



Influence of Abiotic and Biotic Stressors on the Sensitivity Defenses of *Mimosa strigillosa*

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Abstract

In plants, chemical and mechanical traits are involved in the defense against environmental stressors. In the case of *Mimosa* plants, touch-sensitive responses through leaf-closure provide a unique form of mechanical defense. While past research has shown that the impacts of stressors such as competition or short-term drought may cause the chemical defenses in plants to increase or decrease, it is unknown how the interaction of these stressors may influence the efficiency of touch-sensitive responses. In this study, we hypothesized that the touch-sensitive responses of *Mimosa strigillosa* would strongly differ from regularly watered individuals when subjected to short-term drought. Additionally, we hypothesized that competition would hinder the touch-sensitive response, particularly from competitors in closely related taxa. To test these hypotheses, an experimental design was performed with three competition groups of *Mimosa*. In each competition group, half of the individuals were subjected to drought conditions. Plants were then subjected to a tactile stimulus in a greenhouse environment, with the degree of leaf closure being measured as an estimate of recovery. Drought was found to be a significant factor of both the rate of change of leaf closure, and the degree of leaf closure.

Keywords: thigmonasty, plant defenses, abiotic stress, biotic stress

Introduction

Plant defenses broadly describe the group of molecular, biochemical, and morphological mechanisms and barriers that plants use to deter damage from herbivores or pathogens (Skibbe, et al., 2008). Plant defenses can be placed into two categories, chemical and mechanical defenses. Examples of mechanical defenses include trichomes and spikes, while chemical defenses may be observed as hormonal responses or volatile chemicals (Zhou et al., 2010; Becerra et al., 2001). Multiple determinants can contribute to the success of these types of plant defense such as resource availability and allocation (Velzen & Etienne, 2015; Diezel et al., 2011). These determinants play a role in one of the most prominent questions in the field of ecology: how variations in abiotic and biotic factors drive plant defenses (Stamp, 2003; Mckey,

1974). When the availability of abiotic and biotic factors is diminished, they become stressors (Herms & Mattson, 1992).

Water is one well-documented abiotic factor that may negatively alter plant growth and its defenses in instances of drought (Herms & Mattson, 1992). While abiotic stress may severely interrupt or change plant defenses, low water availability has shown to be advantageous in short-term instances. Controlled drought has been observed to provide a greater quantity of high-quality crops, especially when drought conditions are applied to an earlier stage of ontogeny in plants (Carena et. al., 2009; Jongrungrukleng et al., 2013). However, long-term instances of drought may cause more issues for plants that may result in an interruption to plant growth, including structural loss (Li et al., 2013). In arid environments, water-shortages does not cause strong variation or loss of function, as plants may be more drought-tolerant within arid regions (Vincente-Serrano et al., 2013).

To further understand how drought may negatively affect plant defense and growth, recent studies have focused on how drought interacts with biotic stressors. One recognized form of biotic stress, is competition, with its effects on plant defenses varying. Competition has also been further quantified to describe inter- and intraspecific relationships. Each competition relationship may be more intense than the other, with this largely depending on the niche differences between the species in competition (Godoy et al., 2014). However, both intraspecific and interspecific competition has been observed to drive change in resource availability. In intraspecific competition, crowded areas of the same species may decrease available abiotic sources. This results in fewer resources, making many plants unable to meet the proper requirements needed for defense and growth (Agrawal, 2004; Karban et al., 1989). In interspecific competition, plant resources are constrained due to the production of secondary compounds in other plant species. This production of secondary compounds decreases the allocation of resources to chemical defense (Cipollini & Bergelson, 2002).

Plant defenses and their relationship with environmental factors involve complex interactions, but one plant defense that may visualize these interactions is found in thigmonasty. Thigmonasty describes the movement of plant organs (Henning et al., 2018). One category of thigmonasty is defined as nastic responses, which are defined as being independent of the direction of the stimuli and occur in many plants of the *Mimosa* genus (Jensen et. al, 2011). *Mimosa*, a genus of plants in the Fabaceae family, uses its plant defenses through rapid leaf

movement. This rapid leaf movement then results in leaf closure. This defense is thought to make the *Mimosa* less visible to nearby predators, or to startle them (Jensen, Dill, & Cahill, 2011). To test the flexibility of this defense, variations in tradeoffs have been recorded in resource and conditional shifts with light and water (Jensen, Dill & Cahill, 2011). Mechanical leaf damage has also been an indicator of subsequent leaf closure behavior. In some undamaged plants, complete closing time has been recorded to be as quick as 25 seconds or as slow as 159 seconds, while the re-opening time ranges from 71 to 1569 seconds (Cahill et. al, 2012).

As observed in many general plant defenses, abiotic stressors also contribute to the performance of *Mimosa's* defenses. In studies analyzing plants within the same family as *Mimosa*, it was found that drought conditions can greatly inhibit plant growth and production, as well as possibly alter leaf structure (Sun et al., 2020). Additionally, in order for leaf re-opening to occur, the water potential of the system must be able to adapt appropriately. Low turgor pressure introduced from drought conditions in *Mimosa* leaves could increase the level of leaf closure, but hasten the speed of leaf re-opening (Braam, 2004; De Luccia & Friedman, 2011).

While these studies show the effect of abiotic stress on *Mimosa*, none have analyzed the effects of competition on *Mimosa's* leaf defense performance. Studies that analyzed metabolites correlated with plant defenses found significant results for the correlation between decreasing proteins and allelochemicals with increasing competition (Stamp et al., 2004). This may be due to resource allocation and distribution being affected when two or more species are in competition, resulting in lower accessibility to nutrients for the high-cost defenses seen in *Mimosa* (Stamp et al., 2004). However, if acclimated to stress for a certain time, *Mimosa* may also not suffer the expected consequences of the stressor (Sun et al., 2020). Understanding how competition and water stress will affect *Mimosa's* leaf defenses in controlled experiments will help determine the factors that most strongly put *Mimosa* at risk of defense loss and predation.

The first hypothesis studied for this experiment was that the thigmonastic defense response of *Mimosa* will vary among the competition groups. Existing literature does not tell us how *Mimosa* will respond under different competition situations, but some significant variation should be expected, as proteins and allelochemical changes that occur with competition should alter the defense mechanisms of *Mimosa*. The second hypothesis for this study is that *Mimosa* with drought exposure will have a stronger and more efficient degree of closure than wet groups, as a lower turgor pressure can cause leaves to stay closed for longer periods (De Luccia &

Friedman, 2011). Additionally, with more time, the *Mimosa* plant will acclimate to stress and adjust to drought conditions (Sun et al., 2020).

Methods

Plant Systems

Studies that analyzed drought stress in *Mimosa* explored their nastic responses in greenhouse experiments (Sun et al., 2020; Gagliano et al., 2014). For this reason, our experiment took a similar set-up, conducting the primary growth of the individuals in a greenhouse setting and performing the experiment in a growth room. Three species of plants were used, the focal species, *Mimosa strigillosa*, and two competition plants, *Trifolium pratense* (Red Clover) and *Coreopsis basalis* (Golden Wave Tickseed). While *Mimosa pudica* is the most commonly used *Mimosa* species to observe thigmonasty, *M. strigillosa* is the most abundant and local of its species within the Southeastern region.

Plant Collection

M. strigillosa seeds were collected from one population at the University of Florida campus in June 2021 and planted in August 2021. Seeds of competition plants were bought from local nurseries and planted on the same day as the *Mimosa* seeds. All seeds in the experiment were planted half an inch beneath the surface of the soil in a 1:1:1 ratio of sand, germination mix, and fertilizer, as well as grown in 1-inch-wide trays of 4-inch depth, with each tray holding 36 plants. Two to three seeds were initially planted per pot. Plants were initially watered when the soil was dry, however, the protocol had to be changed to daily watering due to increasing temperatures in the later summer.

Transplantation of plants from individual to competition pots was conducted during the last week of September 2021. Competition groups were directed in three treatments, *Mimosa x Mimosa* (MM), *Mimosa x Trifolium* (MT), and *Mimosa x Coreopsis* (MC). Originally, each competition group was composed of eight plants. However, two plants from the MT treatment, and one plant from the MC treatment failed to survive to the start of competition. This left the total number of experimented plants to 21, seven from MC treatments, six from MT treatments, and eight from MM treatments. Plants were left to grow in these competition conditions for three weeks. During this timeframe, some competition plants and one *Mimosa* plant had wilted or

failed to grow, having to be replaced a week before experimental trials. After this period of competition passed, plants were segregated into two groups for the drought experiments, wet and dry.

Measurements and Data Collections

Prior to the drought condition tests, all plants were watered uniformly. To set up the drought conditions, one half of the competition treatments kept the same watering schedule, while the other half was not given water 2 days before the experiments. The day after these conditions were set, a trial run was performed, with the experiment itself conducted a week later. Because plants were in a heated room, the respective drought and moisture conditions could be kept uniform for longer durations. In the trial run of this experiment, a nastic response was elicited by isolating one shoot of a *Mimosa* plant and shaking it five times. However, we instead opted for a more direct form of stimulation, resulting in more consistent nastic responses during trial runs. Rather than shaking the shoot, the flat side of a probe was tapped onto one leaf of a shoot to induce this response. The shaking shoot method was ultimately not used due to leaves growing close to other shoots, which may have led to a strong bias in some leaflets experiencing stronger closure.

Data collection was performed by photographing *Mimosa* at specific, timed intervals. Intervals ranged from 0 min, before being exposed to the touch stimuli, to 30 min after touch stimuli. Plants photographed at 0 min had not yet been in contact with the stimuli. Photos were taken at a consistent height over the plant, with the camera lens focused on the stimulated leaf. Measurements were conducted with the photographed images on ImageJ using the leaf breadth of the tested leaf (Schneider et al., 2012). Leaf breadth at 0 min was taken at the widest point of the leaf in millimeters. This measured leaf breadth would consist of the fullest length of two leaflets. Every successive time interval had their leaf breadth measured at this same location on the leaf. Measurements of the length of the *Mimosa* and its competition plants were taken in centimeters. These measurements were conducted at the end of each trial with a ruler.

Statistical Analyses

Statistical analyses were performed using the programming software R (R Core Team, 2022). *P*-values were estimated using the ANOVA function in the car package, while graphs were visualized using the ggplot2, Rcolorbrewer, and ggpubr packages (Fox & Weisberg, 2019;

Wickham, 2016; Erich Neuwirth, 2014; Kassambara, 2020). To illustrate the intensity of leaf closure, intensity of leaf re-opening, and the rate of leaf breadth change from 1 min to 30 min, measurements were converted into a fraction of the leaf breadth at 1 min and the leaf breadth at 0 min. The same process was conducted for leaf re-opening, replacing the 1 min leaf breadth value with 30 min. Size was also investigated as a factor of stress to eliminate the possibility of additional interactions, as some individuals grew more successfully than others. Size was calculated as the ratio of the competition plant to the focal plant.

Results

Rate of Change of Leaf Reopening

Competition did not influence the rate of change of leaf reopening (Table 1, $p > 0.5$). Drought stress did affect the rate of change (Table 1, $p < 0.05$), with the dry-treatment plants reopening at a faster rate compared to the wet-treatment plants (Figure 1). Other covariates or interactive terms in the model, such as size, did not affect the rate of change (Table 1, $p > 0.1$).

Table 1. p -values of *M. strigillosa* and its stress factors with its respective quantitative methods

Stress Factor	Rate of Change (Slope of 30 min – 1 min)	Reopening Success (30/0 min)	Success of Initial Closure (1-1/0 min)
Competition	.9036	.1129	.7456
Drought	.0264 *	.5665	.0091 **
Size	.9219	.3353	.7610
Competition * Drought	.5090	.9315	.3201
Competition * Size	.2184	.2724	.3607
Drought * Size	.7244	.5570	.7074
Competition * Size * Drought	.2450	.2550	.1170

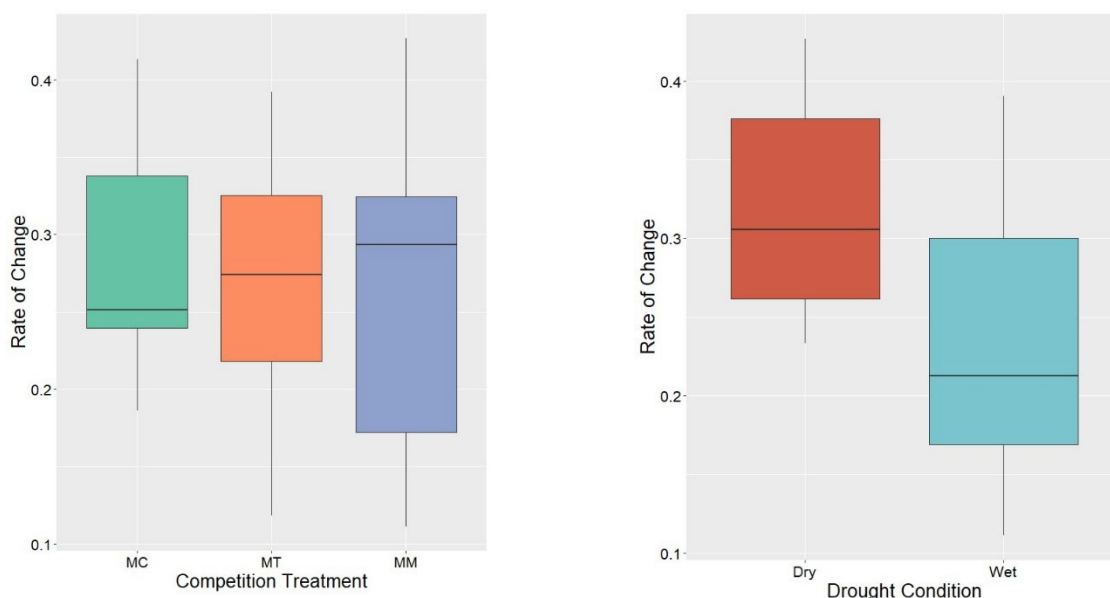


Figure 1. Rate of change from initial closure at the 1 min mark to re-opening at the 30 min mark of *Mimosa* leaves in drought and competition

Leaf Closure

In leaf breadth measurements recorded before the stimulus, and after 1 min of eliciting the closure response to stimulus, a strong degree of significance was observed in the drought experiment ($p < 0.01$, Table 1). The intensity of initial closure was more variable in individuals who were not affected by drought, while those affected by drought had less variation and stronger leaf closure (Figure 2). Competition was not a significant factor in leaf breadth 1 min after experiencing stimuli, but MT replicates did show more variable distribution (Figure 2).

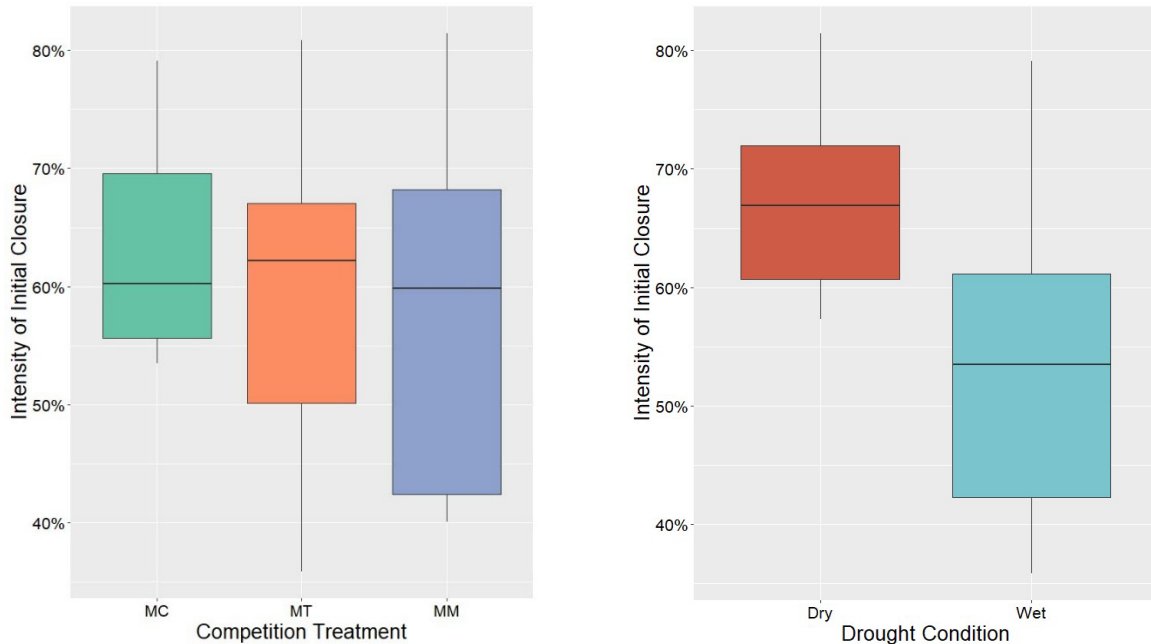


Figure 2. Intensity of initial closure at the 1 min mark in *Mimosa* leaves in competition and drought treatments

The leaf breadth 30 min after the stimulus was close to that of the initial breadth, as indicated by most of the values being close to 0.9 and 1.0 (Figure 3). This indicates that by 30 min, most plants were fully reopened to their original state. Neither group had any statistical significance in being an important factor for this stage of leaf re-opening (Table 1, $p > 0.5$).

Leaf-Reopening

Competition, compared to other stress factors, had a marginal impact on the re-opening intensity of *Mimosa* leaves. variation in the intensity of leaf re-opening, the average was closely within the range of all individuals. When observing drought as a stress factor, individuals experiencing drought had a weaker average re-opening intensity ratio compared to those in the non-drought condition (Figure 3). Both treatments depicted strong variation.

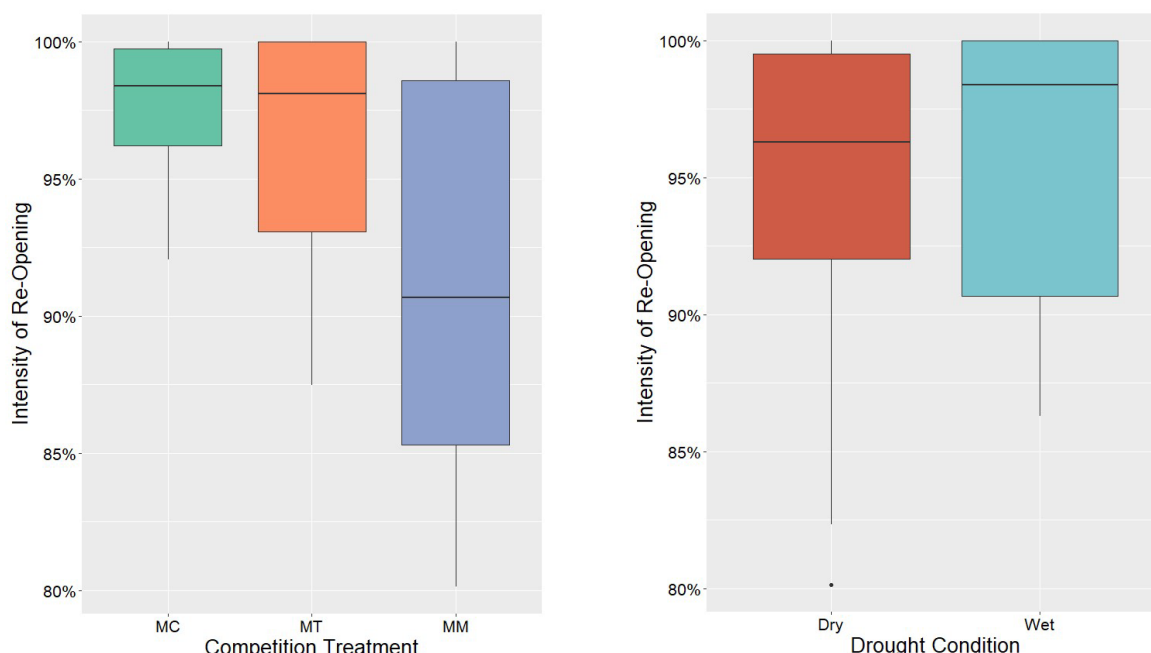


Figure 3. Intensity of re-opening in at the 30 min mark in *Mimosa* leaves in competition and drought treatments

Discussion

Effects of Competition on Leaf Responses

The experimented abiotic factors involved competition and water availability, and a stronger impact of drought over competition on mediating thigmonastic defenses was found. Competition as a factor of stress in the closure, re-opening, and rate of change in *Mimosa* was non-significant. This result contrasted from findings introduced in previously noted literature. Competition should reinforce nutrient deficiencies and a shift in nutrient allocation in plants (Bonser ,1995).

Multiple reasons can be suggested for why the competition data did not depict significant trends. *Mimosa* can have both immediate and gradual responses to changes in abiotic and biotic factors. The long-term effect of competition may have interacted with *Mimosa* 's observed ability to habituate and learn from environmental shifts or stressors (Gagliano et al., 2014). Conversely, the timing of the experiment may have been too short to see the gradual changes that would arise from the competition treatments. Competition may have also shown little variation due to the inherent properties of the focal species. *M. strigillosa* is a notably more resilient, adaptive plant than other *Mimosa* species, due to its ground-covering properties (Chang et al., 1995). Ground-covering and persistent growth may elicit stronger effects in intraspecific competition may have occurred if placed with more individuals to create a crowding effect.

Effects of Drought on Leaf Responses

For the drought hypothesis, the results showed that it impacted the rate of change of leaf closure as well as the initial leaf closure intensity. This supports the hypothesis that individuals who have succumbed to short periods of drought will perform better than individuals who are not succumbed to drought (Sun et al., 2020). *M. strigillosa* may have had a further advantage due to its more frequently observed drought-tolerant properties. Research on this *Mimosa* species highlighted the plant's ability to retain consistent levels of growth even when exposed to prolonged periods of drought (Chang et al., 2017).

Despite this significance, drought was not a major factor of stress on the intensity of leaf-reopening in *Mimosa* leaves. Unlike the high intensity of initial leaf closure, individuals within the drought condition had lower re-opening intensities than those in the non-drought affected condition (Figure 3). While there is no existing literature on drought's effect on *Mimosa*'s thigmonasty, this may have occurred because of the lower water potential found in under the drought condition. *Mimosa*'s leaflets fold quickly because of a loss of water potential, but to re-open the leaves, the uptake of water potential must occur (De Luccia & Friedman, 2011; Telewski, 2006).

Conclusion

This experiment illustrates how *Mimosa*'s sensitivity may be affected or unaffected by an abiotic factor, a biotic factor, and their interactions. Drought was a major factor of stress in this experiment and illustrated an important implication of plant defense variation. While immediate leaf closure may be beneficial against warding off herbivores, longer leaf-reopening times can result in less light availability. This is due to the less frequent appearance of photoreceptors, as well as less nutrient availability due to the additional resource cost of keeping the leaflets close (Simon et al., 2016; Jensen et al., 2011). Future experiments could further analyze how drought in *Mimosa* interacts with herbivory and growth.

While only drought has shown major prominence as a stress factor on *Mimosa*, there is potential in understanding the niche behavior of competition. Signs of intraspecific variation in MM individuals were highly variable and different than those of MC and MT individuals. Future experiments should increase the number of individuals used in MM treatments to analyze how crowding impacts leaf closure and leaf-reopening from resource competition. *Mimosa* species are

an excellent example of thigmonasty and demonstrate a unique plant defense that is unobserved in most other plant species. By looking at how *Mimosa* interacts with other species and stressors, we can determine what parts of these interactions shape *Mimosa's* defenses.

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