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Uptake, Movement, Activity, and Persistence of an Abscisic Acid Analog (8' Acetylene ABA Methyl Ester) in Marigold and Tomato

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ABSTRACT The abscisic acid (ABA) analog 8' acetylene ABA methyl ester (PBI 429) was evaluated for its potential to alter the growth and moisture use of bedding plants during nursery production. Treating seedlings with the ABA analog as a root-dip slowed moisture use and growth of tomato seedlings under greenhouse conditions. In marigolds, comparable ABA analog treatments had no effect on growth and limited effects on plant moisture use. To determine whether these differences in response to treatment with the ABA analog were associated with differences in absorption of the analog and/or its persistence, the ABA analog was applied either as a foliar spray or root-dip, and the resulting concentrations of the ABA analog were monitored over a 10-day interval in both the roots and the leaves. In both crops, the ABA analog was detected in both leaf and root tissues irrespective of the mode of application, suggesting systemic movement of the analog. Tissue concentrations of the ABA analog were consistently lower in the foliar treatment than in the root-dip. The uptake and the retention of the ABA analog over time was similar in leaves of the two test crops, but less of the ABA analog was absorbed and retained in the roots of marigold plants than in the tomatoes. This suggests that the observed differences in responses of these two plant species to application of ABA analogs may be related to differences in retention or accumulation of ABA in the roots rather than to differences in the total amount of ABA analog absorbed or its movement and retention in the plant system. Levels of endogenous ABA were not significantly altered by application of the ABA analog.

Keywords *Lycopersicon esculentum* - *Tagetes petula* - Moisture use - Foliar spray - Root-Dip - Racemic ABA

Meeting the crops' moisture requirements is one of the major maintenance costs incurred during the production, handling, and marketing of nursery plants. The marketing period of nursery-raised seedlings is also limited because of the loss of aesthetic quality associated with undesirable growth and/or excess moisture loss during storage and handling. Plant growth retardants like paclobutrazol, uniconazole, and ancymidol are commonly applied to container-grown ornamental and nursery plants to control plant growth, slow stem elongation, and regulate moisture use, thereby producing more compact, easily maintained, marketable plants (*Gibson and Whipker 2000*; *McDonald and Arnold 2001*; *Whipker and Ingram 2000*; *Whipker and others 2000*). However, these chemicals tend to have long-term negative effects on plant growth and development (*McDonald and Arnold 2001*) and are not registered for use in vegetable crops (*Cantliffe 1993*; *Latimer 1991*)

The phytohormone abscisic acid (ABA) is involved in a range of physiological processes including plant growth and stress physiology (*Addicott 1983*; *Giraudat and others 1994*; *Levitt 1980*). Endogenous ABA levels regulate water loss from the leaves, mainly by regulating the stomatal aperture (*Walton 1980*). Exogenous application of ABA can potentially protect crops from environmental stresses such as drought, chilling, salt, and heat (*Abrams 1999*). Abscisic acid treatments have proven useful in reducing transplant shock in spruce seedlings (*Grossnickle and others 1996*), chilling damage in cucumber seedlings (*Wang 1990*), and freezing damage in *Brassica napus* and *Brassica compestris* seedlings (*Wilen and others 1994*).

Abscisic acid–induced retardation of stomatal gaseous exchange reduces photosynthesis and plant growth (*Arteca and Tsai 1987*; *Kramer 1988*; *Salisbury and Ross 1992*). Foliar-applied ABA significantly decreased stomatal conductance, transpiration rate, and net photosynthesis in potato (*Baricevic and Stopar 1994*). Root-applied ABA inhibited root and shoot growth of sunflower seedlings (*Lenzi and others 1995*). Abscisic acid applied to the rooting medium of intact, hydroponically grown tomato seedlings also inhibited root growth (*Griffiths and others 1997*). Foliar application of ABA decreased transpiration and wilting of pot-grown tomato and cucumber seedlings and also reduced plant growth (*Yamazaki and others 1995*). *Herde and others (1997)* introduced ABA into the transpiration stream of tomato plants and observed that the ABA treatments reduced both transpiration and the net CO_2 assimilation rate. *Leskovar and Cantliffe (1992)* observed that a foliar application of ABA at 10^{-4} M, four weeks after seeding reduced leaf growth, root dry weight, and basal root count of pepper (*Capsicum annuum*) seedlings. The ABA treatments did not influence the subsequent growth of the transplants or total fruit yields. They suggested that exogenous application of ABA might represent a substitute for drought stress to control transplant growth in the nursery.

Agricultural use of ABA is limited due to its poor stability in solution and its rapid deactivation by photoisomerization and/or by metabolism (*Abrams 1999*; *Flores and Dorflling 1990*). Analogs of ABA have been developed that mimic its effects but the analogs are more resistant to degradation and are more rapidly absorbed than ABA itself (*Abrams and others 1997*; *Flores and Dorflling 1990*; *Jung and Grossmann 1985*; *Walton 1983*). A terpenoid analog of ABA (LAB 173 711) was more effective than ABA as a means of improving chilling resistance of rice seedlings (*Flores and others 1993*). *Todoroki and others (1995)*reported that fluorinated ABA analogs (8', 8'-difluoroabscisic acid and 8', 8', 8'-trifluoroabscisic acid) inhibited the elongation of rice seedlings 6 and 30 times more strongly, respectively, than ABA. The ABA analog, 8'methylene ABA was superior to ABA in inhibiting growth of suspension-cultured corn cells, and in reducing transpiration in wheat seedlings (*Abrams and others 1997*). The ABA analog, 8' acetylene ABA was also more persistent than racemic ABA in inhibiting growth of suspension-cultured corn cells (*Cutler and others 2000*).

Greenhouse trials with bedding plant seedlings of various horticultural crops have suggested that foliar or root-zone applications of the ABA analog 8' acetylene ABA methyl ester (developed by PBI/NRC, Saskatoon, Saskatchewan, Canada; Figure 1) have the potential to reduce moisture use and temporarily suppress plant growth during nursery production (*Sharma 2002*, *Waterer 2000*). However, the effects of ABA analog treatments varied with the concentration, mode of application (foliar or root-dip), and crop species (*Sharma 2002*, *Waterer 2000*). Marigold appeared to be less responsive to ABA analog treatments than tomato, snapdragon, and nasturtium (*Sharma 2002*). Similarly, foliar applications of the ABA analog were less effective than root-dip treatments in terms of both the intensity and the duration of efficacy (*Sharma 2002*). The total amount of chemical delivered by foliar application is limited by the moisture-retention characteristics of the leaves (surface area and other characteristics), which, vary with crop species. The volume of ABA analog solution retained by the leaves of tomato seedlings after foliar spray treatment was approximately 10 ml per plant. By comparison, when the analog solutions were applied as a root-dip, the medium associated with each seedling retained approximately 40 ml of solution containing the ABA analog (*Sharma 2002*). Although, root-applied treatments with ABA analogs were more effective than foliar-applied ones, the question arises as to whether the greater efficacy and persistence of root-applied ABA analog reflects better uptake/absorption and movement of the ABA analogs into the plants or simply reflects a dose effect. Studies regarding the mode of action of ABA analogs in plants and whether ABA analogs affect endogenous ABA synthesis or activity are lacking.

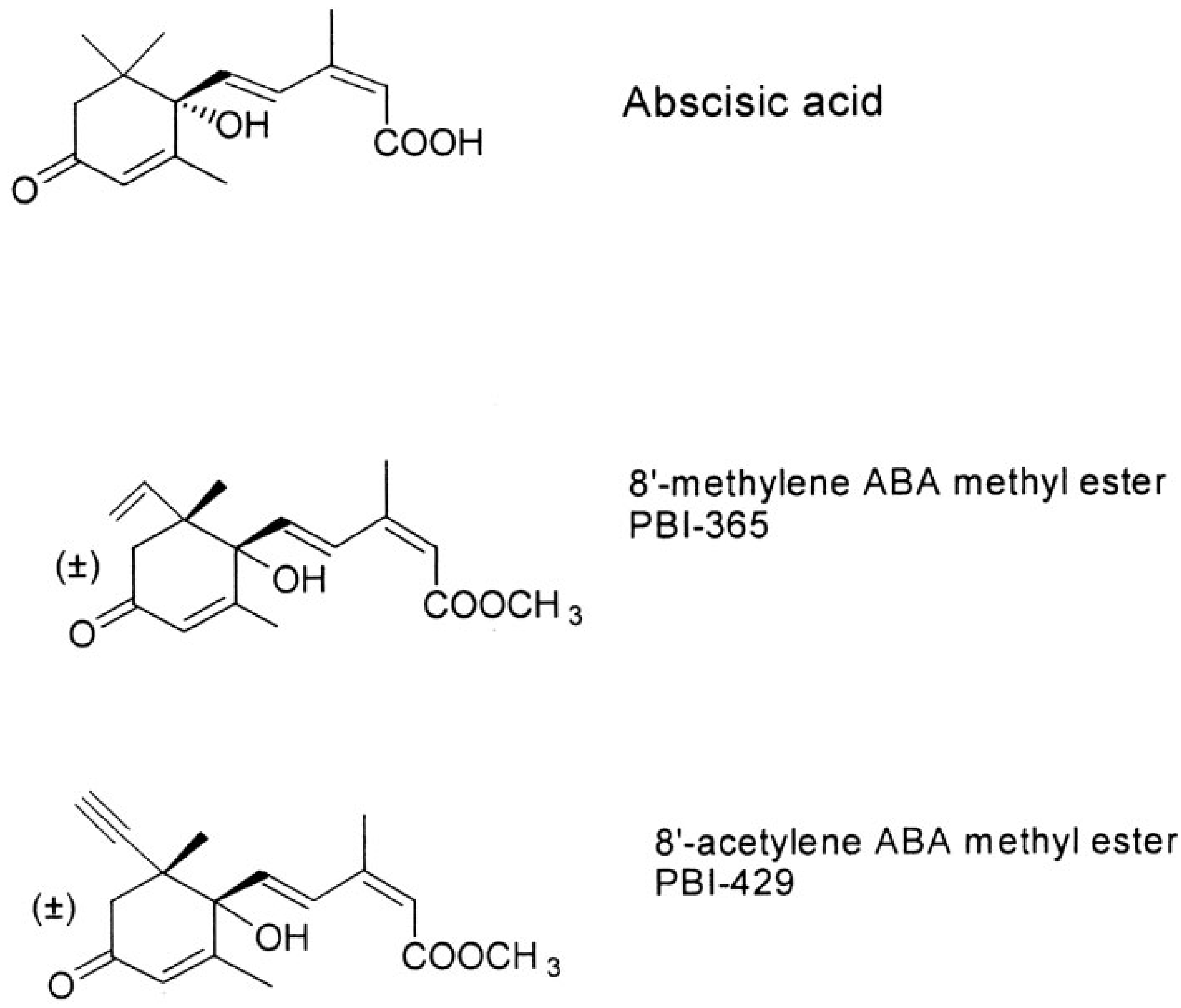

Figure 1 Chemical structures of abscisic acid (ABA) and two ABA analogs developed by Abrams and others (PBI/NRC, Saskatoon Canada).

This study (1) examined the impact of an ABA analog on growth and water use of seedlings of tomato and marigold, (2) studied the uptake, movement, and persistence of foliar and root-applied ABA analog, and (3) determined whether application of ABA analog influenced levels of endogenous ABA.

Materials and Methods

Tomato (*Lycopersicon esculentum* cv. Manitoba) and marigold (*Tagetes petula* cv. Sparky) seedlings were grown in a peat-based soil-less medium under standard greenhouse conditions. To evaluate the effects of the ABA analog on growth and moisture use of the seedlings, three concentrations (10^{-5} M, 5×10^{-5} M, and 10^{-4} M) of 8' acetylene ABA methyl ester (PBI 429) (*Abrams and others 1997*; *Rose and others 1997*) were applied to four-week-old seedlings as root-dip treatments. To study the uptake and movement of the ABA analog, four-week-old seedlings were treated with 10^{-4} M 8' acetylene ABA methyl ester solution either as a root-dip or as a foliar-spray. This concentration of this ABA analog had previously been demonstrated to reduce plant moisture use and plant growth (*Sharma 2002*). The chemical was first dissolved in 1% acetone and then diluted with the required volume of water. A 1% solution of acetone in water served as the control treatment. Following application of the ABA analog, the treatments were arranged in a randomized complete block design on the greenhouse bench, with four replicates. Each treatment replicate consisted of four plants.

Daily water use by the seedlings was determined by weighing each treatment replicate before watering and then subtracting that weight from the weight after watering to field capacity. The trials were terminated (1) when there was no difference between control and treated plants in terms of daily moisture use, suggesting that the effects of the ABA analog treatments had dissipated, or (2) once the control plants had grown to the point where their moisture needs could no longer be met by daily watering. At the termination of the trial, shoot and root fresh weights were measured.

Tissue samples (approx. 2 g) for measurement of levels of endogenous ABA and the ABA analog were collected from the second and third leaves from the top of the plant at 3, 7, and 10 days after treatment. Root samples were collected at the beginning (day 3 after treatment) and the end (day 10 after treatment) of the observation period. The roots were washed and dried with blotting paper before freezing. All leaf and root samples were frozen in liquid nitrogen and stored at - 20°C until analyzed.

Tissue concentrations of the ABA analog and endogenous ABA were determined by high pressure liquid chromatography coupled to electrospray ionization mass spectrometry/mass spectrometry (HPLC ESI-MS/MS) using a deuterated internal standard (*Ross and others 2004*). As HPLC ESI-MS/MS is most sensitive to compounds that are readily ionized, the 8' acetylene ABA methyl ester was hydrolyzed to the acid before analysis.

Plant growth and total water use parameters were subjected to analysis of variance using a RCBD model in the GLM program of SAS (*SAS Institute, 1987*). All F-tests were carried out at $P = 0.05$. Daily moisture-use data were analyzed as a simple RCBD model, using the data from each day as a separate variable. least significant difference (LSD) tests ($P = 0.05$) were used for comparison of treatment means. The following planned contrasts were made in the trials evaluating moisture use and seedling growth of the ABA analog–treated tomatoes and marigolds :(1) ABA analog versus control and (2) ABA analog concentration—linear effects. Levels of endogenous ABA and the ABA analog in the leaves and roots over the 10-day evaluation period were analyzed with SAS GLM, using a repeated measures model described by *Gomez and Gomez (1984)*.

Results

Effects of an ABA Analog on Moisture Use and Growth of Tomato and Marigold Seedlings

Tomato

Moisture use by the ABA analog–treated tomato seedlings was relatively high at the beginning of the evaluation period; it declined for the next 4 days, increased slightly at day 5, and then remained constant for the remainder of the evaluation period (Figure 2). By contrast, water use by the untreated control plants increased through the evaluation period. The ABA analog treatments significantly reduced moisture use of the tomato seedlings relative to the control, beginning 2 days after treatment application and continuing through to the end of the 9-day evaluation period (Figure 2). Total moisture use per plant over the evaluation period was reduced by 39%–62% relative to the control, depending on the ABA analog treatments (Table 1). Total water use declined with increasing ABA analog concentration (Table 1).

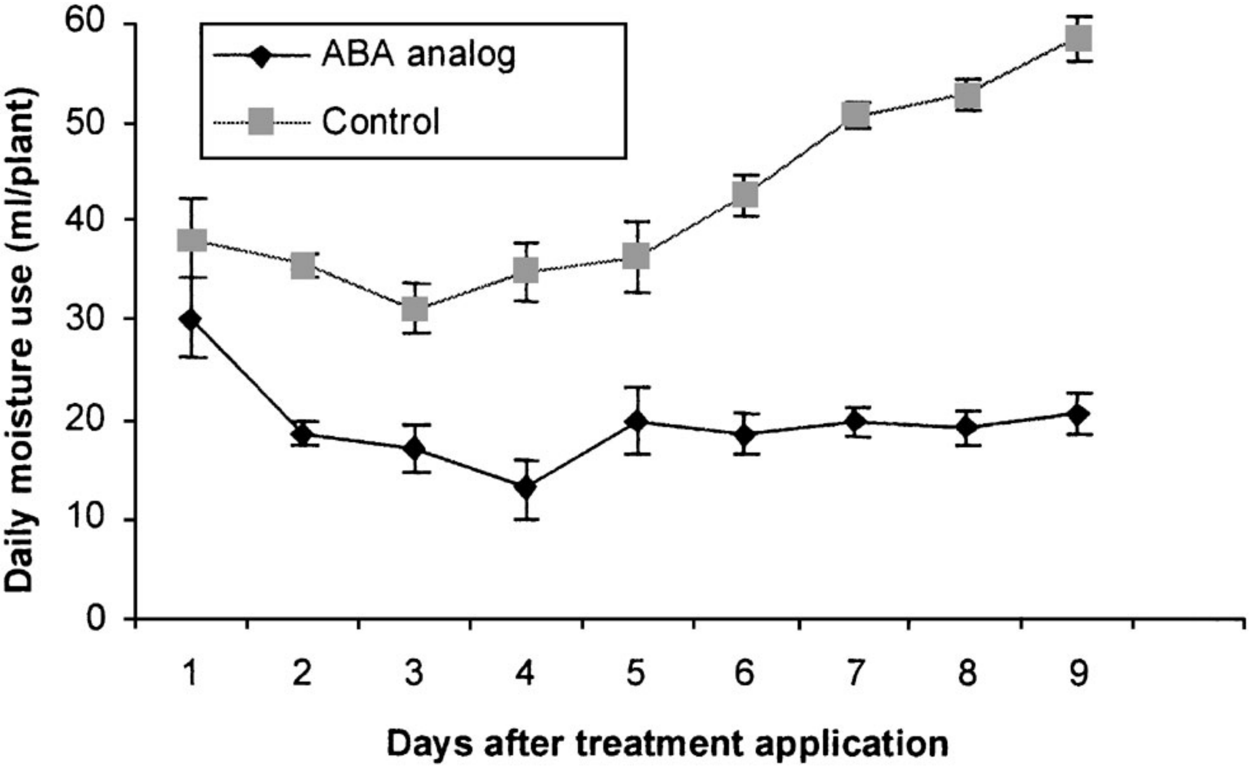

Figure 2 Daily water use by ABA analog-treated tomato seedlings over a 9 day evaluation period. Vertical bars represent ± standard errors. The ABA analog data are the average of 10^{-4} M, 5 × 10^{-5} M, and 10^{-5} M treatments.

Table 1 Moisture Use and Plant Growth Characteristics for Tomato and Marigold Seedlings Treated with an ABA Analog (8' acetylene ABA methyl ester) as a Root-dip

	Tomato			**Marigold**		
	CMU[a] (ml/plant)	**SFW[b] (g/plant)**	**RFW[c] (g/plant)**	**CMU (ml/plant**	**SFW (g/plant)**	**RFW (g/plant)**
ABA analog						
10^{-5} M	265.3	13.4	2.7	479.9	12.7	7.8
5×10^{-5} M	191.7	9.0	2.6	463.4	13.3	7.2
10^{-4} M	163.4	8.0	2.6	449.1	13.6	7.1
Control	434.6	17.3	4.0	505.4	12.3	8.0
Contrast						
Analog vs control	***	***	**	NS	NS	NS
Analog concentration (linear)	***	***	NS	NS	NS	NS

[a] Cumulative water use over 9 (tomato) or 12 (marigold) days.

[b] Shoot fresh weight

[c] Root fresh weight

**Significant at *P* = 0.01. ***Significant at *P* = 0.001. NS: Non-significant at *P* = 0.05.

Shoot fresh weight of the tomato seedlings at the final harvest was 41% higher in the control plants compared to the average of the ABA analog treatments (Table 1). Shoot fresh weight decreased with increasing concentrations of the ABA analog applied (Table 1). Root fresh weights of the tomato seedlings were similarly affected by the ABA analog treatments (Table 1).

Marigold

For the first 3 days, the ABA analog treatments had no significant effect on moisture use by the marigold seedlings (Figure 3). For the next 4 days, the PBI 429 treatments significantly reduced moisture use relative to the controls. From day 8 through to the end of the 12-day evaluation period there was no ABA analog effect on plant moisture use (Figure 3). Total moisture use throughout the evaluation period was reduced, on average, by 9% in ABA analog-treated marigolds, compared to the controls (Table 1). Shoot and root weights for the marigold seedlings were not influenced by the ABA analog treatments (Table 1).

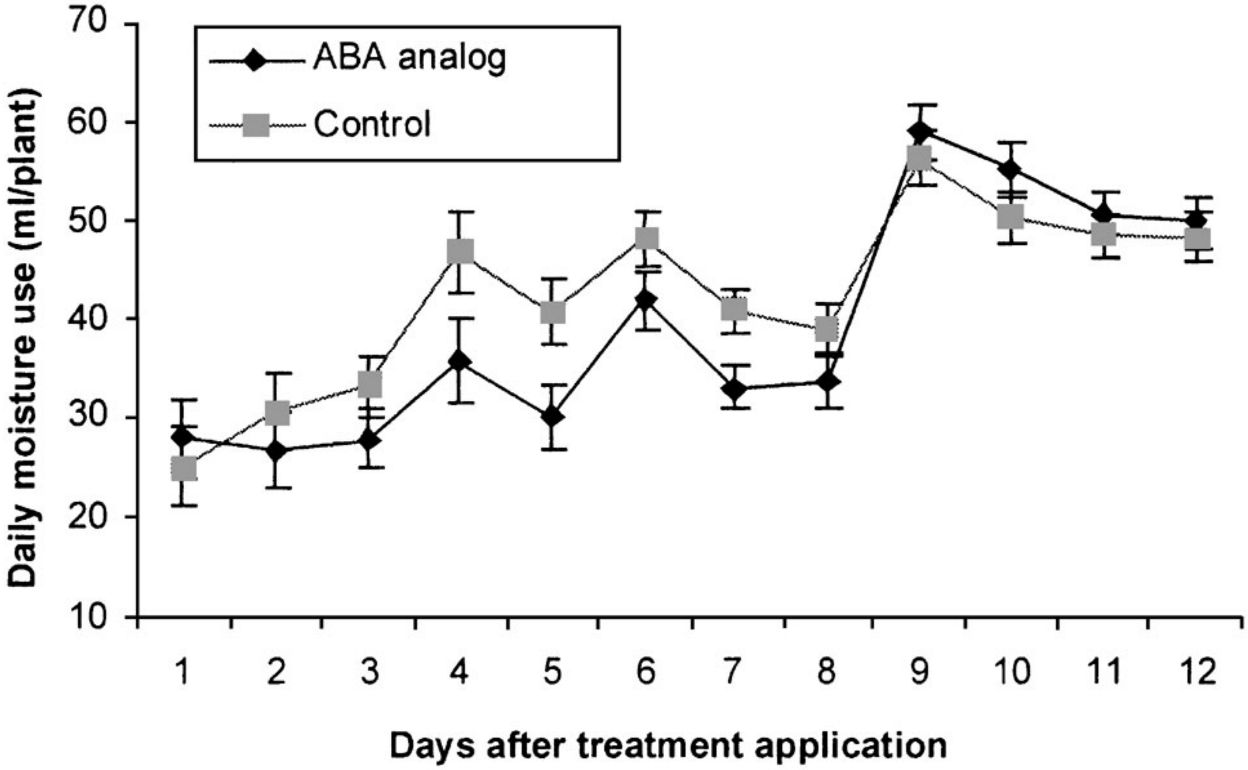

Figure 3 Daily water use by ABA analog-treated marigold seedlings over a 12-day evaluation period. Vertical bars represent ± standard errors. The ABA analog data are the average of 10^{-4} M, 5×10^{-5} M, and 10^{-5} M treatments.

Uptake and Movement of an ABA Analog in Marigold and Tomato

Leaf 8’ Acetylene ABA Concentrations

In both marigold and tomato, the concentration of 8' acetylene ABA in the leaves was highest at the first sampling date (3 days after treatment) and decreased significantly over the evaluation period (Figure 4a and b). At all points in the trial, the concentration of 8' acetylene ABA in leaf samples from the plants receiving the root-dip treatment was significantly higher than the concentration found in the foliar-treated plants. Averaged over the evaluation period, the concentration of 8' acetylene ABA in the leaves of plants given the root-dip treatment was approximately four times higher than in the corresponding foliar application.

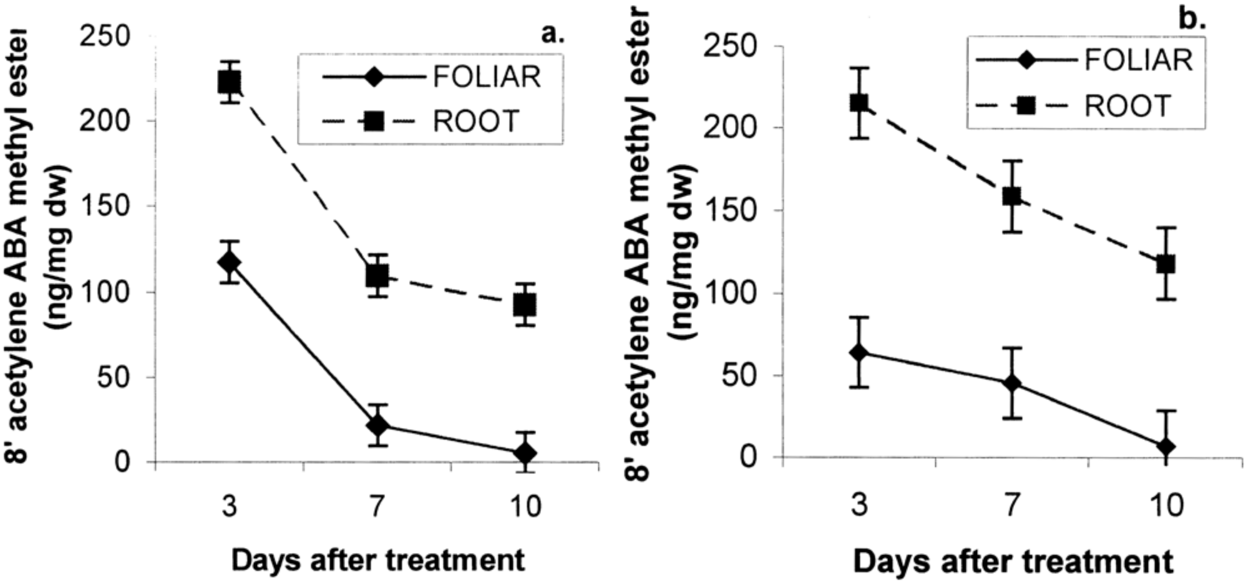

Figure 4 Concentration of 8' acetylene ABA methyl ester in tomato (**a**) and marigold (**b**) leaves over a period of 10 days after application of the ABA analog via foliar or root-dip treatment. Vertical bars represent ± standard errors.

At the first sampling (3 days after treatment), the concentration of 8' acetylene ABA in the leaves of tomato seedlings treated by foliar application was greater than the corresponding samples from marigold (Figure 4a and b). The mean levels of 8' acetylene ABA in the leaves of plants given the root-dip treatment were similar in both crops at that time. The rate of decline over the next 10 days in the 8' acetylene ABA concentrations in both the root and leaf tissues was greater in tomato than marigold, irrespective of the mode of application (Figure 4 a and b).

Root 8' Acetylene ABA Concentrations

The concentrations of 8' acetylene ABA in the roots of both marigold and tomato seedlings was consistently higher in root-dip–treated plants than in those treated via the foliage (Figure 5 a and b). Concentrations of the ABA analog in the roots were approximately four times higher in tomatoes than in marigolds with both modes of application. In both tomato and marigold, the decrease over time in the 8' acetylene ABA content of the roots was more rapid in the foliar-sprayed plants than in those receiving the root-dip treatment (Figure 5 a and 4b).

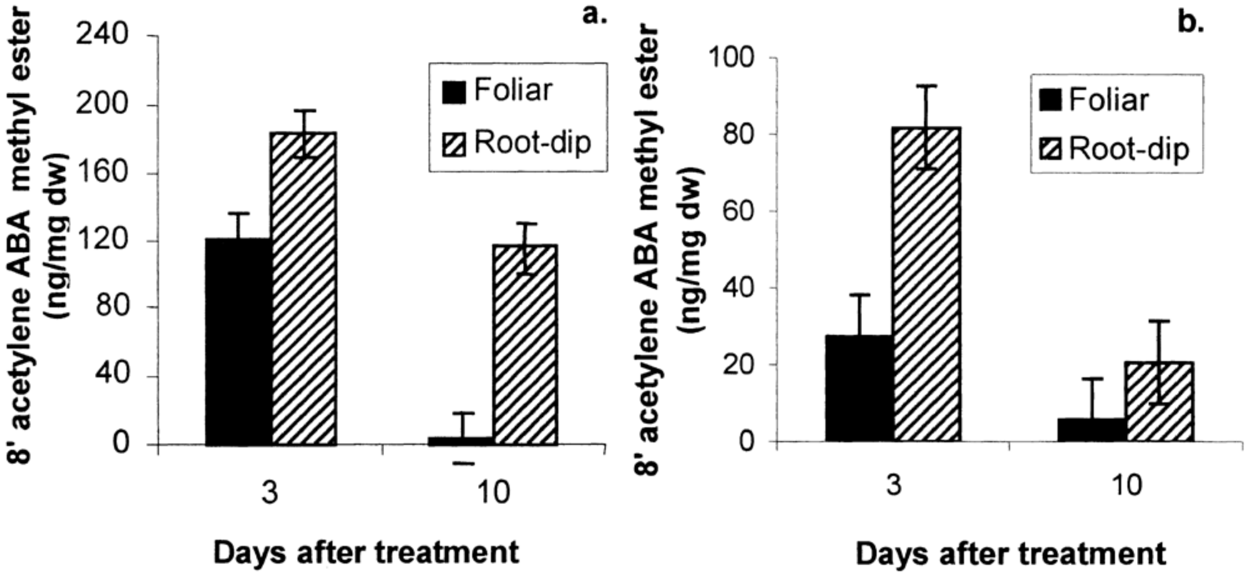

Figure 5 Concentration of 8' acetylene ABA methyl ester in tomato (**a**) and marigold (**b**) roots over a period of 10 days after application of the ABA analog via foliar or root-dip treatment. Vertical bars represent ± standard errors.

Concentrations of endogenous ABA in the roots and leaves were consistent over the sampling period and were not significantly altered by the application of the ABA analog treatments in either crop (data not shown).

Discussion

Chemical holding agents are used by the nursery industry to minimize plant maintenance costs, extend the marketing period, and reduce the risk of dehydration stress during storage and shipping. Ideally, a holding agent checks plant growth at a desired growth stage for a predictable and manageable period of time, with no detrimental effects on plant appearance or long-term performance. Greenhouse studies with various bedding plant crops have indicated that ABA analogs such as 8' acetylene ABA methyl ester might have potential for use as holding agents for bedding plant crops (*Sharma 2002*, *Waterer 2000*). In field and greenhouse experiments, ABA analogs slowed the growth and reduced moisture use and transplanting stress of tomato seedlings more effectively than corresponding treatments with the naturally occurring racemic form of ABA (*Sharma 2002*). In this study, ABA analog treatments again reduced total moisture use by both tomato and marigold seedlings. This reduction in moisture use is likely the result of the ABA analog treatments reducing stomatal conductance (*Grossnickle and others 1996*; Malton 1980) and/or plant growth. The growth and development processes affected by ABA, such as reduction in cell division, cell expansion and growth and ultimate reduction in leaf size, stomatal index, and stomata size are known to have long-term effects on plant water use (*Davies and Jones 1991*; *Quarrie 1991*). Reductions in moisture use were also reported when geraniums were root-drenched with plant-growth retardants such as paclobutrazol or uniconazole (*Whipker and others 2000*), which act by blocking the biosynthesis of growth-promoting hormone gibberellins (*Hartmann and others 1981*).

In marigolds, the ABA analog treatments had no effect on plant growth, and the treatment effects on plant moisture use were weak compared to those observed in tomato. In a previous study, marigolds had also been unresponsive to ABA analog treatments that were effective in other crop species (*Waterer 2000*). Abscisic acid interacts with other plant growth–regulating compounds in a positive or negative manner under differing circumstances (*Davies 1995*; *Van Steveninck and Van Steveninck 1983*). The relatively weak response of marigold to the ABA analog treatments in the present study might have been associated with the limited uptake and/or the presence of high levels of endogenous compounds antagonistic to ABA. It is also possible that the concentration of analogs applied might simply not have been high enough to induce an effect in the marigolds.

Biological activity of any chemical depends on the amount applied, its movement in the plant, and its persistence. In this trial, the amount of ABA analog absorbed in the plant system and its duration of persistence were examined as a function of the mode of application (foliar spray versus root-dips). In both test species, the ABA analog was detected in both the leaves and the roots, irrespective of the site of application. This indicates systemic movement of the ABA analog. Endogenous ABA has been found in both xylem and phloem fluids, as well as in parenchyma cells outside vascular tissues (*Addicott and Carns 1983*), which also suggests systemic movement. Three days after application, the concentration of the ABA analog in the roots was lower than the concentration detected in the leaves, irrespective of the mode of application. This suggests that much of the root-applied ABA is transported to the leaves. Movement of root-derived ABA to the shoots via the transpiration stream is well-documented (*Hartung 1983*; *Hopkins 1999*; *Quarrie 1991*; *Zhang and Davies 1987*). Tissue-specific differences in rates of metabolism could also influence the relative concentrations of ABA analog found in various plant tissues following treatment. The 8' acetylene ABA methyl ester would be expected to be hydrolyzed in plant tissues to the corresponding acid. The relative importance of transport versus metabolism in determining tissue-specific concentrations of the ABA analog could not be assessed in this study.

Root-dip treatment, as compared to foliar application, resulted in greater amounts of the ABA analog in the leaves. *Sharma (2002)* also observed both milder and shorter-term effects of foliar-applied ABA analogs compared to root-dip applications. This difference corresponded to differences in the relative amount of ABA analog solution applied to each plant in the foliar versus the root-dip application. Arteca and Tsai (1987) suggested that the cuticle layer of the leaves and other factors might also hinder the absorption of foliar-applied ABA. There are no corresponding barriers to uptake in the roots. The congruence between the amounts of ABA applied in a root-dip treatment and the amount detected in the plant tissues suggests that the plants rapidly absorb most of the ABA analog solution applied to the medial.

The 8' acetylene ABA methyl ester content was similar in the leaves of the two crops tested, but less of the analog was detected in the roots of marigold than in the roots of tomato. This findings suggests that the observed differences in responses of these two plant species to application of the ABA analog (Sharma 2002) may be related to differences in retention or accumulation of ABA in their roots. A relatively higher concentration of ABA analog may be required in species like marigold to produce effects similar to those observed in tomato. Abscisic acid interacts with other plan growth regulating–compounds in synergistic or antagonistic manners (*Davies 1995*; Van Steveninck and Van Steveninck 1983). Marigolds may produce higher levels of compounds antagonistic to ABA, leading to their limited treatment response. *Quarrie (1991)* suggested that the lack of a receptor system and/or differential sensitivity of the receptors and partitioning of applied ABA away from the receptor sites may also play a role in determining plant responses to applied ABA.

Endogenous ABA levels were not significantly altered by the application of ABA analog. This finding indicated that the 8' acetylene ABA methyl ester induces ABA-like effects without being converted into the natural form, and without affecting ABA synthesis and degradation in the plants. This is in agreement with the results obtained with another ABA analog (FBI 425 or 8' acetylene ABA), which did not alter endogenous ABA levels in wheat embryos (Abrams unpublished data). Similarly, application of the ABA analog Lab 173711 did not alter the levels of endogenous ABA in rice seedlings (*Flores and others 1993*).

This study demonstrated that the 8’acetylene methyl ester analog of ABA moves both acropetally and basipetally in marigold and tomato seedlings. Tissue concentrations of the ABA analog were greater when the compound was applied as a root-dip compared to a foliar application, likely as a consequence of the greater amount of chemical delivered by the root-dip treatment and/or more efficient uptake by the roots. The difference in relative responsiveness of marigold and tomato to applied ABA analog is not related to differences in either the amount of ABA analog absorbed or the rate of degradation of the analog. This study also showed that root-dip treatments with the ABA analog PBI 429 have the potential to slow the growth and reduce moisture use of bedding plants like tomatoes. The ability to hold plants at a particular growth stage may be of economic value during production, storage, and transportation, providing the treatments have no long-term negative effects on subsequent plant performance.

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