutualistic (Hoeksema et al., 2010). For example, in cases where nutrients are

readily available or under low light, the symbiosis can shift toward parasitic as the benefits outweigh the carbon cost of hosting AMF (Johnson et al., 1997; Konvalinková and Jansa, 2016). In addition, the impact of AMF on the plant may vary depending on root characteristics or the stage of plant development. Johnson et al. (1997) suggested that in young seedlings, carbon allocation to the fungi may outweigh the benefit provided by the fungi, resulting in growth depression (Lü et al., 2018).

Sagebrush is a mycorrhizal plant, and AMF colonize its roots in natural habitats (Busby et al., 2013; Carter et al., 2014; Trent et al., 1994). Evidence from pot and field experiments indicates that AMF can improve sagebrush growth and seedling establishment (Allen et al., 1993; Davidson et al., 2016; Dierks et al., 2019; Stahl et al., 1988). However, neutral (Busby et al., 2011; Carpenter et al., 2021; Dettweiler-Robinson et al., 2013) and adverse effects (Prado-Tarango et al., 2021) of AMF on the growth or establishment of sagebrush seedlings have also been reported. Similarly, questions remain about the impact of AMF colonization on the drought tolerance of sagebrush seedlings. Early work by Stahl et al. (1998) showed that AMF seedlings withstood lower soil water potentials than non-mycorrhizal ones. This result indicates that AMF increased drought tolerance, but, to my knowledge, it is the only published study on this aspect of the AMF-sagebrush symbiosis.

In addition to biotic interactions occurring in the soil, alterations in shoot architecture and function mediated by contact with other organisms can also affect drought tolerance (Barton and Shiels, 2020; Canham and Chazdon, 1999; Chen et al., 2017). Because *A. tridentata* is often the only green vegetation during several months, it is an important food source for various animals. These range from large ruminant

ungulates such as pronghorn (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*) to small mammals such as ground squirrels (*Urocitellus endemicus*) and pygmy rabbits (*Brachylagus idahoensis*), and even invertebrates such as harvester ants (*Pogonomyrmex occidentalis subsp. owyheei* Cole) and grasshoppers (*Schistocerca sp.*) (Takahashi and Huntly, 2010; Ulappa et al., 2014; Welch and McArthur, 1986). Even though *A. tridentata* produces many secondary metabolites to deter herbivory, the damage caused by herbivory can be extensive (Dwinnell et al., 2019; McMunn, 2017; Pu et al., 2015).

In previous outplantings of *A.tridentata*, I observed in 1 to 2 years old seedlings herbivory by small mammals (presumably ground squirrels) and to a lesser extent by harvester ants and grasshopper. This damage was often not immediately fatal since seedlings resprouted after herbivory. However, results in other species suggest that herbivory might make *A. tridentata* seedlings more susceptible to abiotic stresses, particularly drought (Barton and Shiels, 2020). Several studies indicate that defoliation caused by herbivory tends to decrease drought tolerance (Barton and Shiels, 2020; Chen et al., 2017; Kolb et al., 2020). This effect may be due to the partitioning of reserves to regrowth new branches rather than roots and an overall decrease in non-structural carbohydrates (Chen et al., 2017). The latter can play an essential role in increasing drought tolerance by contributing to the growth of fine roots, osmotic adjustment, and providing the carbon needed to sustain metabolism when stomata close (Jacquet et al., 2014; Li et al., 2020; Salleo et al., 2006). On the other hand, herbivory reduces the leaf area for transpiration and thus the plant water needs. The extent that reduced transpiration may compensate for plausible adverse effects of herbivory on drought tolerance is not

clear, but assessing how herbivory affects plant water status and survival under drought may provide an insight into this question.

As indicated above, various studies and previous observations suggest that both symbiosis with AMF and herbivory may affect the establishment and drought tolerance of *A. tridentata* seedlings. To further investigate this notion, I conducted two separate but concurrent experiments at a sagebrush site where preliminary data indicated relatively low levels of natural AMF colonization (~ 14%) and the presence of various herbivores, including ground squirrels, harvester ants, and grasshoppers. One experiment aimed to increase the natural levels of AMF colonization in half of the seedlings by adding native AMF inoculum and then assessing the effect of this treatment on plant water status, survival, and reproductive output. The goal of the second experiment was to evaluate the impact of herbivory by placing seedlings under one of three treatments: without tree protectors, with Vexar tree protectors, and with metal-mesh tree protectors. Preliminary experiments indicated that these treatments would lead to different levels of herbivory. I then evaluated the effect of these treatments on seedling survival and variables indicative of plant water stress and shoot development.

Another commonly observed group of root endophytes found in the sagebrush steppe are dark septate endophytic fungi (DSEs)(Carpenter et al., 2021; Gehring et al., 2016; Weber et al., 2015). These fungi are currently known to occur in as many as 600 plant species representing roughly 100 genera (Jumpponen and Trappe, 1998). Due to their ubiquitous nature, colonization of these fungi was likely to occur. Thus during the analysis of AMF colonization, I also quantified the presence of DSE to assess whether AMF inoculation affects DSE colonization.

My hypotheses for the AMF experiment were that *1*) inoculation would increase colonization over the background level naturally occurring in the soil, and *2*) increased colonization would result in reduced drought stress and higher survival. For the herbivory experiment, I hypothesized that compared to seedlings where herbivory was minimal, those that suffered herbivory and resprouted would have lower water potentials and higher mortality during the summer. Apart from testing these hypotheses, an additional goal of the study was to analyze the value of methods used for increasing AMF colonization or protecting plants from herbivory. If any of the methods tested were to prove effective for these purposes, they could be readily employed to increase the establishment of *A. tridentata* seedlings.

Materials and Methods

Experiment 1: Responses to mycorrhizal inoculation

Fungal and plant material

To produce mycorrhizal inoculum, I collected silty-loam soil from a sagebrush community near Kuna Butte, Idaho (43°26.161'N, 116°25.848'W, 908 m a.s.l.). This soil was mixed in a 2:3 ratio with sterilized sand, and then the AMF in the soil were multiplied in trap cultures using *Plantago lanceolata* as a host. Soil and roots from the trap cultures were used as inoculum after three cycles of trap culture cultivation. Previous studies indicated that the trap cultures contained a mixture of AMF within the Glomeraceae family (Serpe et al., 2020).

The plant material used in this experiment was Wyoming big sagebrush seedlings provided by the Bureau of Land Management. Seeds to grow these seedlings had been collected within the Morley Nelson Snake River Birds of Prey National Conservation

Area and sown in 150 ml cone-tainers filled with a 3:1 peat moss to vermiculite mix. Subsequently, growing conditions were as those described by Fleege (2010).

Experimental approach

The experiment was conducted in Kuna Butte, ID (43° 26' 47.32" N, 116° 26' 48.61" W). The soil at this site are Power-McCain silty loams, which are classified as fine-silty, mixed, superactive, mesic Xeric Calciargids ("SoilWeb: An Online Soil Survey Browser | California Soil Resource Lab," n.d.). The outplanting occurred in October 2019. At this time, most of the vegetation at the site was dry and consisted of stalks of non-native plants, mainly crested wheatgrass (*Agropyron cristatum*), cheatgrass (*Bromus tectorum*), and tumble mustard (*Sisymbrium altissimum*). Tumble mustard was particularly abundant, which partly determined the site's selection. I reasoned that the abundance of this non-mycorrhizal plant might reduce the density of AMF present in the soil, increasing the need for inoculation (Bainard et al., 2009). On October 26, 2019, 300 seedlings were randomly assigned to one of two inoculation treatments, control and inoculated. In the control treatment, the seedlings were planted without inoculum. In contrast, 500 ml of soil and roots from the trap cultures were placed beneath and around each seedling for the inoculated treatment. The seedlings were within a 25 m x 30 m plot forming a grid at a distance of 1 to 1.5 m from each other. All seedlings were watered; the water was added through a PVC tube inserted about 20 cm from the soil surface. Each seedling was enclosed within a metal tree protector (6 mm-mesh and close at the top) (Fig. 1.1 A), and the watering was repeated two weeks later.

Data collection

The effects of inoculation and summer drought on the plants were assessed on the following response variables: fungal colonization of roots, plant survival, leaf water potential (Ψ_l), stomatal conductance, and percent of plants with flowers. I collected seedlings to analyze fungal colonization in June and October 2020, eight and twelve months after transplanting, respectively. Six seedlings were harvested for each treatment and sampling time. Colonization was quantified in roots smaller than 2 mm that were cut in roughly 2 cm segments and cleared in 5% KOH for 5 min at 121 °C. Subsequently, the roots were rinsed in water and incubated overnight in a solution containing 0.4 μg ml^{-1} wheat germ agglutinin-horseradish peroxidase (WGA-HRP) and 1% bovine serum albumin in PBS (Kobae and Ohtomo, 2016). Samples were then rinsed in PBS and incubated for 3 to 5 min in a VIP HRP substrate (Vector Laboratories, California, USA). Subsequently, the roots were rinsed in water, mounted on 50% glycerol, and observed through an Olympus BX60 microscope at 200 or 400 magnification. The observed fungi were grouped into two categories: septate and AMF. Within the septate fungi, I recorded the presence of hyaline and melanized hyphae and microsclerotia. For AMF, I quantified the presence of hyphae (diameter of at least 5 μm), arbuscules, and vesicles (Fig. 1.2). The different fungal structures and total colonization by the two groups of fungi were quantified by the intersection method (McGonigle et al., 1990) with about 150 intersections per sample.

Survival was measured biweekly or monthly from November 2019 to September 2020 and bi-monthly afterward. Measurements of midday Ψ_l started in the early summer of 2020 and continued to the late summer of 2021. I measured midday Ψ_l bi-weekly

during the summer and less frequently in fall and spring. In addition, during the summer of 2019, I made measurements of predawn Ψ_l. Midday and predawn Ψ_l measurements were made in 8 plants per sampling day and treatment using a pressure chamber (PMS Instrument Company; Albany, OR, USA). For this purpose, small lateral shoots were wrapped in Saran wrap, excised, and immediately used to determine their Ψ_l. Stomatal conductance was measured during the summer of 2021 in the same plants used to measure midday Ψ_l. Three measurements were made per plant between noon and 2 pm using an SC1 leaf porometer (Meter Group). Plants bearing inflorescences were counted in the late summer of 2020 and 2021. A weather station at the site recorded temperature, precipitation, and soil moisture in the top 20 cm soil.

Data analysis

The effect of the inoculation treatment and sample collection time on fungal colonization was analyzed by two-way ANOVA. The impact of inoculation and day of measurement on midday Ψ_l and stomatal conductance was estimated by comparing box plots of the data. Possible differences in survival or percent of plants with flowers between inoculation treatments were evaluated using a log-rank test and a chi-square test, respectively. All statistical analyses were conducted in R 4.0 (R-Development-Core-Team, 2020)

Experiment 2: Response to herbivory

Plant material and experimental approach

This experiment was conducted in a 35 x 35 m plot immediately adjacent to that used for experiment *1*, which had vegetation and soil characteristics similar to those described earlier. Also, the sagebrush plants were from the same batch that the BLM

provided for experiment *1* and consequently like those used in the mycorrhizal experiment. For this experiment, I outplanted 450 seedlings, which were placed at a distance of about 1.5 m from each other. The seedlings were about 11 months old at outplanting, which occurred on October 26, 2019. All seedlings were watered after outplanting and subsequently randomly assigned to one of three treatments: without tree protector, with plastic tree protector (25.2 mm mesh, 44 cm height, and 10 cm in diameter), and with metal tree protector (6 mm-mesh and close at the top) (Fig. 1.1). Preliminary experiments indicated that these treatments would lead to various levels of herbivory.

Data collection

The efficacy of the tree protectors on reducing herbivory was assessed by counting the plants that showed signs of herbivory, as judged by marked removal of branches or leaves. These observations were made biweekly between March and September 2020 and less frequently during the spring and summer of 2021. In addition, at the end of the 2020 summer, I estimated the shoot area for each treatment. For this purpose, I took pictures from 25 randomly selected seedlings per treatment. These photos were used to measure the shoot areas using ImageJ software.

The effect of the tree protectors was also evaluated on the following variables: leaf water potential (Ψ_l), stomatal conductance, survival, and percent plants bearing flowers. I measured midday and predawn Ψ_l between June 2020 and August 2021 in the same days as in experiment *1*. Similarly, Ψ_l was determined, as noted earlier on eight randomly selected plants per day and treatment. However, I only measured two treatments, no protector and metal protector. This approach reduced work and allowed

me to complete predawn or midday Ψ_l measurements within 2-3 h each. Stomatal conductance was measured during the summer of 2021 using a leaf porometer as described earlier. Survival was monitored biweekly or monthly from November 2019 to September 2020 and bi-monthly afterward. Plants bearing inflorescences were counted in the late summer of 2020 and 2021.

Data analysis

The effect of the tree protectors on survival and the number of plants that experienced herbivory was analyzed using a log-rank test. To examine the impact of the treatments on the shoot area and the number of plants bearing inflorescence, I conducted one-way ANOVAs. Possible differences in Ψ_l and stomatal conductance between the no- and metal protector treatments were evaluated by boxplot comparisons and t-tests.

Results

Experiment 1: Responses to mycorrhizal inoculation

For total AMF, arbuscular, and septate fungi colonization, the two-way ANOVA indicated no significant interaction between inoculation treatment and sampling time on these variables (p values between 0.61 and 0.76). The addition of trap culture beneath the seedlings increased total AMF and arbuscular colonization but did not significantly affect septate fungi colonization (Fig. 1.3 A). The average total AMF colonization was 16.9 and 39.9% for non-inoculated and inoculated seedlings, respectively ($p = 0.0003$). Similarly, inoculation increased arbuscular colonization from 4.8 to 13.4% ($p = 0.016$). In contrast, sampling time only affected total AMF colonization, which decreased from 36 to 17% from late spring to early fall ($p = 0.017$) (Fig. 1.3 B). The two other fungal structures

recorded, vesicles and sclerotia, were not affected by either inoculation treatment or sampling time ($p > 0.05$).

The observed differences in AMF colonization did not impact survival. Seedling mortality was negligible independent of the inoculation treatment (Fig. 1.4). As of December of 2020, approximately one year after transplanting, the percent survival of non-inoculated seedlings was 95.9%, and that of those inoculated 97.2% ($p = 0.5$). Furthermore, by July 2020, many plants had inflorescences. The percent of plants with inflorescences was 37.7 and 44.3 % for the non-inoculated and inoculated seedlings, respectively, but the difference was not significant ($p = 0.25$). Survival remained high during the subsequent winter, spring, and summer (Fig. 1.4). In late July 2021, the percent survival of non-inoculated and inoculated plants was 92.9 and 95.8, respectively ($p = 0.195$). Also, at this time, many plants were in bloom, and the percent of lived plants with inflorescences was lower for the non-inoculated ones (49.2%) than those inoculated (61.2%) ($p = 0.04$). Furthermore, the difference in the presence of inflorescences was somewhat more marked when compared to the initial number of seedlings planted. When expressed in this manner, the percentages were 45.4 and 59.0% for non-inoculated and inoculated seedlings, respectively ($p = 0.028$).

Although the plants showed high survival rates, weather conditions suggest that they had to cope with drought. During 2020, precipitation was minimal between early July and November. This lack of rainfall resulted in a marked decrease in soil moisture in, at least, the upper 20 cm of the soil (Fig. 1.5 B and C). Due to lower precipitation and higher temperatures than in 2020, the summer of 2021 also presented conditions conducive to drought stress (Fig. 1.5). To characterize the degree of water stress the

plants experienced, I measured predawn and midday Ψ_l during the summer of 2020 and the midday Ψ_l and stomatal conductance during the spring and summer of 2021.

During the 2020 summer, the predawn Ψ_l values ranged from -1.0 to -4.1 MPa, while midday Ψ_l ranged between -1.7 and -4.6 MPa (Fig. 1.6). Predawn and midday Ψ_l showed a linear relationship with a slope slightly lower than 1. The variation in predawn or midday Ψ_l primarily reflected differences between plants independent of inoculation treatment; I did not detect differences in Ψ_l between non-inoculated and inoculated seedlings (Fig. 1.7). Between sampling dates, there were some differences in Ψ_l. However, Ψ_l did not show a clear pattern of decreases and increases in Ψ_l; the midday Ψ_l remained relatively constant from July to December, with values between -2 and -3 MPa (Fig. 1.7 B).

In 2021, I began the midday Ψ_l measurements in the spring, when water potentials were high with median values above -1 MPa (Fig. 1.8 A). Subsequently, midday Ψ_l gradually declined until late July and remained relatively constant between -2 and -2.5 MPa for the next month. Similar to 2020, inoculation did not have an effect on Ψ_l since changes in this variable were similar in non-inoculated and inoculated plants. The seasonal decrease in Ψ_l was correlated with a reduction in stomatal conductance that, like Ψ_l, was not affected by the inoculation treatment (Fig. 1.8 B).

Experiment 2: Responses to herbivory

Independent of the protector treatment, damage to or losses of seedlings were minimal during the fall of 2019 and most of the winter of 2020 (Fig. 1.9). In contrast, significant damage due to herbivory occurred in March 2020. In that month, the percent of seedlings that experienced herbivory was about 85%, 25%, and 5% for no-protector,

plastic, and metal protector treatments, respectively ($p < 0.0001$). The extent of damage varied between seedlings that experienced herbivory, but most lost over 60% of their shoot volume (e.g., Fig. 1.10). Herbivory damage markedly declined by April 2020 (Fig. 1.9 and the injured plants began to resprout. However, these plants did not fully recover in size; the median shoot area in the no-protector treatment was less than half of that in the other treatments at the end of the 2020 summer (Fig. 1.11, $p < 0.0001$). In addition, herbivory was low in the second year, and even though I did not quantify plant size, plants damaged in the first year remained smaller through the second year. Apart from the damage observed during the spring, I noted additional damage in the vicinity of harvester ant nests during the summer. These plants were defoliated entirely and did not recover from herbivory. However, only a few were affected, and the loss was similar between treatments.

On May 1, 2020, after most herbivory had occurred, survival was similar between treatments (Fig. 1.9 B). From then on, however, survival rates began to differ. In particular, seedlings without protectors had lower survival at the end of the 2020 summer than those with protectors ($p < 0.001$). This trend continued to the last day of measurement on August 2021, when the survival of seedlings without protectors was 51.9%, the one with plastic protectors 76.7%, and those with metal protectors 89% (Fig. 1.9 B). These differences in survival were significant between each pair of treatments ($p = 0.012$ for the metal vs. plastic protector comparison, $p = 2.9 \times 10^{-9}$ for metal vs. no protector, and $p = 3.56 \times 10^{-5}$ for plastic vs. no protector). Also, herbivory in early spring reduced the proportion of live plants with inflorescences at the end of summer. In August

2020, this proportion was about three times higher in seedlings with protectors than in those without them (Table 1, $p < 0.0001$). Similar results were observed in August 2021.

In 2020, Ψ_l values followed similar trends as those observed in the mycorrhizal experiment. The relationship between predawn and midday Ψ_l was linear, and except for a day in mid-August, median values of midday Ψ_l remained relatively constant from July to December (Fig. 1.12 and 1.13). Differences in predawn or midday Ψ_l between seedlings without and with metal protectors were not significant, and some of the Ψ_l measured in both treatments were very low. These low values led to a broader range of water potentials than in the mycorrhizal experiment. Predawn Ψ_l ranged from -1.4 to -7 MPa, and midday Ψ_l from -1.6 and -8 MPa (Fig. 1.12).

In 2021, midday Ψ_l declined from about -1 MPa in spring to about -2.5 MPa in mid-summer (Fig. 1.14 A). As in 2020, differences in Ψ_l between the no- and metal protector treatments were not significant. However, there were some differences between the years. Although 2021 was drier than 2020, Ψ_l values measured in the 2021 summer were higher than those in the 2020 summer (Fig. 1.13 A and 1.14 A). Furthermore, for comparable periods, the variability in Ψ_l between plants was much less in 2021 than in 2020. Stomatal conductance showed a similar pattern to Ψ_l, with a decline from spring to summer and no apparent differences between the two treatments (Fig. 1.14 B).

Discussion

Responses to mycorrhizal inoculation

The results from the experiment involving inoculation of seedlings with trap cultures supported one of my hypotheses; inoculation increased colonization compared to the levels naturally occurring in the soil. In contrast, the results did not support the

hypothesis that increased colonization would reduce drought stress, as measured by plant water potential, and improve survival. I did not detect significant differences in water potential or survival between treatments. An unexpected result of the study was the development of inflorescences in many of the transplanted seedlings. Moreover, during the second year, the percentage of plants with inflorescences was higher in the inoculated than non-inoculated treatment.

The difference in AMF colonization between inoculated and non-inoculated seedlings was about 23% and 8% for total AMF and arbuscular colonization, respectively. These differences represent more than 100% increases over the colonization levels without inoculation. In *A. tridentata* and other species, similar increases in colonization have been associated with enhancements in growth, nutrient concentrations, tolerance to abiotic stresses, or survival (Caravaca et al., 2003; Davidson et al., 2016; Estrada et al., 2013; Sorensen et al., 2008). Thus, the magnitude of the observed colonization increase can be of biological significance and is typically indicative of successful mycorrhizal inoculation (Faye et al., 2013; Quoreshi and Khasa, 2008).

Apart from the differences in colonization between treatments, the colonization analysis revealed a decrease in colonization from late spring to fall and substantial colonization by dark septate fungi. The seasonal reduction in colonization may be associated with drought and the decline in soil moisture from July to October. In agreement with my results, Clark et al. (2009) and Maitra et al. (2019) observed reduced AMF colonization with drought. However, few studies have investigated the effect of drought on AMF colonization (Cotton, 2018). Moreover, these studies have yielded conflicting results, with some reporting no impact or increased colonization (Staddon et

al., 2003; Zhang et al., 2019). Cotton (2018) proposed that these differences reflect species-specific responses or dissimilarities in drought intensity.

In contrast to AMF colonization which varied with inoculation treatment and seasonally, I did not detect any evident change in colonization by DSE. The trap cultures most likely have some DSE, but inoculation did not increase the levels of these fungi over those observed in non-inoculated seedlings. This result suggests that DSE abundance in the trap cultures was not higher than in the soil. In addition, increased AMF colonization did not affect DSE colonization. While negative and positive effects of AMF on DSE colonization have been reported, the most common response appears to be neutral (Berthelot et al., 2018; Carpenter et al., 2021; Fors et al., 2020), which is consistent with the results in my study. Like with AMF, the effect of drought on DSE colonization has not been well studied (Bueno de Mesquita et al., 2018). The observation that DSE colonization did not decline from June to October suggests that these fungi may be more tolerant to drought than AMF. This notion is consistent with DSE abundance in arid environments, where they may be the most common root fungal symbionts (Porras-Alfaro et al., 2011).

Despite the observed differences in AMF colonization, I did not see a difference in survival between non-inoculated and inoculated seedlings, and mortality was negligible independent of treatment. One year after transplanting, the survival rate was above 95% for both treatments. This survival rate was higher than that reported in other studies (Davidson et al., 2016; Dettweiler-Robinson et al., 2013) and higher than that typically observed following BLM outplantings, where survival rates of 40% are considered a satisfactory outcome (Roger Rosentreter and Craig Carpenter, personal

communication). The weather data indicates that precipitation and temperature during the spring and summer of 2020 were similar to the average for the region (Agrimet Weather Data, http://www.usbr.gov/pn/agrimet/, one in Boise (N 43° 36′ 0.972″, W 116.17694, 831 m) and the other in Nampa (N 43° 26′ 13.992″, W 116° 38′ 42.9714″, 826 m). Thus, weather conditions cannot entirely explain the high rates of survival. Two practices that could have contributed to the successful establishment are deep watering and the use of metal protectors. Water was added immediately after outplanting and two weeks later through a PVC tube inserted in the soil. By providing water deeper into the soil, perhaps this method facilitated the growth of deeper roots during winter, giving access to moister soil layers in summer. Also, in this experiment, each seedling was within a metal-mesh protector that minimized herbivory and increased survival compared to Vexar protectors typically used in many outplantings.

High survival rates could also reflect biotic or soil characteristics of the microsite that somehow favored establishment. Various reports indicate that DSE can increase tolerance to biotic and abiotic stresses (reviewed by Santos et al., 2021). Given the abundance of DSE in *A. tridentata* seedlings, such effects would have improved survival. In addition, due to some edaphic characteristics, the soil may have remained moister at a shallower depth than in neighboring areas. This possibility requires further investigation, but if that was the case, it was not evident in the features of the vegetation. Except for the planted seedlings and a few rabbit brushes, all the other plants, including crested wheatgrass that can develop roots to a depth of more than 2 m (Svejcar, 1990), dried out in early to mid-summer, indicating severe water scarcity. Also, the soil type at the site is

common throughout the area ("SoilWeb: An Online Soil Survey Browser | California Soil Resource Lab," n.d.)

In 2020, I measured Ψ_l from mid-summer to late fall. During this period, I did not observe differences in Ψ_l between non-inoculated and inoculated seedlings. Several studies have reported that AMF colonization contributes to maintaining higher water potentials during water deficits (Augé et al., 1986; Dell'Amico et al., 2002; Porcel and Ruiz-Lozano, 2004). However, these studies were in potted plants comparing non-mycorrhizal vs. mycorrhizal plants, and the lowest water potentials measured were, in general, higher than those in *A. tridentata* seedlings. In maize, Amerian et al. (2001) reported that when non-mycorrhizal plants had Ψ_l of -1.8 MPa and -2.1 MPa, mycorrhizal plants had Ψ_l of -1.0 and -1.8 MPa, respectively. Thus, the effect of AMF on increasing water potentials appears to decrease with drought intensity. Such a trend plus the presence of AMF in non-inoculated seedlings may account for the lack of difference in Ψ_l between treatments in my experiment, where median values of midday Ψ_l were about -2.5 MPa.

Although AMF inoculation did not affect plant water potential or survival, it did seem to increase fitness as seen by the increased number of plants with inflorescences. The higher levels of AMF colonization in the inoculated seedlings may have caused somewhat better nutrient content in the former, promoting flowering (Hartnett et al., 1994; Javaid and Riaz, 2008; Vaingankar and Rodrigues, 2015). Also, the effect on flowering appeared to be cumulative. Albeit not significant, some differences were observed during the first year, and these augmented and became significant during the second year. These results were unexpected because the plants were relatively close to

each other. Thus, differences in AMF colonization were likely to have decreased over time. It would be interesting to determine whether dissimilarities in flowering between the two treatments continue in subsequent years. Such a result would indicate that early benefits of AMF colonization have a long-lasting competitive advantage (Zaiats et al., 2021).

Effects of herbivory

The results of the herbivory experiment did not support one of my hypotheses; plants that suffered herbivory did not have lower water potentials than the protected ones. In contrast, the results support my second hypothesis: seedlings that endured herbivory were more susceptible to summer mortality. In addition, herbivory markedly reduced the percentage of plants that developed inflorescences.

Between late winter and early spring, ground squirrels come out from hibernation. Most of the herbivory observed in this experiment occurred during the early spring of 2020. This observation and the type of cut noted in the seedlings (Fig. 1.10.C) strongly suggest that herbivory was mainly caused by ground squirrels. Interestingly, this damage did not continue during spring and early summer when ground squirrels were still active. Moreover, herbivory was minimal during 2021, despite the abundant presence of ground squirrels in spring. This last result suggests changes in plant chemistry or structure that discouraged herbivory. Herbivory may have triggered some of these changes, but they could also have resulted from development processes (Barton and Hanley, 2013; Karban and Thaler, 1999). Some observations support this notion; by March 2021, many plants had branches extending out of the protectors. These branches experienced minimal herbivory.

While most plants recovered from herbivory, the damage caused affected subsequent survival. Differences in survival between treatments increased during the first summer and continued augmenting during the fall and winter. By late July 2021, the degree of herbivory in each treatment was negatively correlated with survival. In plants within metal protectors, survival was 12 and 39% higher than in those within plastic and no protectors, respectively. Whether a difference of 12% in survival justifies the use of metal over plastic protectors is arguable. In plants smaller than those used in this study, recovery from herbivory may be more difficult. Under these circumstances, metal protectors may have a higher impact on survival. In addition, the metal protectors have two other advantages. They can provide some protection against grasshoppers in years with high herbivory by these insects and are much more durable than the plastic ones. Consequently, the former can be used in multiple succeeding outplantings.

The reasons for higher mortality following herbivory are not clear. During the summer of 2020 and extending into the fall, most plants kept water potentials between -2.0 and -4.0 MPa that, while low, are above those that cause hydraulic failure in this species (Kolb and Sperry, 1999). Nevertheless, some plants died, suggesting that their Ψ_l declined further. Detecting this decline was very unlikely because I only measured randomly selected lived plants at approximately biweekly intervals. Thus, even though I did not note differences in Ψ_l between treatments, the frequency of plants that reached Ψ_l below the threshold for hydraulic failure (-6 to -7 MPa in *A. tridentata*) might have been higher in those that suffered herbivory.

Water potentials between -2 and -4 MPa corresponded with low to minimal stomatal conductance (Fig. 1.15). This decrease in stomatal conductance was likely an

important factor in maintaining plants at Ψ_l above the threshold for hydraulic failure (Martínez-Vilalta and Garcia-Forner, 2017). Still, it may have led to periods when plants had a negative carbon balance (McDowell et al., 2008). Under this scenario, a possibility is that plants that suffered herbivory had fewer storage carbohydrates and were less able to maintain metabolic processes that contribute to drought tolerance, such as osmotic adjustment, xylem embolism repair, and root growth maintenance (De Baerdemaeker et al., 2017; Jacquet et al., 2014). Reduced capacity for any of these processes could have increased the chances of hydraulic failure and plant death (McDowell, 2011). Storage carbohydrates also play an important role in cold tolerance (Pommerrenig et al., 2018). Consequently, fewer storage carbohydrates in plants that suffered herbivory could also explain their higher mortality during the winter.

For the surviving plants, the percentage that developed inflorescences was approximately threefold higher in plants with protectors than those without them. A decrease in the production of structures involved in sexual reproduction is a common observation in plants that suffer herbivory (Brys et al., 2011; Marquis et al., 1997). The loss of vegetative structures due to herbivory can delay the transition from juveniles to adults and decrease the production of internal signals and photosynthates that promote flowering (Brys et al., 2011; Huijser and Schmid, 2011; Marquis et al., 1997). Both of these effects may have mediated the differences between treatments observed.

Responses to summer conditions independent of treatment

Independent of the treatment applied, some of the results collected during the summer are informative of seedlings' physiological characteristics and developmental differences in their ability to cope with drought. The relationship between predawn and

midday Ψ_l was close to one. Based on the work of Martínez-Vilalta et al. (2014), a slope of 1 indicates strict anisohydric stomatal behavior. An anisohydric behavior means that as drought develops, plants keep a relatively constant soil to leaf Ψ gradient, allowing them to maintain high g_s and photosynthesis (Martínez-Vilalta and Garcia-Forner, 2017; McDowell et al., 2008). Assuming that the predawn Ψ_l represents the water potential of the soil from where the roots took water (Donovan et al., 2003), *A. tridentata* seedlings showed a behavior close to anisohydric. However, they did not maintain high g_s with declining midday Ψ_l(Fig. 1.15). In *Eucalyptus gomphocepala*, Franks et al. (2007) observed similar results, a constant plant water potential gradient with increasing water deficits but decreased stomatal conductance. They described this behavior as anisohydric but hydrodynamic and indicative of parallel reductions of g_s and plant hydraulic conductivity with drought (Franks et al., 2007). Whether *A. tridentata* seedlings followed this behavior requires further experimentation, but it would explain the apparent discrepancies between water potential gradients and changes in g_s.

The measurements of midday Ψ_l also showed that plants maintained higher water potential during the summer of 2021 than in the summer of 2020. For comparable periods (mid-July to late-August), the average midday Ψ_l in the mycorrhizal and herbivory experiment was respectively 0.3 ($p = 0.02$) and 1.4 MPa ($p < 0.0001$) higher in 2021 than in 2020. These increases occurred even though the weather was more conducive to drought in 2021 than in 2020. Such results are not entirely unexpected. The plants were larger in 2021 than 2020 and presumably had a larger root system to extract more water from moister and deeper soil. Another possibility is that older plants had tighter control of stomatal opening. However, this is unlikely; in other experiments, I observed younger

seedlings closing their stomata at higher water potentials than older ones (Geisler and Serpe, 2021).

In summary, the results of this study indicate that inoculation with mycorrhizal trap cultures increased AMF colonization over the background level naturally occurring in the soil. Survival was very high independent of the inoculation treatment, but increased AMF colonization was associated with a higher proportion of plants bearing inflorescences. Protecting plants from herbivory had a more beneficial effect on survival and inflorescence development than mycorrhizal inoculation. Most plants that suffered herbivory did not die directly from this disturbance. Instead, herbivory increased mortality during the subsequent year. Neither AMF inoculation nor herbivory had apparent effects on plant water potential. However, questions remain whether herbivory increased the proportion of plants with water potentials below the threshold for hydraulic failure. Water potentials decreased from early spring to early summer. Subsequently, Ψ_l varied between plants, particularly during 2020, but average values remained relatively constant through the summer despite the lack of precipitation and a continued decrease in soil moisture. The causes that determined this seasonal homeostasis in water potential require further investigation, but one contributing factor was a reduction in stomatal conductance.

Figures and Tables

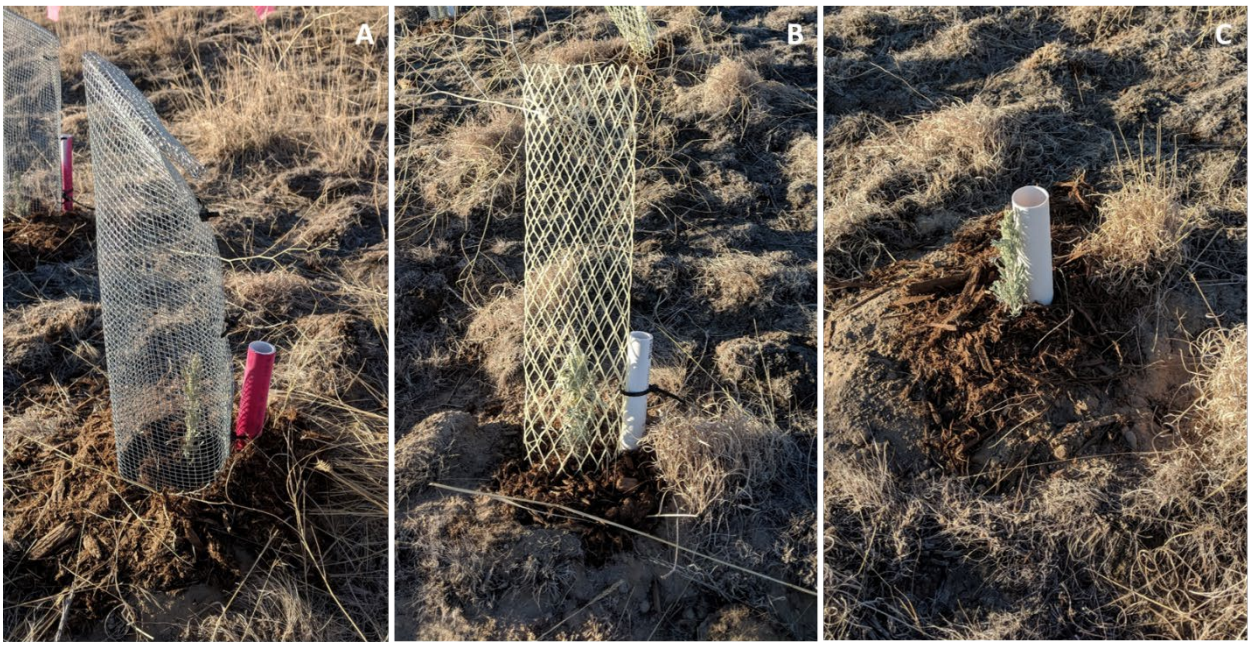

Figure 1.1. Seedlings after outplanting covered with a metal (A) or plastic (B) tree protector or without a protector (C).

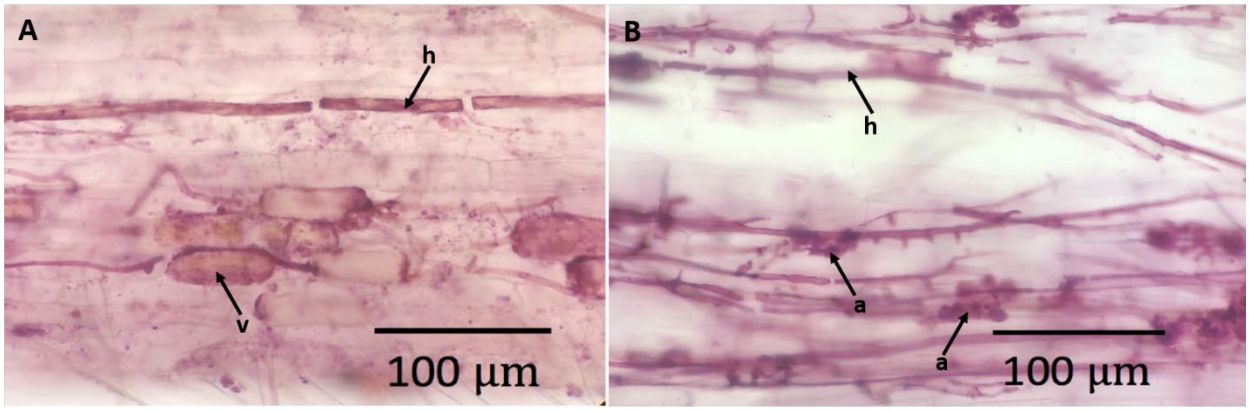

Figure 1.2. Arbuscular mycorrhizal structures recorded during the study: a, arbuscules; h, hyphae; v, vesicles.

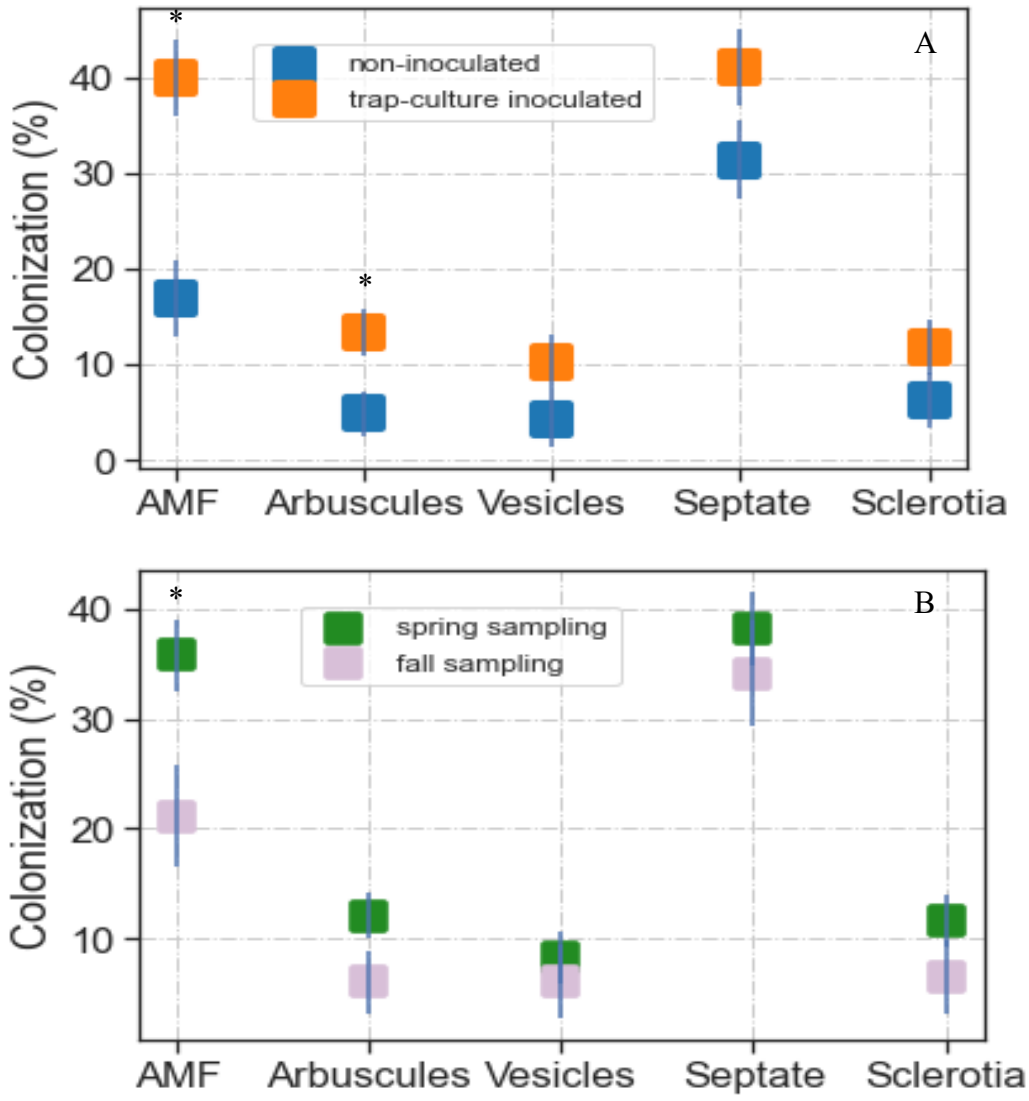

Figure 1.3. Colonization of *Artemisia tridentata* roots by arbuscular mycorrhizal and sepate fungi in field collected samples. A, Colonization in non-inoculated and inoculated plants averaged over the two sampling times; means and experimental errors of 12 plants. B, Colonization in plants harvested in early spring (6/12/2020) and early fall (10/5/2020) averaged over the two inoculation treatments; means and experimental errors of 8 plants. For a particular variable, means labeled by an asterisk (*) are significantly different ($p < 0.05$) based on t-tests.

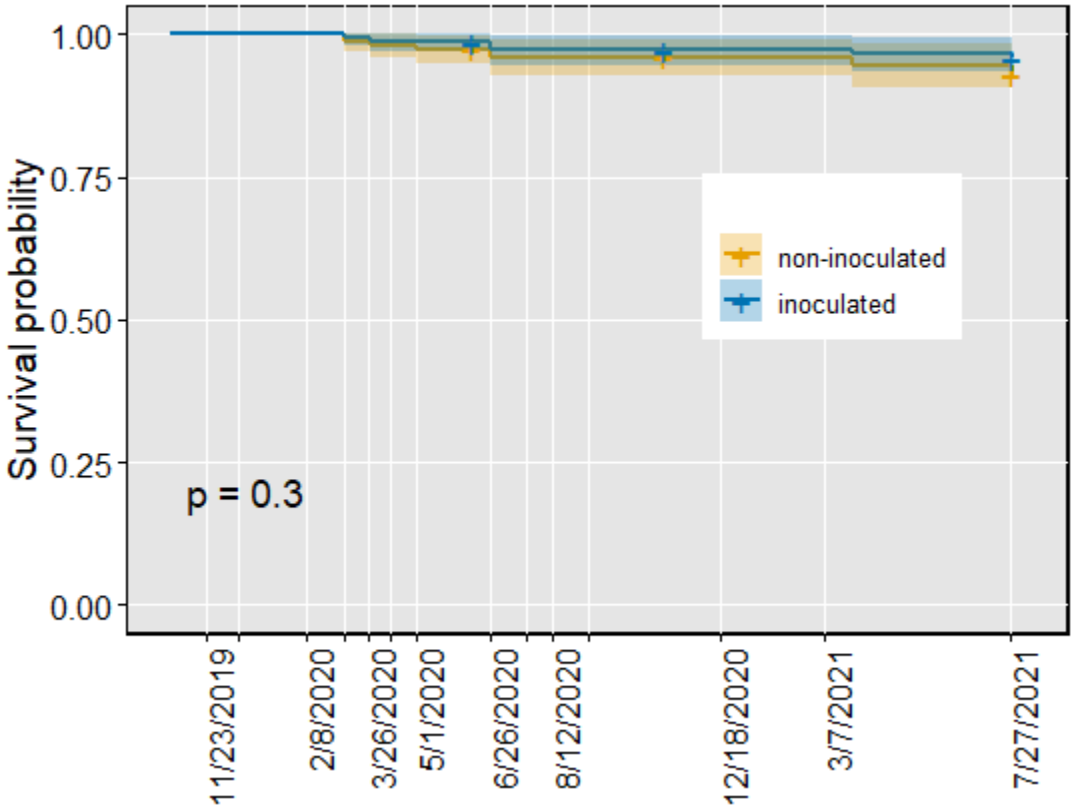

Figure 1.4. Survival of *Artemisia tridentata* seedlings since outplanting on 10/26/2019. Survival curves indicate median survival and 95% confidence intervals; *p*-value estimated based on a log-rank test.

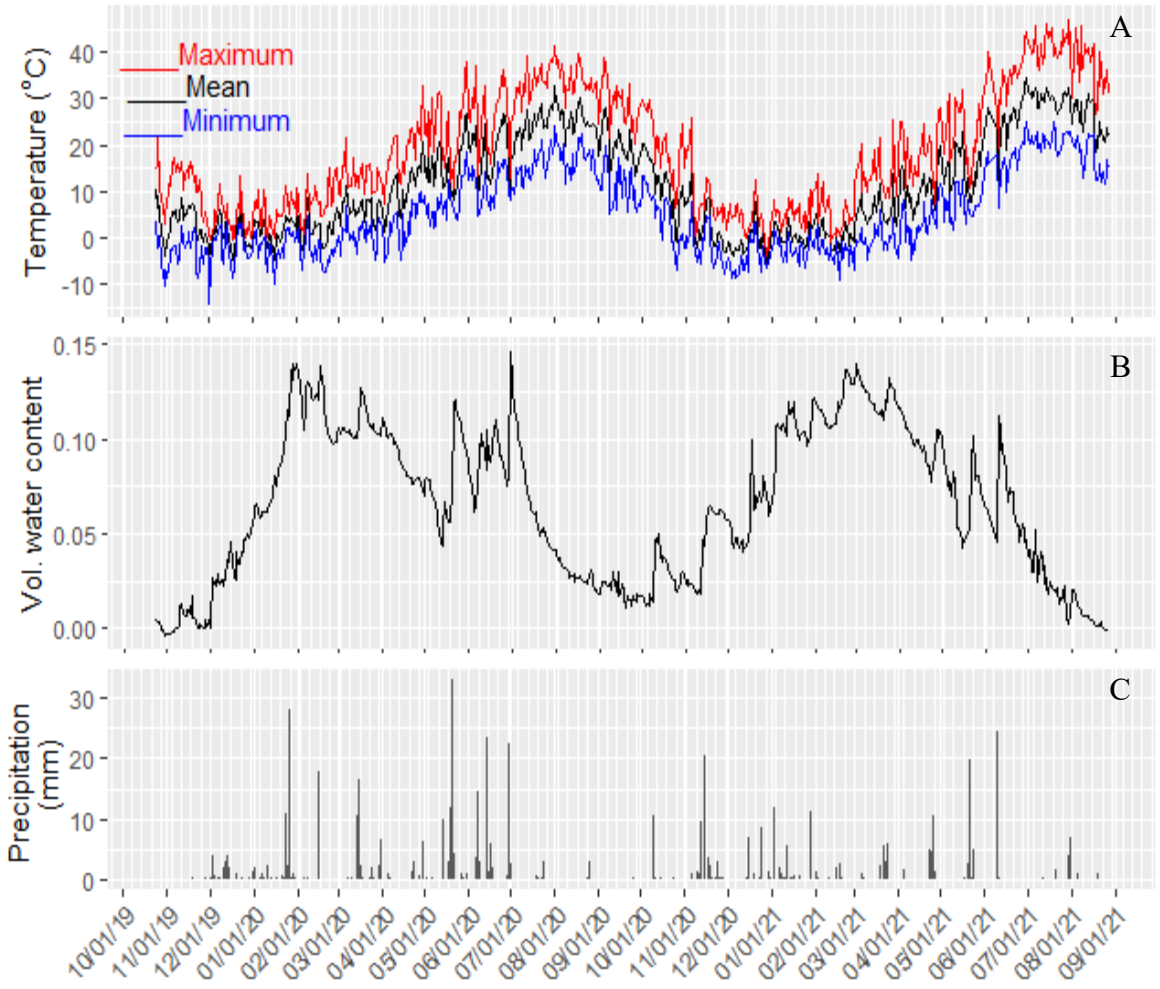

Figure 1.5. Weather conditions during the experimental period. A, Temperature; B, Volumetric water content in the upper 20 cm of the soil (water volume over soil volume); C, Precipitation.

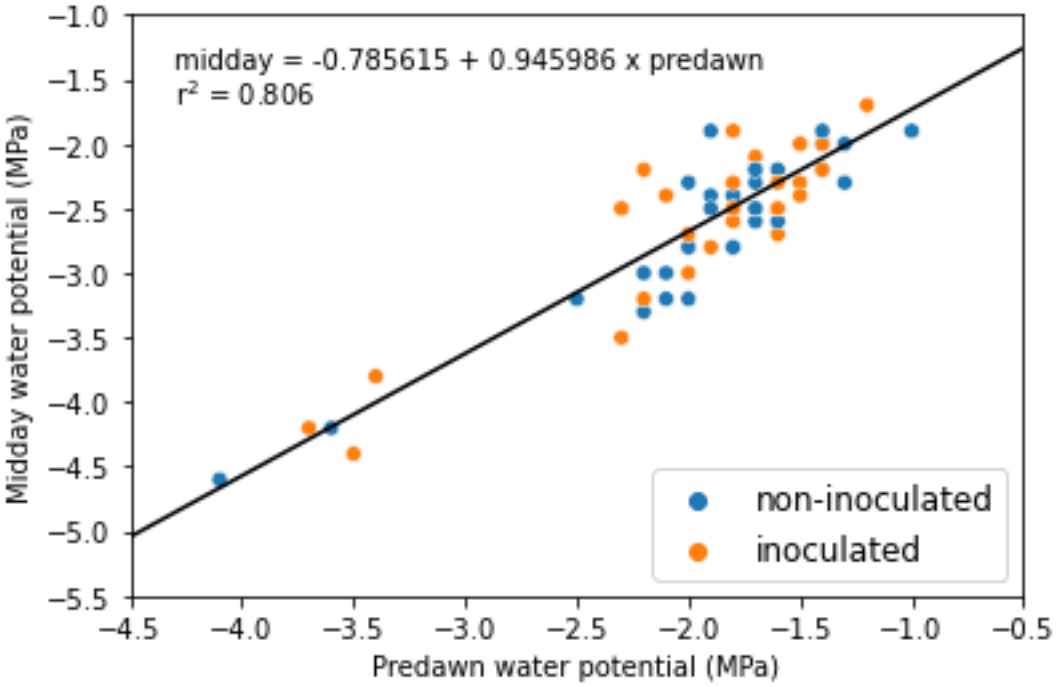

Figure 1.6. Relationship between predawn and midday water potential.

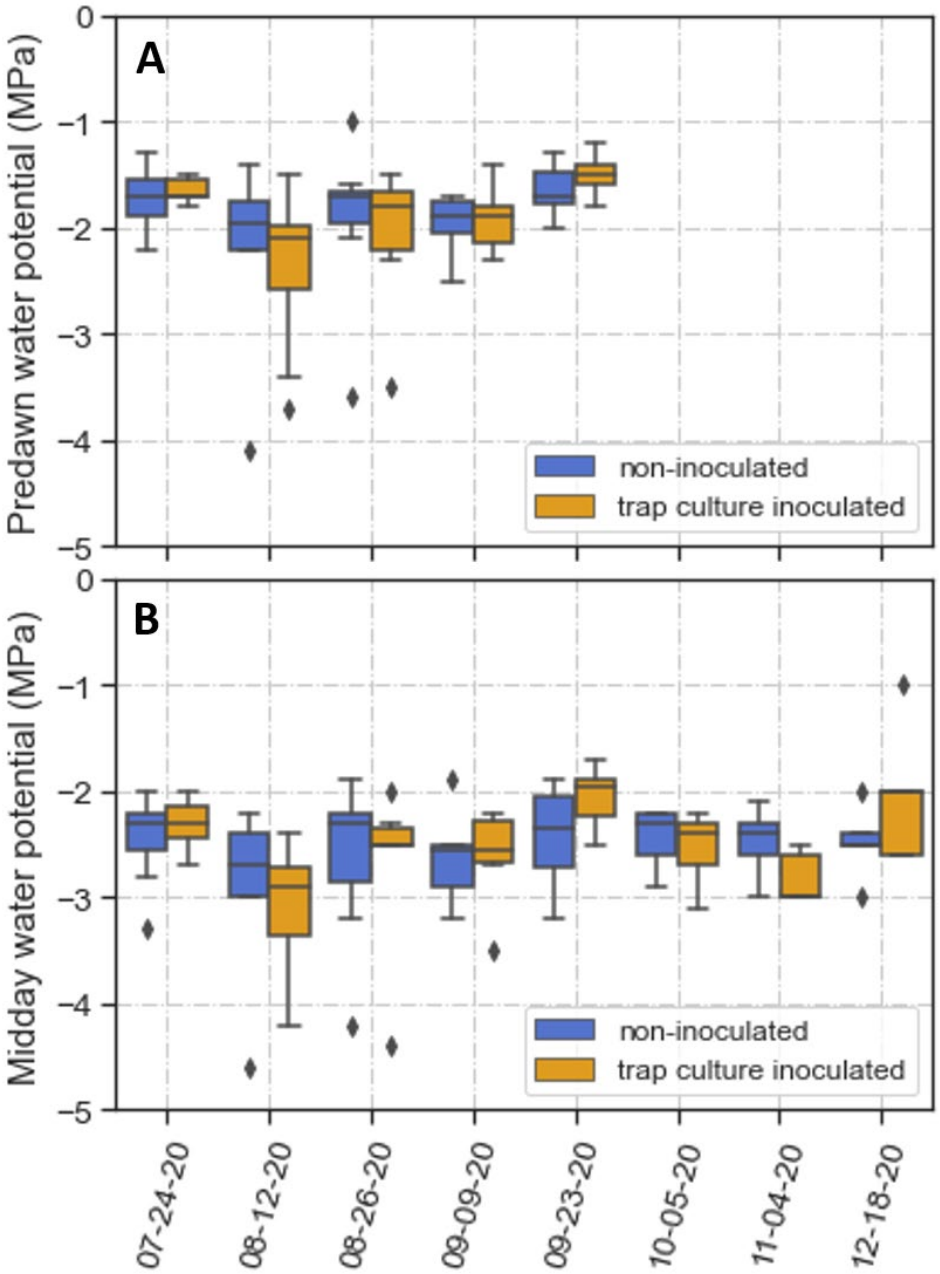

Figure 1.7. Predawn (A) and midday (B) leaf water potential of *Artemisia tridentata* plants during the summer and fall of 2020. Boxplots of eight measurements. Diamonds indicate outliers. Note: The days when water potentials were measured are plotted as categorical variables rather than a continuous time sequence to make the boxplots more noticeable.

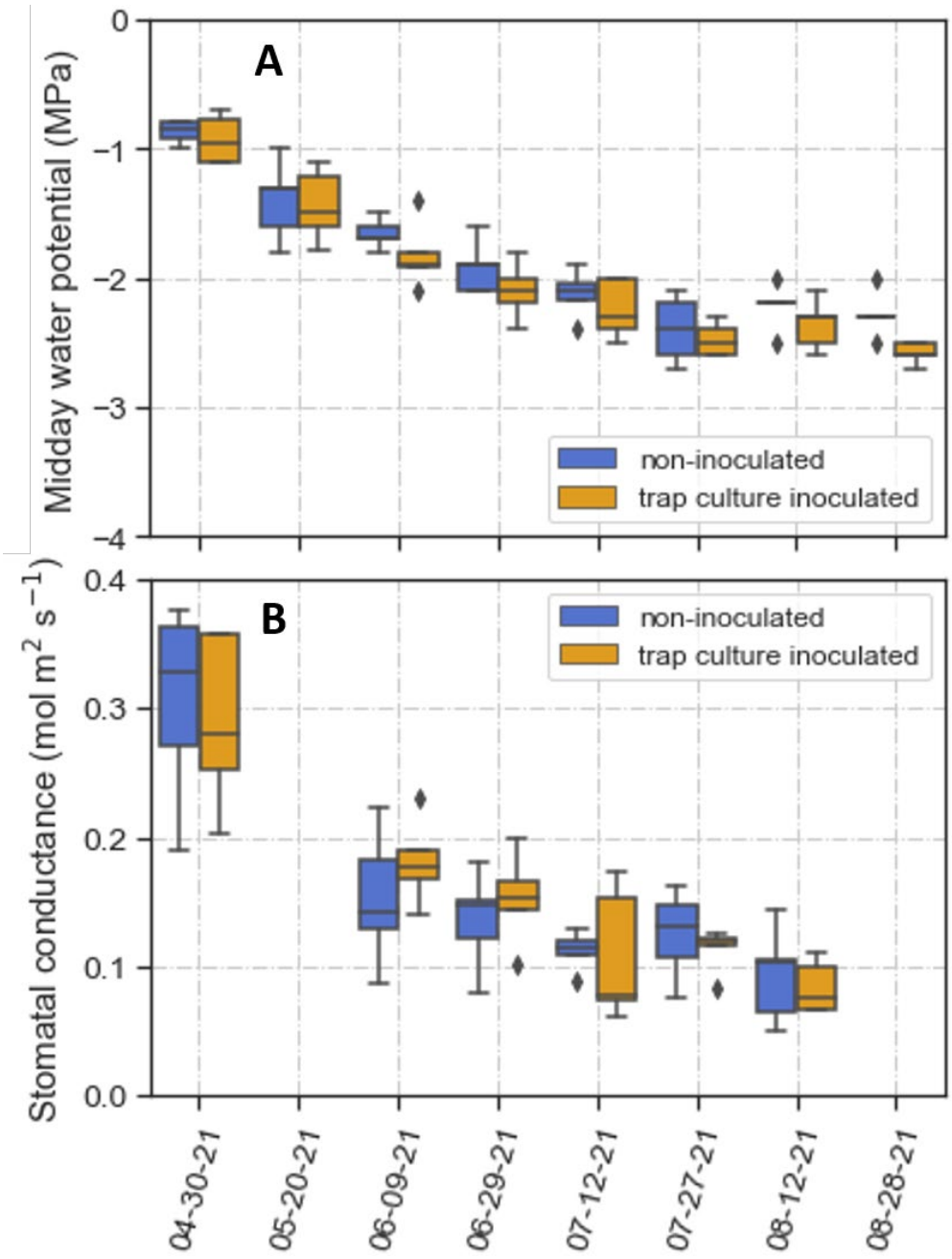

Figure 1.8. Midday leaf water potential (A) and stomatal conductance (B) of *Artemisia tridentata* plants during the spring and summer of 2021. Boxplots of eight measurements. Diamonds indicate outliers. Note: The days when water potentials were measured are plotted as categorical variables rather than a continuous time sequence to make the boxplots more noticeable.

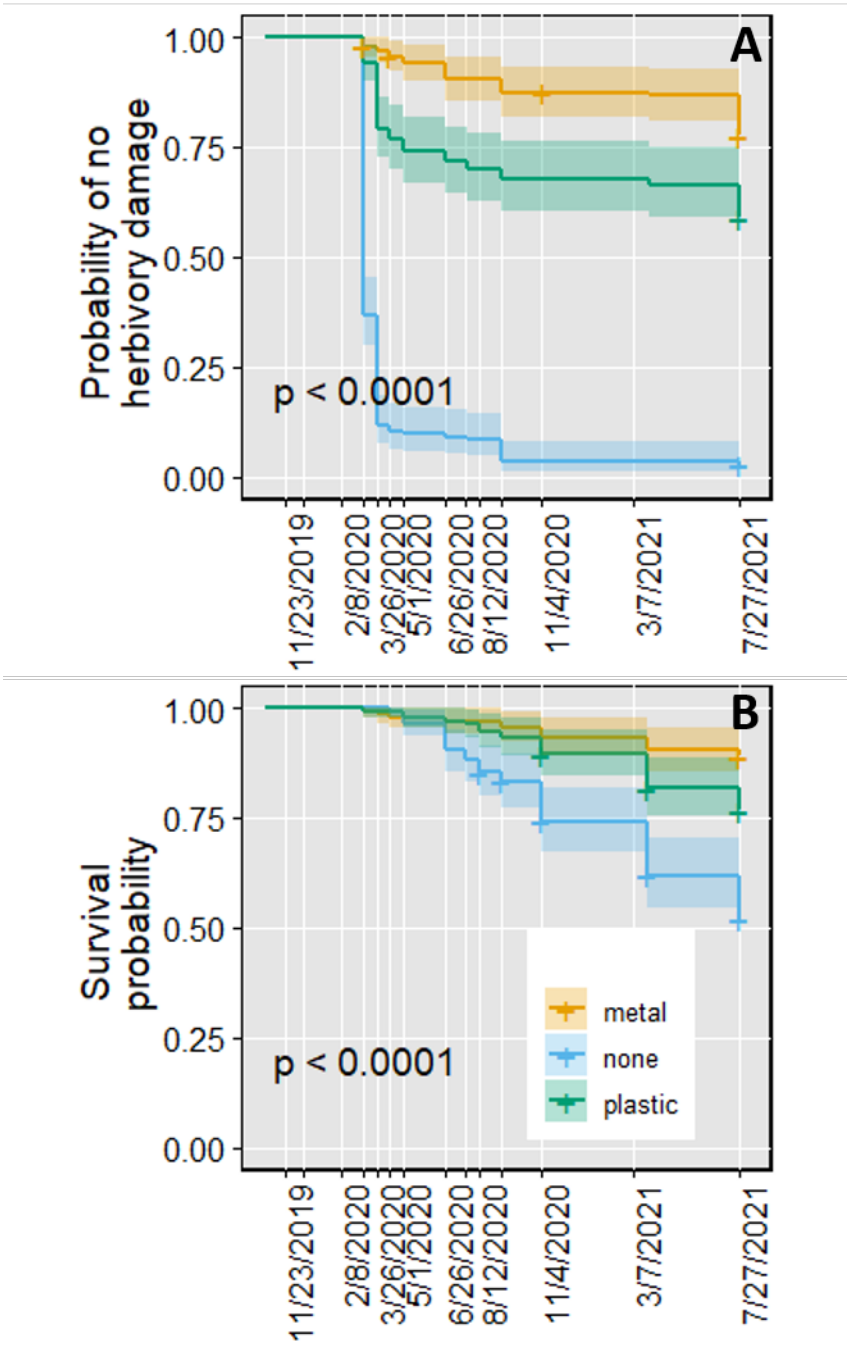

Figure 1.9. Probability of no herbivory damage (A) and survival (B) of *Artemisia tridentata* seedlings under the different tree protector treatments. Curves indicate medians and 95% confidence intervals.

Figure 1.10. Representative pictures of *Artemisia tridentata* plants in early spring 2020. A, Plant within a metal protector. B, Plant without protector showing herbivory damage. C, Close view of a cut stem.

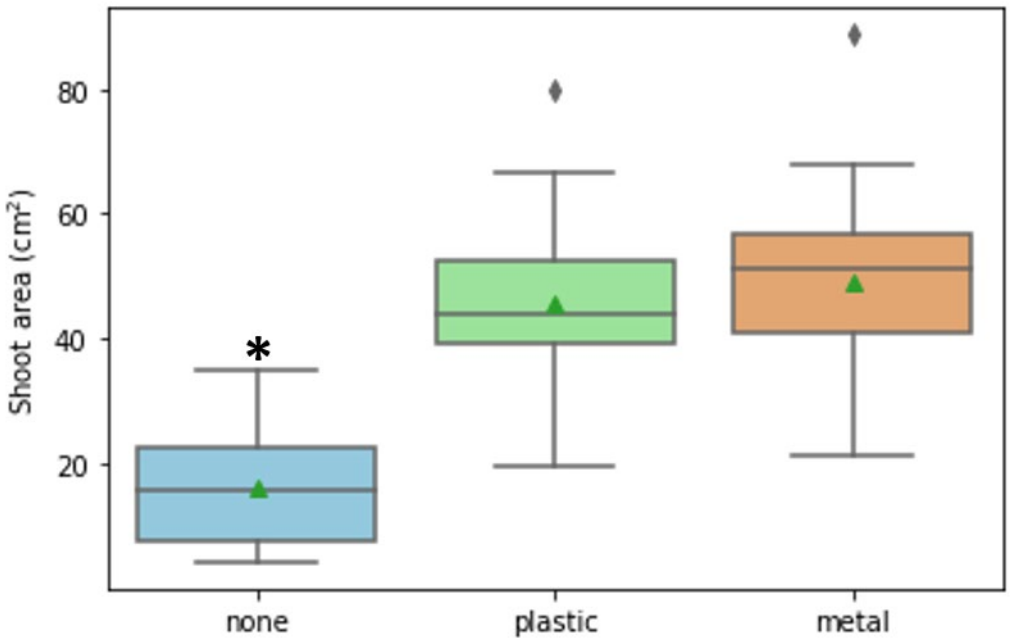

Figure 1.11. Effect of tree protectors on shoot size. Boxplots of 25 randomly selected seedlings from each treatment. The boxplot marked by an asterisk is significantly different from the other treatments.

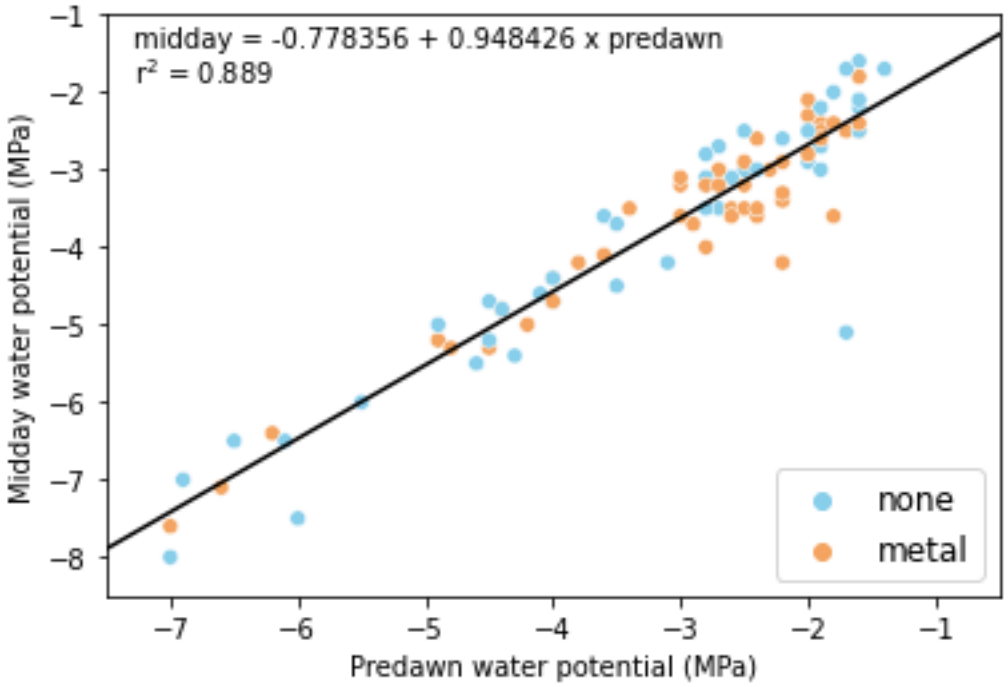

Figure 1.12. Relationship between predawn and midday water potential.

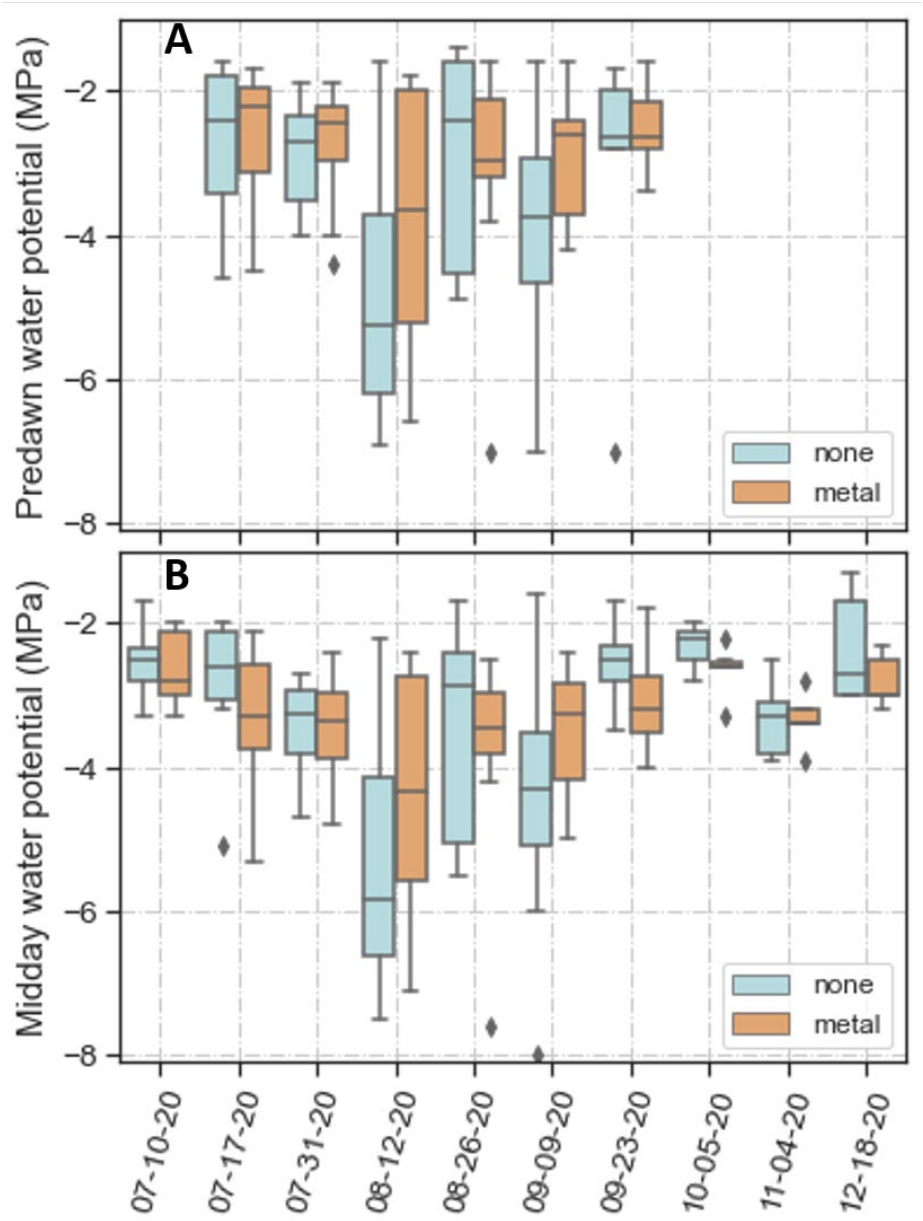

Figure 1.13. Predawn (A) and midday (B) leaf water potential of *Artemisia tridentata* plants during the summer and fall of 2020. Boxplots of eight measurements. Note: The days when water potentials were measured are plotted as categorical variables rather than a continuous time sequence to make the boxplots more noticeable.

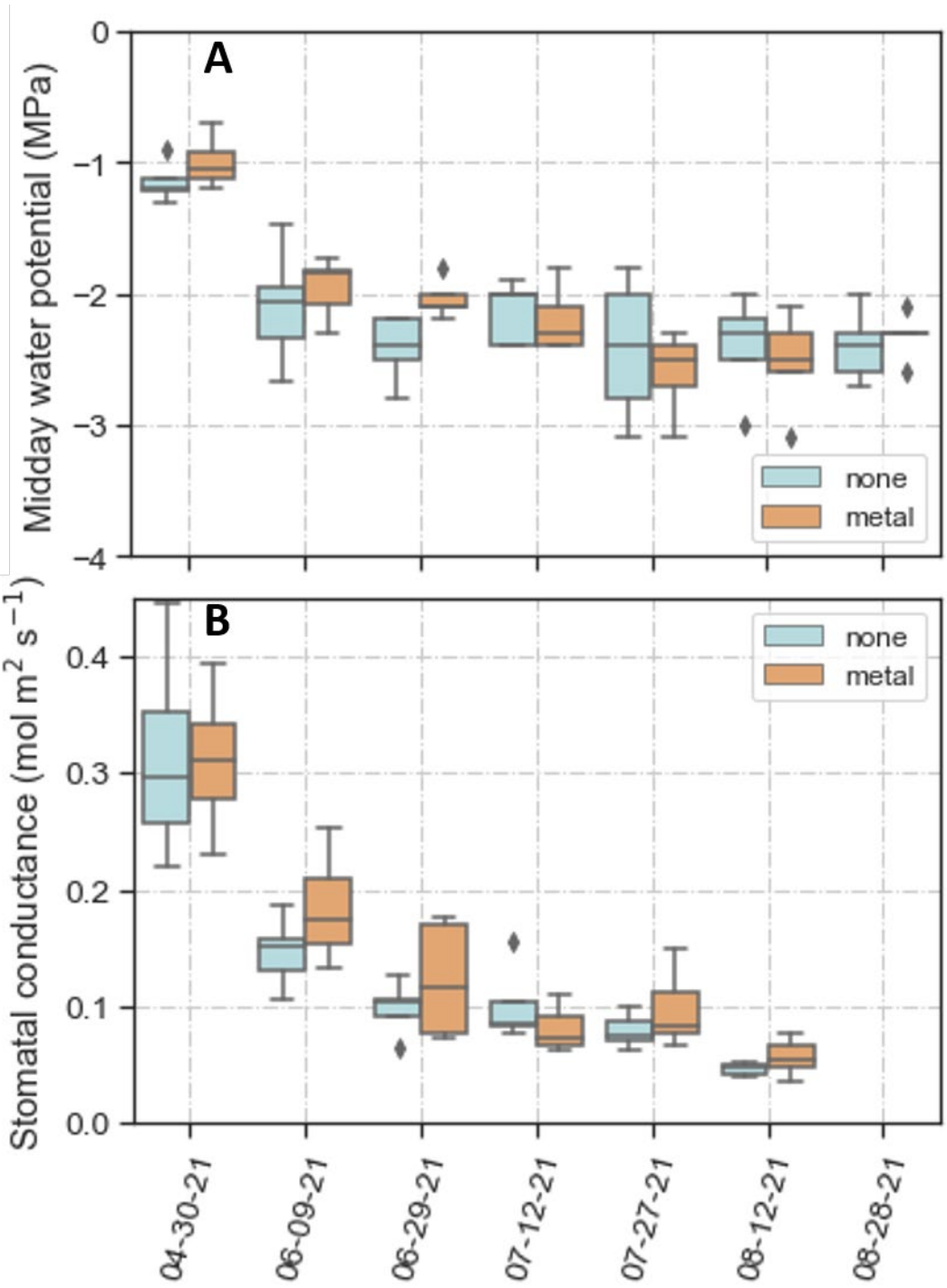

Figure 1.14. Midday leaf water potential(A) and stomatal conductance (B) of *Artemisia tridentata* plants during the spring and summer of 2021. Boxplots of eight measurements.

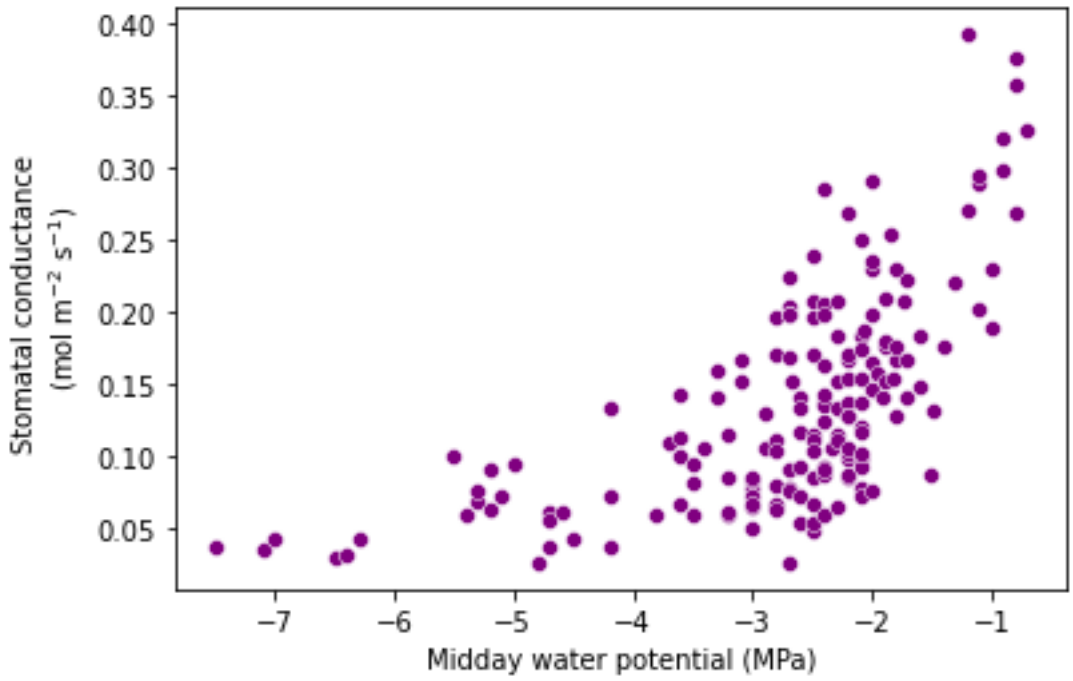

Figure 1.15. Field measurements of stomatal conductance and midday Ψ_l. The data combine results obtained in the mycorrhizal and herbivory experiment.

Table 1.1 Percent of *Artemisia tridentata* plant bearing inflorescence at the end of the 2020 and 2021 summers. Values labeled by different letters are significantly different within a column based on χ^2 tests.

Treatment	2020	2021
no-protector	11[b]	12[b]
plastic protector	31[a]	41[a]
metal protector	29[a]	30[a]

CHAPTER TWO: DROUGHT-INDUCED DECLINE IN PHOTOSYNTHESIS IN NON-MYCORRHIZAL AND MYCORRHIZAL ARTEMISIA TRIDENTATA SEEDLINGS

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that form associations with about 80% of land plants (Wang and Qiu, 2006b). These associations are widespread in terrestrial ecosystems, occurring in wetlands to deserts and the tropics to the low Arctic (Gardes and Dahlberg, 1996; Vasar et al., 2021; Xu et al., 2016). Numerous studies have shown that plants can increase their growth by establishing symbiotic associations with AMF (Rodriguez et al., 2008). In this symbiosis, AMF provides mineral nutrients to the plant, particularly those with low mobility in the soil, while plants provide carbohydrates and lipids to their fungal partners (Keymer et al., 2017; Smith and Smith, 2011). In addition, other beneficial effects of AMF on plants have been reported. These include less susceptibility to pathogens and increased tolerance to abiotic stresses caused by salinity, toxic metals, and drought (Miransari, 2011; Ruiz-Lozano et al., 2012; Ruiz-Lozano, 2003; Sikes, 2010).

Although many plants form associations with AMF, the effect of the association on the plant is not always beneficial but ranges from somewhat parasitic to mutualistic (Smith and Smith, 2012). This variation depends on the environmental conditions where the symbiosis occurs and genetic differences within and among species (Konvalinková and Jansa, 2016; Maherali, 2014; Tawaraya, 2003). Because a primary role of AMF is to

increase P transfer from the soil to the plant, root biochemical or structural characteristics that facilitate the solubilization and acquisition of P tend to reduce mycorrhizal dependency (Shen et al., 2011; Tawaraya, 2003). Similarly, plants with high P use efficiency due to their ability to mobilize and translocate this nutrient may be less dependent on AMF (Tawaraya, 2003; Wang et al., 2010). The variable AMF effects on plants are not limited to mineral nutrition and growth but also have been reported for biotic and abiotic stresses (Chandrasekaran et al., 2014; Del Fabbro and Prati, 2014; Jongen et al., 2022; Sikes, 2010).

An aspect of the plant-AMF symbiosis that has received much attention is its impact on drought tolerance. Numerous studies indicate that AMF enhances drought tolerance (Jayne and Quigley, 2014). This effect can result from various factors and responses (Ruiz-Lozano et al., 2012). As soil moisture declines, the role of AMF in P uptake becomes more critical due to the low mobility of this element and the more tortuous pathway of water movement to the root surface (Suriyagoda et al., 2014). Also, the symbiosis can lead to changes in plant gene expression that increase root hydraulic conductivity and water use efficiency and reduce drought-induced oxidative damage (Ruiz-Lozano *et al.*, 2006; Barzana *et al.*, 2014; Zou *et al.*, 2021). Water transport through the extraradical hyphae to the plant is often described as a means by which AMF alleviates drought stress. However, there are conflicting results about the significance of this pathway. Some studies indicate that water transport through extraradical hyphae is negligible compared to the direct uptake by the roots (Auge, 2001; Khalvati et al., 2005). On the other hand, Li et al. (2014) showed that colonization by *Rhizophagus intraradices*

compensated for the absence of root hairs in a bald root barley mutant under mild water stress, thus suggesting that water uptake via AMF can affect plant water status.

Independent of the mechanisms involved, there has been an increased interest in using AMF to mitigate plant water stress in crops as well as seedlings transplanted to forest plantations and disturbed natural habitats (Liu et al., 2015; Ruiz-Sánchez et al., 2010; Asmelash et al., 2016). In western North America, an ecosystem that has experienced widespread human disturbances is the sagebrush steppe (Davies et al., 2011). In particular, the introduction of non-native annual grasses has increased wildfire frequency (Baker, 2006). Fires tend to remove various native species, including the dominant shrub *Artemisia tridentata subsp. wyomingensis* (Big sagebrush) (Baker, 2006; Dettweiler-Robinson et al., 2013). Success in re-establishing this species has been limited by multiple factors, but a significant one is summer drought (Applestein et al., 2021; Maier et al., 2001; Schlaepfer et al., 2014).

Big sagebrush forms associations with AMF and results from pot and field experiments indicate that this symbiosis can improve sagebrush growth and seedling establishment (Allen et al., 1993; Davidson et al., 2016; Dierks et al., 2019; Stahl et al., 1988). However, neutral (Busby et al., 2011; Carpenter et al., 2021; Dettweiler-Robinson et al., 2013) and adverse effects (Prado-Tarango et al., 2021) of AMF on the growth or establishment of sagebrush seedlings have also been reported. Also, questions remain about the impact of AMF colonization on the drought tolerance of sagebrush seedlings. Early work by Stahl et al. (1998) showed that AMF seedlings withstood lower soil water potentials than non-mycorrhizal ones. This result indicates that AMF increased drought

tolerance, but, to my knowledge, it is the only published study on this aspect of the AMF-sagebrush symbiosis.

In a previous study, I conducted a field experiment to investigate how inoculation with AMF affects the water status and survival of outplanted *Artemisia tridentata* seedlings. My experiment showed that inoculation increased AMF colonization over the natural levels occurring in the soil and that the plants experienced water stress through most of the summer. However, increased AMF colonization did not affect plant water potential or survival, which was over 90% in both non-inoculated and inoculated seedlings. Even though enhanced colonization did not affect water potential and survival, it does not exclude possible effects on variables that I did not measure. For example, higher osmotic adjustment and activity of antioxidant defenses in inoculated seedlings could have led, at the same water potential, to more photosynthesis in inoculated than non-inoculated plants (Augé et al., 1986; Ruiz-Lozano, 2003). Such an effect could have, in turn, affected growth or reproductive development (Jayne and Quigley, 2014). Interestingly, the percentage of plants with inflorescences was higher in inoculated than non-inoculated plants.

Field studies are ultimately the most relevant for determining the value of AMF inoculation in improving restoration (Karban et al., 2014). However, detecting differences in the field is complicated by factors such as spatial differences in the immediate vegetation or microheterogeneity in soil's physical and biotic characteristics. These differences increase variations in plant growth and water status independent of treatment, making any effect of AMF on plants more challenging to detect. To complement the field experiments and assess whether AMF affects the physiology of *A.*

tridentata seedlings under drought, I conducted experiments under more controlled conditions in a greenhouse.

A typical physiological response to drought is a decrease in stomatal conductance (g_s) and an associated reduction in photosynthesis and transpiration (Gupta et al., 2020). Because the reduction in g_s tends to lower transpiration more than photosynthesis, decreased g_s increases water use efficiency (WUE), although not all plants behave in this manner (Lawson and Blatt, 2014; Tomeo and Rosenthal, 2017). There are contrasting reports on the effects of AMF on g_s, but a meta-analysis of more than 400 studies indicates that overall, AMF increases g_s, particularly under drought stress (Augé et al., 2014). Despite this increase in g_s, several studies have also reported AMF-induced enhancements in WUE (Birhane et al., 2012; Querejeta et al., 2003; Ruiz-Sánchez et al., 2010). The gain in photosynthesis resulting from better mineral nutrition and reduction in oxidative damage in mycorrhizal plants appears to outweigh the effect of a higher g_s on WUE (Ruiz-Sánchez et al., 2010). Thus, in many water-stressed plants, symbioses with AMF contribute to keeping the stomata open and photosynthesis rates higher for longer periods. At least under moderate water stress, this effect can be beneficial; the additional photosynthates can support root or hyphal growth in still moist soil patches or help sustain metabolism when more severe drought causes stomatal closure (Mathimaran, 2017; Sade et al., 2012a).

The studies cited above indicate that AMF colonization tends to increase g_s, photosynthesis, and WUE. Whether similar effects occur in *A. tridentata* seedlings is, however, unclear. A primary purpose of the present study was to investigate such plausible effects. For this purpose, I exposed non-inoculated and inoculated seedlings to

progressive drought and measured the time course of changes in g_s, photosynthesis, and transpiration. Prolonged photosynthesis or increased WUE in the AMF treatment would indicate that these fungi help the seedlings cope with drought (Ruiz-Lozano et al., 2012). Such a result would be an important reason for further testing AMF under field conditions. Plants were inoculated using two methods. The first method involved inoculation with isolated spores, which typically leads to high colonization and is viable for multiple AMF species (Auge, 2001; Balzergue et al., 2011; Klironomos and Hart, 2002). This method allowed to create a layer rich in inoculum that the plants would encounter as their roots grew. However, this method is laborious because it requires the separation of spores from soil and root material and is not well suited to inoculate the thousands of plants needed for restoration. As an alternative method, I also inoculated seedlings using soil and roots from trap cultures (Morton et al., 2004). Previous research has shown that not all inoculation methods provide the same levels of colonization, and using inoculum from trap cultures may limit colonization rates (Klironomos & Hart, 2002, Morton et al., 2004).

Apart from the primary purpose of assessing the impact of AMF on drought tolerance, an additional motivation for the present study was to investigate how drought affects AMF colonization. As shown in chapter *1*, *A. tridentata* can maintain low water potentials and minimal g_s for several months. Under these conditions, the maintenance of arbuscles could result in a parasitic symbiosis because the plant has limited ability to gain any carbon but would still be supporting AMF (Smith and Smith, 2013). To prevent parasitism, I hypothesized that stomatal closure would cause a decrease in arbuscular

colonization. This hypothesis was investigated by comparing colonization in seedlings with high and negligible g_s.

Materials and Methods

Experiment 1: Inoculation with extracted spores

Fungal and plant material

To produce mycorrhizal inoculum, I collected silty-loam soil from a sagebrush community near Kuna Butte, Idaho (43°26.161'N, 116°25.848'W, 908 m a.s.l.). This soil was mixed in a 2:3 ratio with sterilized sand, and then the AMF in the soil were multiplied in trap cultures using *Plantago lanceolata* as a host. After three cycles of trap culture cultivation, spores were extracted from these cultures by wet sieving and sucrose gradient centrifugation (Morton et al., 2004). Subsequently, the spores were surface-sterilized in 0.5% sodium hypochlorite for ten minutes, rinsed in sterile water, and stored at 4°C in an aqueous solution containing 200 mg l^{-1} streptomycin and 100 mg l^{-1} gentamycin. The spores were resuspended in water and used as inoculum within a month. Previous studies indicated that the trap cultures contained a mixture of AMF within the Glomeraceae family (Serpe et al., 2020). The Wyoming big sagebrush seeds used in this study (*Artemisia tridentata* ssp. *wyomingensis*, hereafter referred to as *A. tridentata*) were gifts from the Bureau of Land Management. The seeds had been collected within the Morley Nelson Snake River Birds of Prey National Conservation Area in southwestern Idaho.

Growing conditions

Artemisia tridentata seeds were planted in 150 ml cone-tainers filled with a 3:2 sand to soil mix, which has been autoclaved twice for 1h. The soil used in this experiment

was silty loam collected at a sagebrush steppe community in Kuna Butte, Idaho (43°26.161'N, 116°25.848'W). Before mixing it with sand, the soil was screened through a 1 mm mesh to remove leaf litter and roots. Two months after seeding, the pots were thinned to one seedling per cone-tainer and randomly assigned to either of two treatments: non-inoculated or inoculated. The inoculated cone-tainers received an aqueous suspension containing about 700 spores extracted from the trap cultures. The latter were placed 6 to 7 cm from the soil surface, while the non-inoculated cone-tainers did not receive spores. After inoculation, plants were grown in a greenhouse for eight months. Since seeding and throughout the experiment, the plants were under a 15- hour photoperiod with day/night conditions of 23/18 ±3°C. Until the beginning of the drought treatment, the sand/soil mix was kept close to field capacity and fertilized monthly with a 1/8 strength Hoagland's solution.

Experimental approach

Eight months after inoculation, the *A. tridentata* seedlings were used to investigate the effect of AMF colonization on plant physiological responses to drought. I used ten plants for this experiment, five non-inoculated and five inoculated ones. These plants were similar in size, about 10 cm in height, and with similar projected leaf areas. Drought was imposed by withholding watering, and subsequently, I made gas exchange measurements of all plants every day until stomatal conductance was minimal for at least one week. Using ten plants allowed me to complete the gas exchange measurements in 3 to 4 h. I followed this approach to reduce variations in photosynthesis due to circadian rhythms or differences in light intensity before the measures (Dodd et al., 2014).

The parameters measured daily were: CO_2 assimilation per unit leaf area (*A*), transpiration per unit leaf area (*Tr*), stomatal conductance (g_s), and photosystem II operating efficiency (ΦPSII). These parameters were measured using a LI-6400-40 leaf chamber fluorometer connected to a LI-COR LI-6400XT portable photosynthesis system (LI-COR Inc, Lincoln, NE). The leaves were arranged to cover the leaf chamber area fully. Net photosynthesis, *Tr*, and g_s were measured at an incoming airflow of 200 µmol s^{-1}, a CO_2 concentration of 400 µmol mol^{-1}, ambient temperature, and 500 µmol m^{-2} s^{-1} light intensity. This light consisted of a 90% red and a 10% blue fraction. Values of *A* and *Tr* were recorded after the CO_2 assimilation rates, and stomatal conductance values became stable; the infrared gas analyzer was matched before each measurement. After completing the gas exchange measurements, ΦPSII was determined in the same leaves by measuring the steady-state fluorescence (*F′*) and the maximal fluorescence (*Fm′*). The latter was determined following a light saturating pulse of 8,000 µmol m^{-2} s^{-1}. The above measurements were conducted for about twenty-three days.

To estimate the change in plant water status the plants experienced during the drought period, I also made measurements of leaf water potential (Ψ_l) in non-inoculated and inoculated seedlings under well-watered conditions and after drought-induced stomatal closure. Ψ_l was determined using a pressure chamber (PMS Instrument Company; Albany, OR, USA). For this purpose, the whole shoot or small lateral shoots were wrapped in Saran wrap, excised, and immediately used to determine their Ψ_l. Due to the small size of the seedlings and the partially destructive nature of the measurement, Ψ_l for well-watered seedlings were assessed in different seedlings than those exposed to drought.

Before initiating the drought experiment, five non-inoculated and five inoculated seedlings were harvested and used to analyze the extent of AMF colonization. Similarly, after completing the drought period, I collected roots from each plant and used them to quantify fungal colonization. Colonization was quantified in roots smaller than 2 mm that were cut in roughly 2 cm segments and cleared in 5% KOH for 5 min at 121 °C. Subsequently, the roots were rinsed in water and incubated overnight in a solution containing 0.4 µg ml^{-1} wheat germ agglutinin-horseradish peroxidase (WGA-HRP) and 1% bovine serum albumin in PBS (Kobae and Ohtomo, 2016). Samples were then rinsed in PBS and incubated for 3 to 5 min in a VIP HRP substrate (Vector Laboratories, California, USA). Subsequently, the roots were rinsed in water, mounted on 50% glycerol, and observed through an Olympus BX60 microscope at 200 or 400 magnification. The observed fungi were grouped into two categories: AMF and septate. For AMF, I quantified the presence of hyphae (diameter of at least 5 µm), arbuscules, and vesicles. Within the septate fungi, I recorded the presence of hyaline and melanized hyphae and microsclerotia. A few thin non-septate fungi, having a hyphal diameter of about 1 µm, were also observed but were not recorded in this experiment. The different fungal structures and total colonization by the two groups of fungi were quantified by the intersection method (McGonigle et al., 1990) with about 150 intersections per sample.

Data analyses

The effectiveness of inoculating with AMF spores on colonization was determined by comparing the colonization boxplots of the non-inoculated and inoculated seedlings. A lack of overlap between the boxes was taken as evidence of statistical differences between treatments. I used this approach rather than a t-test or Wilcoxon

rank-sum test because the prevalence of zeros in the non-inoculated seedlings resulted in non-normal and heteroscedastic data.

In plants exposed to drought, the relationship between time and *A*, *Tr*, g_s, and ΦPSII was not linear but followed a negative sigmoidal curve. This response was modeled using the following equation:

$$\text{Response variable} = I \times (1/(1 + e^{(S \times (\text{time} - t1/2))})) \qquad (1)$$

Where the response variable is the particular parameter measured (i.e., *A*), *I* is the parameter's initial value, *S* is a factor that accounts for the shape of the curve, and t1/2 is the time when the parameter reached half its initial value. The data for each plant was fitted to equation *1* to obtain the values of *I*, *S*, and t1/2. For this purpose, I used the Non-Linear Least-Square Minimization and Curve-Fitting library (LMFIT) in Python. Figure 2.1 shows an outcome of the curve fitting process. The values of *I* and t1/2 estimated for each parameter were compared between inoculation treatments by t-test or Wilcoxon test.

The instantaneous water use efficiency (WUE) is the ratio between *A* and *Tr*. WUE did not show a clear pattern of increase or decrease during the experiment (data not shown). As an estimate of the WUE of each plant, I used the median of the daily values. Differences in the median values between treatments were analyzed by t-test.

Values of Ψ_l between non-inoculated and inoculated seedlings under well-water conditions and after stomatal closure were compared by Welch's ANOVA. Except for the curve fitting process, all statistical analyzes were implemented using basic functions in R 4.0 (R-Development-Core-Team 2020).

Experiment 2: Inoculation with soil and roots from trap cultures

In this experiment, the growing conditions and the procedures used to measure fungal colonization and physiological responses to drought were the same as those described for experiment *1*. Also, as before, plants were grown from seeds. However, I tested a different approach to inoculate the seedlings. Instead of using extracted spores, the seedlings were inoculated with the soil and roots from the trap cultures. While inoculation with isolated spores proved effective in causing AMF colonization, the method was very laborious and rather impractical for many pots. For the non-inoculated treatment, the 150 ml cone-tainers had a 3:2 sand: silty loam soil mix that had been autoclaved twice for 1 h and supplemented with an AMF-free microbial wash. This wash was obtained by blending soil and roots from the trap cultures with water in a 1:15 (w/v) ratio and passing the suspension through a 35 μm nylon mesh. I used the filtrate to drench the sterilized sand-soil mix (Mummey et al., 2009). For the inoculated treatment, the cone-tainers were instead filled with the soil/sand and roots from the trap cultures.

After a year of growth, I selected 24 plants similar in height and projected leaf area, 12 non-inoculated and 12 inoculated ones. After withholding watering, I made measurements of A, Tr, g_s, and ΦPSII daily from 10 am to 3 pm. Following stomatal closure, five non-inoculated and inoculated plants were randomly selected to assess fungal colonization. Comparisons between treatments for fungal colonization and physiological parameters were made as described under experiment *1*.

Experiment 3: Inoculation with soil and root from the trap cultures in large pots

Plant material and treatments

The plant material used in this experiment was Wyoming big sagebrush seedlings provided by the Bureau of Land Management. Seeds to grow these seedlings had been collected within the Morley Nelson Snake River Birds of Prey National Conservation Area and sown in 150 ml cone-tainers filled with a 3:1 peat moss to vermiculite mix. Subsequently, growing conditions were as those described by Fleege (2010). I received the seedlings when they were about twelve months old. The seedlings had firm root balls at this stage, and I transplanted them to 1 L cone-tainers filled with either soil and roots from the trap cultures (inoculated seedlings) or a 3:2 sand-soil mix (non-inoculated seedlings). The latter had been autoclaved twice for 1 h and subsequently supplemented with an AMF-free microbial wash obtained as described earlier. After transplanting to the 1 L cone-tainers, plants were grown in a greenhouse for an additional six months under a 15 h photoperiod and day/night conditions of 23/18 ± 3 °C. Plants were fertilized monthly with a 1/8 strength Hoagland's solution.

Six months after transplanting to the 1L cone-tainers, the *A. tridentata* seedlings were used to conduct a completely randomized factorial combination experiment consisting of two watering treatments (well-watered and drought-stressed) and two inoculation treatments (non-inoculated and inoculated plants). The well-watered plants had four plants per inoculation treatment, and the drought-stressed plants had six plants per inoculation treatment. The plants were similar in height, about 20 to 25 cm, and with comparable projected leaf area.

The well-watered plants received water to field capacity every other day, while for the drought treatment, drought was imposed by stopping watering. In both the well-watered and drought treatments, a 7.62 cm foam collar covered the top of the cone-tainers to minimize evaporative water loss from the soil. The collars reduced the rate of water loss from the cone-tainers resulting in more gradual drought development. In the drought treatment, water was withheld until stomatal conductance was minimal for at least one week.

Data collection

Every other day I measured *A*, *Tr*, g_s, and ΦPSII as described under experiment *1*, except that the light intensity was 1500 μmol m^{-2} s^{-1}. For these plants, preliminary light curves indicated that CO_2 assimilation was saturated at this light intensity. Differences in CO_2 assimilation and chlorophyll fluorescence between plants with different degrees of drought tolerance or treatments that affect drought tolerance tend to increase with increasing light intensities (de Sousa et al., 2017). Thus, I reasoned that measurements at saturating light intensity might facilitate the detection of differences between non-inoculated and inoculated plants.

In addition to the gas exchange measurements, I also weighted the pots every other day. Particularly for the drought treatment, I used these values to determine the ratio of the pot weight to the weight at pot capacity (rPC). The latter represented the pot weight after watering the pot to saturation and letting it drain for one day (Turner, 2019). At the end of the experiment, I also sampled aliquots of the sand/soil mix in each cone-tainer. The samples were used to determine the water-holding capacity of the mixture and its moisture release curve, which depicts the relationship between the moisture content of

the mix and its water potential. The water holding capacity was calculated as (weight at field capacity – dry weight)/ dry weight. The weight at field capacity represented the weight after saturating the sand/soil mix with water and letting it drain for two days, while the dry weight was the weight after drying in an oven at 120° C until no change in weight occurred over 24h. For estimating the moisture release curve, the sand/soil mix at field capacity was transferred to a sample cup and placed in a water potential meter (WP4T Dewpoint WaterPotential Meter, Metergroup) to determine its water potential. The sand/soil mix in the cup was gradually dried, weighted, and its water potential was again determined. I repeated this procedure until the water potential readings reached values below -50 MPa. I then plotted the relationship between soil moisture content ((soil weight - dry soil weight)/dry soil weight) and water potential to determine whether AMF-inoculation affected this relationship.

Fungal colonization was analyzed before and after completing the drought period. After the drought, I harvested the roots from both the well-watered and drought treatments. Colonization was analyzed as described earlier, except that I quantified three groups of fungi: septate, AMF, and thin-non-septate fungi. I considered thin non-septate fungi as those having a hyphal diameter of about 1 µm, and only the hyphae were quantified in this group.

Data analyses

The effect of inoculation and watering treatments on fungal colonization was analyzed by two-way ANOVA or Welch's ANOVA. The latter analysis allowed to model unequal variance between treatments in response variables that showed heteroscedasticity. Following ANOVA or Welch's ANOVA, significant differences

between treatments were determined by the Tukey Honest Significant differences and the Games-Howell test, respectively. Some data were not normally distributed but homoscedastic. In this case, differences between treatments were evaluated using the Wilcoxon rank-sum test for two-level analyses and the Kruskal Wallis test for more than two levels. Nonparametric pairwise multiple comparisons were made using the Dunn's test. Comparisons are reported as means when using parametric tests and as medians when using nonparametric ones.

Physiological responses to the treatments were first analyzed using linear or non-linear regression. I used linear regression to assess whether the physiological parameters measured in well-watered plants significantly changed during the experiment and determine whether they differed between the two inoculation treatments. For this purpose, the intercepts and slopes estimated for each parameter were compared between inoculation treatments by t-test.

The plant physiological responses to drought were analyzed using two approaches. One approach was the same as in the previous experiments. I used equation *1* to estimate *I* (initial value) and t1/2 for each parameter. These values were then compared between inoculation treatments by t-test. The second approach involved analyzing the change in A, Tr, g_s, and ΦPSII in relation to the ratio of the pot weight to the weight at pot capacity (rPC). The equation used to model this relationship was similar to equation *1*.

$$\text{Response variable} = I \times (1/(1 + e^{(S \times ((1 - \text{rPC}) - (1 - \text{rPC1/2}))}))) \qquad (2)$$

where *I* is the parameter's initial value at pot capacity, *S* is a factor that accounts for the shape of the curve, rPC is the ratio of the pot weight to the weight at pot capacity, and $rPC_{1/2}$ is the value of rPC when the parameter reaches half its initial value.

I substrated rPC and $rPC_{1/2}$ from 1 in the equation to model a decrease in the response variable as the abscissa value increases. This approach allowed me to obtain a good fit of the data using an equation similar to equation *1* (e.g., Figure 1.2). The values of $rPC_{1/2}$ estimated after curve fitting were then compared between non-inoculated and inoculated plants using the Wilcoxon test because the data were homoscedastic but did not follow a normal distribution.

To correlate changes in rPC with plant water status, I also made water potential measurements (Ψ_w) with a pressure chamber (PMS Instrument Company; Albany, OR, USA). Between noon and 2 pm, small lateral shoots were wrapped in Saran wrap, excised, and immediately used to determine their water potential. Due to the destructive nature of these measurements, only 3 or 4 measurements were made per plant throughout the experiment.

The WUE of each plant was estimated based on the median value throughout the experiment. Differences in these values between watering and inoculation treatments were evaluated by two-way ANOVA.

Meta-analysis

In addition to analyzing the effect of AMF inoculation on g_s, *A*, and *Tr* in individual experiments, I conducted meta-analyses to assess average effects across experiments. The meta-analysis was performed using a random model because differences in the inoculum and pot size could have influenced the impact of inoculation

on the dependent variables (Hunter and Schmidt, 2000). Within each experiment, the effect size of inoculation on the $t_{1/2}$ of g_s, A, and Tr was estimated as the natural log of the response ratio (RR), which was the mean of the inoculated seedlings divided by the mean of the non-inoculated ones (Hedges et al., 1999). The variance within each study was the standard error of ln RR. The effect size and its variance were calculated using the function escalc in the metaphor package in R (R-Development-Core-Team 2013, Viechtbauer, 2010).

Results

Experiment 1: Inoculation with extracted spores

Fungal Colonization

Before withholding watering, inoculated plants had higher levels of AMF colonization than non-inoculated ones (Fig. 2.3 A). The mean value for total AMF colonization was 6.1 and 58.8% for non-inoculated and inoculated plants, respectively ($p < 0.0001$). There was also a difference in arbuscular colonization, which was 5.1% in non-inoculated plants and 37.4% in inoculated ones ($p < .001$). Vesicles were less common, with an average of 11.0% in inoculated plants. I also measured colonization of dark septate endophytes. Colonization by these fungi was low and not significantly different ($p = .3167$) between non-inoculated and inoculated plants, 5.7 and 6.1%, respectively.

Following the drought period, I measured colonization in each plant used in the experiment. Similar to before the drought, inoculated plants had higher levels of AMF than non-inoculated ones (Fig. 2.3 B). Total AMF colonization post-drought in inoculated plants was 48.1% and 2.6% in non-inoculated plants ($p < .0001$). Albeit higher on

average, total and arbuscular colonization before the drought was not significantly different from that post-drought ($p > .05$). Also, withholding watering did not cause changes in DSE colonization ($p = .7$).

Sensitivity of physiological parameters to inoculation

Before and after drought, Ψ_w was measured in both inoculated and non-inoculated plants. Before the drought, there was no significant difference between well-watered inoculated plants, with mean values of -1.0 and -1.1 MPa, respectively ($p > .05$). After stomatal closure, Ψ_l was about 2.5 MPa lower than before the drought (Fig 2.4). The mean Ψ_l was -3.4 MPa for non-inoculated plants and -3.9 MPa for inoculated ones; these differences were not significant ($p > .05$).

The initial values of *A*, *Tr*, and g_s estimated using equation *1* indicate the rates of these parameters when the plants were still well watered. There was no difference in these initial values between non-inoculated and inoculated plants (Table 2.1). The operating efficiency of photosystem II (ΦPSII) did not decline to half of its initial value during the experiment. Consequently, the initial values were not estimated using equation *1* but as the average of the first three measurements. No difference in ΦPSII between non-inoculated and inoculated plants was detected (Table 2.1). As the drought progressed, values of *A*, *Tr,* and g_s markedly declined, and this decline occurred sooner in non-inoculated plants. The $t_{1/2}$ estimated using equation *1* indicates the number of days since withholding watering that caused *A*, *Tr*, and g_s to fall to half of their initial value. The average $t_{1/2}$ for stomatal conductance (g_s) was 6.3 d in non-inoculated plants and 10.1 d for inoculated plants ($p = 0.002$) (Fig. 2.5). Similarly, the average $t_{1/2}$ for CO_2 assimilation (*A*) in non-inoculated plants was 5.5 d and 11.4 d for inoculated ones ($p =$

0.004). The average $t_{1/2}$ for *Tr* was 5.39 d for non-inoculated plants and 8.8 d for inoculated plants ($p = 0.004$).

Water use efficiency was similar between non-inoculated and inoculated seedlings. The average of the medians observed for each plant during the experiment was 2.02 and 2.10 µmol CO_2 per mmol H_2O for non-inoculated and inoculated seedlings, respectively ($p = 0.72$).

Experiment 2: Inoculation with soil and roots from trap cultures

Fungal Colonization

After the drought treatment, inoculated plants had higher levels of AMF colonization than non-inoculated ones (Fig. 2.6 A). The mean value for total AMF colonization was 0.5 and 29.1% for non-inoculated and inoculated plants, respectively. There was also a difference in arbuscular colonization, which was 0% in non-inoculated plants and 5.5% in inoculated ones. Storage structures, consisting of vesicles and intraradical spores, were more common than arbuscules. In inoculated plants, storage structures were, on average, found in 15.8% of the intersections. After the drought, I also measured the presence of dark septate endophytes (DSE) and found no difference between non-inoculated and inoculated plants. Total DSE colonization was 15.74% in non-inoculated plants and 17.8% in those inoculated (Fig. 2.6B, $p = 0.99$). Microsclerotia were also common; they were present in 12.9 and 12.5 % of the intersections in non-inoculated and inoculated plants, respectively ($p = 0.7$).

Sensitivity of physiological parameters to inoculation

As mentioned earlier, the initial values of *A*, *Tr*, g_s, and *ΦPSII* estimated using equation *1* indicate the rates of these parameters when the plants were still well watered.

There were no differences in these initial values between non-inoculated and inoculated plants (Table 2.2). After stomatal closure, measurements in experiment *2* continue for a more extended period than in experiment *1* to better detect the decline in *ΦPSII*. As the drought progressed, values of *A*, *Tr*, g_s, and *ΦPSII* markedly declined, but this decline did not differ between inoculated and non-inoculated plants ($p > 0.05$). There were, however, differences in $t_{1/2}$ between parameters. The $t_{1/2}$ for *ΦPSII* was significantly longer than that of the other parameter ($p < 0.05$) (Fig. 2.7). As in experiment 1, inoculation did not affect WUE. Non-inoculated seedlings had an average WUE of 2.05 µmol CO_2 per mmol H_2O, and those inoculated 1.81 µmol CO_2 per mmol H_2O ($p = 0.35$).

Experiment 3: Inoculation with soil and roots from trap cultures in large pots

Fungal Colonization

Before withholding watering, plants inoculated with soil and roots from the trap cultures had higher levels of AMF colonization than non-inoculated ones (Fig. 2.8 A). The median value for total_AMF colonization was 0 and 20.6% for non-inoculated and inoculated plants, respectively ($p = 0.008$). There was also a difference in arbuscular colonization, which was negligible in non-inoculated plants and 7.7% in inoculated ones ($p = 0.03$). Vesicles were less common, with an average of 2.0% in inoculated plants. Apart from enhancing AMF colonization, the trap-culture inoculum increased colonization by septate fungi (Fig. 2.8B). The median value for total DSE colonization in non-inoculated and inoculated plants was 0.8 and 8.2%, respectively ($p = 0.046$), while no significant difference was detected for microsclerotia ($p = 0.09$).

Following the drought period, I measured colonization in each plant used in the experiment. Total AMF colonization in inoculated well-watered plants was nearly

identical to those inoculated and water-stressed plants, 29.6 and 30.5%, respectively (p = 0.90, Fig. 2.9A). However, drought reduced arbuscular colonization (Fig. 2.9A), which in inoculated plants was 19.7% and 6.6% in well-watered and drought-stressed plants, respectively (p = 0.02). No differences were observed in the percentage of vesicles, which were present in less than 2.5% of the intersections (data not shown). As before the drought treatment, AMF colonization in non-inoculated plants was minimal (Fig. 2.9A).

In addition to AMF, the roots have septate and thin non-septate fungi (Fig. 2.9B). Septate fungi, in particular, were present at levels comparable to those observed for AMF in inoculated plants (Fig. 2.9A). However, no difference in either septate or thin-non-septate fungal colonization was apparent between inoculation or watering treatments. Septate fungi showed a large variation in total colonization and the abundance of microsclerotia within each treatment. Microsclerotia ranged from 0 to 18% between plants but differences between treatments were not significant (data not shown).

Sensitivity of physiological parameters to inoculation

The effect of inoculation on A, Tr, g_s, and $\Phi PSII$ was investigated in well-watered and drought-stressed plants. In plants kept well-watered, I observed daily variations in A, Tr, g_s, and $\Phi PSII$ within a plant (e.g., Fig. 2.10). However, this variation did not show a trend; the slope of the linear relationship between time and the various parameters measured was not significantly different than zero. Given these results, I estimated the average rates of A, Tr_{leaf}, g_s, and $\Phi PSII$ for each plant during the 60 days of the experiment and used them to compare the effect of inoculation on these parameters. Table 2.3 summarizes the results of these comparisons; under well-watered conditions, inoculation did not affect any of the physiological parameters measured (Table 2.3).

For plants exposed to drought stress, the initial values of *A*, *Tr*, g_s, and *ΦPSII* estimated using equation *1* indicate the rates of these parameters when the plants were still well-watered. I did not detect differences in these initial values between non-inoculated and inoculated plants, supporting the notion that inoculation did not affect *A*, *Tr*, g_s, or *ΦPSII* under well-watered conditions (Table 2.4). As the rate of these parameters declined with drought, there was considerable variability between plants in the decline rates. However, this variability was not associated with the inoculation treatment. For a particular parameter, the $t_{1/2}$ value of non-inoculated plants was not significantly different from that of inoculated ones (Fig. 2.11).

Part of the variability in $t_{1/2}$ values was likely attributed to the different transpiration rates between plants. To account for this variability, I also analyzed physiological responses as a function of the pot weight to the pot capacity weight (rPC). In particular, I used equation *2* to estimate the ratio of pot weight to weight at field capacity at which each of the physiological parameters measured declined to half its initial value ($rPC_{1/2}$). Due to outliers, significant variation in rPC1/2 occurred within treatment (Fig. 2.12). Nevertheless, for the four parameters measured, $rPC_{1/2}$ in inoculated plants was significantly lower than that of non-inoculated ones ($p < 0.05$ based on Wilconox test). Thus, in inoculated plants, the decline in g_s, CO_2 assimilation, *Tr*, and *ΦPSII* occurred at a lower soil water content than in non-inoculated ones. A similar trend was observed for plant Ψ_w. The initial decrease in rPC did not affect plant Ψ_w, which was comparable in non-mycorrhizal and mycorrhizal plants. However, as rPC continued declining, the reduction in plant Ψ_w tended to occur at lower rPC in mycorrhizal plants than in non-mycorrhizal ones (Fig. 2.13).

The difference in $rPC_{1/2}$ between the non-inoculated and inoculated treatment could have been caused by an effect of AMF on the water holding capacity of the potting mix or its moisture release curve. The water holding capacity of the mix was nearly identical between treatments 39.5% and 39.8% from non-inoculated and inoculated cone-tainers, respectively ($p = 0.93$). For the moisture release curve, some samples showed a decrease in soil water potential at higher soil moisture content than others. However, these differences occurred within each treatment, without any apparent dissimilarity between non-inoculated and inoculated pots.

For WUE, the two-way ANOVA indicated no significant interaction between watering and inoculation treatment on this parameter. Watering affected WUE; well-watered and drought-stressed seedlings had an average of 1.95 and 2.31 µmol CO_2 per mmol H_2O, respectively ($p = 0.03$). In contrast, the effect of inoculation on WUE was not significant; the average WUE in non-inoculated seedlings was 2.04 µmol CO_2 per mmol H_2O, and that in inoculated ones 2.30 µmol CO_2 per mmol H_2O ($p = 0.1$).

Meta-analysis

Across the three experiments, AMF inoculation delayed stomatal closure during drought by an average of 38% (Fig. 2.15 A, $p = 0.014$). However, heterogeneity between experiments was high, with a T^2 of 0.024 and a confidence interval from 7 to 79%. Photosynthesis yielded similar results; the average delay in decreasing CO_2 assimilation was 59% (Fig. 2.15 B, $p = 0.008$). The effect size variability between experiments was high, with a T^2 of 0.033 and a confidence interval between 13 and 123%. In contrast to stomatal conductance and CO_2 assimilation, the effect of inoculation on the average transpiration rate was not significant (Fig. 2.15 C, $p = 0.28$).

Discussion

Physiological responses to AMF colonization

This study's two general goals were to analyze the effect of AMF on WUE and the drought-induced decline in stomatal conductance and photosynthesis. Water-stressed plants had higher WUE than well-watered plants, but AMF colonization did not alter WUE. In contrast, AMF colonization delayed the drought-induced decline in g_s, CO_2 assimilation, and ΦPSII, or this decline occurred at a lower soil water content. A secondary goal of this experiment was to analyze the efficacy of different inoculation methods and I found that inoculation with isolated spores was more efficient, but more laborious, than pot culture inoculation.

Even though the meta-analysis indicates that AMF contributed to maintaining the stomata open and photosynthesis during drought, differences in the extent of this effect were apparent between experiments. These differences may be due to variations in AMF colonization. In the three experiments, inoculation increased AMF colonization compared to non-inoculated seedlings. However, the largest difference between inoculation treatments (~52% difference in total AMF colonization) was observed in experiment *1*. This experiment was also the one where the effect of AMF on delaying the drought-induced decline in g_s was noticeable. In experiment *1*, the inoculum was surface-sterilized spores obtained from trap cultures. In contrast, I used all the material from the trap cultures for the two other experiments, resulting in a difference between inoculated and non-inoculated seedlings of about 30%. The reduced effect of AMF on g_s with less colonization is consistent with the result reported by Augé et al. (2014); their meta-

analysis of stomatal responses to AMF revealed that the AMF effect on g_s was about ten times higher in heavily than lightly colonized plants.

Another potential factor contributing to differences in the AMF impact on g_s and photosynthesis between experiments was the abundance of DSE fungi. DSE colonization was lower in experiment *1* (~ 6%) than in the other two experiments (between 15 to 30%). While many studies have reported the co-occurrence of AMF and DSE, the extent to which the presence of DSE alters the plant-AMF symbiosis is not clear (Chaudhry et al., 2009; Postma et al., 2007; Priyadharsini et al., 2012). A recent study reported that in *A. tridentata* seedlings, the DSE *Darksidea* sp. reduced arbuscular colonization by *R. irregularis* (Carpenter et al., 2021). Such an outcome would tend to diminish the beneficial effects of AMF (Smith et al., 2011). However, in my experiments, the ratio of arbuscular to total colonization under well-watered conditions was not significantly different between experiments or plants with varying levels of DSE colonization (data not shown), suggesting that DSE did not reduce arbuscular colonization.

The drought-induced decline in g_s and photosynthesis were measured against time in the three experiments. Time, however, was probably not the best indicator of the drought stress the plants were experiencing. Although the seedlings were similar in size, they showed marked differences in their transpiration rates independent of treatment. Such differences likely resulted in different rates of soil water depletion and water potential decrease, causing considerable variability in g_s and other parameters when plotted against time. In experiment *3*, I also measured the daily decline in soil water content to account for some of this variability. Interestingly, I observed no difference in the time it took for gas exchange and chlorophyll fluorescence parameters to reach half

their initial values. However, when I considered the ratio of percent pot weight to weight at pot capacity (rPC) as the independent variable, the decline in gas exchange parameter and *ΦPSII* occurred at lower rPCs in mycorrhizal than non-mycorrhizal plants. Thus, in inoculated plants, the decrease in g_s, CO_2 assimilation, *Tr*, and *ΦPSII* happened at a lower soil water content than in non-inoculated ones. These results suggest that using a similar approach in experiments *1* and *2* may have revealed wider differences between treatments than those observed using time as the independent variable.

Observations of decreases in transpiration and photosynthesis at lower soil water content in mycorrhizal than non-mycorrhizal plants are not uncommon (Auge, 2001; Bitterlich et al., 2018b). Various mechanisms can be responsible for such an effect. AMF can increase osmotic adjustment in plants exposed to drought (Augé et al., 1986; Wu and Xia, 2006). Through opposite effects on plant Ψw and turgor, osmotic adjustment can prolong water uptake and photosynthesis as the soil dries out (Turner, 2018). Although I did not measure the osmotic potential of the plants, a higher osmotic adjustment in mycorrhizal than non-mycorrhizal plants does not appear to explain my results. For example, in experiment *1*, inoculation delayed stomatal closure. If osmotic adjustment had caused this delay, it would have resulted in stomatal closure at lower plant Ψw. However, the plant Ψw at which stomata closed were similar between treatments (Fig. 2.4).

Another means by which AMF can affect plant water status is via changes in soil and plant hydraulic conductivity (Barzana et al., 2012; Bitterlich et al., 2018a). Bitterlick et al. (2018c) showed that under increasing drought, mycorrhizal tomato plants reduced transpiration at lower potting substrate Ψw than non-mycorrhizal ones. Interestingly, the

plant Ψw at which transpiration decreased was the same for AMF and non-AMF plants (Bitterlich et al., 2018c). These results were attributed to the extraradical hyphae, which presumably enhanced liquid continuity through air gaps, thereby increasing the potting substrate's hydraulic conductivity. The notion that AMF increase hydraulic conductivity seems consistent with results in experiment *3*. Compared to non-mycorrhizal plants, AMF colonization decreased the rPC1/2 of transpiration and the rPC at which the leaf Ψw declined (Fig. 2.13 and 2.14). Since rPC is related to soil Ψw, these results suggest that AMF plants maintained transpiration and leaf Ψw with a narrower gradient in the soil to leaf Ψw. Based on fundamental principles of water transport, such a situation would require an increase in soil or plant hydraulic conductivity (Barzana et al., 2012; Boanares et al., 2020).

A consistent result in the three experiments was that under well-watered conditions, AMF did not affect the gas exchange parameters measured nor ΦPSII. Enhancements in photosynthesis by AMF are often associated with improved nutrient uptake, particularly P (Parádi et al., 2003; Suriyagoda et al., 2014). The presence of arbuscles suggests that the plants took P via mycorrhizae because arbuscules tend to collapse without P transport (Javot et al., 2007). However, a remaining question is whether AMF colonization increased the uptake of P and other nutrients. In response to AMF colonization, some plants downregulate direct P uptake through the root epidermis and root hairs, resulting in mycorrhizal plants having a P concentration similar to non-mycorrhizal ones (Smith et al., 2003). It is unknown the extent that *A. tridentata* behaves in this manner, but such behavior would have resulted in comparable mineral nutrient concentrations and, therefore, a lack of an effect on metabolic processes such as

photosynthesis. Alternatively, mycorrhizal plants may have taken more P than non-mycorrhizal ones. However, in the latter, mobilization from older leaves could have maintained nutrient levels, resulting in a lack of differences in their concentrations in the relatively younger leaves where I conducted the measurements. Independent of the reason involved, the lack of an effect of AMF on the rate of photosynthesis seems to explain the similarities in WUE between mycorrhizal and non-mycorrhizal plants. There have been conflicting reports on the impact of AMF on WUE, but when positive outcomes were observed, they were related to higher net photosynthetic rates in mycorrhizal plants (Birhane et al., 2012; Delavaux et al., 2017; Querejeta et al., 2003; Ruiz-Lozano and Aroca, 2010). Such an AMF-induced increase in photosynthesis did not occur in my experiments.

Another secondary goal of the experiments was to analyze the effect of drought and, in particular, drought-induced stomatal closure on AMF colonization. The levels of total AMF colonization in plants with high g_s were similar to those of drought-stressed plants where g_s was negligible. On the other hand, drought decreased arbuscular colonization, although this effect was only significant in experiment *3* (Fig. 2.9 A). In this experiment, the pots were larger, and the drought developed more gradually, which may have provided sufficient time to induce arbuscular collapse. This collapse often occurs under water stress, perhaps triggered by a reduction in the exchange of nutrients (Gehring et al., 2017; Kiers et al., 2011; Sun et al., 2017). The observed tendency to decrease arbuscules following stomatal closure likely reduced carbon losses, which may be critical to maintaining basic metabolism when seedlings are experiencing severe drought (Chuste et al., 2020; McDowell, 2011).

Ecological and practical considerations

This study indicates that AMF colonization delayed the drought-induced decline in g_s, CO_2 assimilation, and ΦPSII, or this decline occurred at a lower soil water content. While the results demonstrate that AMF can postpone typical symptoms of drought in *A. tridentata* seedlings, questions remain regarding the practical implications or ecological significance of the findings. The effect of AMF on delaying drought symptoms was more noticeable in plants inoculated with surface-sterilized spores. This method resulted in high AMF colonization and low levels of other fungal endophytes. Presently, the laborious nature of this procedure makes it impractical for the thousands of plants typically used in restoration. However, there are ongoing efforts to mass-produce AMF spores in aeroponics and in vitro (Dalpé and Monreal, 2004; Singh et al., 2014). These efforts may open the possibility of generating clean and locally adapted inoculum economically in the future (Rillig et al., 2020).

Currently, inoculation with trap culture either in the nursery or at the time of transplanting is a more feasible option. Although colonization was lower with this method, the levels achieved were sufficient to allow mycorrhizal plants to maintain photosynthesis at lower soil water content than non-mycorrhizal ones. Mechanistically, this effect appears to be mediated by an AMF increase in soil or plant hydraulic conductivity. The significance of this effect on increasing *A. tridentata* survival during summer drought remains to be investigated. Compared to non-mycorrhizal plants, the additional water extracted from the pots by mycorrhizal plants before stomatal closure was relatively small, between 40 to 50 ml. This estimate comes from the weight at pot capacity (~1300 g) times the difference in rPC1/2 between non-mycorrhizal and

mycorrhizal plants (~3 to 4%). Forty to 50 ml is a trivial amount to cause changes in the physiology of the plants under field conditions. However, water uptake can occur over a much more extended soil volume in the field. Consequently, a similar phenomenon of soil water depletion in natural settings is likely to result in the extraction of larger volumes of water. The extent of this effect needs to be determined. Still, if it helps the plants prolong photosynthesis without decreasing plant Ψw, it may help maintain water homeostasis. The plant could use the additional photosynthates to grow fine roots and hyphae toward moist soil patches, facilitating water uptake and preventing drought-induced damage and death.

In summary, the results of this study indicate that AMF colonization affected the seedlings' responses to drought. Postponing stomatal closure with increased drought severity may be considered risky behavior, particularly if associated with decreased plant Ψw (Sade et al., 2012b). However, the effect of AMF on maintaining g_s and photosynthesis did not appear to be related to a lower Ψw threshold for stomatal closure but rather to an increased ability to extract water from the potting substrate. This manner of prolonging photosynthesis and potentially root growth would be valuable in many sagebrush habitats where water progressively dwindles during the summer, but some water remains deeper into the soil. Overall, the results suggest that AMF increases the drought tolerance of *A. tridentata* seedlings. Yet, much work is still needed to ascertain the significance of the observed effects on increasing survival under field conditions.

Figures and Tables

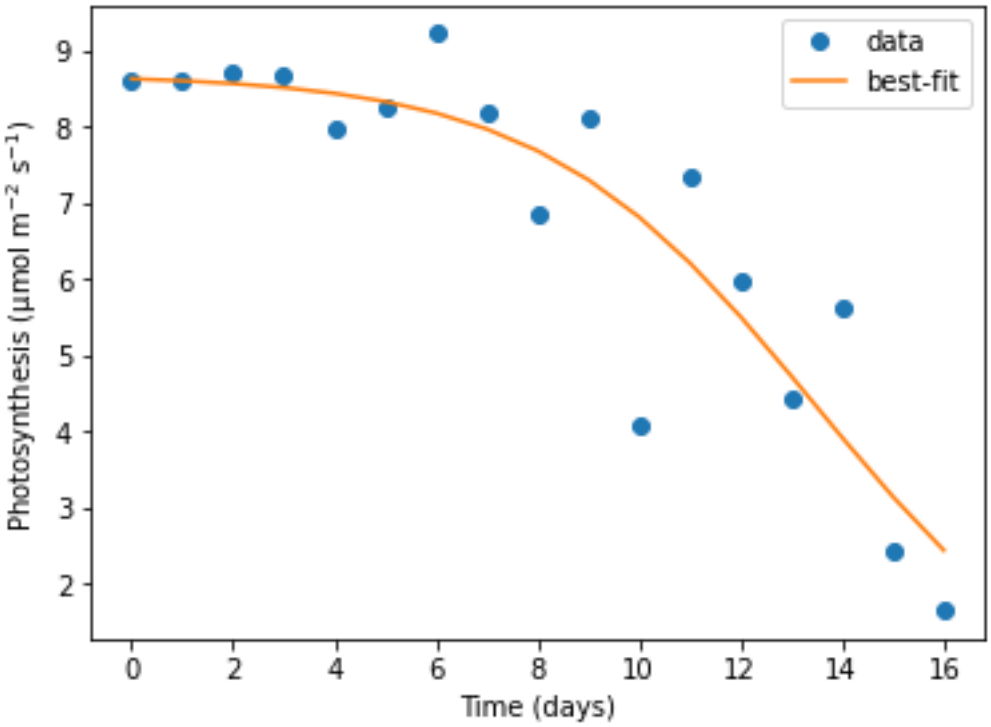

Figure 2.1. Example of the time course of changes in CO_2 assimilation during the progression of drought. For this plant, the value of *I*, *S*, and t1/2 was 8.69 µmol CO_2 m^{-2} s^{-1}, 0.37, and 13.4 days, respectively.

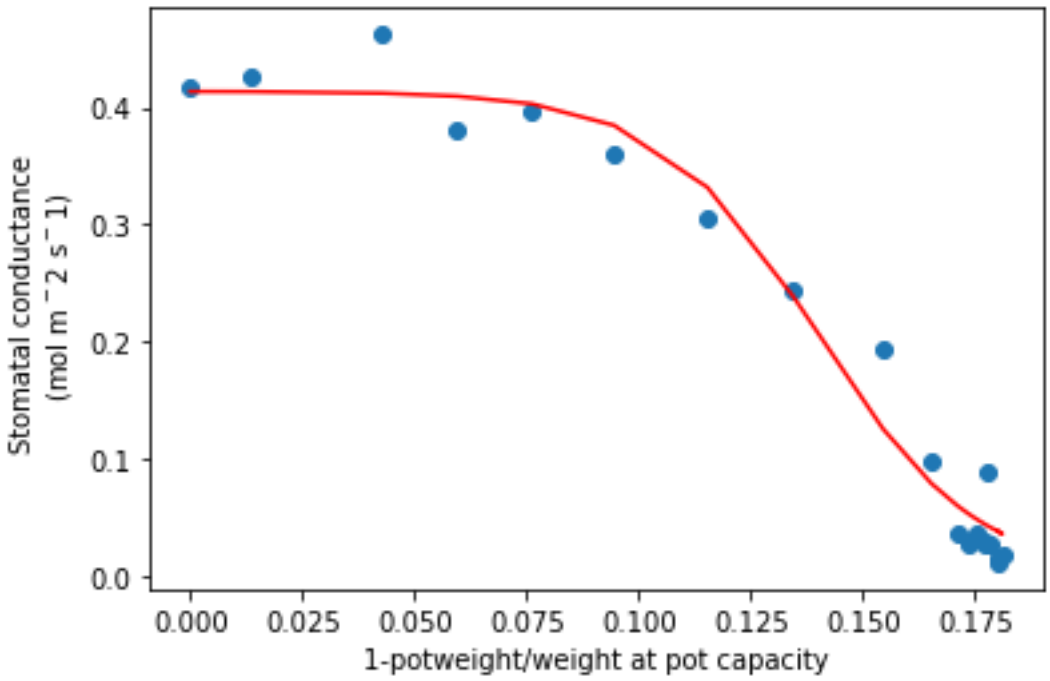

Figure 2.2. Example of changes in stomatal conductance as the soil dries out. For this plant, the value of *I*, *S*, and $rPC_{1/2}$ was 0.41 mol H_2O m^{-2} s^{-1}, 56.9, and 0.86, respectively.

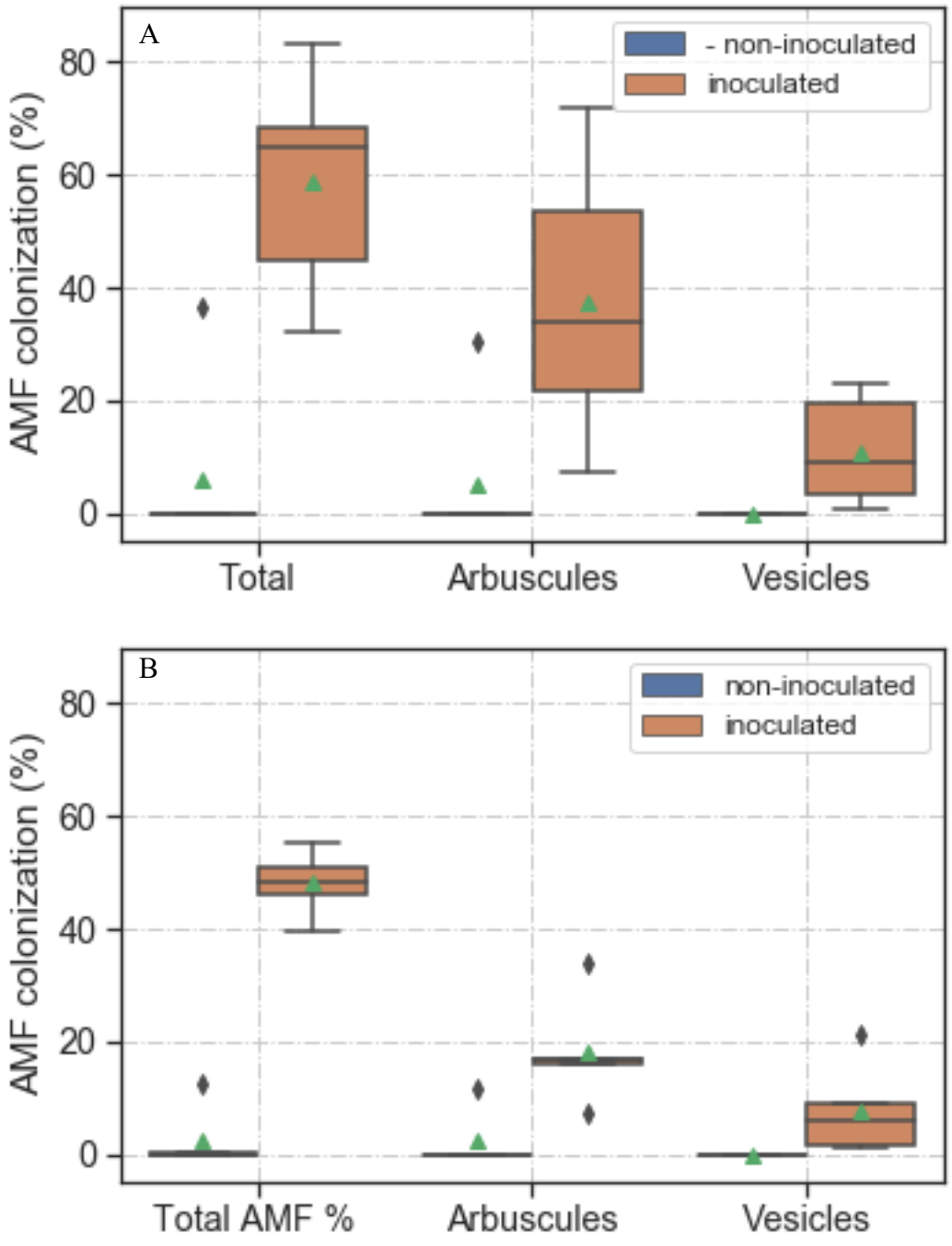

Figure 2.3. Colonization of *Artemisia* tridentata roots by arbuscular mycorrhizal fungi before (A) and after the imposition of drought (B). Boxplot of 5 replications per treatment. Triangles and diamonds indicate the mean and outliers within each treatment, respectively.

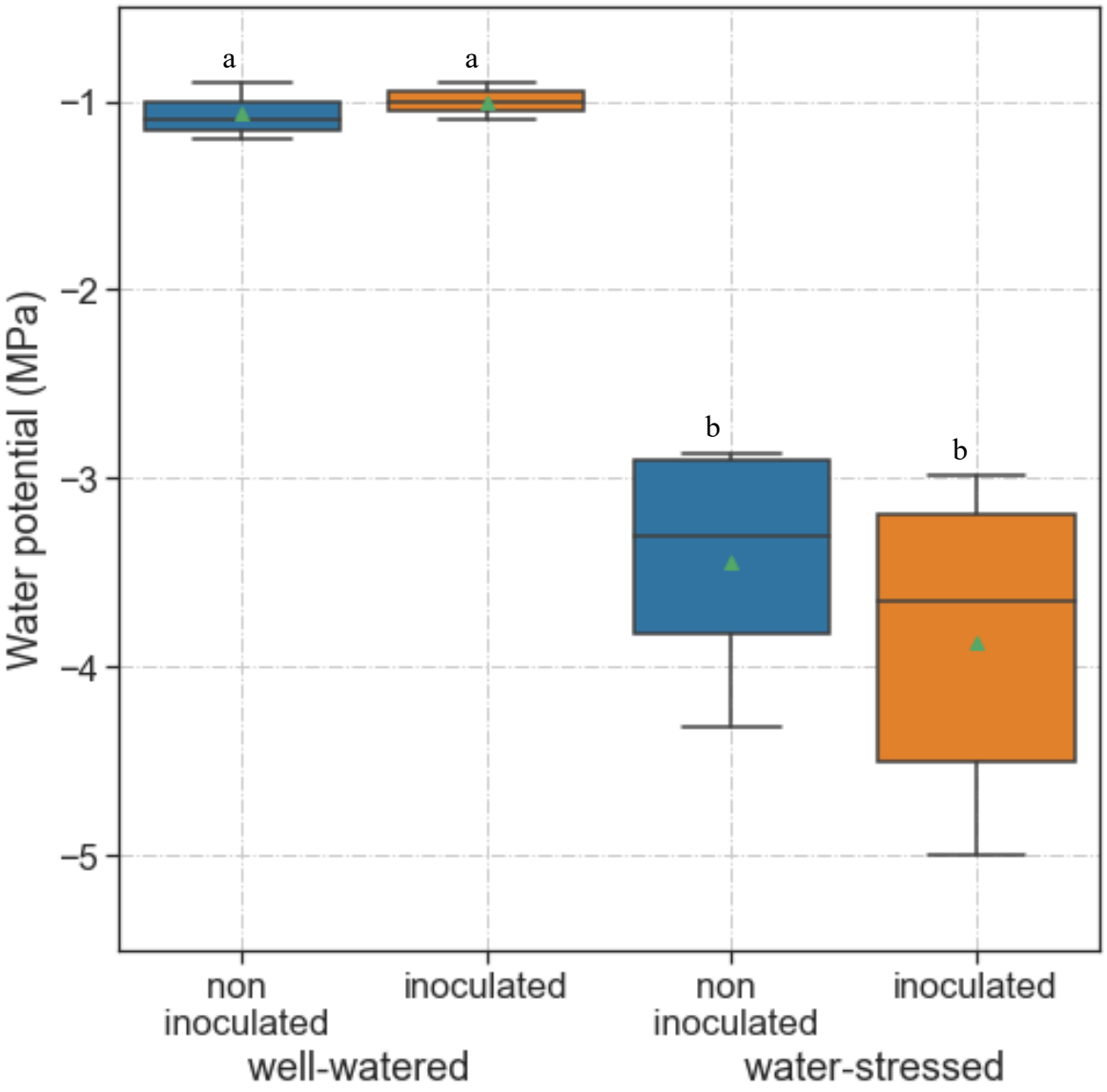

Figure 2.4. Water potential of well-watered and water-stressed *Artemisia tridentata* seedlings. In water-stressed seedlings, plant water potential was determined after stomatal closure (g_s < than 0.03 mol m^{-2} s^{-1}). Boxplots not labeled by the same letter are significantly different (p < 0.05) based on Welch's ANOVA test.

Table 2.1. Comparison of initial values of stomatal conductance (g_s), CO_2 assimilation (A), leaf transpiration (tr_{leaf}), and operating efficiency of photosystem II ($\Phi PSII$) in non-inoculated and inoculated *Artemisia tridentanta* plants before the onset of drought. Mean (±SE) of 5 plants; initial values were estimated using equation *1*. *p*-values based on t-tests

Parameter	Non-inoculated	inoculated	*p*-value
g_s (mol H_2O m^{-2} s^{-1})	0.28 (±0.02)	0.26 (±0.02)	0.53
A (µmol CO_2 m^{-2} s^{-1})	10.52 (±0.95)	9.68 (±0.88)	0.53
tr_{leaf} (mol H_2O m^{-2} s^{-1})	6.51 (±0.70)	6.17 (±0.39)	0.67
$\Phi PSII$	0.46 (±0.014)	0.45 (±0.015)	0.54

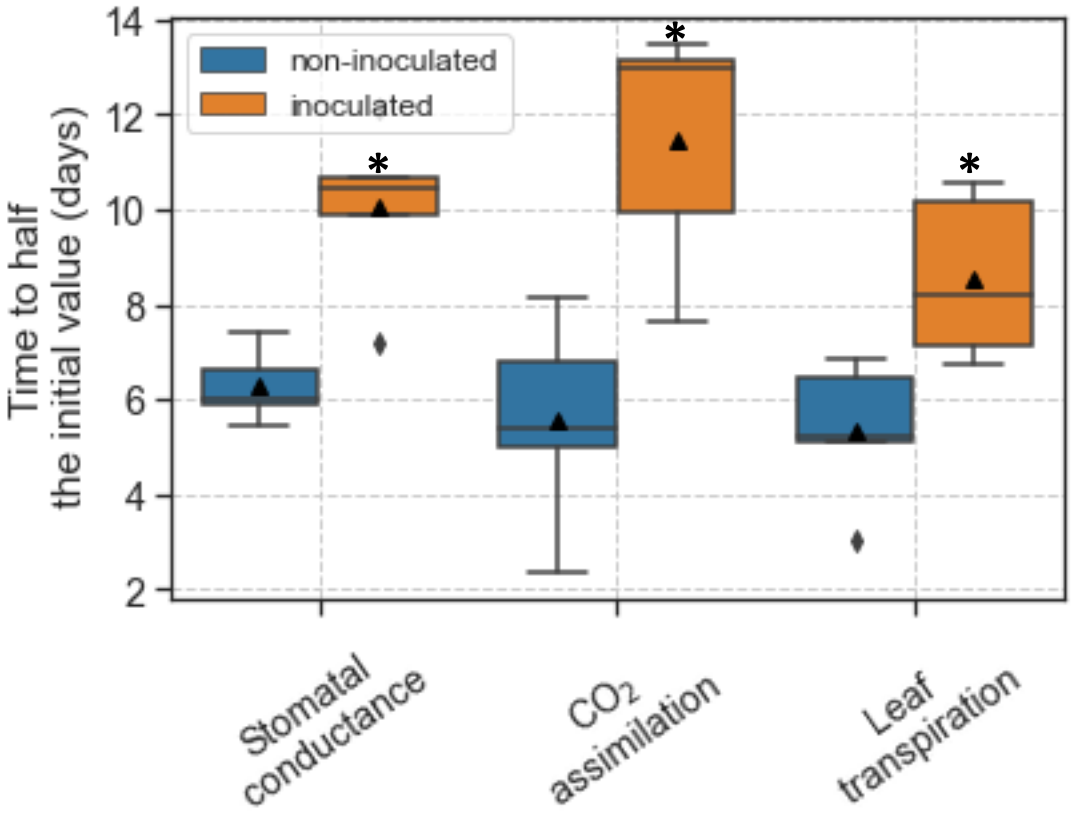

Figure 2.5. Time for stomatal conductance, CO_2 assimilation, and leaf transpiration to reach half of their initial values after withholding water. Boxplot of 5 plants per inoculation treatment Triangles and diamonds indicate the mean and outliers within each treatment, respectively. For a particular variable, boxplots labeled by an asterisk (*) are significantly different ($p < 0.05$) based on t-tests.

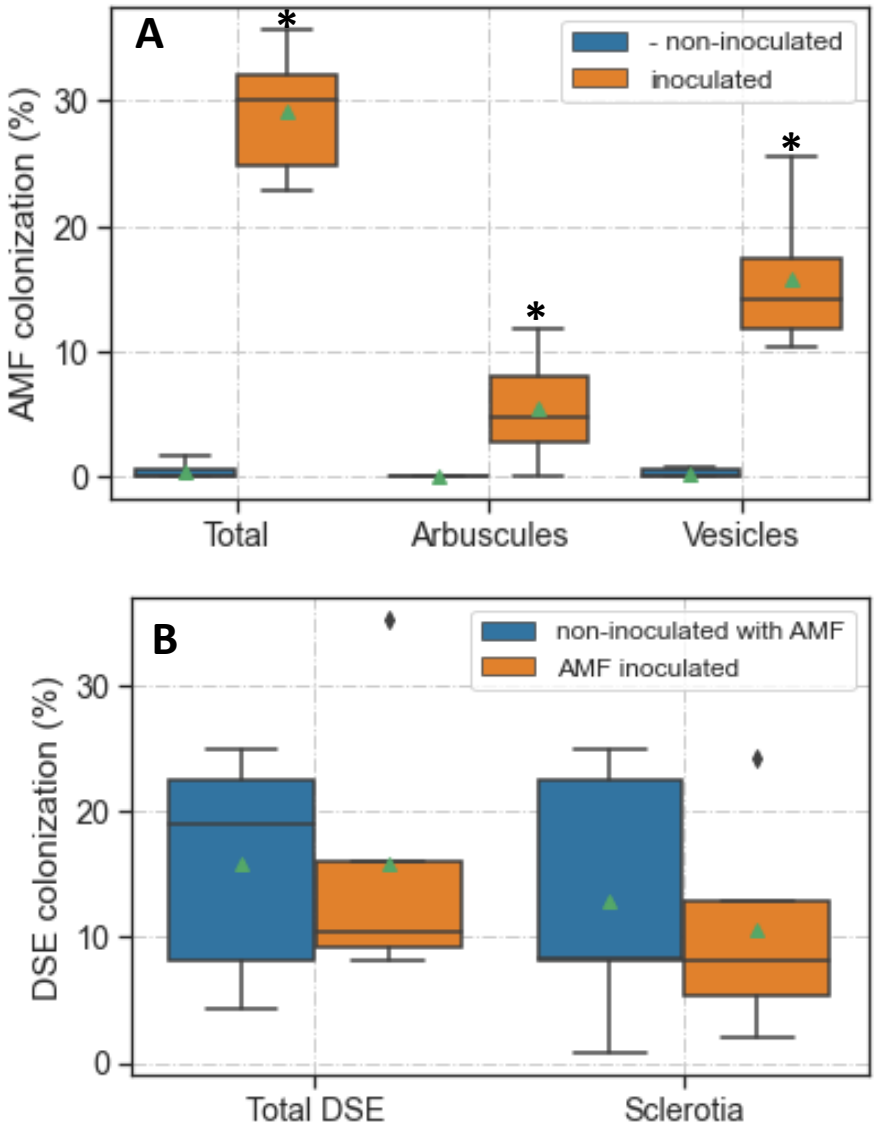

Figure 2.6. Fungal Colonization of *Artemisia* tridentata roots after the imposition of drought stress. A, AMF colonization. B, Colonization by septate fungi. Boxplot of 5 replications per treatment. For a particular variable (total colonization, arbuscules, vesicles, or microsclerotia), boxplots labeled by an asterisk (*) are significantly different ($p < 0.05$) based on Wilcoxon rank-sum test.

Table 2.2. Comparison of stomatal conductance (g_s), CO_2 assimilation (A), leaf transpiration (tr_{leaf}), and operating efficiency of photosystem II ($\Phi PSII$) in non-inoculated and inoculated *Artemisia tridentanta* plants before the onset of drought. Mean (±SE) of 10 to12 plants. *p*-values based on t-tests

Parameter	Non-inoculated	inoculated	*p*-value
g_s (mol H_2O m^{-2} s^{-1})	0.26(±0.02)	0.32 (±0.03)	0.10
A (µmol CO_2 m^{-2} s^{-1})	11.1 (±0.4)	10.3 (±0.5)	0.21
tr_{leaf} (mol H_2O m^{-2} s^{-1})	4.21 (±0.3)	4.8 (±0.4)	0.31
$\Phi PSII$	0.47 (±0.01)	0.47 (±0.01)	0.77

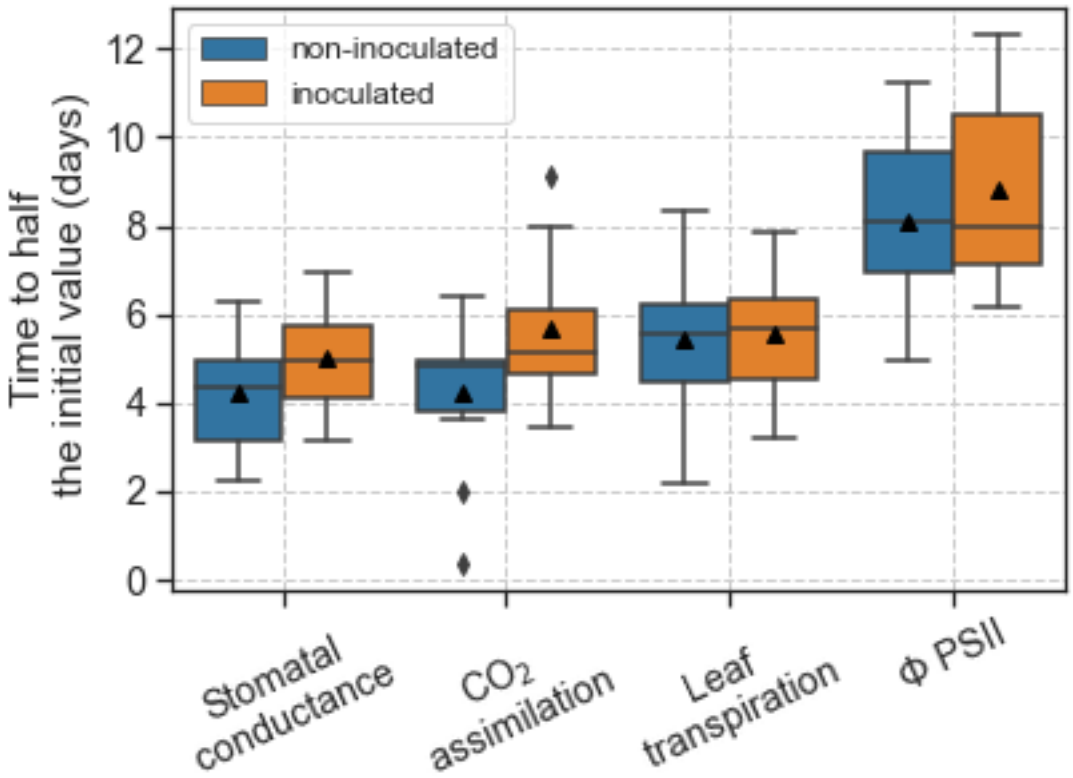

Figure 2.7. Time for gas exchange and chlorophyll fluorescence parameters to reach half of their initial values after withholding water. Boxplot of 10 to 12 plants per inoculation treatment. For a particular parameter, differences between non-inoculated and inoculated plants were not significant.

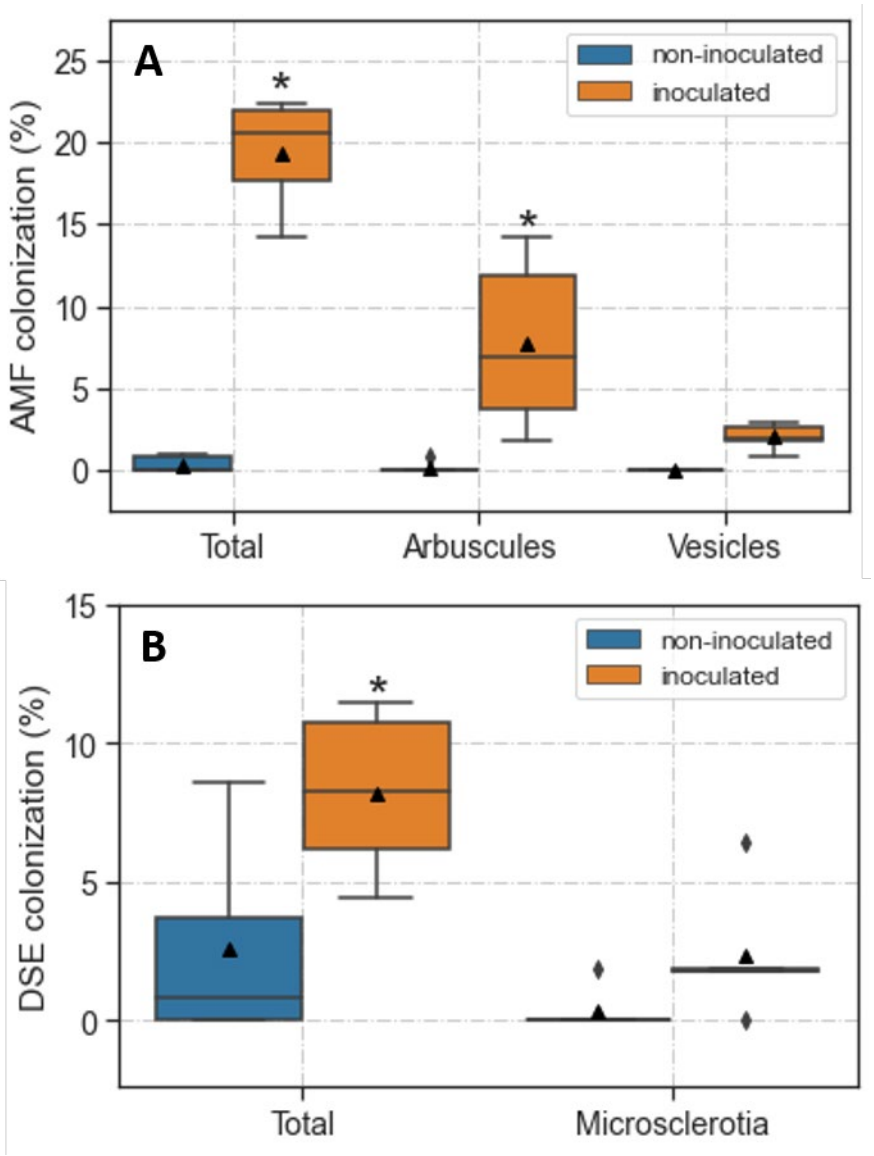

Figure 2.8. Fungal Colonization of *Artemisia* tridentata roots before the imposition of drought stress. A, AMF colonization. B, Colonization by septate fungi. Boxplot of 5 replications per treatment. Each replication consisted of root fragments collected from two plants. Triangles and diamonds indicate the mean and outliers within each treatment, respectively. For a particular variable (total colonization, arbuscules, vesicles, or microsclerotia), boxplots labeled by an asterisk (*) are significantly different ($p < 0.05$) based on Wilcoxon rank-sum test.

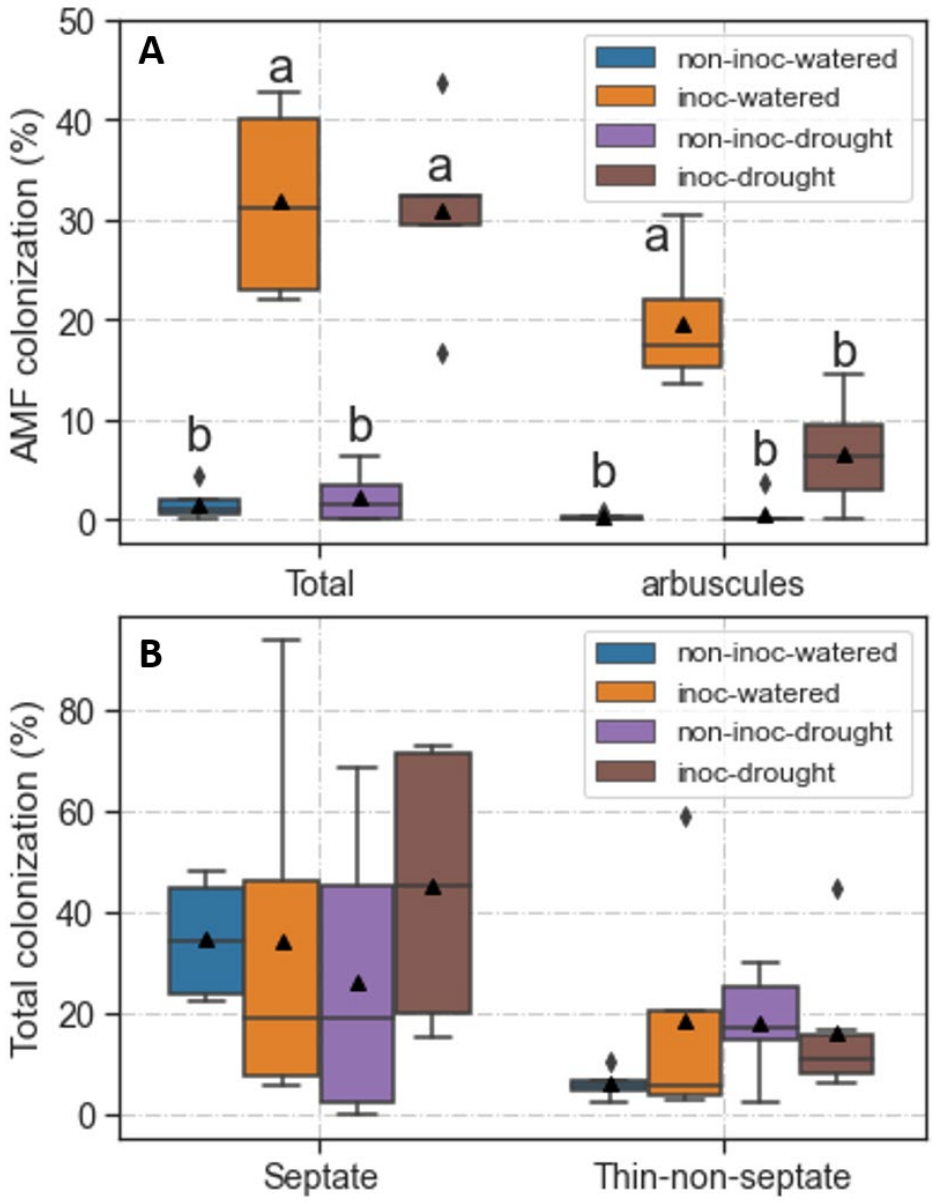

Figure 2.9. Fungal colonization of *Artemisia* tridentata roots after the drought experiment. During this experiment, some plants were maintained well-watered while others were exposed to terminal drought. A, AMF colonization. B, Colonization by septate and thin non-septate fungi. Boxplot of 4 (well-watered plants) or 6 (drought-stressed plants) replications per treatment. Triangles and diamonds indicate the mean and outliers within each treatment, respectively. For a particular variable, boxplots not labeled by the same letter are significantly different ($p < 0.05$) based Welch ANOVA test.

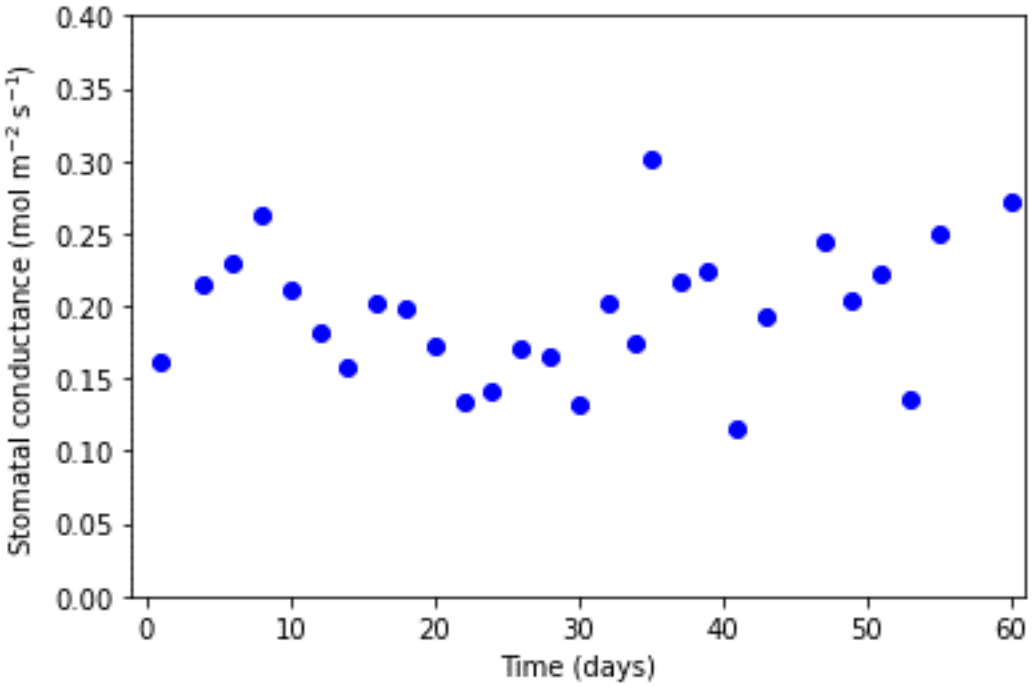

Figure 2.10. Example of daily variations in stomatal conductance observed in well-watered plants. Similar patterns were observed for CO_2 assimilation, leaf transpiration, and the operating efficiency of photosystem II.

Table 2.3. Comparison of stomatal conductance (g_s), CO_2 assimilation (A), leaf transpiration (tr_{leaf}), and operating efficiency of photosystem II ($\Phi PSII$) in non-inoculated and inoculated *Artemisia tridentanta* plants growing under well-watered conditions. Mean (±SE) of 4 plants; each plant was measured 30 times at 2d intervals over 60 days. *P*-values based on t-tests

Parameter	Non-inoculated	inoculated	*p*-value
g_s (mol H_2O m^{-2} s^{-1})	0.24 (±0.03)	0.25 (±0.01)	0.75
A (µmol CO_2 m^{-2} s^{-1})	11.4 (±0.6)	10.6 (±0.7)	0.38
tr_{leaf} (mol H_2O m^{-2} s^{-1})	4.8 (±0.1)	5.1 (±0.4)	0.48
$\Phi PSII$	0.18 (±0.01)	0.17 (±0.01)	0.33

Table 2.4. Comparison of initial values of stomatal conductance (g_s), CO_2 assimilation (A), leaf transpiration (tr_{leaf}), and operating efficiency of photosystem II ($\Phi PSII$) in non-inoculated and inoculated *Artemisia tridentanta* plants before the onset of drought. Mean (±SE) of 5 or 6 plants; initial values were estimated using equation *1*. *P*-values based on t-tests

Parameter	Non-inoculated	inoculated	*p*-value
g_s (mol H_2O m^{-2} s^{-1})	0.27 (±0.04)	0.26 (±0.03)	0.88
A (µmol CO_2 m^{-2} s^{-1})	11.2 (±1.7)	12.14 (±1.7)	0.71
tr_{leaf} (mol H_2O m^{-2} s^{-1})	5.4 (±0.7)	4.83 (±0.4)	0.51
$\Phi PSII$	0.21 (±0.01)	0.21 (±0.01)	0.60

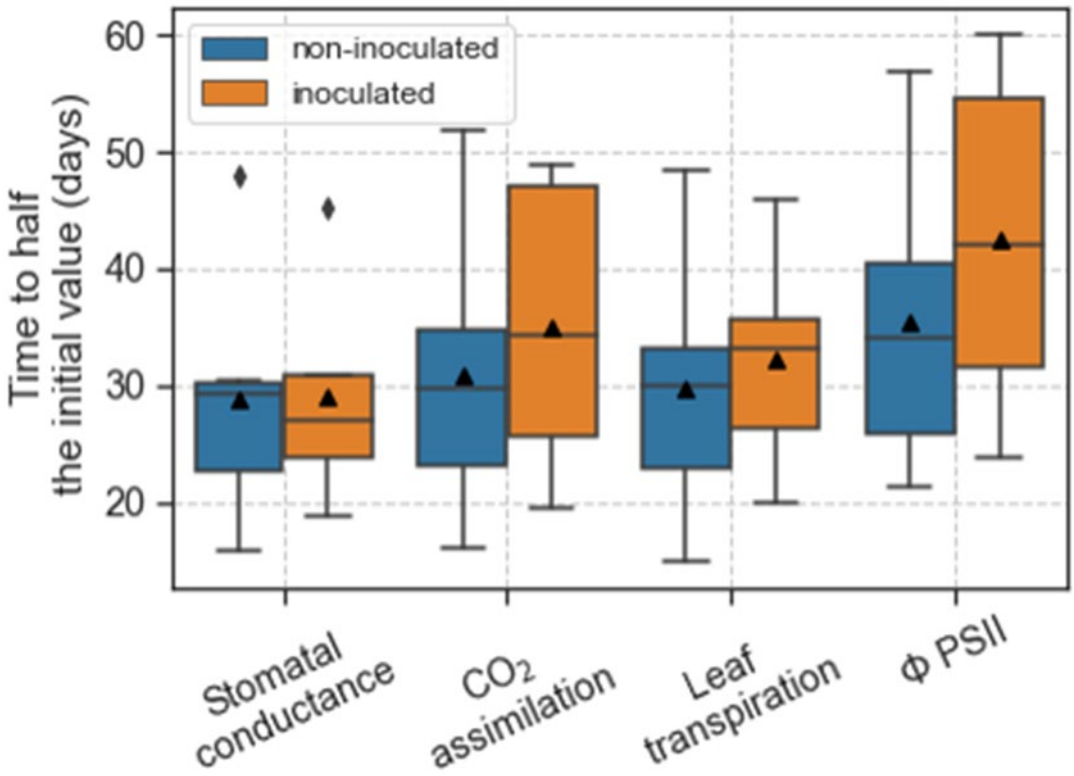

Figure 2.11. Time for gas exchange and chlorophyll fluorescence parameters to reach half of their initial values after withholding water. Boxplot of 5 or 6 plants per inoculation treatment. For a particular parameter, differences between non-inoculated and inoculated plants were not significant.

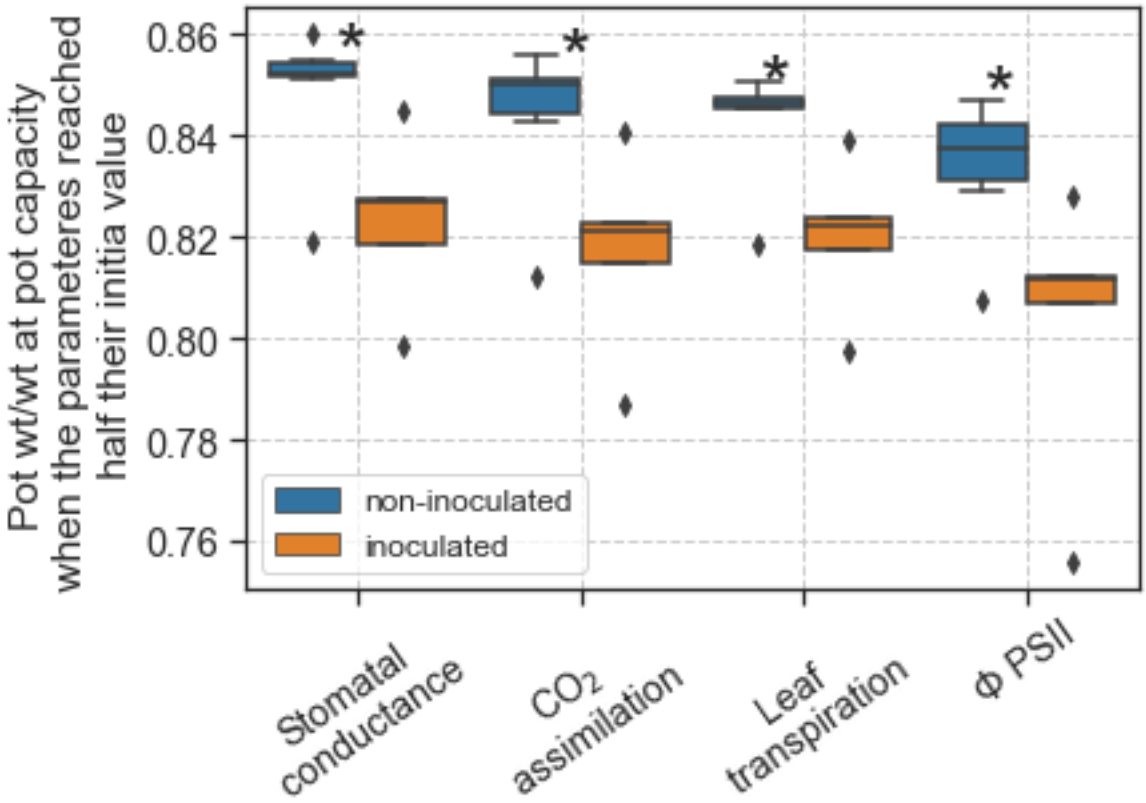

Figure 2.12. Ratio of pot weight to weight at pot capacity at which stomatal conductance, CO2 assimilation, leaf transpiration, and PSII operating efficiency declined to half of their values under well-watered conditions. Boxplot of 5 or 6 plants per inoculation treatment. For a particular variable, boxplots labeled by an asterisk (*) are significantly different ($p < 0.05$) based on Wilcoxon tests.

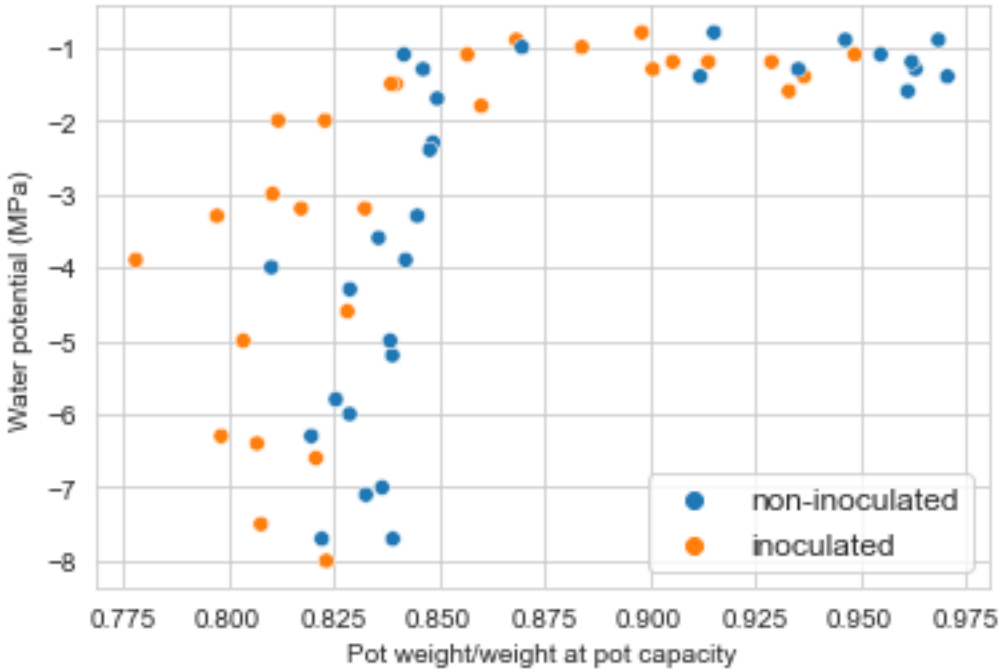

Figure 2.13. Relationship between decreases in soil water content and plant water potential. Measurements of water potential were conducted in randomly selected plants throughout the experiments.

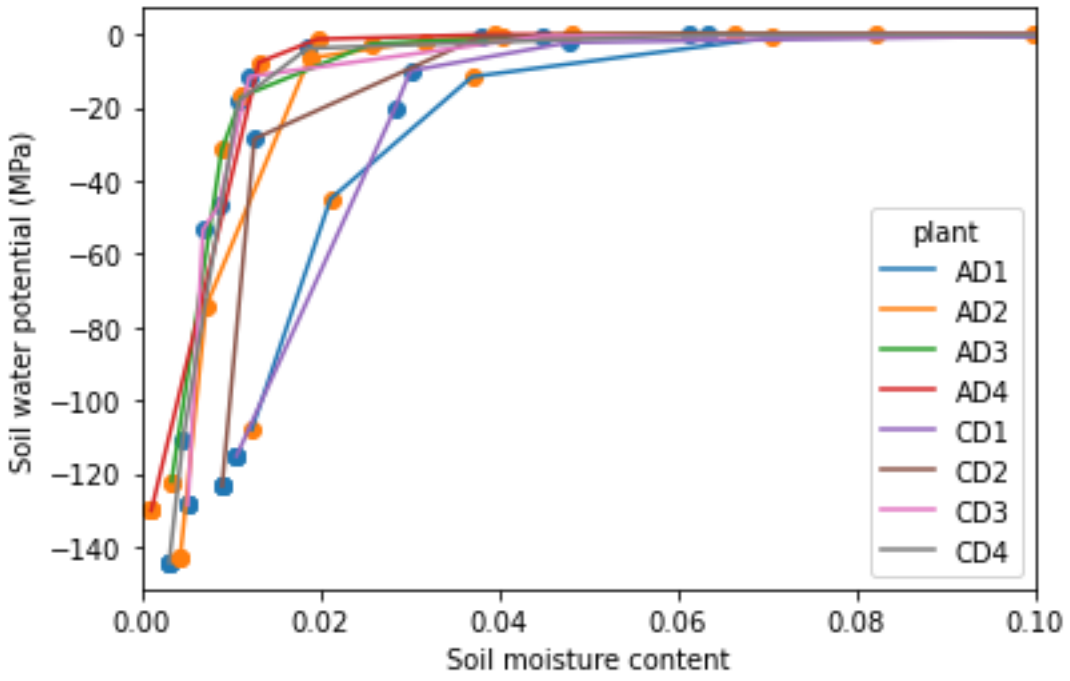

Figure 2.14. Relationship between soil moisture content ((soil weight – dry soil weight)/dry soil weight) and soil water potential. Each line represents a different plant. Lines with blue dots correspond to samples collected from non-inoculated cone-tainers. Lines with orange dots correspond to samples collected from inoculated cone-tainers.

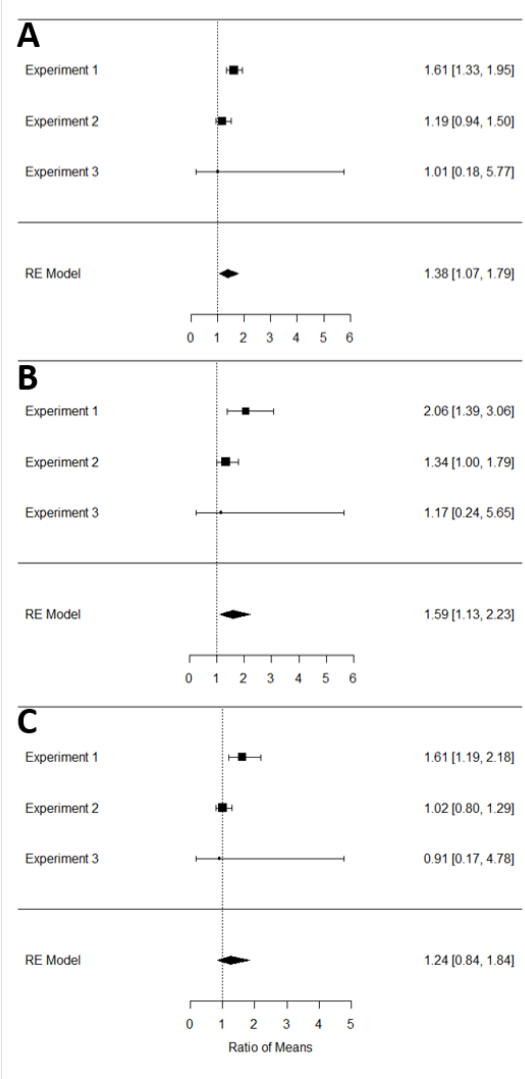

Figure 2.15. Weighted and summary responses of *Artemisia tridentata* seedlings to inoculation with arbuscular mycorrhizae estimated by meta-analysis. Response ratio between inoculated and no-inoculated seedlings on time for stomatal conductance (A), CO_2 assimilation (B), and leaf transpiration (C) to reach half of their initial values after withholding water ($t_{1/2}$) and 95% confidence intervals. A response ratio > 1 indicates that inoculation delayed $t_{1/2}$. Summary responses based on the DerSimonian-Laird estimator for random effects (RE model).

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