Growth Regulators

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Effects of Plant Growth Regulators on Growth and Reproduction of *Humulus lupulus*

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Index Words: Plant growth regulator (PGR), Sumagic, *Humulus lupulus* 'Cascade', hydroponic table.

Significance to Industry: Hops are one of the four main ingredients used in beer production. Over ninety-four million pounds of hops (including 'Cascade') were produced in the United States during the 2009 growing season. The hop cultivar Cascade selected for this study is grown primarily in Idaho, Oregon, and Washington State where environmental conditions of the Northwestern U.S. are most favorable for its production. The use of plant growth regulators (PGR) in greenhouse for hop production makes it possible to control their height along with conditions that can be manipulated in greenhouses such as temperature and light to best suit the exact needs of a specific cultivar. Hydroponics technology allows for precise nutrient formulation to be applied to plants in increments or continuously to satisfy the requirement of a specific cultivar. Thus, combining hydroponics and the proper application of PGR allows for a northern cultivar to be grown anywhere. For example, nurseries in Tennessee that do not use their greenhouses during the summer months could use them to grow hops by using the techniques described in this paper to supplement income.

Nature of Work: *Humulus lupulus* 'Cascade' is an herbaceous perennial grown annually in the Northwest under field conditions. In 2009 over two thousand acres were planted in Washington State and Oregon (1). Applications of PGRs have only been recommended for use in greenhouse production of vegetable and ornamental crops. A previous evaluation of PGRs on hops indicated that uniconazol (Sumagic) suppressed hop vine growth better than A- rest, B-9, and Cycocell (C. Rowland, Unpublished data). The objective of this study was to reduce the height of "Cascade" hop vines to allow it to be grown under greenhouse conditions using PGR while maintaining adequate yield.

Randomized complete block design was used in this experiment. There were four blocks each was a 4'x 8' hydroponic table. Each table was connected to a reservoir containing 170 gallons of nutrient solution. The solutions were maintained at 1000 ppm, (total concentration for all elements in the solution) and a pH at 6.0. All reservoirs contained the same fertilizer solution. Cuttings were taken from large 'Cascade' mother plants and rooted in the same nutrient solution. PGR treatments were Sumagic applied at 0, 0.62, 1.25, 2.5, and 5 ppm. A total of 16 cuttings were randomly chosen from a group of plants for each treatment. Sumagic was applied with a hand sprayer.

Treatments were applied using a hand sprayer. Two applications were applied prior to flowering. The first application was applied two weeks after rooting on May 10, 2010. The second application was applied sixteen days following the first application after internodes on vines were measured. A trellis system was used to support the vines. When cones were matured, all vines were harvested and evaluated for length and fresh weight yield. The total length measurements were done after harvesting the vine using a standard 30' carpenter's tape.

Results and Discussion: Sumagic was effective in controlling the height of 'Cascade'. The most effective concentration was the 5 ppm that reduced vine height without affecting the mean yield of the crop. The average height of vines treated with 5 ppm Sumagic was 165.525 inches (Graph 1). However, the mean yield was slightly less than that of the control. Plants treated with 2.5 ppm Sumagic were slightly taller than plants treated with 5 ppm and had a slightly lower mean yield than the control treatment (Fig 2).

In summary, plants that were treated with the highest concentration of Sumagic had the shortest lateral branching length. Shorter lateral growths made it easier to harvest and manage the vines during the experiment. By using PGR, up to three vines could be grown in the space of one vine without PGR. More vines in production would result in increase yield.

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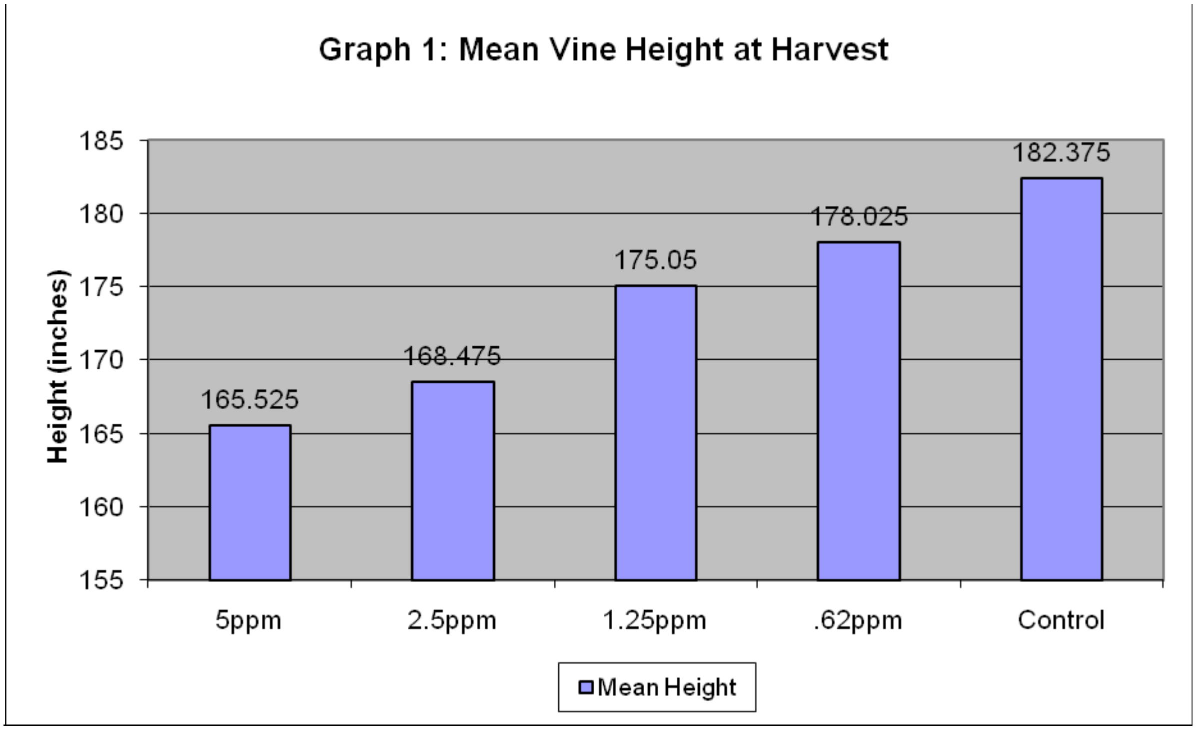

Fig.1. Mean height of hop vines treated with Sumagic at various concentrations.

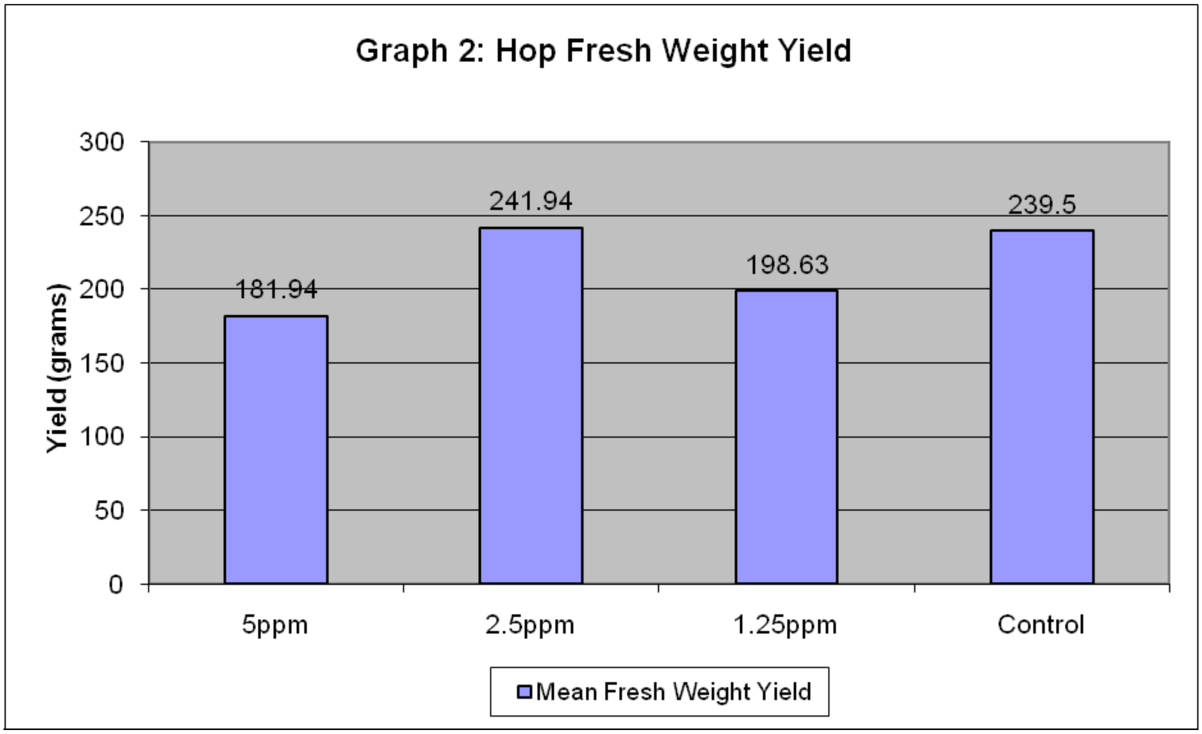

Fig. 2. Hop yield (as fresh weight) of vines treated with various concentrations of Sumagic.

Unexplained Wilting of Tomatoes after Exposure to Large Doses of Exogenous Abscisic Acid (ABA)

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Index Words: hydraulic conductance, stomatal conductance, transpiration

Significance to Industry: It is common for plants in the retail market to receive inadequate water and lose aesthetic value within a short period of time. The plant hormone abscisic acid (ABA) is a natural growth regulator that has recently presented itself as a commercially feasible and effective method to help plants maintain their salability longer. However, it has the potential to impart negative side effects. One of the more peculiar side effects is wilting that may occur when plants are given high doses of ABA, even though the substrate is wet. This study examined the physiological effects of ABA on tomato (*Solanum lycopersicum*). We found that ABA reduces the hydraulic conductance of root systems, but not of the stems. We also found that the relative water content of leaves is not affected by ABA, even if those leaves exhibit wilting symptoms. Although the ABA effect on roots suggest that the cause of the wilting symptoms is related to root functionality, the finding that wilted leaves still have a high water content casts doubt on this theory. Since wilting symptoms generally do not appear when ABA is applied as a spray, that application method allows growers to get the benefits of ABA without the risk of wilting symptoms.

Nature of Work: Inadequate watering rapidly diminishes a plant's salability and drastically shortens its shelf life (1). The hormone ABA has the potential to act as a transpiration inhibitor and extend the shelf life of plants during retail. Normally, ABA is produced by plants in response to drought conditions, accumulating in the leaves and causing the guard cells to respond by either closing stomates or preventing them from opening (5, 6), thus reducing transpiration (4). The effects of exogenous ABA applications on shoots have been well-documented. However, comparatively little work has been done to evaluate the effects of high concentrations of ABA on roots and stems. Previous research has reported contradictory results. Hose et al (3) examined excised cells of maize with cell pressure probes and concluded that ABA transiently increased hydraulic conductance of roots for a few hours by stimulating water channels (aquaporins) in the cell membranes. Conversely, earlier work indicated that ABA limits root hydraulic conductance as a means of protecting the membranes from cold stress damage (7).

Recent studies have demonstrated that an unexplained wilting can occur when certain plants are drenched with high concentrations of exogenous ABA (2). The plants will begin to wilt, even though the substrate is still moist. The cause of this wilting is not understood, but may be related to an inhibition of water transportation to the leaves.

The objectives of this study were to evaluate the effects of various ABA rates on the hydraulic conductance of stems and roots and to gather preliminary information on leaf physiology in an attempt to elucidate the cause of the unexplained wilting. Tomatoes were used as the model crop because they are sensitive to ABA applications and wilt rapidly when exposed to high ABA concentrations.

Study 1: Root and stem conductance. Tomatoes ‘Supersweet 100’ were seeded in 72-cell trays. Following germination, plants were transplanted into 4” round pots filled with soilless substrate (Fafard 2P, Conrad Fafard Inc., Agawam, MA) and grown under an overhead irrigation system. The plants each received 2.6 grams of Osmocote 14-14-14 (Scotts, Marysville, OH).

An ABA stock solution (10% w/v s-ABA, the biologically-active form of ABA, VBC-30101, Valent BioSciences, Long Grove, IL) was diluted with deionized water to yield concentrations of 0, 62.5, 125, 250, 500, and 1000 ppm. At the commencement of the experiment, the pots were well-watered. Shoots were cut off approximately 5-6 cm above the substrate surface, and then about 4 cm long stem sections were cut off from the remaining shoots. The stem sections and root systems were then inserted into plastic tubes connected to a vacuum pump which served to simulate the effects of transpirational pull (Fig. 1). The stems and root systems were sealed to the inside of the tubing with a silicone gel (3140 RTV coating, Dow Corning, Midland, MI) and then wrapped with Parafilm to prevent leaks. The base of the stem sections was placed in beakers with 100 ml of deionized water. Once the pump was turned on, initial readings of water movement through the root systems and stem sections were taken prior to ABA application to determine an untreated conductance baseline. All ABA applications to the root systems were made as a drench, with each pot receiving 100 ml of solution. For the stem sections, the deionized water in the beakers was replaced with 100 ml of ABA solution. The water levels in the tubes were marked at various times, and these marks were used to determine the cumulative water flow through the roots or stems. At the end of the study, the root systems were washed off and examined for any detrimental side effects that may have been caused by ABA. The experiment was a randomized complete block design with a split plot. The main treatment factor was the ABA concentration (6 rates) and the split consisted of root systems versus stem sections. Each treatment was replicated twice and individual root systems or stem sections were the experimental unit. The study was conducted twice, and both trials returned similar results. Data were analyzed using regression analysis.

Study 2: Leaf water relations. Tomatoes were seeded and grown as described above. Two plants were drenched with 100 mL of 2,000 ppm ABA, while two other plants served as controls. This concentration was chosen because it quickly imparts the wilting symptoms in the plants. Plants were placed in a growth chamber (21 °C, photosynthetic photon flux: 406 $\mu mol \cdot m^{-2} \cdot s^{-1}$) approximately 1 day after ABA application. Leaf gas exchange (photosynthesis, transpiration, stomatal conductance) was then measured with a leaf photosynthesis system (Ciras-2, PP Systems, Amesbury, MA) every 5 minutes for a 4 hour period. Data from this 4-hour period were averaged.

Leaf discs were taken from the plants at the end of the leaf gas exchange study. A cork borer was used to sample leaf discs ½" in diameter. The fresh weight of the discs was recorded, after which the discs were floated on deionized water in Petri dishes overnight. The discs were then blotted dry with filter paper and their turgid weight was recorded. Finally, the discs were oven dried at 80 °C and their dry weights were recorded. Relative water content was determined as (fresh weight – dry weight) / (turgid weight – dry weight) × 100 %. Two replications were used for this initial study. The experimental unit was an individual plant. Additionally, one plant was treated with ABA 30 minutes after the leaf photosynthesis measurements were started. Gas exchange was measured every 5 minutes, and the objective was to determine how quickly the stomates closed following ABA application.

Results and Discussion: As ABA concentration increased, cumulative water flow through the roots decreased (Fig. 2, $P = 0.00001$), indicating a decrease in root conductance. Treatment effects were obvious within 12 hours. These findings corroborate our previous study that showed an ABA-induced reduction in the hydraulic conductance of root systems (2). At the termination of the experiment, the cumulative flow through roots of the control plants was 27.4 mL, whereas the cumulative flow of plants treated with 1,000 ppm ABA was only 4.8 mL, a reduction of 83 %. There were no visible symptoms of ABA-induced side effects on the appearance of the roots. Unlike the root systems, the stem sections were unaffected by the ABA (Fig. 3, $P = 0.26$). Cumulative water flow through the stem sections was between 29.6 and 36.5 mL, with no trend of an ABA-related effect. By the termination of the experiment, some of the stem sections were beginning to break down and decompose. The combination of the reduction of root conductance without an ABA effect on stem conductance suggests that the cause of the ABA-induced unexplained wilting may be in the root system.

Results from the preliminary leaf physiology study yielded promising data. One day after the application of 2000 ppm ABA, plants showed wilting symptoms, as well as mild chlorosis and abscission of lower leaves. The results show a clear difference between the ABA-treated and control plants regarding stomatal conductance, transpiration, and photosynthesis (Table 1). The control plants averaged a transpiration rate of 1.97 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas the ABA treatments averaged 0.27 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 86% less. This effect on transpiration is related to changes in stomatal conductance, with the control plants averaging 121.8 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, compared to 12.6 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the ABA-treated plants, a 90% reduction. Net photosynthesis of ABA-treated plants was negative, indicating that respiration exceeded photosynthesis. The ABA is clearly causing the stomates of the plants to close tightly, thus limiting transpiration and photosynthetic rates.

There were no changes in the relative water content of the leaf discs as a result of ABA applications. Leaves of ABA-treated plants had a relative water content of 75.2%, compared to 77.3% in control leaves. Because the relative water content was similar in both treatments, the wilting of ABA treated plants does not seem to be caused by a decrease in leaf water content. Interestingly, the fresh and turgid weights of the leaf discs from the ABA treatment were approximately 30% greater than those of the controls (results not shown), indicating that ABA-treated leaves contained more water. However, the wilting symptoms were apparent in ABA-treated plants, suggesting that their turgor pressure was reduced. This could indicate that the water in the leaves of ABA-treated plants was not contained within cells, pointing to a potential decrease in cell membrane integrity.

Results from the study where a plant was treated 30 minutes after the start of the gas exchange measurements show that ABA reduced stomatal conductance quickly, within 15 minutes after application (Fig. 4). Although this research suggests that reductions in root conductance may hinder water transportation to the shoots, our results also suggest that the unexplained wilting may not be caused by a lack of water in the leaves. To confirm these ABA effects on leaf physiology, a full scale study will be conducted. Further studies will need to look at the relationship between exposure to high levels of exogenous ABA and its effect on cell membrane integrity to determine whether or not this has contributed to the unexplained wilting being noticed in past research.

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Table 1: Transpiration rate, stomatal conductance, and photosynthetic rate for control plants and plants drenched with 1200 ml of a 2,000 ppm ABA solution. Values represent the mean ± sd (n=2).

Treatment	Transpiration rate ($mmol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)	Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
ABA treatment	0.27 ± 0.05	12.6 ± 3.2	-0.48 ± 0.20
Control	1.96 ± 1.45	121.8 ± 94.3	4.3 ± 4.1

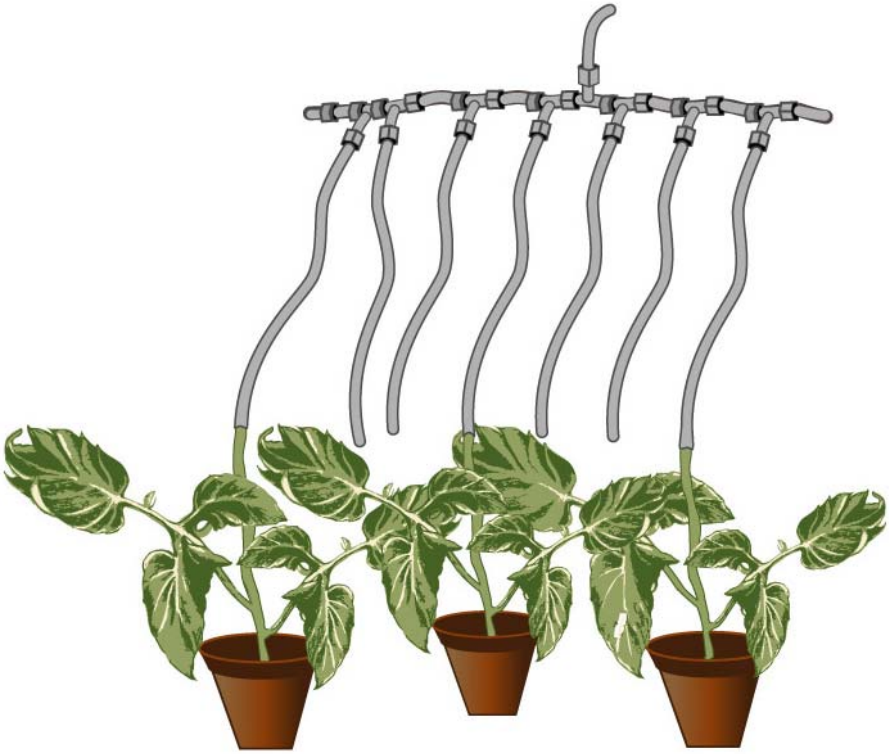

Figure 1: An image of the tube and vacuum system used to simulate the effects of transpirational pull on stem sections and root systems. Note: all leaves were removed for the experiment and the vacuum pump is not shown.

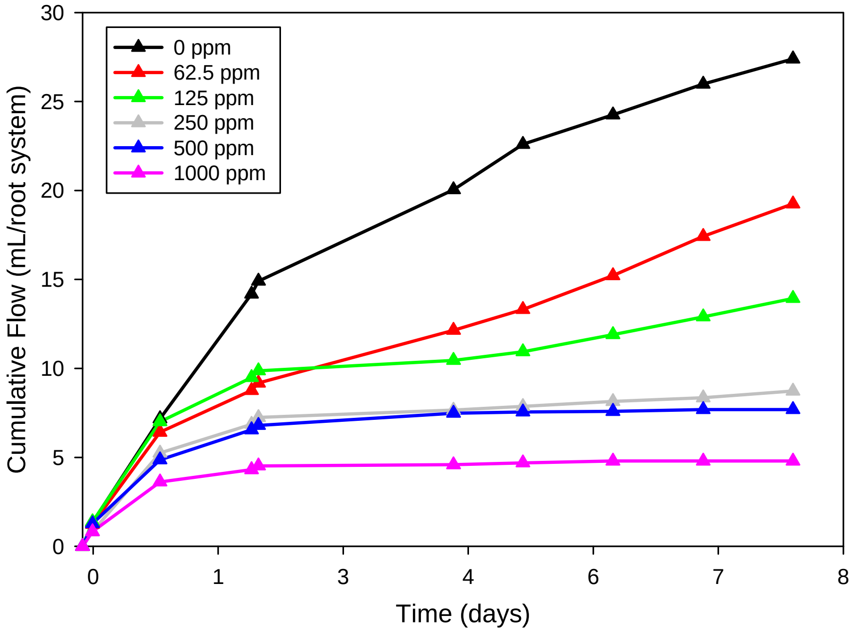

Figure 2: Cumulative water flow through decapitated root systems during an eight day period following drenches with various ABA concentrations. Flow was reduced by ABA (*P* = 0.00001).

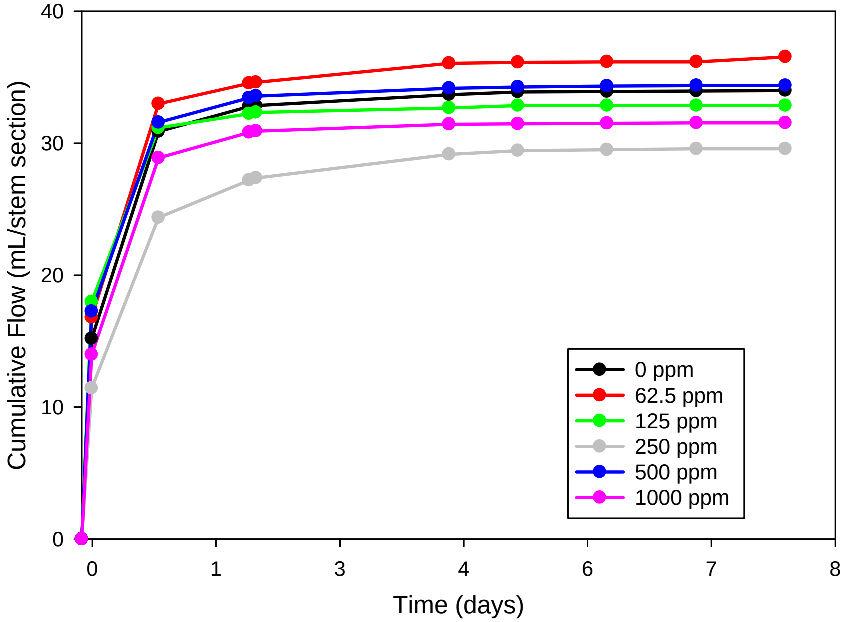

Figure 3: Cumulative water flow through stem sections during an eight day period following drenches with various ABA concentrations. There was no treatment effect (*P* = 0.26).

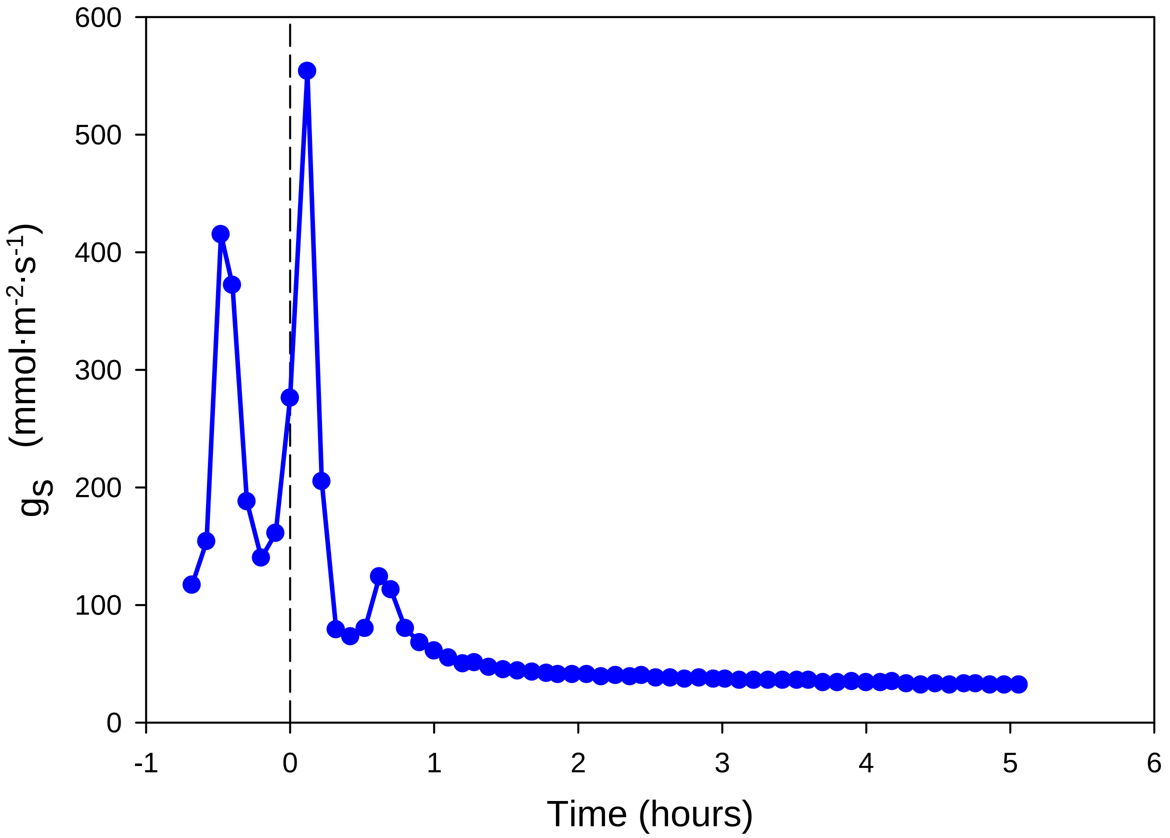

Figure 4: Stomatal conductance (g_s) over time for a plant drenched with 100 ml of 2,000 ppm ABA solution. The ABA was applied at time 0.