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Chemical Priming with Brassinosteroids to Mitigate Responses of Avocado (*Persea americana*) Trees to Flooding Stress

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Abstract: Priming involves the exposure of plants to a mild stress to increase tolerance to a more intense stress in the future. Chemical priming with brassinosteroids reduces the negative effects of flooding on the physiology and survival of some plant species. Avocado trees are very susceptible to flooding, and flooding susceptibility is related to the rootstock, which is often derived from seeds. In this study, the effects of chemical priming with two brassinosteroids, 28-homobrassinolide or 24-epibrassinolide, to improve the tolerance of avocado (*Persea americana* Mill.) trees to short-term flooding was assessed in two separate experiments with seedling trees of avocado cultivars Monroe (Experiment 1) and Reed (Experiment 2). In each experiment, trees were treated with a soil drench of 28-homobrassinolide, 24-epibrassinolide, or deionized water as a control. Trees in each chemical treatment were divided into two flooding treatments: flooded by submerging potted trees in water to above the soil surface or nonflooded. After the flooding treatments had begun, we measured the leaf gas exchange (net CO₂ assimilation (*A*), stomatal conductance (*g_s*), transpiration (*Tr*) and water use efficiency (*WUE*)) and the maximal potential quantum efficiency of photosystem II (the ratio of variable to maximum chlorophyll fluorescence; *F_v/F_m*) daily during the flooding period as indicators of plant stress. After the trees had been unflooded, leaf gas exchange and *F_v/F_m* were measured periodically during the recovery period. In both experiments, flooding reduced leaf gas exchange and *F_v/F_m* of trees in the chemical control and 28-homobrassinolide treatments beginning 2–3 days after flooding began until the end of the experiment. However, there was a negligible effect of flooding on leaf gas exchange or *F_v/F_m* of trees treated with 24-epibrassinolide. In both experiments, a greater percentage of the flooded plants treated with 24-epibrassinolide survived compared to plants in the two other chemical treatments. The results of this study indicate that pretreatment (priming) with 24-epibrassinolide as a soil drench prior to flooding reduces flooding stress of avocado trees.



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Keywords: avocado; *Persea americana*; flooding; chemical priming; brassinosteroids; leaf gas exchange; chlorophyll fluorescence

1. Introduction

Avocado (*Persea americana* Mill.) trees are extremely sensitive to low soil oxygen content, with net CO₂ assimilation (*A*), transpiration (*Tr*), stomatal conductance (*g_s*), water use efficiency (*WUE*), growth, and survival negatively impacted by flooded soil conditions [1–5]. In several avocado-growing regions of the world, there is an increasing risk of periodic flooding as a result of the disruption of normal precipitation patterns; climate change models predict greater global variation and severity of weather events [6,7]. If avocado orchards are to continue to be productive in increasingly flood-prone regions, there is a need to develop strategies to mitigate the effects of flooding on tree physiology, growth, yield, and survival.

Priming is a management strategy that involves exposing plants to a mild stress to increase tolerance to more intense stress in the future [8]. Studies of priming have mainly focused on short-lived annual species such as *Arabidopsis thaliana* (L.) Heynh., peanuts (*Arachis hypogaea* L.), and peppers (*Capsicum* spp. L.); less is known about the long-term

effects of primed acclimation on perennial crops and tree species [9]. Volatile signaling plays a significant role in activating systemic responses to abiotic stresses that are retained over time [10]. Naturally occurring volatile elicitors such as methyl jasmonate and salicylate enable signaling between plant organs or even neighboring plants, activating signaling cascades given the appropriate environmental conditions and receptors [10]. Priming can be performed using the same stressor as the anticipated stress, such as a mild drought to prepare for severe or prolonged drought, or a different stressor, such as biochemically relevant compounds like abscisic acid treatments to prime plants for salinity or drought stress [8,11].

The developmental stage at which priming occurs is important in determining the resilience of the crop in the face of more severe stress. For example, peanuts primed with a mild water deficit in the early and middle part of the crop cycle were able to maintain acceptable yields throughout the season [12]. In addition to temporal considerations, the sites of application and concentration are critical when applying chemicals as priming agents. As an example, auxins, a major class of plant growth regulator (PGR), have very distinct consequences when applied in high concentrations to different plant parts: auxins promote cell elongation and growth when applied to shoots, but have the opposite effect on roots [13]. Plant growth, development, and responses to external stimuli are regulated by a complex network of environmental cues and internal signals [14]. Abscisic acid, auxin, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonates, salicylic acid, and strigolactones are nine primary classes of PGRs. In conjunction with environmental stimuli, these PGRs form complex cross-regulatory networks, up- and downregulating one another on the basis of context and localization [15]. Beyond basic developmental patterns and tropisms, PGRs play critical roles in stress signaling.

Brassinosteroids are a class of PGRs comprising more than 60 compounds noted for their pleiotropic effects and roles in stress responses [16]. While they are associated with regulatory pathways influencing germination, root growth, and senescence, brassinosteroids are also noted for their capacity to elicit antioxidant defense systems in response to elevated reactive oxygen species (ROS) levels [17]. Brassinosteroid applications improved flooding stress tolerance of cucumbers (*Cucumis sativus*), oilseed rape (*Brassica napus*), and soybeans (*Glycine max*) exposed to flooding stress through the modulation of the antioxidant defense system, increasing the expression of superoxide dismutase (SOD) and peroxidase (POD) [18–20]. Identifying chemical priming treatments that could ameliorate the damage caused by anticipated flooding events could improve the resilience of avocado production in marginal, flood-prone areas. The objective of this study was to determine if chemical priming with either 28-homobrassinolide or 24-epibrassinolide could reduce the negative impacts of flooding on tree physiology and survival. Measurements of leaf gas exchange variables (A , g_s , Tr , WUE) and Fv/Fm were used to evaluate plant physiological responses because they are effective nondestructive indicators of the flooding stress of avocados prior to the appearance of visible stress symptoms [1–4,21].

2. Materials and Methods

2.1. Plant Material and Experimental Design

This study comprised two separate experiments. ‘Monroe’ seedling trees were used in Experiment 1. To determine if there were cultivar differences in the responses of flooded trees to the chemical priming treatments, ‘Reed’ seedling trees were used in Experiment 2 (Table 1). In both experiments, three-year-old avocado trees were grown from seed in 11.3 L plastic nursery pots filled with a commercial potting mix (PRO-MIX Premier Tech, Rivière-du Loup, QC, Canada). Seeds were collected from mature trees at the University of Florida, Tropical Research and Education Center (TREC) in Homestead, FL, USA.

Table 1. Plant material, treatments, and number of replicates used for brassinosteroid priming of seedling avocado trees used in Experiments 1 and 2.

Experiment	Cultivar	Chemical Treatment	Flooding Treatment	Number of Replicates
1	Monroe	24-Epibrassinolide	Flooded	5
			Nonflooded	5
		28-Homobrassinolide	Flooded	5
			Nonflooded	5
		Deionized water (control)	Flooded	5
			Non-flooded	5
2	Reed	24-Epibrassinolide	Flooded	10
			Nonflooded	10
		28-Homobrassinolide	Flooded	10
			Nonflooded	10
		Deionized water (control)	Flooded	10
			Nonflooded	10

Both experiments were conducted in a fan and pad-cooled greenhouse. Prior to initiating treatments, trees were hand-watered daily to field capacity. In each experiment, trees were divided into three chemical priming treatments: (1) 28-homobrassinolide applied as a soil drench, (2) 24-epibrassinolide applied as a soil drench, or (3) deionized water applied as a soil drench (control treatment). Within each chemical priming treatment, there were two flooding treatments: (1) flooded or (2) nonflooded (control) (Table 1).

Each experiment was arranged as a 3 (chemical priming treatments) \times 2 (flooding treatments) factorial and organized in a randomized complete block design. In Experiment 1, there were a total of five single-tree replicates (blocks) for each chemical priming \times flooding treatment combination. On the basis of the results from Experiment 1, the total number of replicates (blocks) was increased to 10 in Experiment 2 because leaf gas exchange and F_v/F_m were measured on the surviving plants, and by the end of Experiment 1, the number of replicates was reduced due to tree mortality. Therefore, the number of replicates was increased to 10 in Experiment 2 to reduce within-treatment variation if some of the plants died by the end of the experiment.

2.2. Chemical Priming Treatments

Trees designated for chemical priming were not watered for 24 h before a root drench was applied. For both the 24-epibrassinolide (MedChem Express, Monmouth Junction, NJ, USA) and 28-homobrassinolide (Gold Biotechnology, St. Louis, MO, USA) treatments, a 1.0×10^{-6} M solution was prepared using deionized water as described by Kang et al. [19] and Ikekawa and Zhao [22]. For the chemical control treatment, deionized water was applied. For all chemical treatments, 850 mL was applied to each pot.

2.3. Flooding Treatments

Well water was used to fill 18.9 L plastic utility buckets, and the water was left in the buckets for 3–4 days at ambient temperature to allow for all the dissolved oxygen to dissipate, which was monitored using a YSI Pro 20 galvanic dissolved oxygen probe (YSI Inc., Yellow Springs, OH, USA). For trees in the flooded treatment, nursery pots containing the trees were then placed in the plastic utility tubs, so that the pot was submerged to 2.5 cm above the soil surface. Trees in the nonflooded treatment were not submerged and irrigated manually daily during the flooding period.

The duration of the flooding treatment was determined through daily leaf gas exchange measurements, as described below. Trees were unflooded after there had been significant statistical difference ($p \leq 0.05$) between the flooded and control treatments for any leaf gas exchange variable for two consecutive days or when visible symptoms of stress (loss of turgor) appeared.

Trees were unflooded by removing the plant pots from the plastic utility buckets and allowing the soil to drain for 2 h. Beginning with the day after unflooding, leaf gas exchange and chlorophyll fluorescence were measured every day for 5 days, and thereafter every 3–7 days until recovery had been determined to have occurred. Recovery was defined as no significant statistical difference ($p \leq 0.05$) in any leaf gas exchange variable between the flooded and nonflooded treatments. Recovery was determined to be unlikely if it had not been reached by 14 days after unflooding, and/or flooded trees had died. During the recovery period, all trees were manually irrigated daily to field capacity.

2.4. Leaf Gas Exchange

Net CO₂ assimilation (A), stomatal conductance of water vapor (g_s), and transpiration (Tr) were measured with a CIRAS-3 portable gas analyzer (PP Systems, Amesbury, MA, USA) as previously described [1–4]. For each tree, leaf gas exchange was measured in two fully expanded leaves with fully developed cuticles. The CIRAS-3 was set to a reference CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$, a light saturated photosynthetic photon flux of 1000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, and an air flow rate of 200 mL min^{-1} into the leaf cuvette. Water use efficiency (WUE) was calculated as $A \div Tr$.

2.5. Chlorophyll Fluorescence

The maximal potential quantum efficiency of Photosystem II (the ratio of variable to maximal chlorophyll fluorescence; Fv/Fm), as an indicator of flooding stress [21], was measured on the adaxial surface of two leaves per tree with an OS-30p portable fluorescence meter (Opti-Sciences Inc., Hudson, NH, USA). Leaves were acclimated in the dark for 30 min prior to measurements.

2.6. Statistical Analyses

Statistical interactions between the chemical-priming and flooding treatments were determined by two-way analysis of variance (ANOVA). Differences among means of chemical priming treatments within each flooding treatment were determined with repeated-measures ANOVA. All statistical analyses were conducted with SAS Statistical Software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Experiment 1

There was a significant statistical interaction ($p \leq 0.05$) between flooding and chemical priming treatments for several of the measured dependent variables. Therefore, flooding effects were analyzed separately within each chemical priming treatment.

After two consecutive days of flooding, all trees were unflooded (on the basis of a significant decrease in leaf gas exchange between flooded and nonflooded trees), and the experiment was halted 28 days after flooding treatments had begun. All nonflooded trees survived in all chemical priming treatments (Table 2). In the flooded treatment, 40% of the chemical control trees survived, and 60% of the trees treated with 28-homobrassinolide survived (Table 2). However, 100% of the trees treated with 24-epibrassinolide survived (Table 2).

Table 2. Survival of ‘Monroe’ avocado seedling trees treated with brassinosteroids and either flooded or nonflooded (Experiment 1).

Chemical Priming Treatment	Flooding Treatment	
	Nonflooded	Flooded
	Plant survival (%)	
Control	100	40
28-Homobrassinolide	100	60
24-Epibrassinolide	100	100

Flooding reduced A in the chemical control and the 28-homobrassinolide treatments, and differences between flooding treatments were significant on several measurement dates (Figure 1A,B). However, there were no significant effects of flooding on A of trees treated with 24-epibrassinolide (Figure 1C).

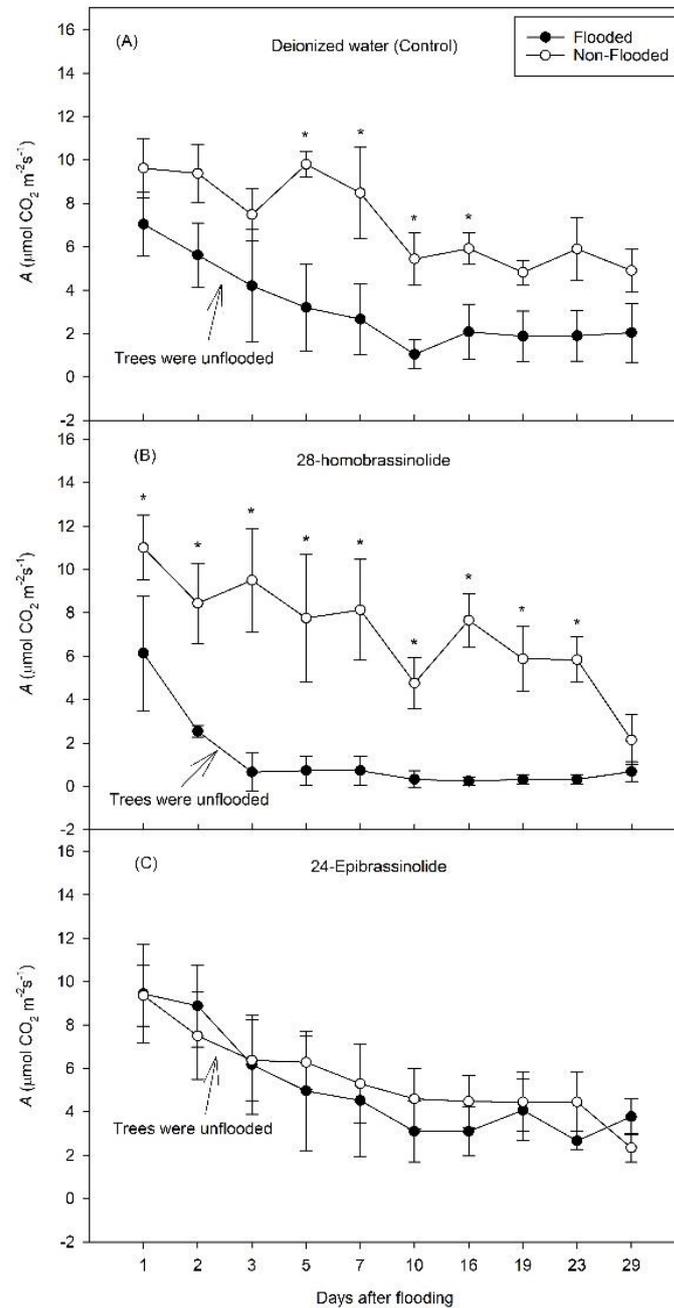


Figure 1. Effect of flooding on net CO₂ assimilation (A) of ‘Monroe’ avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide and flooded or nonflooded (Experiment 1). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. ‘Trees were unflooded’ indicates the day on which trees were removed from the flooding treatment to start the recovery period.

For trees in the chemical control and 28-homobrassinolide treatments, g_s was significantly lower in the flooded than that in the nonflooded treatment on most measurement dates until 3 days before trees were harvested for trees in the chemical control treatment,

and 1 day before trees were harvested for trees in the 28-homobrassinolide treatment (Figure 2A,B). There were no significant differences in g_s between flooded and nonflooded trees in the 24-epibrassinolide treatment (Figure 2C).

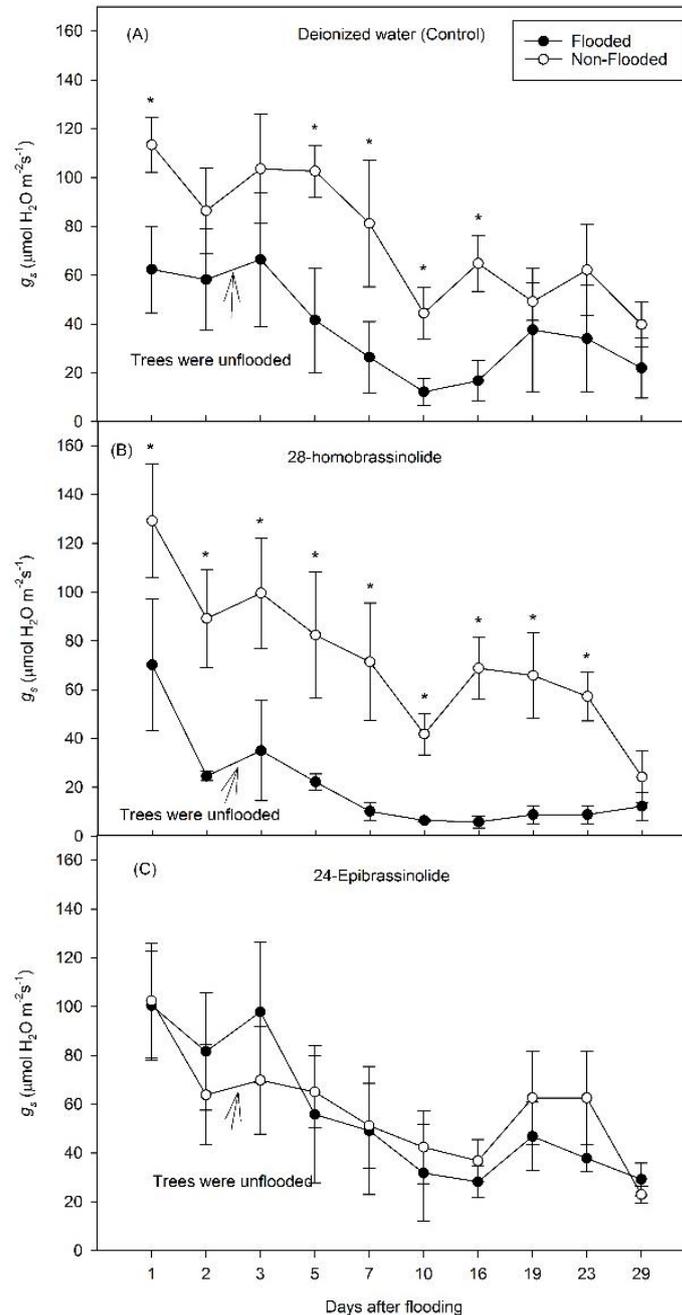


Figure 2. Effect of flooding on stomatal conductance (g_s) of 'Monroe' avocado trees treated with or (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide (Experiment 1). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. 'Trees were unflooded' indicates the day on which trees were removed from the flooding treatment to start the recovery period.

Flooding significantly reduced Tr in the chemical control and 28-homobrassinolide treatments on most measurement dates after the flooding treatments had begun. However, on the last three measurement dates for the chemical control treatment and the last measurement date for the 28-homobrassinolide treatment, there were no significant differences in

Tr between the flooded and nonflooded treatments (Figure 3A,B). There was no significant difference in Tr between flooding treatments on any measurement date for trees treated with 24-epibrassinolide (Figure 3C).

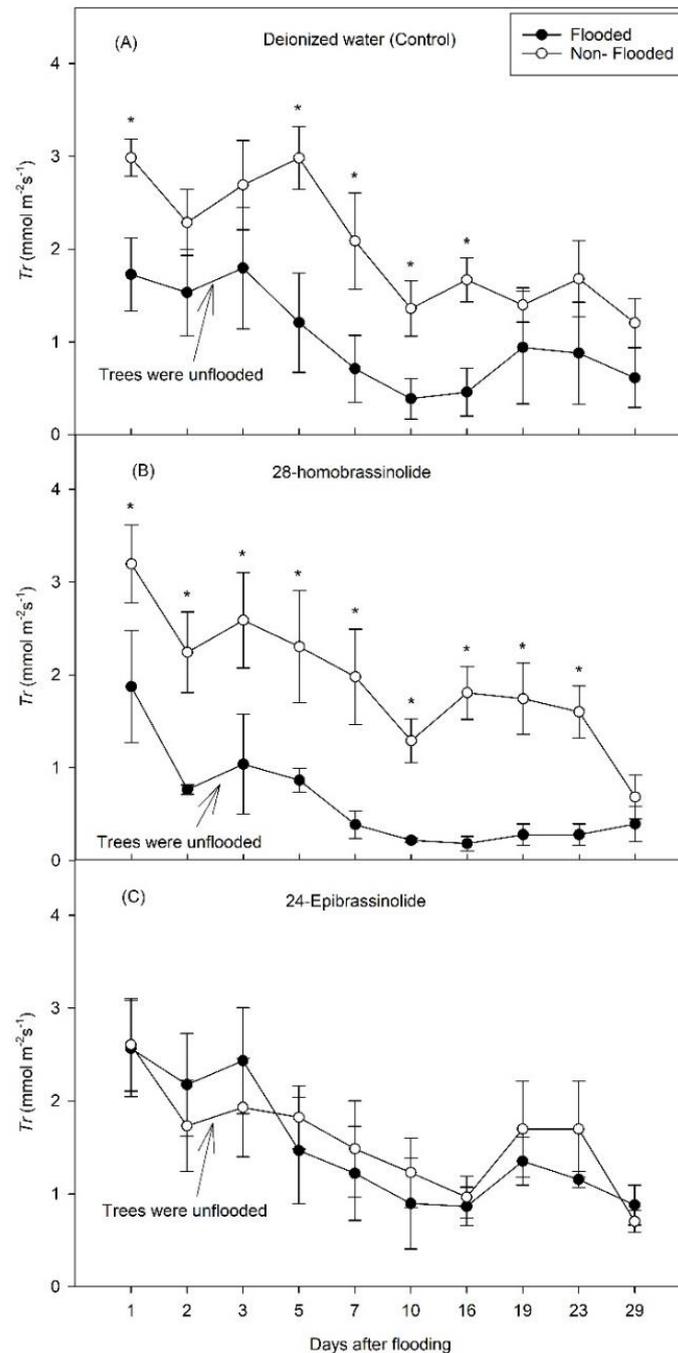


Figure 3. Effect of flooding on transpiration (Tr) of ‘Monroe’ avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide (Experiment 1). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. ‘Trees were unflooded’ indicates the day on which trees were removed from the flooding treatment to start the recovery period.

For trees in the chemical control and 28-homobrassinolide treatments, WUE was significantly lower in the flooded than that in the nonflooded treatment on every measurement date, beginning at 2 days after plants were flooded until the end of the experiment

(Figure 4A,B). For trees in the 24-epibrassinolide treatment, there were no significant differences in *WUE* between flooding treatments on any measurement date (Figure 4C).

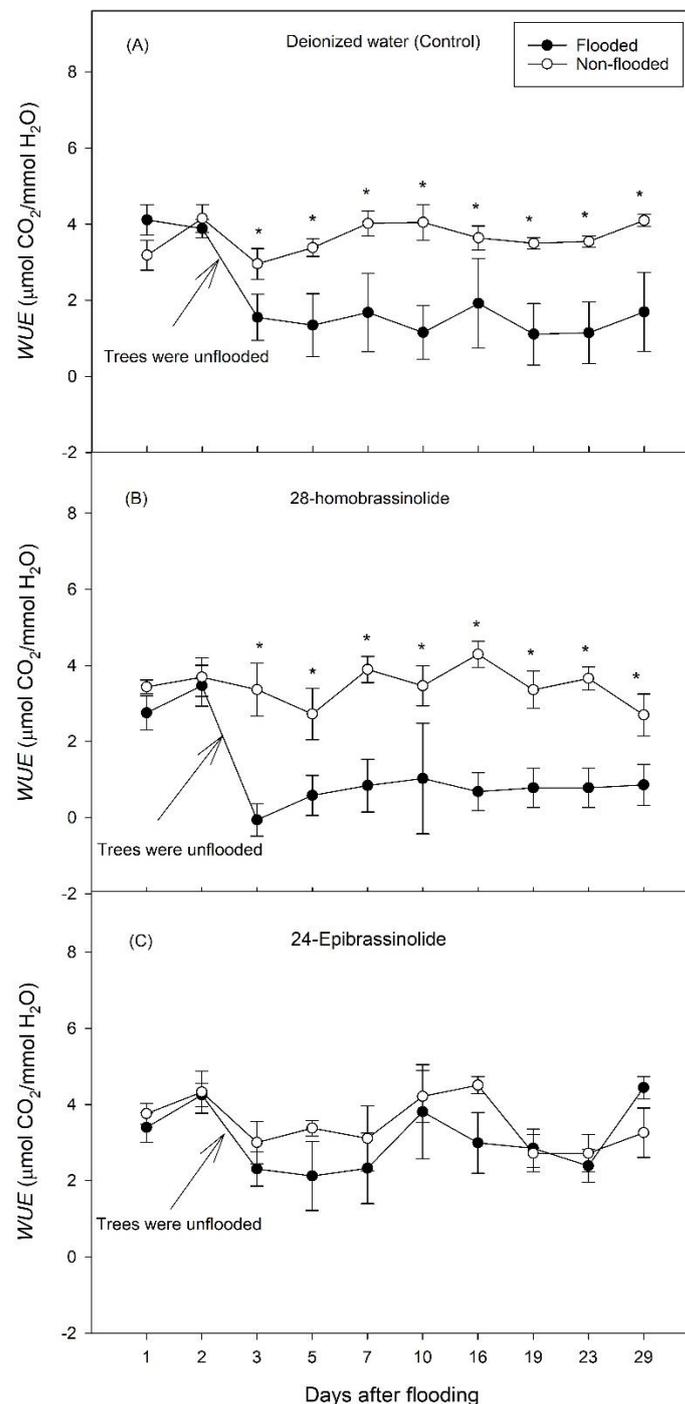


Figure 4. Effect of flooding on water use efficiency (*WUE*) of ‘Monroe’ avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 1). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. ‘Trees were unflooded’ indicates the day on which trees were removed from the flooding treatment to start the recovery period.

For trees in the chemical control and 28-homobrassinolide treatments, flooding significantly reduced Fv/Fm , beginning at 10 or 16 days after flooding treatments had be-

gun for trees in the chemical control and 28-homobrassinolide treatments, respectively (Figure 5A,B). Flooding had no effect on Fv/Fm for trees in the 24-epibrassinolide treatment (Figure 5C).

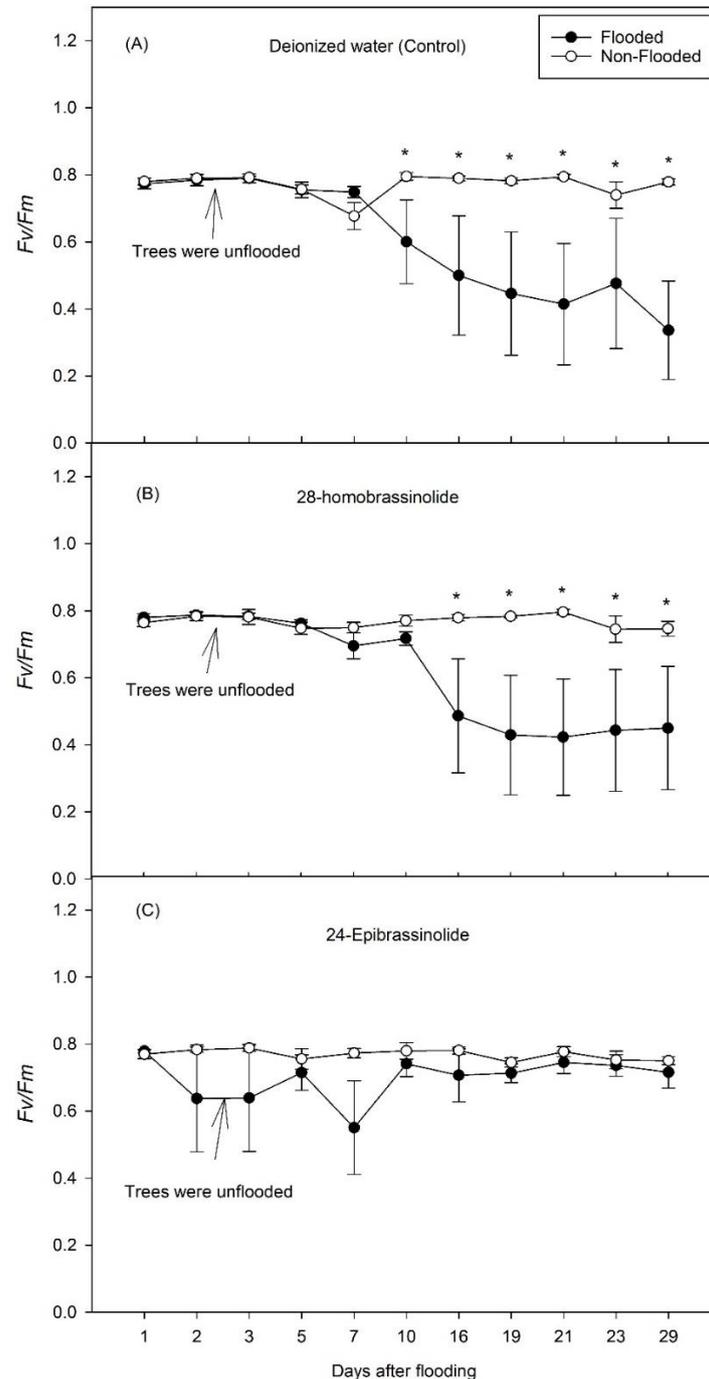


Figure 5. Effect of flooding on the maximal potential quantum efficiency of photosystem II (Fv/Fm) of 'Monroe' avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 1). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. 'Trees were unflooded' indicates the day on which trees were removed from the flooding treatment to start the recovery period.

3.2. Experiment 2

There was a significant statistical interaction ($p \leq 0.05$) between flooding and chemical priming treatments for several of the measured dependent variables. Therefore, flooding effects were analyzed separately within each chemical priming treatment.

Trees were unflooded after 3 days (on the basis of a significant difference in leaf gas exchange between the flooded and nonflooded treatments), and the experiment was ended 21 days after flooding treatments had begun. After 21 days, all plants in the nonflooded treatment survived in all chemical priming treatments (Table 3). However, in the flooded treatment, only 20% of trees in the chemical control treatment survived, whereas 40% of trees treated with 28-homobrassinolide and 50% of the trees treated with 24-epibrassinolide survived (Table 3).

Table 3. Survival of ‘Reed’ avocado seedling trees treated with brassinosteroids and either flooded or nonflooded (Experiment 2).

Chemical Priming Treatment	Flooding Treatment	
	Nonflooded	Flooded
	Plant survival (%)	
Control	100	20
28-Homobrassinolide	100	40
24-Epibrassinolide	100	50

Overall, leaf gas exchange rates were low compared to those in Experiment 1 regardless of the chemical treatment for trees in both the flooded and nonflooded treatments. After 3 days of flooding, all trees were unflooded. Though visible symptoms of stress such as wilting and the desiccation of younger leaves were observed prior to Day 3, no significant differences in leaf gas exchange were noted until Day 3. By Day 11, several leaves on the surviving flooded plants abscised. Therefore, after measurement Day 9, we waited until there was a sufficient number of leaves for the final gas exchange and Fv/Fm measurements, which was on Day 21.

From 3 days after flooding treatments had begun to the end of the experiment, A , g_s , Tr , and WUE were significantly lower for flooded than nonflooded trees in the chemical control and 28-homobrassinolide treatments (Figure 6A,B, Figure 7A,B, Figure 8A,B and Figure 9A,B). However, in the 24-epibrassinolide treatment, A , g_s , Tr , and WUE were only significantly lower for flooded than nonflooded trees on Day 3, the day at which trees were unflooded. After the trees had been unflooded, A , g_s , Tr , and WUE of flooded trees returned to values close to those of trees in the nonflooded treatment (Figures 6B, 7B, 8B and 9B).

Fv/Fm was significantly lower for flooded than nonflooded trees only on Day 3 in the chemical control treatment (Figure 10A) and Day 9 in the 28-homobrassinolide treatment (Figure 10B). There was no significant difference in Fv/Fm between flooding treatments on any measurement date (Figure 10C).

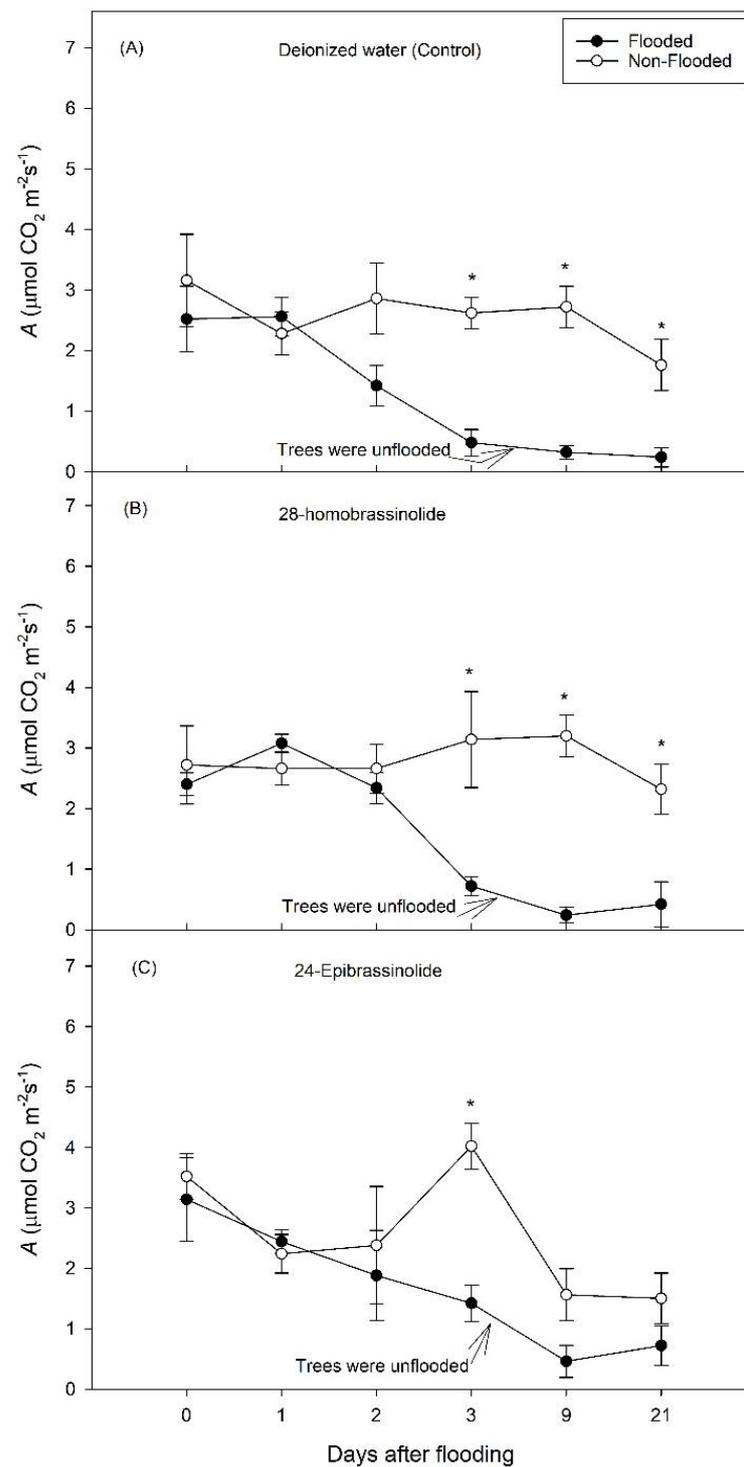


Figure 6. Effect of flooding on net CO₂ assimilation (A) of ‘Reed’ avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 2). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with a single asterisk and were determined by repeated-measures ANOVA. ‘Trees were unflooded’ indicates the day on which trees were removed from the flooding treatment to start the recovery period.

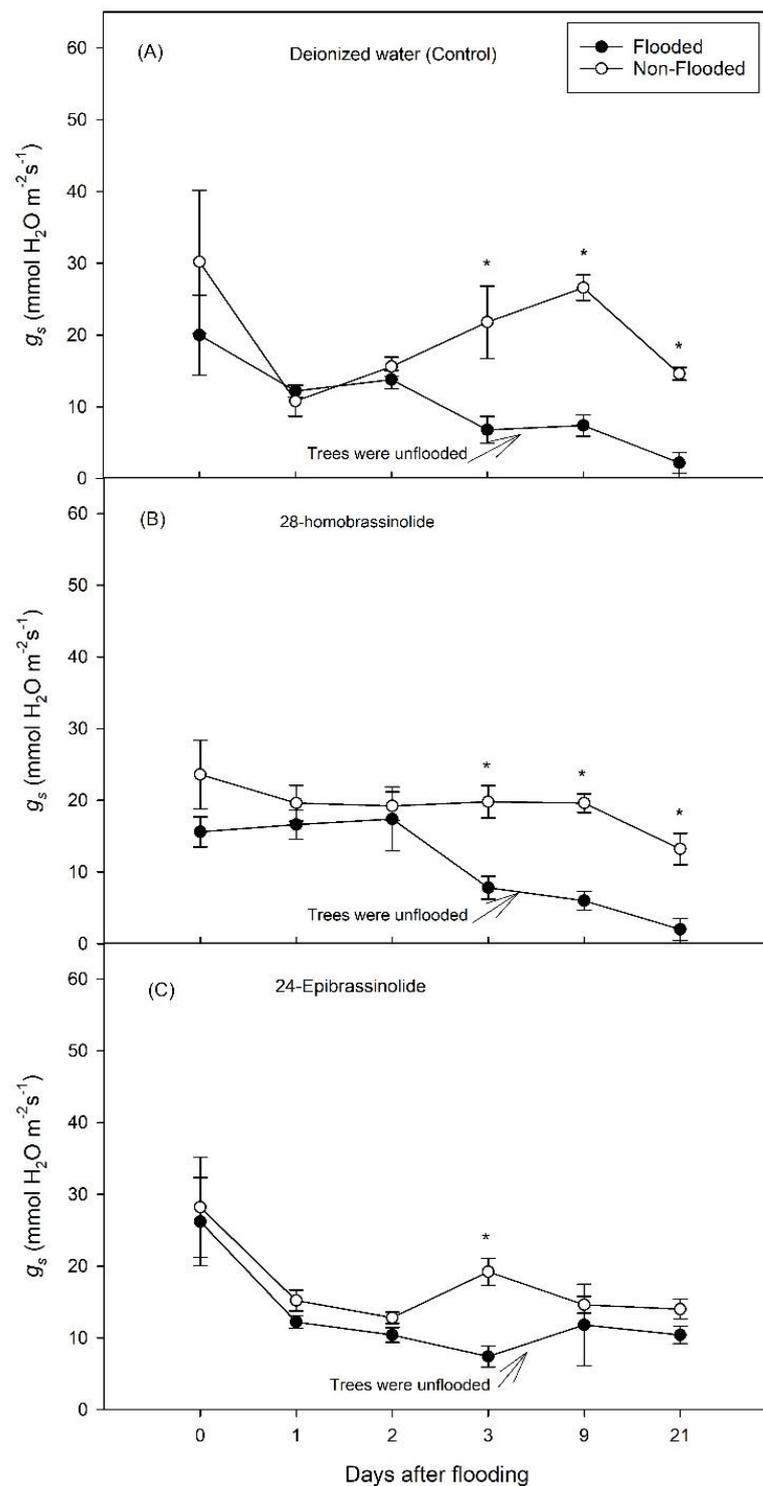


Figure 7. Effect of flooding on stomatal conductance (g_s) of 'Reed' avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 2). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and where determined by repeated-measures ANOVA. 'Trees were unflooded' indicates the day on which trees were removed from the flooding treatment to start the recovery period.

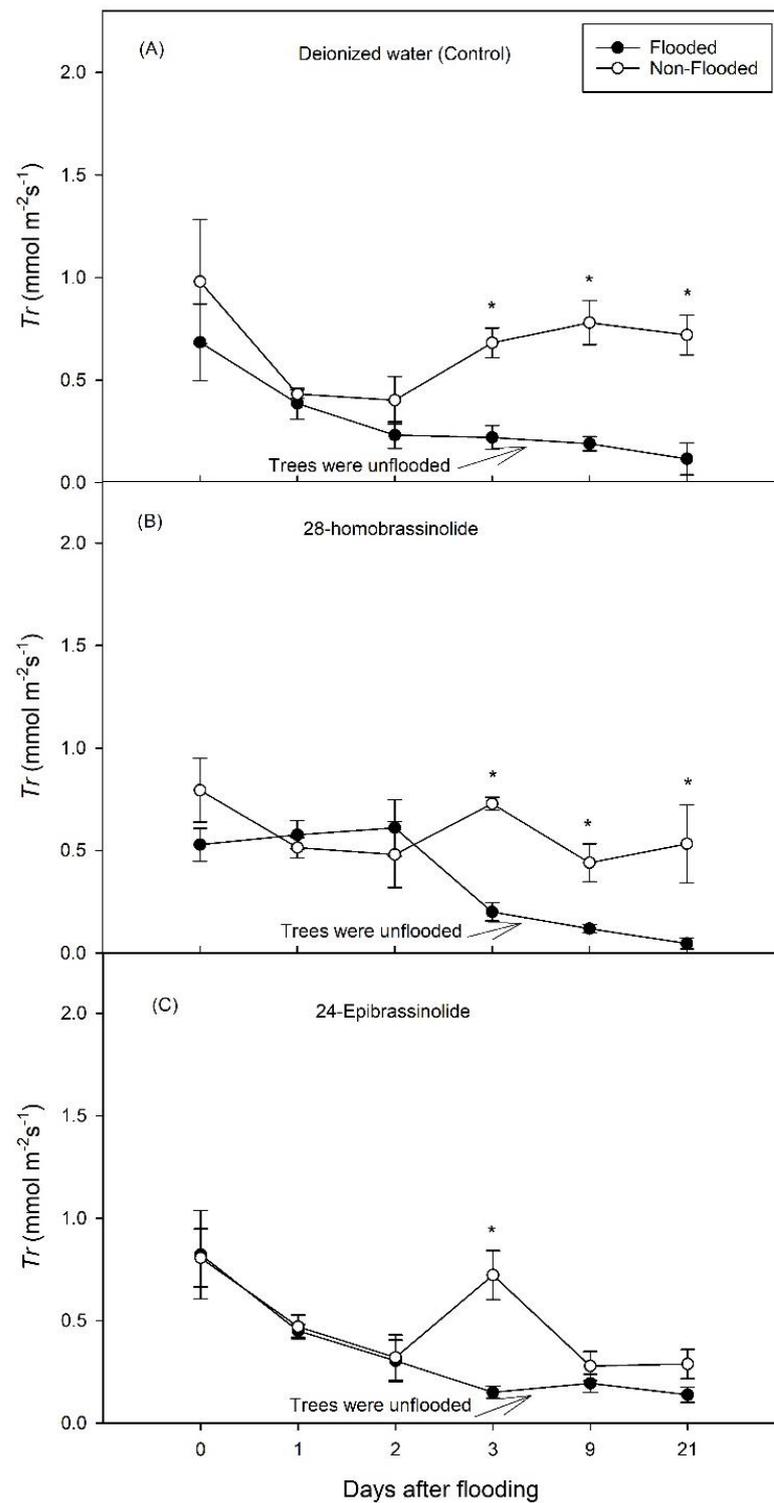


Figure 8. Effect of flooding on transpiration (Tr) of 'Reed' avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 2). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. 'Trees were unflooded' indicates the day on which trees were removed from the flooding treatment to start the recovery period.

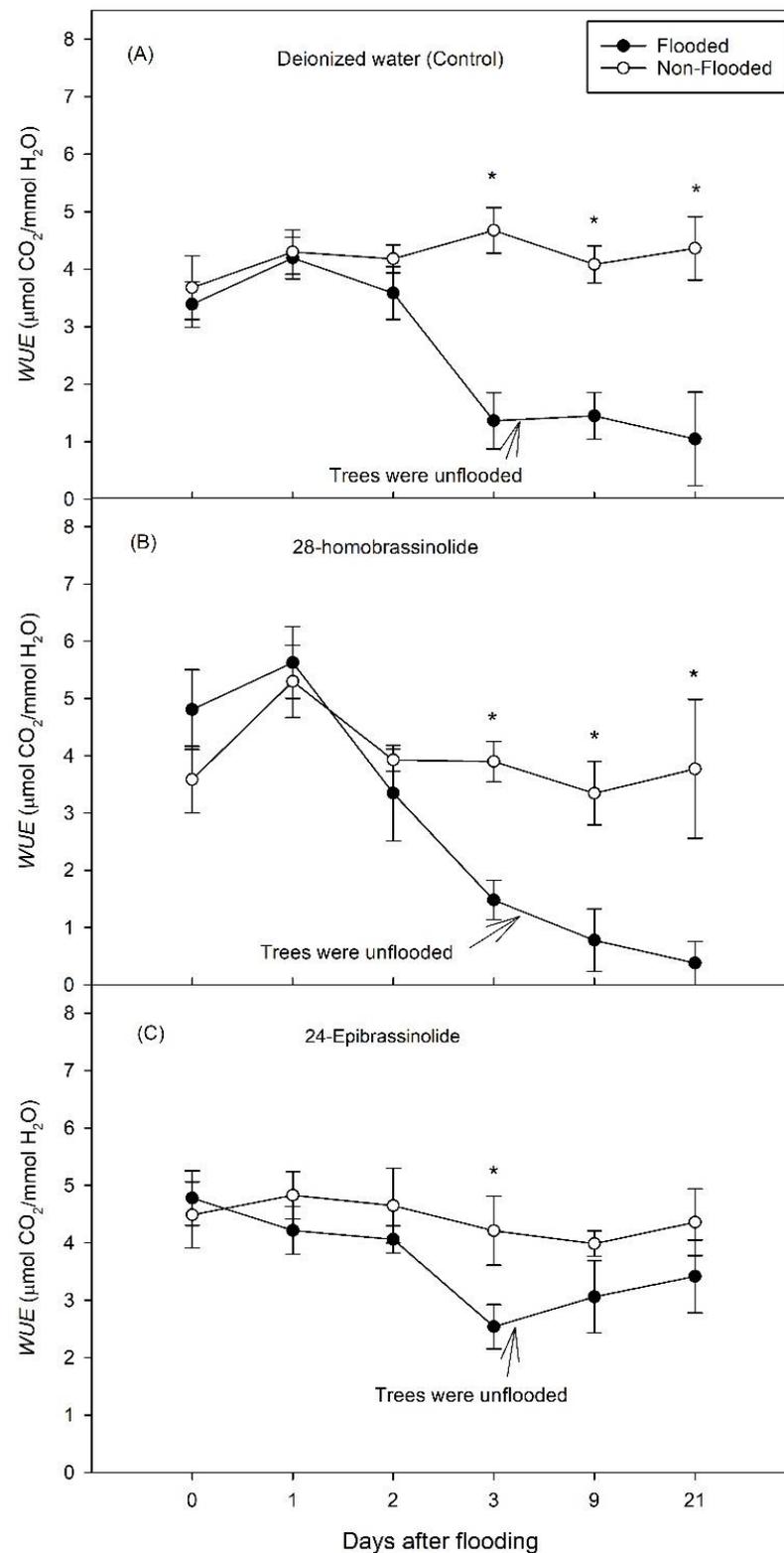


Figure 9. Effect of flooding on water use efficiency (*WUE*) of ‘Reed’ avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 2). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. ‘Trees were unflooded’ indicates the day on which trees were removed from the flooding treatment to start the recovery period.

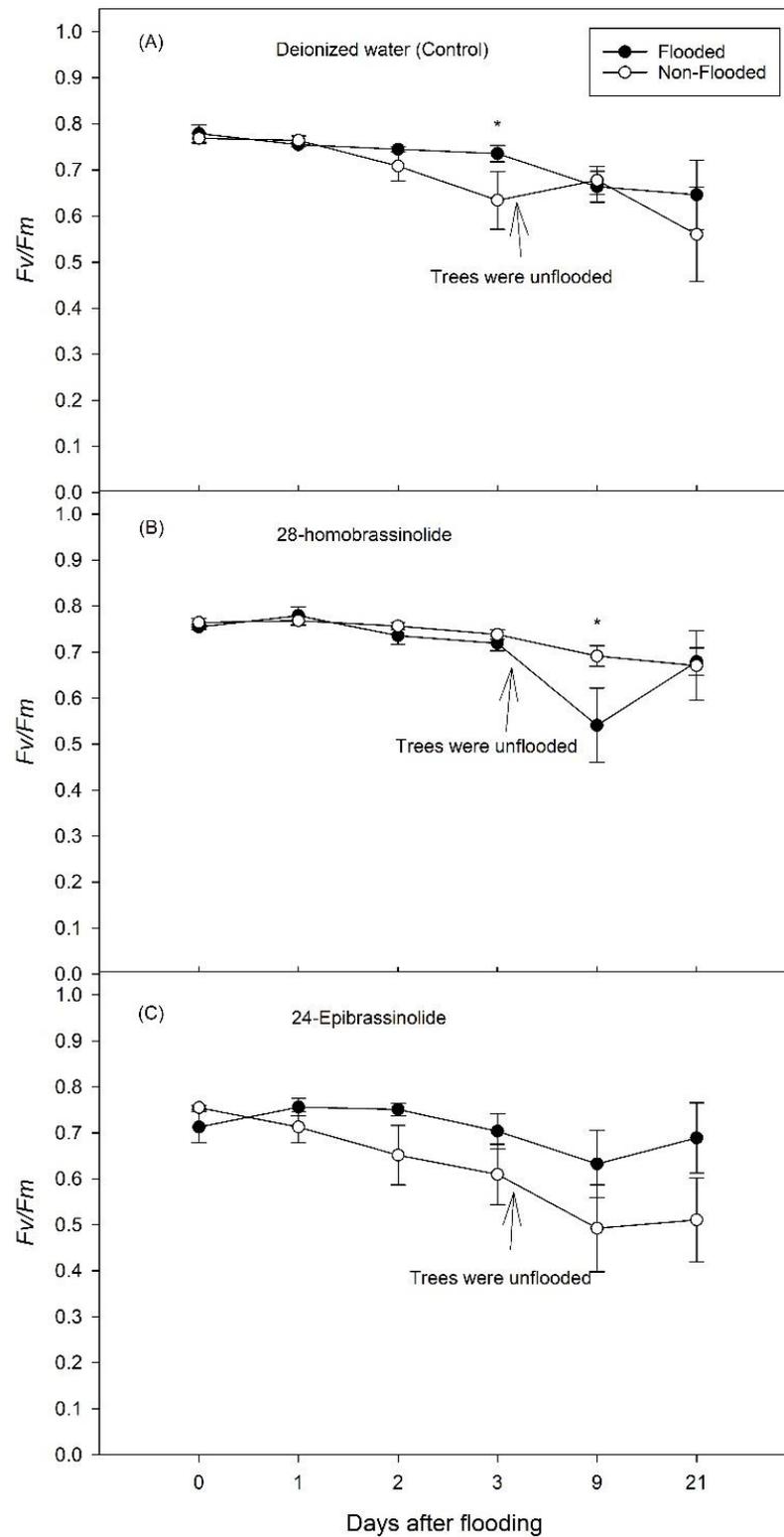


Figure 10. Effect of flooding on the maximum potential quantum efficiency of photosystem II (F_v/F_m) of 'Reed' avocado trees treated with (A) deionized water as a control, (B) 28-homobrassinolide, or (C) 24-epibrassinolide, (Experiment 2). Symbols and bars indicate means \pm std. err. Significant differences ($p \leq 0.05$) between flooding treatments are indicated with an asterisk and were determined by repeated-measures ANOVA. 'Trees were unflooded' indicates the day on which trees were removed from the flooding treatment to start the recovery period.

4. Discussion

Chemically priming avocado trees with 24-epibrassinolide as a soil drench prior to flooding, improved flood tolerance in both experiments. However, the effects were more pronounced in Experiment 1 than in Experiment 2. This may have been related to the cultivar used for each experiment. Seedling rootstocks are used in many avocado growing regions throughout the world [23]. Studies have indicated that the susceptibility of avocado trees to flooding is more related to the rootstock than to the scion [24]. There are three botanical races of avocado: The West Indian, Guatemalan, and Mexican [25,26]. All three races are sexually compatible, and hybridization occurs among them [26]. A Previous study has shown that cultivars of the Guatemalan race are more sensitive to flooding than cultivars of the West Indian or Mexican race are [27]. The Monroe cultivar used in Experiment 1 is a West Indian × Guatemalan hybrid [28], whereas the Reed cultivar used in Experiment 2 is a pure Guatemalan race cultivar [28,29]. Thus, the greater susceptibility of 'Reed' to flooding compared to 'Monroe' may have resulted in the 24-epibrassinolide treatment being less effective for mitigating flooding stress of the more flood-sensitive 'Reed' compared to 'Monroe'. Brassinosteroids are a class of plant growth regulators with a multitude of effects impacting plant growth, development, organ differentiation, reproduction, and abiotic stress tolerance mechanisms [30]. While the complexities of hormonal crosstalk between brassinosteroids and other plant growth regulators are still not completely known, brassinosteroids were implicated in the antioxidant defense systems of maize (*Zea mays*), rice (*Oryza sativa*), and many other agriculturally important plant species [17,29]. Both brassinosteroids, 28-homobrassinolide and 24-epibrassinolide, tested in this study were previously associated with improved tolerance to hypoxia; the exogenous applications of which are associated with upregulating antioxidant defense systems, including scavenging enzymes, osmolytes, and antioxidants [18–20]. The primary role of brassinosteroids in alleviating flooding stress has been attributed to decreased oxidative damage because of the increased activities of the antioxidants superoxide dismutase (SOD) and peroxidase (POD) [17]. Although we did not measure antioxidants in the present study, in a previous study, where we measured antioxidant activity in avocado trees in response to flooding, principal component analyses showed that flooded trees of the Guatemalan race clustered separately from Mexican × Guatemalan hybrid trees, which was primarily influenced by several antioxidants, including SOD and POD [27]. Thus, differences in the intensity of the response to 24-epibrassinolide observed between the Guatemalan × Mexican hybrid cultivar Monroe used in Experiment 1 and the pure Guatemalan cultivar Reed used in Experiment 2 may have been related to varying effects of 24-epibrassinolide on antioxidant activity among avocado genotypes.

The performance of 'Monroe' trees primed with 24-epibrassinolide was improved overall, with generally no decrease in leaf gas exchange and a 100% survival rate of flooded trees. The leaf gas exchange and F_v/F_m of 'Reed' trees treated with 28-homobrassinolide recovered after plants were unflooded, with a detrimental or neutral effect observed with the application of 24-epibrassinolide. However, the leaf gas exchange values for all trees in Experiment 2 were relatively low in general, hindering assessing whether the plants had recovered.

Previously, 28-homobrassinolide was shown to mitigate cold, drought, heat, and salinity stresses in agronomic crops [31,32]. Our previous studies indicated that the upregulation and increased expression of enzymes and ROS-mitigating factors may be related to survival and recovery from short-term flooding stress in avocados [26]. Future studies should investigate ROS activity, and the antioxidant capacity of 24-epibrassinolide-treated trees should be evaluated. Though the results of this study are promising, additional studies with additional cultivars may identify the true survival rate for select seedling types and brassinosteroid combinations when exposed to short-term flooding and may be useful for the anticipatory mitigation of flooding events.

5. Conclusions

The pretreatment of avocado trees with 24-epibrassinolide as a soil drench prior to flooding reduced the flooding stress of avocado trees. In two experiments, each with a different cultivar, treatment with 24-epibrassinolide reduced the stress of flooded plants, as indicated by no significant differences in leaf gas exchange (A , g_s , Tr , WUE) and maximal potential quantum efficiency of Photosystem II (Fv/Fm) between flooded and nonflooded plants, whereas flooding reduced these variables in the control (deionized water) and the 28-homobrassinolide treatments. In both experiments, the survival of flooded plants was greater in the 24-epibrassinolide treatment compared to the control or 28-homobrassinolide treatments. Although treatment with 24-epibrassinolide reduced flooding stress and increased survival in both experiments, the effects were more pronounced for ‘Monroe’ than for ‘Reed’. Thus, the effects of priming plants with 24-epibrassinolide on mitigating flooding stress may be related to the relative flooding sensitivity of different avocado cultivars.

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References

- Ploetz, R.C.; Schaffer, B. Effects of flooding and Phytophthora root rot on net gas exchange and growth of avocado. *Phytopathology* **1989**, *79*, 204–208. [[CrossRef](#)]
- Sanclemente, M.A.; Schaffer, B.; Gil, P.M.; Davies, F.S.; Crane, J.H. Leaf removal before flooding influences recovery of avocado (*Persea americana* Mill.) trees from flooding stress. *Sci. Hortic.* **2013**, *150*, 154–163. [[CrossRef](#)]
- Sanclemente, M.A.; Schaffer, B.; Gil, P.M.; Vargas, A.I.; Davies, F.S. Pruning after flooding hastens recovery of flood-stressed avocado (*Persea americana* Mill.) trees. *Sci. Hortic.* **2014**, *169*, 27–35. [[CrossRef](#)]
- Schaffer, B.; Andersen, P.C.; Ploetz, R.C. Responses of fruit crops to flooding. *Hortic. Rev.* **1992**, *13*, 257–313.
- Schaffer, B. Flooding responses and water-use efficiency of subtropical and tropical fruit trees in an environmentally-sensitive wetland. *Ann. Bot.* **1998**, *81*, 475–481. [[CrossRef](#)]
- National Academies of Sciences, Engineering, and Medicine. *Attribution of Extreme Weather Events in the Context of Climate Change*; The National Academies Press: Washington, DC, USA, 2016. [[CrossRef](#)]
- Her, Y.; Boote, K.; Migliaccio, K.W.; Fraisse, C.; Letson, D.; Mbuya, O.; Swamy, A.A.; Chi, H.; Ngatia, L.L.; Asseng, S. Climate change impacts and adaptation in Florida’s agriculture. In *Florida’s Climate: Changes, Variations, and Impacts*; Chassignet, E.P., Jones, J.W., Misra, V., Obeyesekera, J., Eds.; Florida Climate Institute, CreateSpace Independent Publishing: Gainesville, FL, USA, 2017; pp. 235–267.
- Bruce, T.J.; Matthes, M.C.; Napier, J.A.; Pickett, J.A. Stressful “memories” of plants: Evidence and possible mechanisms. *Plant Sci.* **2007**, *173*, 603–608. [[CrossRef](#)]
- Vincent, C.I.; Rowland, D.; Schaffer, B.; Bassil, E.; Racette, K.; Zurweller, B. Primed acclimation: A physiological process offers a strategy for more resilient and irrigation-efficient crop production. *Plant Sci.* **2019**, *295*, 110240. [[CrossRef](#)] [[PubMed](#)]
- Baldwin, I.T.; Halitschke, R.; Paschold, A.; Von Dahl, C.C.; Preston, C.A. Volatile signaling in plant-plant interactions: “talking trees” in the genomics era. *Science* **2006**, *311*, 812–815. [[CrossRef](#)] [[PubMed](#)]
- Jakab, G.; Ton, J.; Flors, V.; Zimmerli, L.; Métraux, J.-P.; Mauch-Mani, B. Enhancing Arabidopsis salt and drought stress tolerance by chemical priming for its abscisic acid responses. *Plant Physiol.* **2005**, *139*, 267–274. [[CrossRef](#)] [[PubMed](#)]
- Rowland, D.L.; Faircloth, W.H.; Payton, P.; Tissue, D.T.; Ferrell, J.A.; Sorensen, R.B.; Butts, C.L. Primed acclimation of cultivated peanut (*Arachis hypogaea* L.) through the use of deficit irrigation timed to crop developmental periods. *Agric. Water Manag.* **2012**, *113*, 85–95. [[CrossRef](#)]

13. Teale, W.; Paponov, D.I.A.; Palme, K. Auxin in action: Signaling, transport and the control of plant growth and development. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 847–859. [[CrossRef](#)] [[PubMed](#)]
14. Depuydt, S.; Hardtke, C.S. Hormone signaling crosstalk in plant growth regulation. *Curr. Biol.* **2011**, *21*, R365–R373. [[CrossRef](#)]
15. Santner, A.M.; Estelle, M. Recent advances and emerging trends in plant hormone signaling. *Nature* **2009**, *459*, 1071–1078. [[CrossRef](#)] [[PubMed](#)]
16. Peres, A.L.; Soares, G.J.S.; Tavares, R.G.; Righetto, G.; Zullo, M.A.; Mandava, N.B.; Menossi, M. Brassinosteroids, the sixth class of phytohormones: A molecular view from the discovery to hormonal interactions in plant development and stress adaptation. *Int. J. Mol. Sci.* **2019**, *20*, 331. [[CrossRef](#)] [[PubMed](#)]
17. Vardhini, B.V.; Anjum, N.A. Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. *Front. Environ. Sci.* **2015**, *2*, 67. [[CrossRef](#)]
18. Lu, X.; Chen, Y.; Gong, W.; Chen, Y. Effect of brassinolide on the seedling growth and waterlogging resistance of soybean. *Chin. Agric. Sci. Bull.* **2006**, *23*, 37–38.
19. Kang, Y.-Y.; Guo, S.-R.; Li, J.; Duan, J.-J. Effect of root applied 24-24-epibrassinolide on carbohydrate status and fermentative enzyme activities in cucumber (*Cucumis sativus* L.) seedlings under hypoxia. *Plant Growth Reg.* **2009**, *57*, 259–269. [[CrossRef](#)]
20. Lu, X.; Guo, S. Effects of brassinolide on the polyamines, ATPase activity, and inorganic ion content in roots of cucumber seedlings under hypoxia stress. *Chin. J. Ecol.* **2013**, *32*, 611–614.
21. Lin, S.-Y.; Chen, P.-A.; Zhuang, B.-W. The stomatal conductance and Fv/Fm as the indicators of stress tolerance of avocado seedlings under short-term waterlogging. *Agronomy* **2022**, *12*, 1804. [[CrossRef](#)]
22. Ikekawa, N.; Zhao, Y.-J. Application of 24-24-epibrassinolide in agriculture. In *Brassinosteroids, Chemistry, Bioactivity, and Applications*; Cutler, H.G., Yokota, T., Adam, G., Eds.; ACS Symposium Series 474; American Chemical Society: Washington, DC, USA, 1991; pp. 280–291.
23. Crane, J.H.; Douhan, D.; Faber, B.A.; Arpaia, M.L.; Bender, G.S.; Balerdi, C.F.; Barrientos-Priego, A.F. Cultivars and rootstocks. In *The Avocado: Botany Production and Uses*; Schaffer, B., Wolstenholme, B.N., Whiley, A.W., Eds.; CABI Press: Waddington, UK, 2013; pp. 200–233.
24. Schaffer, B.; Gil, P.M.; Mickelbart, M.V.; Whiley, A.W. Ecophysiology. In *The Avocado: Botany, Production and Uses*; Schaffer, B., Wolstenholme, B.N., Whiley, A.W., Eds.; CABI Press: Waddington, UK, 2013; pp. 168–199.
25. Bergh, B.; Ellstrand, N. Taxonomy of the avocado. *Calif. Avocado Soc. Yearb.* **1986**, *70*, 135–145.
26. Chandrabali, A.; Soltis, D.; Soltis, P.; Wolstenholme, B. Taxonomy and botany. In *The Avocado: Botany Production and Uses*; Schaffer, B., Wolstenholme, B.N., Whiley, A.W., Eds.; CABI Press: Waddington, UK, 2013; pp. 31–50.
27. Yin, M.H. Physiological and Biochemical Responses of Avocado to Short-Term Flooding: Effects of Ecotype and Brassinosteroid Treatments. Ph.D. Thesis, University of Florida, Gainesville, FL, USA, 2022.
28. Schnell, R.J.; Brown, J.S.; Olano, C.T.; Power, E.J.; Krol, C.A. Evaluation of avocado germplasm using microsatellite markers. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 881–889. [[CrossRef](#)]
29. Ge, Y.; Zhang, T.; Wu, B.; Tan, L.; Ma, F.; Zou, M.; Chen, H.; Pei, J.; Liu, Y.; Chen, Z.; et al. Genome-wide assessment of avocado germplasm determined from specific length amplified fragment sequencing and transcriptomes: Population structure, genetic diversity, identification, and application of race-specific markers. *Genes* **2019**, *10*, 215. [[CrossRef](#)] [[PubMed](#)]
30. Vardhini, B.V. Modifications of morphological and anatomical characteristics of plants by application of brassinosteroids under various abiotic stress conditions—A review. *Plant Gene* **2017**, *11*, 70–89. [[CrossRef](#)]
31. Arora, N.; Bhardwaj, R.; Sharma, P.; Arora, H.K. Effects of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. *Acta Physiol. Plant.* **2008**, *30*, 833–839. [[CrossRef](#)]
32. Kaur, H.; Sirhindi, G.; Bhardwaj, R.; Alyemeni, M.; Siddique, K.H.; Ahmad, P. 28-homobrassinolide regulates antioxidant enzyme activities and gene expression in response to salt-and temperature-induced oxidative stress in *Brassica juncea*. *Sci. Rep.* **2018**, *8*, 8735. [[CrossRef](#)] [[PubMed](#)]