

EFFECTS OF POLYETHYLENE GLYCOL ON THE MORPHOLOGY OF
ORNAMENTAL SEEDLINGS

by

STEPHANIE ELAINE BURNETT

(Under the Direction of Paul Thomas and Marc van Iersel)

ABSTRACT

Osmotic compounds such as polyethylene glycol 8000 (PEG-8000) may control seedling elongation by imposing physiological drought. *Tagetes patula* (French marigold) and *Impatiens wallerana* (impatiens) were grown hydroponically in modified Hoagland solutions containing 0 to 62.5 g·L⁻¹ PEG-8000. Marigolds and impatiens were a maximum of 68% shorter when grown in PEG-8000. In a separate experiment, PEG-8000 was incorporated into a growing medium from 0 to 83 g·L⁻¹. Marigold and *Salvia splendens* (salvia) grown in 15-50 g·L⁻¹ of PEG-8000 emerged later than non-treated controls, and were up to 53% shorter, for salvia, or 38% shorter, for marigolds at harvest. PEG-8000 reduced leaf water and turgor potential, and marigolds grown in PEG-8000 photosynthesized less than non-treated seedlings. Finally, when PEG-8000 was applied to seedlings as a drench, 15-50 g·L⁻¹ of PEG-8000 reduced elongation. It appears that PEG reduces elongation by reducing turgor potential and photosynthesis, not by delaying seedling development.

INDEX WORDS: *Tagetes patula*, *Salvia splendens*, *Impatiens wallerana*, Polyethylene glycol, Drought, Bedding plant, Seedling, Height

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B.S., Auburn University, 1997

M.S., Auburn University, 2000

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2004

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For my parents and my sister,
who never stopped believing in me

ACKNOWLEDGMENTS

I'd like to thank both of my major advisors, Marc van Iersel and Paul Thomas for their assistance and advice during this research project. Thank you also to my committee members, Bodie Pennisi, Peter Hartel, Hugh Earl, and Hazel Wetzstein for their insightful comments on this manuscript and their assistance with my research throughout my time at the university of Georgia. I'd also like to thank all of the people who assisted me in laboring through all of the data collection and maintenance involved in this project. Thanks especially to Carrie Radcliffe for spending hundreds of hours helping me collect data and for always reliably helping me take care of my plants when I was out of town. Matt Hawkins, Beth Babbit, and Matthew Talenski, thank you for helping me with data collections, and with construction projects that were vital and essential for this project. I'd also like to thank all of the technicians and in the horticulture department, especially Deirdre Duncan, Chris Hussey, and Gwen Hirsch for their advice and assistance during my time at the University of Georgia. And, I'd like to thank Edgar Vinson, from Auburn University, for his technical assistance. Thanks also to Carl Lasco for all of his assistance in the greenhouse. Thank you to all of the graduate students I've had the pleasure of working with at the University of Georgia. Finally, I'd like to thank my family and friends for their patience and support as I pursued my degree.

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CHAPTER 1

INTRODUCTION

Ornamental and vegetable plants grown by horticulturists are a source of joy and beauty for many people. Greenhouses are often used to grow these plants from seed at a fast pace under optimal light and temperature conditions. However, many of these seedlings often elongate too quickly and become leggy, unattractive, difficult to ship, and less able to survive transplanting. In particular, height control is often an immense problem for producers of seedlings, because seedlings tend to stretch during hypocotyl elongation. These seedlings are usually sold to other growers who transplant and grow them until they are marketable. Growers are sophisticated customers who demand quality, consistency, and uniformity. Leggy, low quality transplants do not meet these demands.

The obvious solution to control height would be to prune the transplants, but this can be costly, time-consuming and would prolong the production period. As an alternative, many growers apply chemical plant growth retardants (PGRs) to reduce plant growth (Anderson and Hartley, 1990; Karlsson and Werner, 1991; Keever and Gilliam, 1994; Latimer et al., 1998). Most PGRs reduce internode elongation by inhibiting biosynthesis of gibberellins, a group of natural plant hormones involved in cell elongation.

Soaking seeds in growth retardants has been explored as a possible new method of applying PGRs (Fletcher and Hofstra, 1990; Fletcher and Kraus, 1995; Pasian and Bennett, 1999; Pill and Gunter, 2001). However, there is little research indicating whether growth reduction in

plants grown from treated seeds is due to the presence of PGRs or is an artifact of the soaking process. Researchers have long observed that plants grown from seeds soaked in water tend to be stunted (Lang, 1965; Maiti and Moreno, 1995; Orphanos and Heydecker, 1968).

An alternative method to control growth of plants is to restrict water availability. Most cell expansion, which is responsible for visible growth, is a result of turgor pressure. Therefore, decreasing the water supply to plants decreases growth (Frensch, 1997). This method of controlling growth will also reduce excessive use of water. However, drought stress can be dangerous. If growers allow plants to wilt, the plants may suffer aesthetic and physiological damage. Severe water stress may result in uneven growth across a crop (Ludolph, 1993). Currently, there is no research available that quantifies the amount of water necessary to slow the growth of plants without adversely affecting plant quality and health. There is little research that measures drought stress in ornamental seedlings. This is because most ornamental seedlings are grown in substrate volumes that are too small (approximately 8 mL) for most analytical equipment. It is plausible to measure the gravimetric water content of the substrate or water potential of seedlings periodically (Birnbaum and Versluys, 1998; Turner, 1981). However constant, in situ measurement of water status is difficult in small substrate volumes.

It may be possible to grow seedlings with minimal water availability by adding osmotic compounds to the growing medium which reduce the osmotic potential of water (Michel, 1983). Osmotic compounds, such as polyethylene glycol (PEG) form hydrogen bonds with water, making it unavailable for plant uptake (Kjellander and Florin, 1981). These compounds have been used to prime seeds and to examine the effects of drought in physiological studies (Bradford, 1986). PEG-4000 was observed to reduce leaf elongation, shoot dry weight, and leaf area

(Lawlor, 1970). However, the effects of these compounds on overall plant growth and morphology have not been well documented, especially for ornamental plants.

The objective of my research was to explore a new method of controlling seedling growth using PEG-8000 (PEG with an average molecular weight of 8000). This could result in plants with acceptable quality that are shorter and more compact than non-treated plants. Also, these plants should exhibit normal morphology, physiology, anatomy, and performance after transplant.

Salvia splendens F. Sellow. ex Roem. & Shult. 'Bonfire' (annual salvia) was chosen as a model crop. This plant was selected because it is a popular spring annual with attractive red, purple, or coral flowers and appears to respond to restricted water by growing compactly (Eakes et al., 1991). In addition, the effects of PEG-8000 on the growth of *Tagetes patula* L. (marigolds) and *Impatiens wallerana* Hook.f. [*I. Holstii* Engl. & Warb.; *I. Sultanii* Hook.f.] (impatiens), two popular annual bedding plants, were explored. Marigolds were chosen because they exhibit rapid hypocotyl elongation. If growers apply growth retardants according to label recommendations (Uniroyal Chemical, 2000), they will be applied too late to reduce hypocotyl elongation. Impatiens were selected as a substitute for salvia in studies conducted in hydroponics, because salvia survival rate in hydroponics was approximately 30% (Burnett et al., unpublished results).

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CHAPTER 2

LITERATURE REVIEW

Plug Culture

Horticulturists can propagate plants vegetatively from cuttings or grafts, or sexually from seeds. Most annual flowering plants and vegetables are produced from seed. Until the mid 1960s, most growers propagated seedlings in large trays without individual cells (Thomas, 1993). After seedlings were large enough, 'bare-root' seedlings were transplanted into larger containers. After the mid 1960s, growers began to rely on seedlings grown as small, individual plugs.

Plugs are common because they avoid several disadvantages associated with bare-root seedlings. Root hairs are usually damaged during transplant of bare-root seedlings. This causes growth to cease until the seedlings grow new root hairs, and damaged roots are a point of entry for root rot pathogens (Styer and Koranski, 1997). Bedding plants grown from plugs are usually marketable 7 to 10 d sooner than plants grown from bare-root seedlings (Ball, 1991). Also, bare-root seedlings cannot be held in the greenhouse as long as plugs (Styer and Koranski, 1997). Finally, plants grown from plugs tend to be more uniform and survive transplanting better than bare-root seedlings (Thomas, 1993).

Although plugs are superior in many ways, some growers still prefer bare-root seedlings. Since most growers buy plugs from commercial suppliers, it is difficult to find a source for unusual, specialty crops. Plugs also have smaller rooting zones than bare-root seedlings grown in

traditional flats (Biernbaum and Versluys, 1998). Despite these drawbacks, most growers currently prefer plugs over bare-root seedlings because they are convenient and perform consistently.

Since the practice of growing plugs is relatively new, there are still a number of problems that must be addressed. One of the biggest problems with transplant production is that plants tend to out-grow their small growing medium volumes quickly and become too tall (Styer and Koranski, 1997). Overgrown transplants are difficult to water, more expensive to ship, and less aesthetically pleasing than compact transplants. This is a serious problem for plug producers who sell their products to other growers. Purchasers of plugs have more exacting expectations of plant quality than the average consumer.

Height Control

A common assumption about horticulturists is that they desire to grow the largest possible plant as quickly as possible. However, rapid growth can lead to such problems as plants out-growing their pots, excessive drying between irrigations, increased shipping costs, and leggy, unmarketable plants. For these reasons, many growers use some form of growth control during plant production. Height control results in plants that are more compact, less expensive to ship, and more aesthetically pleasing (Davis and Curry, 1991).

The most obvious form of growth control is pruning shoot growth to the desired form. Although this is an option for many growers of bedding plants, most bedding plants are rarely pruned because pruning requires a large input of labor and can be costly. One particular problem for seedlings is that they are produced in as few as four weeks and are sold when they have very

few true leaves (Styer and Koranski, 1997). Pruning such small seedlings may decrease photosynthesis and delay growth after transplanting.

Other methods to keep plant growth in check include manipulating the growing environment or plant genome. Plant breeding techniques have the potential, in the future, to produce plants that are genetically designed to grow to an optimum size. Wheat plants, for example, have been manipulated to include dwarfing genes (Roberts and Hooley, 1988). Plant size can also be restricted by growing plants in smaller containers, thereby restraining root growth and subsequent uptake of the water and nutrients necessary for shoot growth.

Yet another method is the use of plant growth retardants, defined as, '[any] chemical that slows cell division and cell elongation in shoot tissues and regulates plant height physiologically' (Cathey, 1963). Chemical plant growth retardants are one common, relatively inexpensive method for greenhouse and nursery growers to produce compact plants, induce branching, and control production time (Anderson and Hartley, 1990; Karlsson and Werner, 1991; Keever and Gilliam, 1994; Latimer et al., 1998; Pisarczyk and Splittstoesser, 1979; Whipker and Dasoju, 1998). Additionally, growth retardant applications have been associated with beneficial plant attributes such as drought and pollution tolerance, and possibly protection from pests, including whiteflies (Larson, 1985). Some plant growth retardants have also been shown to increase plant quality, producing plants with darker green foliage (Davis et al., 1986).

The mode of action for the most common chemical plant growth retardants is to block one of the many points on the gibberellin biosynthesis pathway (Davis and Curry, 1991). Gibberellins are a group of natural plant growth regulators, and one of their functions is to stimulate cell elongation (Mohr and Schopfer, 1995). Because chemical growth retardants block the biosynthesis of gibberellins, they essentially interfere with cell elongation; a growth retardant-

treated plant should have cells that divide at a normal rate, but elongate less. Therefore, plants treated with plant growth retardants should be shorter and more compact than those not treated with growth retardants. However, many growers are reluctant to use chemical plant growth retardants on young, tender seedlings which are sensitive and often exhibit stunted growth unless growth retardant application is precise. The use of chemical plant growth retardants also increases the overall cost of a plant and they are illegal to use on edible crops (Erwin and Heins, 1990).

There are many nonchemical ways to control height. Light quality, intensity, and duration affect plant height and quality. Generally, plants grown under high intensity light are shorter at maturity; plants grown under low light tend to stretch towards available light and have a leggy, less compact, and lower quality appearance. Spectral filters in greenhouse coverings containing CuSO_4 manipulate light quality to encourage compact growth. These filters decrease transmission of far-red light into greenhouses, and decrease height and leaf area of *Lycopersicon esculentum* L. (tomato), *Capsicum annuum* L. (bell pepper), *Citrullus lanatus* (Thunb). Matsum. and Nakai (watermelon), and *Dendranthema × grandiflorum* Ramat. Kitamura (chrysanthemum) (Rajapakse and Kelly, 1995; Rajapakse et al., 1991; Cerny and Rajapakse, 1999). This technology is still being developed, and while it may offer options for many growers, it decreases the flexibility of growing several species within one greenhouse that is needed in many greenhouse operations.

Day and night temperatures may also affect plant growth; in particular the difference between the night and day temperatures (DIF = day - night temperature) is an important measure of how temperature affects plant growth. A negative DIF is often associated with reduced stem elongation, and hence, a shorter plant (Erwin and Heins, 1989). Negative DIF can be impractical

and costly in warmer areas of the country, and is even seldom used in regions where weather is not as prohibitive.

Mechanical conditioning, commonly done by brushing shoot tissue with a wooden or metal rod, is another nonchemical control of plant growth (Mitchell, 1996; Latimer 1998; Garner and Björkman, 1996). Brushing is labor-intensive, and can be impractical and expensive for many growers. Another problem is that brushing is destructive for some plants such as pepper (Latimer, 1994). Plants are also sometimes grown in smaller containers to restrict root and shoot growth (NeSmith and Duval, 1998). However, it is difficult to manage the water supply to plants that are pot-bound.

Decreasing the amount of fertilizer supplied to plants decreases growth. However, levels of fertility that are low enough to slow growth are also associated with decreased plant quality. Recently, it has been reported that decreasing the level of phosphorus supplied to plants decreases elongation (Liptay and Sikkema, 2000). Unfortunately, seedlings grown with a low phosphorus fertilizer do not grow normally for extended periods of time after they are transplanted (Liptay and Sikkema, 2000).

While these methods of nonchemical growth control may be feasible in specific situations, most of them are not cost-effective, easy to use, or flexible under different production regimes. Therefore, growers who wish to control growth non-chemically are left with few effective options. However, there is yet another nonchemical means of height control that may be cost effective, easy to use, and compatible with different production regimes.

Growing plants with minimal water supply is currently one of the most common methods of controlling growth of horticultural crops. Ideally, plants are not allowed to visibly wilt and are never completely saturated with water (Liptay et al., 1998). This method of controlling growth

has the added benefit of reducing waste consumption of water, a non-renewable resource. However, if growers restrict water too much, they risk sacrificing plant quality. Currently, only experienced growers successfully grow plants with minimal water to achieve compact plant growth.

Previous research suggests that vegetable transplants grown using minimal water are smaller than non-stressed controls and have thicker cuticles (Latimer, 1992; Latimer and Severson, 1997). Plants grown with minimal water tend to resist mechanical stress and would likely survive transplanting more readily (Liptay et al., 1998). Such plants also have fewer basal roots (Leskovar, 1998).

Currently, drought in transplants is determined visually by most researchers. This is partly because transplant substrate volumes are so small that there is no room for analytical equipment, such as tensiometers (Liptay et al., 1998). Methods of estimating plant water stress include observing leaf color change or leaf rolling (Turner, 1981). Visual assessments of the plant water status have allowed researchers to make generalizations about how growing plants with minimal water affects plants. However, visual assessments can be inaccurate and inconsistent. Sometimes, researchers even allow plants to wilt, and this is not the best way of growing plants under water deficit stress (Biernbaum and Versluys, 1998). Because there are currently no precise recommendations, growers would benefit from specific guidelines on limiting water supply to plugs.

Salvia

Salvia is a genus of over 700 annual and perennial plants in the Lamiaceae (mint) family (Walters and Keil, 1996; Armitage, 1997). Members of the genus are characterized by four-sided (square) stems, opposite leaves, and bi-labiate flowers in a variety of colors including red, white, pink, and blue (Walters and Keil, 1996; Armitage, 1997). Many salvias are also characterized by foliar glands which exude characteristic scents that may or may not be considered pleasant (Walters and Keil, 1996; Armitage, 1997).

Salvia splendens F. Sellow. ex Roem & Shult., or annual salvia, is possibly the most-commonly grown and sold ornamental salvia. It is a perennial in USDA Hardiness Zones 9 and 10, but for the majority of people in the United States, it is strictly planted as an annual (Armitage, 1997). Breeding has provided consumers with a variety of sizes, from the more common dwarf cultivars, like the miniature 'Rhea' (maximum height = 10 cm), 'Red Hot Sally' and 'Scarlet Queen' (maximum height = 30-36 cm) to taller cultivars like 'Bonfire' and 'America' (Nau, 1998). Annual salvia flower colors include red, white, lilac, salmon, burgundy wine, and blue (Nau, 1998). Reliable form and perpetual spring and summer flowers in varieties of colors make annual salvia a popular plant for growers, landscapers, and gardeners. It is also quickly produced from seed for transplanting in 5-6 weeks (Styer and Koranski, 1997).

One problem with the production of salvia seedlings is that plants grow so rapidly that they can appear leggy and of low quality in plug flats. Growth retardants exist for salvia, but over-application, particularly on young seedlings, may result in stunted plants. Excessively reduced growth would result in increased cost to the grower because plants must reach a set size before they are considered marketable.

Annual salvia seedlings are a good candidate for height control using minimal water mainly because they exhibit such rapid growth. Mature *Salvia splendens* 'Bonfire' also decreased shoot dry weight and leaf area after exposure to drought (with leaves reaching water potentials of -1.1 to -1.4 MPa on four separate occasions) (Eakes et al., 1991). Stressed plants recover from drought to exhibit normal photosynthesis and increased tolerance to stress (Eakes et al., 1991). Unfortunately, the precise range of water potentials at which plants may be grown to exhibit reduced growth without decreased quality is unknown for salvia seedlings. It is also unknown for any other plants while they are at the plug stage. In addition, even if the ideal water potential for reduced elongation without seedling damage was known, it would be impractical for commercial growers to continually monitor leaf water status.

Marigold

The genus *Tagetes* (marigold) is a summer flowering annual with yellow and orange flowers in the family *Asteracea*. There are three marigold species that are typically used in horticulture: *Tagetes erecta* L. (African marigold), *Tagetes patula* L. (French marigold), and *Tagetes erecta* × *patula* (triploid marigolds). African marigolds are the tallest of the three; some varieties are 75 cm in height (Nau, 1991). They flower approximately two weeks later than French marigolds, and require short days for earliest flowering (Nau, 1991). Height of triploid marigolds ranges from 40-70 cm, and these plants tend to germinate at lower rates than French or African marigolds (Nau, 1991). French marigolds are the shortest of the three species, and, unlike African marigolds, some cultivars have red or crimson flowers (Nau, 1991).

Although marigolds are popular and attractive plants, there are two problems with the production of marigolds seedlings. First, seeds have a 'tail', which prohibits automatic seeding (Ball, 1991). Second, marigold hypocotyls elongate rapidly, so height control of seedlings is difficult. For this reason, marigolds would benefit from height reduction before hypocotyl elongation, and would be a good candidate for height control using minimal water. French marigolds were chosen as a second model crop because triploid marigolds germinate at lower percentages than French and African marigolds. French marigolds also may be more popular than African marigolds, since they are available in more colors.

Impatiens

Impatiens wallerana Hook.f. (impatiens or busy lizzie) are in the Balsaminaceae family and are the best selling bedding plant in the world (Armitage, 2001; Corr, 1998). They are annuals that flower in the shade throughout the spring and summer, and flowers may be single or double depending on the cultivar (Armitage, 2001). Dwarf impatiens are 38-64 cm tall, and tall impatiens are 64-85 cm in height (Armitage, 2001).

In commercial settings, impatiens seedlings grow rapidly and some form of growth regulation is recommended (Corr, 1998). Impatiens also grow in naturally moist soils. This makes them ideally suited to hydroponic culture, and, in fact, the survival percentage of impatiens in hydroponics is near 100% (Burnett et al., unpublished data). For this reason, and because they are such a popular bedding plant, impatiens were chosen as the model crop species for studies conducted in hydroponics.

Drought

Methods of Measurement

The two most common methods of quantifying soil or substrate water content use tensiometers or lysimeters. Tensiometers measure the tension water exerts on a ceramic tip that is inserted in a plant's growing medium. They are commonly used to measure the matric potential of the growing medium in experimental research, and are also used to schedule irrigation timing for agronomic and horticultural crops (Norrie et al., 1994; Glen and Peterson, 1996). Micro-tensiometers have been used to measure drought stress in smaller (16.5 cm) pots (Karlovich and Fonteno, 1986). However, tensiometers have two restrictions that may discourage researchers from using them. First, the ceramic tip in tensiometers only measures water tension where it is inserted. Often, the moisture content in a pot varies so that some regions are drier than others due to air pockets, soil depth, and small inconsistencies in water holding capacity through the medium. For this reason, tensiometers do not always provide accurate measurements of substrate matrix potential. Second, it is impossible to use tensiometers to measure the matric potential of small growing medium volumes in which seedlings are often grown.

Weighing lysimeters measure soil water content by actually weighing pots containing soil, water, and plants. This measurement may be adjusted for the known weight of the dry soil and the plant so that the weight of water in a container is known. This allows researchers to know how much water is in a pot, regardless of soil depth or inconsistencies in the soil microclimate (Young et al., 1996). However, lysimetry would be impractical to scale down for measuring water content of plants with small volumes.

There is one method of controlling the matrix potential of growing media that is not commonly used, but that may be adapted to quantify drought stress in small seedlings. Osmotic compounds, such as polyethylene glycol (PEG) may be added to water so that the water potential may be adjusted to a known level (Michel, 1983). PEG of various molecular weights is commonly used to 'prime' seeds so that they germinate in a synchronized manner (Bradford, 1986). PEG compounds of varying molecular weights are also used to study the physiology of drought stress in hydroponic culture or in growing media (Pérez-Alfocea and et al., 1993; Chazen, 1995; Zhang and Kirkham, 1995; Zhang and Outlaw, 2001; Zhang et al., 2001; Zou et al., 2000).

It has been reported that *Oryza sativa* L. (rice) grown in PEG-1500 are shorter than non-treated controls, and *Pinus radiata* D. Don [*P. insignis* Dougl. ex Loud.] seedling roots elongate less when grown in PEG-4000 compared to non-treated seedlings (Choi et al., 2000; Zou et al., 2000). *Zea mays* L. plants grown in PEG-4000 have lower rates of leaf elongation, and *Sorghum bicolor* (L.) Moench [*S. vulgare* Pers.] seedlings grown in PEG-8000 had lower shoot and root dry weights than non-treated plants (Gill et al., 2001; Lawlor, 1970). Hypocotyl elongation of *Colophospermum mopane* seedlings was reduced when plants were grown in PEG (Johnson et al., 1996). The molecular weight of PEG used in this experiment was not reported, however, hypocotyl elongation was reduced in PEG concentration with water potentials from -0.2 MPa to -2.0 MPa (Johnson et al., 1996). Although it appears that PEG-8000 reduces elongation of roots, and shoots, the use of PEG-8000 as a growth retardant has not been explored.

Physiology of Drought and Elongation

When plants are exposed to drought, the most visible difference is that they tend to be smaller in size. There are several theories about the physiological mechanism that causes this size reduction, but the main theory involves turgor pressure (Taiz and Zeiger, 1998). Plant cell

elongation increases with increasing turgor potential (Carpita and McCann, 2000; Cosgrove, 1997; Frensch, 1997; van Volkenburgh 1999). This is explained by the Lockhart equation (Lockhart, 1965). A simplified version of the Lockhart equation suggested by Ray et al. (1972) is: $dV/dt=L(\Psi_p-Y)$ where dV/dt is the change in cell volume over time, L represents the yield coefficient, Ψ_p is turgor potential, and Y is the yield threshold for turgor (Lockhart, 1965). Plants grown in substrates with low water potential typically have low leaf turgor potential. Root and shoot elongation decreases as turgor potential decreases, although root elongation is usually less affected than shoot elongation (Jones, 1992; Kramer and Boyer, 1995).

The plant growth regulator abscisic acid (ABA) is involved in triggering many physiological reactions to stress (Bray et al., 2000). Most importantly, ABA triggers closure of stomata in response to water stress (Crozier et al., 2000; Tabaeizadeh, 1998). Closure of stomata decreases transpirational water losses within plants (Taiz and Zeiger, 1998). Unfortunately, this defensive measure also decreases photosynthesis in drought-stressed plants since they take up less CO_2 to convert to carbohydrates.

Effects of Drought on Plant Anatomy and Morphology

Plants exposed to drought adapt overall anatomy to minimize the impact of water stress and maximize the efficiency of use of available water. Shoot growth tends to be reduced so that drought-stressed plants have smaller leaf areas (Taiz and Zeiger, 1998). Some plants, particularly succulents, have adapted their anatomy so that they have reduced leaf surface to volume ratios. This not only decreases the transpirational surface area, but thicker leaves also allow plants to have more volume for storage of water reserves (Mauseth, 2000). This evolutionary advantage does not mean that plants always respond to drought by increasing leaf thickness. For example,

leaves of *Peperomia carnevalii* exposed to drought were thinner than those of non-stressed plants due to shrinkage of the hydrenchyma but not the mesophyll layer (Herrera et al., 2000).

Shoots and roots of corn seedlings exposed to drought had lower dry weights than those grown under normal water regimes (Stasovski and Peterson, 1990). Shoot and root elongation decreases when plants are drought stressed, but root elongation tends to be less sensitive to drought stress than shoot elongation (Frensch, 1997). Two sorghum cultivars had smaller root length densities (estimated as in Newman, 1966) when grown in soil with minimal water than when grown in moist soil (Salih et al., 1999). The remaining root growth of drought-stressed corn seedlings appeared to be biased towards seminal lateral root growth (Stasovski and Peterson, 1990). Stressed seedlings eventually resumed normal root growth if exposed to 21 d or less of stress, but roots did not resume growth if they were exposed to excessive stress (at least 34 d of water stress). According to Taiz and Zeiger (1998), roots shrink and slow the addition of new root hairs when plants are exposed to rapid drought. However, if drought occurs slowly, plants tend to increase root growth deeper into the soil (Taiz and Zeiger, 1998). Roots of drought-tolerant species, such as *Paspalum dilatatum* Poir. tend to decrease root metaxylem vessel diameter when grown under water stress (Vasellati et al., 2001). Decreasing root diameter would decrease the possibility that the capillary action of water within xylem would be disrupted (Taiz and Zeiger, 1998).

Some plants contain protective anatomical structures which appears to help them survive drought more readily. In shoots, a thicker cuticle layer is associated with decreased water loss in more drought-tolerant species or individual plants (Latimer and Severson, 1997; Helbsing et al., 2000; Taiz and Zeiger, 1998). In roots, a suberized exodermis is sometimes associated with increased drought tolerance. (Taiz and Zeiger, 1998; Taleisnik et al., 1999). On a cellular level,

Arabidopsis appears to upregulate transcription of the genes responsible for an aquaporin protein in response to drought. Since aquaporins allow water to pass through the cell membrane, increased numbers of aquaporins should be associated with increased water uptake (Bray et al., 2000).

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CHAPTER 3

PEG-8000 REDUCES GROWTH OF HYDROPONIC IMPATIENS AND MARIGOLDS¹

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Abstract.

It is desirable to grow short bedding plants without the use of chemical growth retardants since these retardants may stunt young plants. The use of osmotic compounds such as polyethylene glycol 8000 (PEG-8000) may control growth by imposing a controlled osmotic stress. *Tagetes patula* 'Boy Orange' L. (French marigold) and *Impatiens walleriana* 'Dazzler Pink' Hook. F. (impatiens) were grown hydroponically in modified Hoagland solutions containing 0, 10, 17.5, 25, 32.5, 40, 47.5, 55, or 62.5 g·L⁻¹ PEG-8000. Marigolds and impatiens were up to 68% shorter when grown in PEG-8000. Additions of PEG-8000 at rates above 10 or 25 g·L⁻¹ resulted in plants that were either of low quality and had damaged roots, or were not much shorter than seedlings treated with 10 or 25 g·L⁻¹ of PEG-8000. Electrical conductivities of Hoagland solutions were up to approximately 1 dS·m⁻¹ lower in solutions containing PEG-8000 when compared to non-treated Hoagland solutions. Foliar tissue of impatiens contained significantly less nitrogen, calcium, zinc and copper, but significantly more phosphorus and nickel when grown in PEG-8000. However, PEG-8000 did not appear to induce nutrient deficiencies. Therefore, 10 or 25 g·L⁻¹ PEG-8000 reduces growth of marigolds and impatiens, respectively, without visibly reducing plant quality, when foliar and root color were considered.

Tagetes patula (marigold) and *Impatiens walleriana* (impatiens) are popular summer annuals in the United States and are grown for their diverse colorful flowers and growth forms. Impatiens are also well known in the landscape since they flower in shade. Unfortunately, under commercial greenhouse conditions, both plants elongate rapidly, especially as seedlings. Resultant plants are leggy, more difficult to water, more expensive to ship, and less attractive than smaller plants.

Several solutions are available to greenhouse growers who wish to keep bedding plants short. Many growers choose chemical plant growth retardants (Bailey and Whipker, 1998). However, if greenhouse workers over-apply plant growth retardants, then the plants will be stunted, and the sale date of plants may be delayed or plants may not be saleable.

Some growers choose to slow plant growth using non-chemical methods. These options include brushing the leaves (Latimer, 1991; Mitchell, 1996), withholding nutrients (Huang et al., 2002), or applying negative DIF (DIF=day temperature-night temperature) (Erwin et al., 1989). Unfortunately, non-chemical growth control tends to be expensive or, if used improperly, may damage plants (Amsen and Nielson, 1991; Latimer, 1994, Huang et al., 2002).

One option for greenhouse growers is drought-stressing plants to slow growth. Plants grown with minimal water tend to grow more slowly than those with replete water because the rate of cell elongation is partially controlled by turgor potential. In addition to other factors, cell water potential determines turgor potential (Van Volkenburgh, 1999). Since the cell water potential is influenced by the soil water potential, decreasing soil water potential may decrease cell elongation and plant growth.

For this reason, horticultural plants are often allowed to wilt or are grown in soils close to the point where plants would wilt to reduce growth (Latimer and Severson, 1997; Liptay et al.,

1998). Water requirements for most horticultural crops are unknown. This means that drought stressing plants to reduce growth often requires guesswork for growers. This can be deleterious for plants because, if water is restricted too much, stomata will close, and photosynthesis will decrease (Mohr and Schopfer, 1995). On a morphological level, severe symptoms of drought stress may include leaf abscission, scorching, or in extreme cases, plant death. Often, only experienced growers are able to drought stress plants without causing damage.

It is desirable to find a method of objectively restricting water to control plant growth that may be used by growers regardless of experience. One option is the use of osmotic compounds to reduce substrate water potential. One osmotic compound, polyethylene glycol 8000 (PEG-8000), forms hydrogen bonds with water (Kjellander and Florin, 1981). PEG-8000 is inert and is generally accepted to be too large for plant uptake, although there is some evidence that PEG-8000 may be taken up (Krizek, 1985). Since PEG-8000 bonds with water, water is less available for plants. Models have been developed for predicting the water potential of any given PEG-8000 solution (Michel, 1983). For these reasons, PEG-8000 is one choice as an osmotic compound for imposing controlled drought.

Currently, there is no research directly examining the use of PEG compounds to reduce plant growth. However, PEG reduces plant height: Rice seedlings grown in PEG-1500 were shorter than non-treated controls (Choi et al., 2000). PEG-1500 has a lower molecular weight than PEG-8000 and is more likely to be absorbed by plants (Krizek, 1985).

The effects of any PEG compound on growth of commercial bedding plants is unknown. Therefore, we determined the ability of PEG-8000 to control elongation of hydroponically grown marigolds and impatiens. We also determined which range of PEG-8000 concentrations controls growth without damaging plants.

Materials and Methods

Tagetes patula ‘Boy Orange’ (marigold) (Pan American Seed Company, West Chicago, Ill.) and *Impatiens walleriana* ‘Dazzler Pink’ (impatiens) (Ball Seed Company, West Chicago, Ill.) seeds were planted in open trays containing vermiculite on April 3 (marigold) or September 2, 2003 (impatiens). Marigold seeds were germinated in a growth chamber (20 °C) and transferred to a glass greenhouse (temperature minimum = 19 °C ± 2 °C; maximum = 33°C ± 2 °C) 17 d after seeding (75% of seeds had emerged and first true leaves were visible). Impatiens seeds were germinated on a mist bench irrigated 5 s every 5 min from 8:00 am until 5:00 pm and grown in a glass greenhouse (temperature minimum = 17.6 °C ± 2.3 °C; maximum = 29.7 °C ± 4.4 °C; Maximum *PPF* levels = 802±212 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Marigolds were allowed to acclimate to greenhouse conditions for 2 days and then were transplanted into plastic tubs (4.5 L capacity) containing modified Hoagland solution (Hoagland and Arnon, 1950). Impatiens were transplanted into similar plastic tubs 45 d after seeds were planted, when seedlings roots were at least 5 cm long. The seedlings were supported within trays by polyester batting. Subdue Maxx fungicide (Mefonoxam, Syngenta Chemical, Greensboro, N.C.) was added bi-weekly at a rate of 0.125 mL·L⁻¹ of Hoagland solution to all plastic tubs to prevent *Pythium* infection.

PEG-8000 was added to containers after plants were acclimated for 2 or 13 d for marigolds or impatiens, respectively. Impatiens were allowed to acclimate longer since they have a slower growth habit than marigolds. PEG-8000 rates were: 0, 10, 17.5, 25, 32.5, 40, 47.5, 55, or 62.5 g·L⁻¹. PEG was added at dusk and in a step-wise manner over a period of eight days. On the first evening, the lowest PEG concentration was added to all containers except controls. On

the following days, $7.5 \text{ g}\cdot\text{L}^{-1}$ of PEG was added to containers each day as necessary to attain the appropriate PEG concentrations. After eight days, PEG had been added to all containers in the appropriate concentrations. PEG was added in this manner to allow seedlings to slowly acclimate to the presence of PEG.

Hoagland solutions were changed 41 d after seeding marigolds, or 58 and 78 d after seeding impatiens to reduce the risk of fungal contamination. The new solutions contained appropriate amounts of PEG-8000 for each treatment. Electrical conductivity (EC) and pH were measured twice weekly as long as seedlings were grown hydroponically with a Corning M90 meter with interchangeable sensors for measuring EC and pH (Corning Inc., Corning, N.Y.). Liquid flowable lime (Limestone F, Cleary Chemical, Dayton, N.J.) or dilute sulfuric acid were added to the solutions to maintain a pH within the range of 5.5 - 7.5.

Plant height and width were recorded 42, 56, and 70 d after seeding for marigolds or 84 and 98 d after seeding for impatiens. Plant width was the maximum horizontal distance between two opposite leaf tips. Marigolds were harvested 70 d and impatiens harvested 98 d after seeding. At this time, control plants were of marketable size. Leaf area was measured with a leaf area meter (Li-3100, Li-Cor, Inc., Lincoln, Nebr.), and root length was noted. Roots and shoots (stems and leaves), were dried in an oven at $80 \text{ }^{\circ}\text{C}$ for at least three days, and then tissue dry weights were recorded. Compactness was calculated from these data (compactness = leaf area/height or shoot dry weight/height). Finally, at the termination of the experiment, foliar tissue of impatiens was analyzed at the University of Georgia Soil, Plant, and Water Analysis Lab for mineral nutrient content.

Plants were arranged in a randomized complete block design with two blocks and six subsamples in each block. A single experimental unit consisted of a plastic container with six

plants (subsamples). Data collected for the subsamples were averaged within each experimental unit before statistical analysis. Both blocks were combined for foliar tissue analysis because plants grown in the treatments with the four highest PEG concentrations were too small to be analyzed for each block individually. Data were analyzed by testing for significant linear and quadratic effects of the PEG concentrations on the various parameters using general linear models in Statistical Analysis System (SAS, Cary, N.C.). Height data were analyzed with and without the data from control plants to determine if there were any differences among PEG-8000 treatments.

Results

Morphology

Marigolds and impatiens were shorter throughout both experiments when grown in solutions containing increasing quantities of PEG-8000 (Figs. 1, 2). Plants were also less wide (Figs. 3, 4). Plant heights were up to a maximum of 68% less for both species at harvest, and widths were a maximum of 71% and 67% less at harvest for plants treated with PEG for marigolds and impatiens, respectively. When height data were analyzed without data from the control plants included, there were no differences in height of PEG-treated plants for the first two measurement dates for marigolds. At harvest, marigolds treated with PEG-8000 from 10-47.5 gL⁻¹ had similar heights, but PEG-treated plants were significantly different from each other. Height of impatiens decreased significantly with increasing PEG-8000 concentrations, independent of whether data from the control plants were included in the analysis.

At harvest, PEG-treated plants of both species were less compact than non-treated controls when compactness was measured as either the ratio of leaf area or shoot dry weight to

height (data not shown). For marigolds, PEG-treated plants were a maximum of 86% (leaf area) or 79% (dry weight) less compact when compared to control plants (leaf area/height = $64-1,236x+6,402x^2$, $R^2 = 0.77$, $P = 0.0001$; dry weight/height = $.224-2.675x$, $r^2 = 0.50$, $P = 0.0052$; x = PEG-concentration in $\text{g}\cdot\text{L}^{-1}$). Compactness of impatiens treated with PEG-8000 decreased quadratically up to 76% (leaf area) or 72% (dry weight) (leaf area/height = $38-783x+4969x^2$, $R^2 = 0.84$, $P = 0.0001$; dry weight/height = $0.094-0.610x-7.001x^2$, $R^2=0.77$, $P=0.0001$; x = PEG-concentration in $\text{g}\cdot\text{L}^{-1}$).

Both species had shorter roots (reduced up to 82% or 84% for marigolds and impatiens, respectively) at harvest when grown in increasing concentrations of PEG-8000 (Fig. 5). Marigold root dry weight decreased linearly up to 91% with increasing PEG concentrations (data not shown). Also, shoot dry weight for both species and inflorescence dry weight (impatiens) was lower with more PEG added to Hoagland solutions (data not shown). Leaf area for both species significantly decreased by up to 94% or 92%, for marigolds or impatiens at harvest (data not shown, marigold leaf area (cm^2) = $1066-32,052x-279,086x^2$, $R^2=0.72$, $P=0.0004$; impatiens leaf area = $311-11,587x+115,272x^2$, $R^2=0.90$, $P=0.0001$; x = PEG-concentration in $\text{g}\cdot\text{L}^{-1}$).

Electrical conductivity, pH, and nutrient analysis

pH and EC during the latter half of the impatiens experiment (after Hoagland solutions and PEG-8000 were changed the second time) are represented graphically (Figs. 6, 7). Electrical conductivities of Hoagland solutions decreased significantly, with the addition of PEG-8000 (Figure 6). Over time, EC increased in the control treatment, stayed the same in the lower level PEG treatments (10, 17.5, 25, and 40 $\text{g}\cdot\text{L}^{-1}$), and decreased in high level PEG treatments (32.5, 47.5, 55, and 62.5 $\text{g}\cdot\text{L}^{-1}$) (Figure 7). Data for marigolds and the first half of the experiment for impatiens showed similar trends.

Hoagland solutions containing higher PEG concentrations had a higher pH than non-treated controls near the end of both experiments (data not shown), despite regular pH adjustments. This trend was not as pronounced as the effect of PEG on EC; however, it was statistically significant. There was a general trend for pH to increase over time in all treatments (data not shown).

Foliar tissue analysis revealed that nitrogen, calcium, zinc, and copper concentrations decreased with increasing PEG-8000 concentrations, while nickel and phosphorus increased with increasing concentrations of PEG-8000 in the Hoagland solutions (Figure 8).

Discussion

Morphology

Both impatiens and marigolds were shorter, narrower, and had smaller leaf areas and lower shoot dry weights when grown in Hoagland solutions containing PEG-8000 compared to non-treated controls. These results are similar to rice (Choi et al., 2000). For marigolds, there was little or no additional height reduction from adding more than 10 gL⁻¹ PEG-8000. For impatiens there was little additional effect on plant height at PEG-8000 concentrations above 25 gL⁻¹.

Although PEG-treated plants were smaller, non-treated plants were more compact (i.e. more leaf area or dry weight per unit plant height). Compactness traditionally has not been measured in growth retardant studies; however, van Iersel and Nemali (2004) observed that drought-stressed marigolds were also smaller, but less compact, than controls because leaf expansion is affected more by drought than stem elongation. It may be more important to

decrease height than to increase compactness, since shorter plants are cheaper to ship and generally of higher quality than tall, leggy plants.

It is important to note, though, that the two highest concentrations of PEG-8000 adversely affected growth. Impatiens and marigold height in the highest concentration increased no more than 2 cm during both experiments. Impatiens roots harvested from plants grown in the highest concentrations appeared black and did not grow much, if at all, after the addition of PEG to Hoagland solutions.

The addition of more than 25 gL⁻¹ PEG-8000 for impatiens or 10 gL⁻¹ PEG-8000 for marigolds offered little or no additional height control. At harvest, adding PEG-8000 up to 47.5 gL⁻¹ decreased heights and widths by no more than 10 mm, as compared to 25 gL⁻¹ PEG-8000 (impatiens) or 10 gL⁻¹ (marigold). This additional effect would probably not improve aesthetic quality, decrease shipping costs, or improve ease of watering and production of marigolds or impatiens. In addition, since root lengths decreased with the addition of more PEG, the lowest quantity possible is best to avoid this detrimental effect. Therefore, when considering root damage and overall aesthetic appeal, marigolds treated with 10 gL⁻¹ PEG-8000 were of the highest quality. Similarly, impatiens treated with 10-25 gL⁻¹ PEG were shorter than controls and of high quality.

Electrical conductivity, pH, and nutrient analysis

One surprising aspect of this research was the observed decrease in electrical conductivity of the nutrient solution after the addition of PEG. This effect has not been reported previously. The conductivity of solutions increases with increasing mineral nutrient concentration (Whipker and Cavins, 2000). Since PEG-8000 is a polar compound, it likely forms bonds with ionic mineral nutrients in addition to water in Hoagland solutions. Undoubtedly, the relationship among

nutrient uptake, PEG-8000, mineral nutrients, and water in Hoagland solution is more complex than a simple ionic bonding relationship. For example, if mineral nutrients form chemical bonds with PEG-8000, are they unavailable for plant uptake?

PEG-4000 decreased nutrient content in hydroponically grown corn (Izzo et al., 1989). However, nutrient quantities were expressed on a per plant basis, not per unit dry weight. Since we observed that PEG decreased growth, nutrient contents would naturally be lower in smaller plants. Unfortunately, height and dry weight were not measured by Izzo et al. (1989), so it is unclear if nutrient content was lower because of lower nutrient concentrations in the tissue, or a decrease in plant size.

To address this question, we submitted impatiens tissue for nutrient analysis. Nitrogen, calcium, zinc, and copper concentrations were significantly lower with the addition of PEG-8000 to Hoagland solutions. Zinc and copper were present at concentrations below the recommended range (zinc: 57-67 $\mu\text{g}\cdot\text{g}$, copper: 20-37 $\mu\text{g}\cdot\text{g}$) for impatiens in all treatments (Mills and Jones, 1996). Some of the effect of PEG on zinc and copper concentrations may be explained by significantly higher pH present in solutions containing PEG. Copper and zinc are less available in mineral soils with pH levels greater >7 (Truog, 1948). In some cases, PEG-containing solutions attained pH levels above eight before they were adjusted with sulfuric acid. However, nutrient exchange likely is different in Hoagland solutions than in mineral soils, where soil colloids bond with nutrients through cation exchange. According to Marschner (1995), symptoms of copper and zinc deficiencies include reduced growth; however, levels of these nutrients were deficient in control plants as well, where pH levels were never above 7. Nitrogen and calcium levels were also significantly lower with increasing PEG concentrations; however, since levels never dropped below recommended rates, this effect probably did not affect plant growth (Mills and Jones,

1996). So, the observed height differences may not be explained from PEG-induced nutrient deficiencies, especially since plants did not show deficiency symptoms typical for zinc or copper (Mills and Jones, 1996). All plants, including controls, had slight interveinal chlorosis early in the experiment. These symptoms were not due to the presence of PEG-8000 since they were visible in all treatments.

Phosphorus rates increased with PEG, which may be related to the low zinc concentrations. Zinc deficiencies are associated with increased phosphorus uptake and translocation to shoot tissues (Marschner, 1995). Little research has determined the effects of nickel on impatiens growth; in fact, no recommendations for impatiens are available in the literature. However, nickel levels in two of the higher PEG treatments are above general recommended levels for Ni-sensitive species (Marschner, 1995). PEG-8000 in this experiment contains trace amounts of nickel ($\leq 0.0005\%$), which may explain the increase in nickel with increasing PEG concentrations. Also, the observed increase in both nickel and phosphorus concentrations in the impatiens may simply be an artifact of the reduced growth. The increased growth at low PEG concentrations may have resulted in a dilution of these nutrients.

Observed decreases in EC with increasing PEG concentration are not likely to be explained only by possible binding of zinc, copper, and calcium to PEG-8000, since these materials only represent a small component of the total nutrient content in Hoagland solution. Since this trend was observed even in newly prepared PEG-8000 solutions, the decrease in EC is probably related to nutrient absorption to polar sites on PEG. It is possible that nutrients are bound by PEG-8000 in solution, but are still available for plant uptake, much like cations are temporarily bound to soil colloids. However, the interaction of PEG-8000 and plant micro- and

macronutrients is beyond the study and this topic must be explored in greater detail in future research.

Electrical conductivity increased over time in the control treatment, but generally decreased over time in plants grown in PEG-8000 quantities greater than 32.5 g L^{-1} . This likely is related to differences in the ratio between water and nutrient uptake among treatments. Stomata will close when water availability decreases (Taiz and Zeiger, 1998) (i.e., at high PEG concentrations), thus reducing transpiration and water uptake by the plants. Since nutrient uptake is not directly related to water uptake, PEG may affect transpiration more than nutrient uptake. A decrease in water uptake without a concomitant decrease in nutrient uptake will result in a decrease in the nutrient concentration of the solution, and thus a decrease in EC.

Conclusions

PEG-8000 concentrations of 10 g L^{-1} or 25 g L^{-1} reduce growth of marigolds and impatiens, respectively, in nutrient culture without damaging roots or reducing plant quality. Rates $> 47.5 \text{ g L}^{-1}$ PEG-8000 should be avoided because plants had small, black root systems and did not exhibit new shoot growth. Future research in this area should determine the effects of PEG-8000 on plant nutrient uptake and availability in hydroponic culture and in typical greenhouse media. Ultimately, it is of more practical interest to determine if PEG-8000 reduces growth of bedding plants in greenhouse media. Future research should determine if PEG-8000 also reduces growth of bedding plants in soilless mixes, and if so, what rates and cultural conditions would be appropriate.

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Figure 1. Effects of PEG-8000 concentrations in Hoagland solution on height of marigold 42, 56, and 70 d after seeding. Data points are the mean of two blocks with standard error.

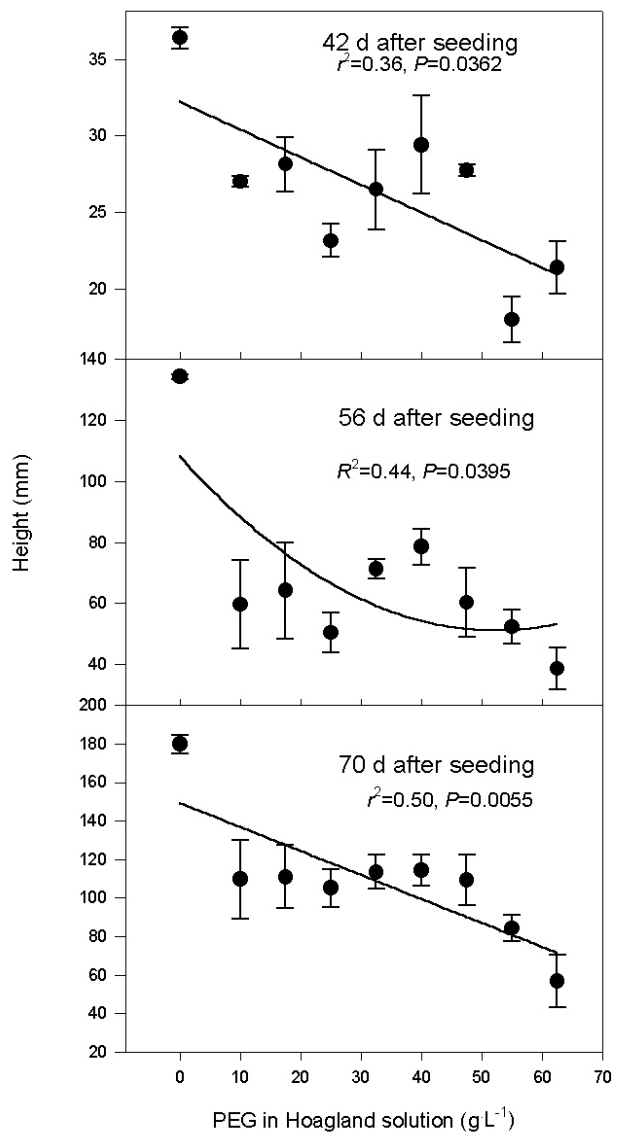


Figure 2. Effects of PEG-8000 concentrations in Hoagland solution on height of impatiens 84 and 98 d after seeding. Data points are the mean of two blocks with standard error.

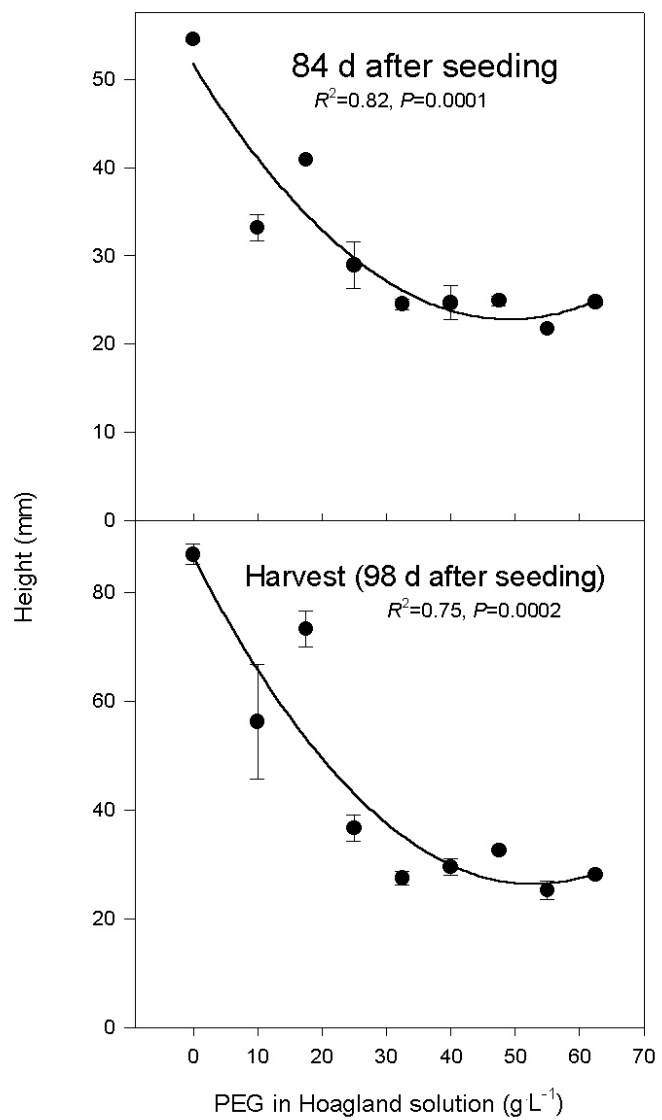


Figure 3. Effects of PEG-8000 concentrations in Hoagland solution on width of marigolds 42, 56, and 70 d after seeding. Data points are the mean of two blocks with standard error.

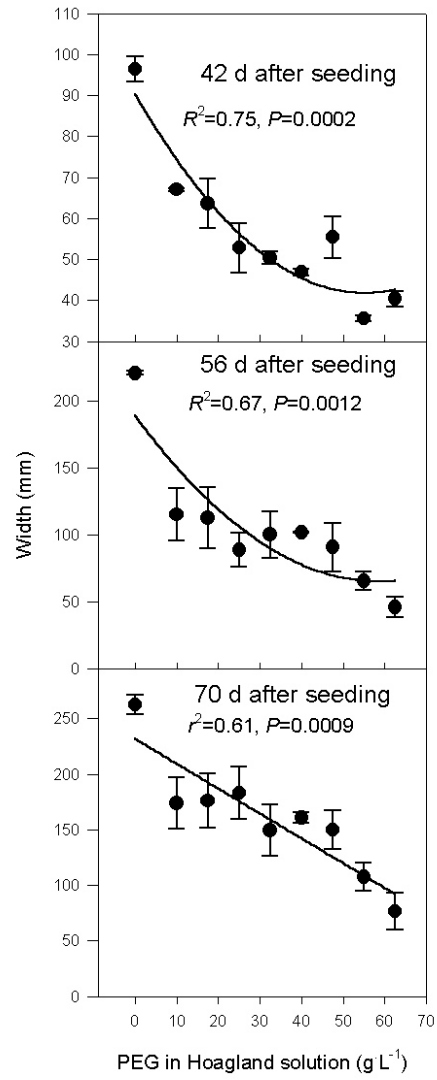


Figure 4. Effects of PEG-8000 concentrations in Hoagland solution on width of impatiens 84 and 98 d after seeding. Data points are the mean of two blocks with standard error.

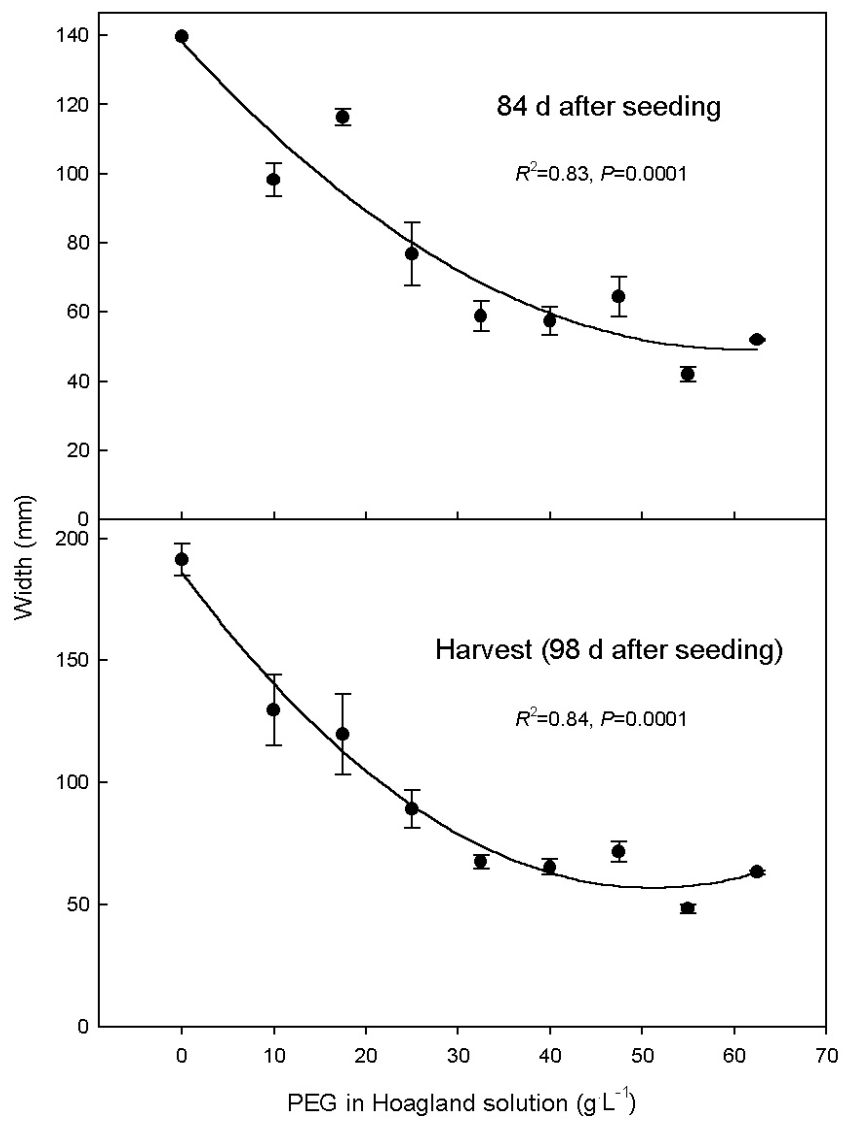


Figure 5. Effects of PEG-8000 concentrations in Hoagland solution on root length of marigolds and impatiens at harvest (70 or 98 d after seeding, for marigolds and impatiens, respectively) Data points are the mean of two blocks with standard error.

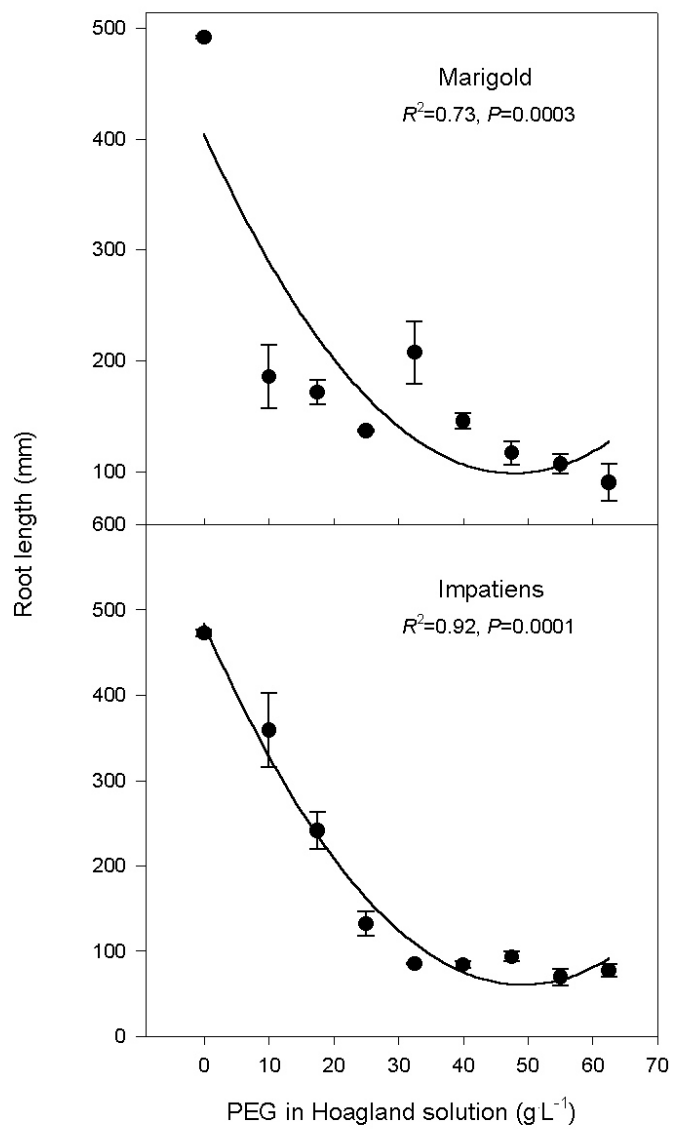


Figure 6. Effects of PEG-8000 concentrations on Hoagland solution pH. Data are presented for impatiens' Hoagland solutions 78, 82, 85, 87, 93, and 97 d after seeding. Data points are the mean of two blocks with standard error.

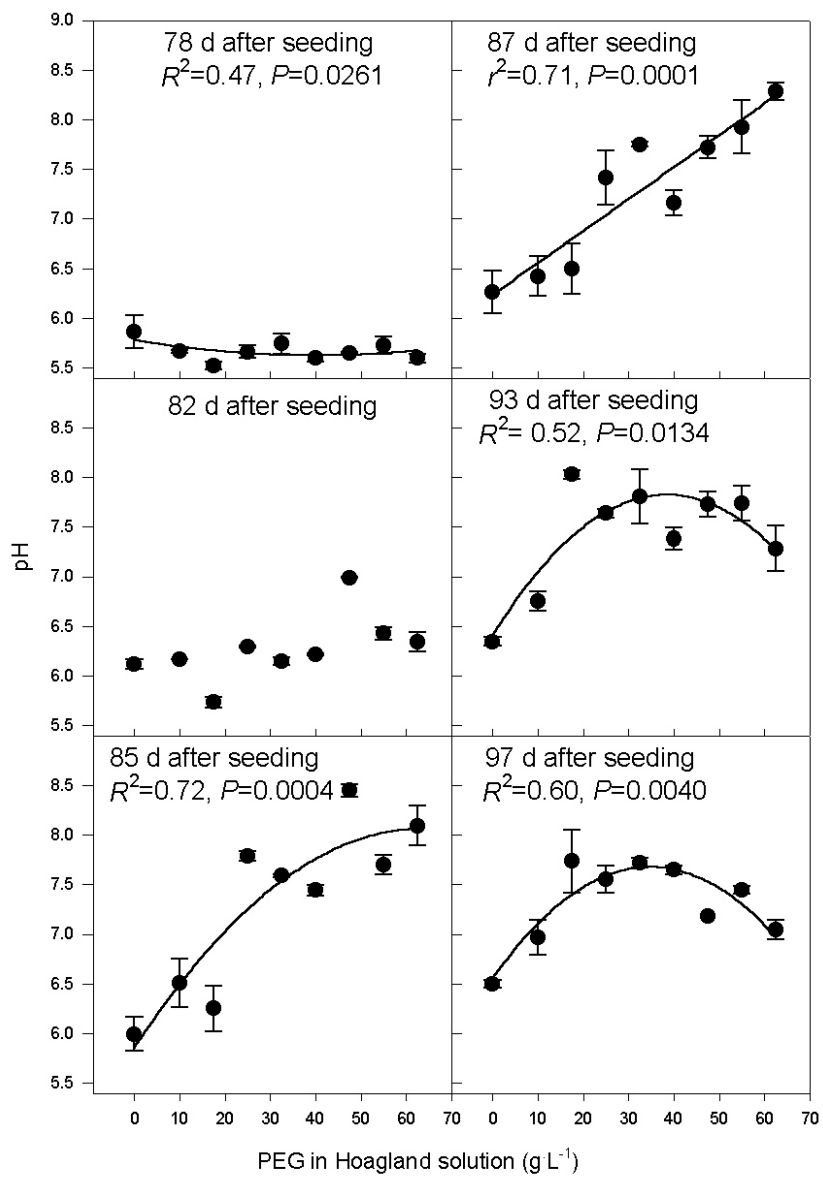


Figure 7. Effects of PEG-8000 concentrations on Hoagland solution electrical conductivity. Data are presented for impatiens' Hoagland solutions 78, 82, 85, 87, 93, and 97 d after seeding. Data points are the mean of two blocks with standard error.

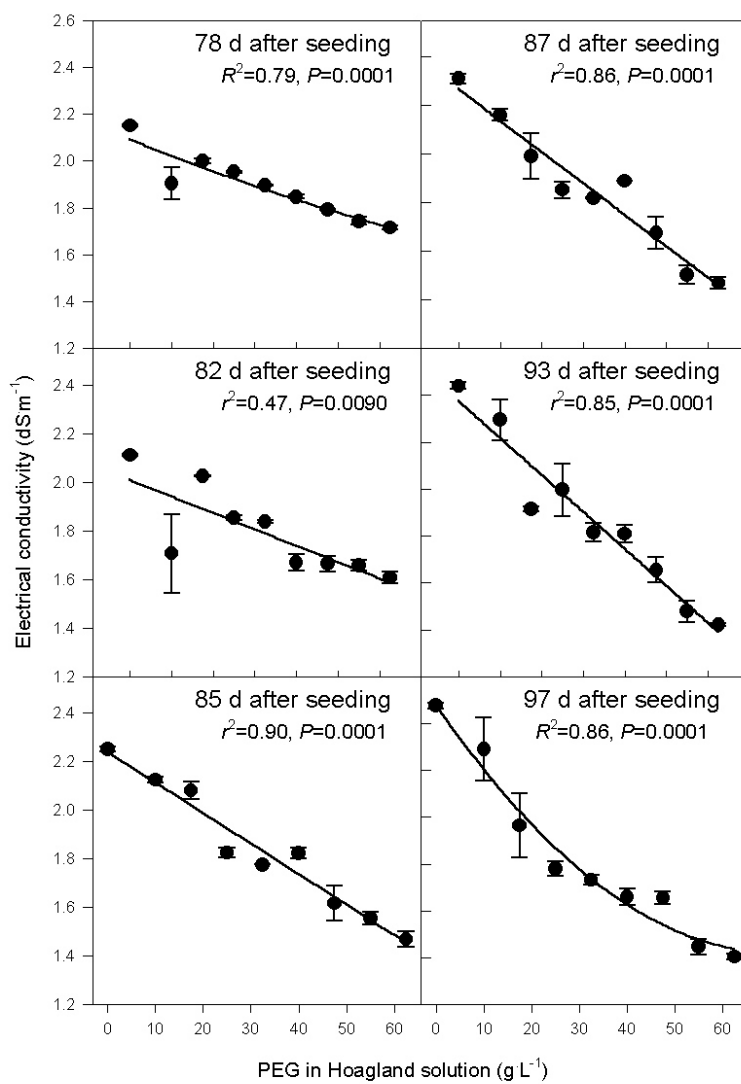
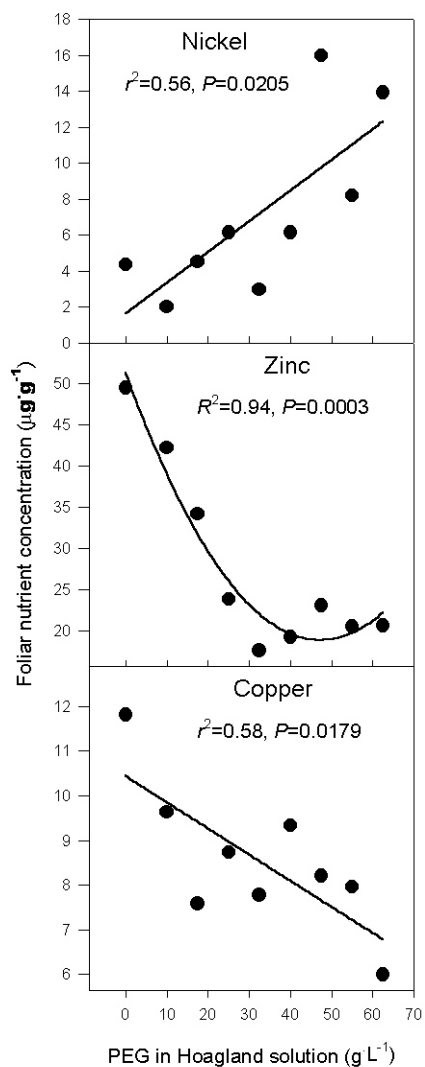
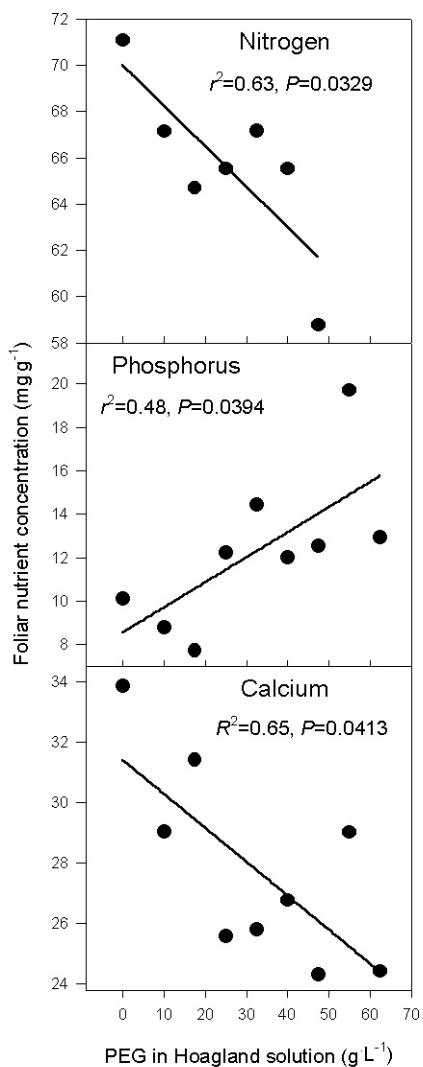


Figure 8. Effects of PEG-8000 concentrations in Hoagland solution on impatiens' foliar nutrient concentrations. Data are only presented for those macro- and micronutrients that were significantly affected by PEG-8000 concentration. Foliar samples from both blocks were combined for nutrient analysis to obtain adequate material for analysis.



CHAPTER 4

EFFECTS OF PEG-8000 ON GROWTH, MORPHOLOGY, AND ANATOMY OF *SALVIA*
SPLENDENS 'BONFIRE' SEEDLINGS²

²Burnett, S.E., S.V. Pennisi, M.W. van Iersel, and P.A. Thomas. To be submitted in *The Journal of the American Society for Horticultural Scientists*.

Abstract.

Osmotic compounds slow growth of hydroponically grown ornamental bedding plants. However, it is unknown if the same is true in growing media. For this reason, polyethylene glycol 8000 (PEG-8000) was applied to a soilless growing medium in June, 2003 at the following concentrations in the growing medium: 0, 15, 20, 30, 42, 50, 60, 72, or 83 g·L⁻¹. *Salvia splendens* F. Sellow. ex Roem & Shult. (salvia) seeds were planted in the growing medium to determine if the osmotic compound, PEG-8000, may reduce hypocotyl height and elongation of seedlings. Such reduction may improve plant quality. Emergence percentage was below 50% in the three highest PEG-8000 concentrations but there was no difference among the remaining treatments. For salvia treated the five lowest concentrations, hypocotyl height was reduced up to 35% (21 d after seeding), and salvia were a maximum of 53% shorter and 20% more narrow than non-treated seedlings 70 d after seeding. Leaf water potential (Ψ_w) was significantly reduced up to -0.8 MPa by PEG-8000 treatments. Reductions in leaf Ψ_w were correlated with height reductions 21 d after seeding and were correlated with reductions in leaf Ψ_s 35 d after seeding. On an anatomical level, stem diameter was reduced mainly due to reductions in vascular cross-sectional area. Xylem cross-sectional area decreased more relative to stem and phloem cross-sectional area. PEG-treated plants elongated more slowly because of reduced leaf water potential, and possibly because of reduced stem xylem development.

Growing annual seedlings comprises a large portion of the national floriculture market. In 1998, seedling production was estimated to be worth approximately \$107 million (National Agricultural Statistics Service, 1998). Over the past 20 years, there has been an increasing trend to grow seedlings in trays with separate compartments for each seedling rather than use bare-root seedlings. There are many advantages to growing seedlings separately, but possibly the biggest advantage for commercial greenhouse growers is that seedlings grown in individual cells are marketable as much as 7 to 10 d earlier than bare-root seedlings (Ball, 1991). However, commercial production has just shifted from using bare-root seedlings within the past 20 years, there is a need for research on the production of seedlings in separate compartments. Quality is a significant issue in seedling production since seedlings are usually sold to other growers who have high quality standards. One important aspect of quality is the overall appearance of the plant, which is often decided in part by the plant size (Styer and Koranski, 1997). Short, compact seedlings are considered to be of high quality because they are cheaper to ship, survive transplanting more easily, and are more aesthetically pleasing than tall seedlings (Davis and Curry, 1991).

Chemical growth retardants, which reduce synthesis of gibberellin (Davis and Curry, 1991), may seem like the most obvious method of controlling height of seedlings. However, many growers hesitate to use chemical growth retardants on young seedlings because these chemicals are applied at low rates to seedlings ($5 \text{ mg}\cdot\text{L}^{-1}$ for paclobutrazol; Uniroyal Chemical, 2000), and over-application can stunt growth. In some cases, applications of chemical growth retardants delayed growth of plants after they were transplanted into the landscape up to 4 to 6 weeks (Latimer and Baden, 1994).

One other common method of reducing elongation of plants is to reduce the substrate water content or Ψ_w . Plants grown in substrates that have a low Ψ_w typically have low leaf Ψ_w and turgor potential (Ψ_p). Turgor potential, in excess of the minimum ‘yield’ threshold, is the driving force for cell elongation (Carpita and McCann, 2000; Cosgrove, 1997; van Volkenburgh 1999; Frensch, 1997). Drought stress is commonly used to slow elongation of commercially grown plants. For example, drought-stressed vegetable transplants are shorter than non-stressed transplants (Latimer, 1992; Latimer and Severson, 1997).

The disadvantage of using drought to reduce growth of ornamental seedlings is that it can cause damage, including leaf abscission (Mohr and Schopfer, 1995). Even minor damage, such as marginal leaf scorching, would decrease seedling quality. Further complicating the matter, seedlings are grown in small substrate volumes (approximately 8 mL for 288-trays) that dry out quickly between irrigations. It would be desirable for growers to have a method of controlling drought stress so that seedlings are shorter and of high quality.

Osmotic compounds, such as polyethylene glycol (PEG) impose controlled drought via osmotic stress. PEG forms hydrogen bonds with water and decreases the matric potential of substrates (Kjellander and Florin, 1981; Steuter et al., 1981). Hydroponically grown marigolds (*Tagetes patula* L.) and impatiens (*Impatiens walleriana* Hook. F.) treated with PEG-8000 (8000 stands for molecular weight) were shorter and of equivalent or greater quality to non-treated plants (Burnett, 2004). Other plants that exhibited less shoot or root growth when grown in PEG compounds of varying molecular weight include *Oryza sativa* L. (rice), *Pinus radiata* D. Don [*P. Insignis* Dougl. ex Loud.] (Monterey pine), *Zea mays* L. (corn), and *Sorghum bicolor* (L.) Moench [*S. Vulgare* Pers.] (sorghum) (Choi et al., 2000, Gill et al., 2001, Lawlor, 1970, Zou et al., 2000).

It is unknown, however, if PEG-8000 reduces growth of ornamental seedlings in soilless growing media. In addition, it would be of interest to examine the effects of PEG-8000 on plant morphology. It would be important, further, to correlate morphological information with anatomical data, in order to describe macroscopic changes that occur. For these reasons, the two objectives of this experiment were to determine if PEG-8000 reduces shoot elongation of a popular summer annual, *Salvia splendens* F. Sellow. ex Roem & Shult. (salvia), and to determine the mechanism by which plant morphology is modified.

Annual salvia was chosen as the model crop for several reasons. Firstly, salvia grows rapidly, and chemical growth retardants are usually recommended for commercial greenhouse production (Nau, 1998). Secondly, previous research reported that drought-stressed salvia (leaf $\Psi_w = -1.1$ to -1.4 MPa) were shorter and more compact than non-stressed salvia (Eakes et al., 1991). For these reasons, annual salvia is a good candidate for height control using osmotic compounds.

Materials and Methods

Polyethylene glycol 8000 was mixed with water and added to a commercial peat-based growing medium specifically formulated for germinating seedlings (a mixture of sphagnum peat, perlite, and vermiculite; Germinating mix, Fafard, Anderson, S.C.) at the following concentrations in the growing medium: 0, 15, 20, 30, 42, 50, 62, 70 and 83 g·L⁻¹. Each PEG-8000 and growing medium combination was shaken vigorously in a large plastic container for 10 minutes to produce a homogenous mix. Treated growing medium was placed in 6×6 cell sections cut from 288-trays (cell volume = 8.5 mL). Each 6×6 section was one experimental unit. Substrate Ψ_w of three

samples from each treatment was measured using a vapor pressure osmometer (Model 5520 Vapro Vapor Pressure Osmometer, Wescor, Inc., Logan, Utah). The volumetric water content of the growing medium during these measurements was approximately 29%, which was the target water content for the duration of the experiment.

Two salvia 'Bonfire' (Ball Seed Company, West Chicago, Ill.) seeds were planted in each cell on June 6, 2003. Salvia seeds were grown until emergence in a Conviron growth chamber (E-15, Conviron, Winnipeg, Canada; light levels $\approx 300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature $\approx 24 \text{ }^\circ\text{C}$). While in the growth chamber, plants were misted overhead as needed to maintain a constant volumetric water content ($29\pm 3\%$) using a mist nozzle hose attachment. Fourteen d after seeding, plants were grown on a mist bench in a glass greenhouse (temperature = $25\pm 4.4 \text{ }^\circ\text{C}$) and irrigated 20 s every 20 min from 6:00 am until 6:00 pm (EST) 15 d after seeding. The mist timing was changed to 20 s every 30 min from 6:00 am until 6:00 pm (EST) on 19 d after seeding, to obtain appropriate irrigation levels.

The smaller seedling was removed from cells containing more than one plant 27 d after seeding. All seedlings were fertilized twice weekly with a 20N:8.7P:16.6K fertilizer (20-20-20 General Purpose, Scotts Company, Marysville, Ohio) solution with a nitrogen concentration of $200 \text{ mg}\cdot\text{L}^{-1}$, beginning when the first true leaves were visible (23 d after seeding). When plugs were fertilized, they were removed from the mist bench and hand-misted overhead with a fertilizer solution using a mist nozzle for 20 s. Electrical conductivity (EC) of the pore water in the growing medium of one cell/replication was measured at the beginning of the experiment and bi-weekly afterwards using a ECK-1 Basic SigmaProbe (Delta-T Devices, Ltd., Cambridge, UK), inserted directly into the medium. Granular Marathon (Imidacloprid, Olympic Horticultural

Products, Mainland, Pa.), for whitefly and thrips control, was applied to seedlings at a rate of 5 mL for each experimental unit.

Experimental unit weight was recorded twice daily throughout the experiment and plants were removed from mist or not watered in the growth chamber when necessary to maintain constant water contents ($29\% \pm 3\%$). Since PEG is water soluble, this also prevented leaching which would result in loss of PEG and thus change treatment levels. Empty tray weights were noted at the beginning of the experiment. After emergence, one representative seedling from the outermost row was harvested bi-weekly. The weight of this seedling was multiplied by the number of plants in each tray to account for changes in plant weight throughout the experiment. Each tray contained approximately 307 mL of growing medium, and the same volume of excess treated growing mix was dried in a drying oven at approximately 80 °C. The amount of water in each tray was calculated by subtracting the tray, seedling, and dry growing medium weight from the experimental unit weight. The drying oven did not completely remove all the water bound to PEG-8000 from the growing medium. Thus, the estimated dry weight of the growing medium included some water, which was permanently bound to the PEG-8000 and therefore not available for plant uptake.

Data collected include number of days to emergence (cotyledons perpendicular to hypocotyl) and percentage emergence. Plants in the outermost rows of the 6×6 cell sections were not measured for data collection, except after transplanting as discussed below, to prevent edge effects. Height and width were measured at emergence and then bi-weekly until the termination of the experiment (21, 35, 56, and 70 d after seeding). Plant width was the maximum horizontal distance between any two leaf tips. Hypocotyl height was measured 21 d after seeding. At harvest (70 d after seeding) leaf area was measured using a leaf area meter (Li-3100, Li-Cor, Inc.,

Lincoln, Nebr.). Longest root length was measured. Shoot (stems and leaves) and root tissues were dried in an oven at 80 °C for at least three days; then dry weights were measured.

Compactness (leaf area/height at harvest), stem density (stem dry weight/stem length) and specific leaf weight (leaf dry weight/leaf area) were calculated from these data. All living seedlings except those from the border were harvested for shoot data, but, for roots, only four representative plants were harvested.

Leaf Water Relations

Midday leaf Ψ_w [$\Psi_w = \Psi_p + \text{solute potential } (\Psi_s)$] of the second acropetal pair of leaves was measured 21, 35, and 56 d after seeding using leaf-cutter thermocouple psychrometers (J.R.D. Specialty Equipment, Logan, Utah). Representative plants from three blocks were selected for psychrometer measurements. Leaf samples enclosed in the psychrometer chambers were equilibrated in a water bath at 25 °C for 4 h before measurement. Ψ_w of intact leaves was measured first using a microvoltmeter. Then, leaf samples were frozen to disrupt cell membranes and remove Ψ_p . Samples were then re-equilibrated as described above and Ψ_s was measured. Finally, Ψ_p was calculated by subtracting Ψ_s from Ψ_w .

Anatomy

At harvest, salvia leaves, hypocotyls, and roots from four representative blocks were fixed in Histochoice (Amresco, Solon, Ohio), an aldehyde-based fixative, under vacuum conditions. Tissue from the second acropetal leaves, and roots and hypocotyls within 2 mm of the root-shoot junction were used. The main (only) hypocotyl and the longest root from each plant were chosen. The tissue samples were dehydrated in an ascending alcohol series (25%, 50%, 75%, 90%, and 100%) and embedded in Spurr's resin (Spurr, 1969) using standard light microscopy protocols. Tissue samples were sectioned to a thickness of 3 μm with a microtome (Leica, Wetzlar,

Germany). Cross-sections were stained with 0.5% Toluidine blue, mounted on poly-lysine coated slides, and examined with a Zeiss (Thornwood, N.Y.) light microscope. Morphological data from plants treated with 0, 15, 30, and 50 g·L⁻¹ of PEG-8000 were analyzed. Hypocotyl diameter, xylem cross-sectional area, phloem cross-sectional area, pith cross-sectional area, xylem element number, and the average cross-sectional area of ten randomly selected xylem elements was measured. The ratios of xylem to phloem cross sectional, xylem to hypocotyl cross-sectional area, and phloem to hypocotyl cross-sectional area within stems were calculated from this data. Hypocotyl cross-sectional area was calculated from the hypocotyl diameter. Root xylem and pith cross-sectional area were measured, and leaf, palisade, and spongy mesophyll thickness were noted. Tissues were photographed with a digital camera, and a micrometer was photographed under the same magnification as each section. Then, diameters were calculated using Adobe Photoshop software (Adobe Systems, Inc., San Jose, Calif.) and measurements collected with the micrometer. Cross-sectional areas were measured by calculating the number of pixels in a grid with sides of known lengths (from the micrometer). Cross-sectional area was then calculated by dividing the pixels in a measured cross-sectional area by the number of pixels in an area of known dimensions.

Post Transplant Morphology

When the seedlings were harvested, six representative plants from outermost rows were transplanted into six-cell packs (cell volume = 380 mL) containing a peat-based growing medium (Fafard 3M, Fafard, Anderson, S.C.). Plants were grown on ebb and flow benches irrigated daily with a 20N:8.7P:16.6K fertilizer (20-20-20 General Purpose, Scotts Company, Marysville, Ohio) solution with a N concentration of 150 mg·L⁻¹. After transplanting, no effort was made to keep PEG-8000 in the growing medium. Height was measured bi-weekly until plants were flowering

and considered marketable (four weeks after transplant). The number of days to the appearance of the first flower (the calyx of the first flower was open and bright red) was noted.

The experimental design was a randomized complete block design with six blocks, 16 subsamples/treatment before seedling harvest, and 6 subsamples/treatment after transplanting. For root harvest data, there were 4 subsamples/treatment. Three blocks were measured for water relations data, and four blocks were measured for anatomical data. Data were analyzed using regression analysis (Proc GLM, Statistical Analysis Systems, Cary, N.C.). Volumetric water contents were averaged over all measurements before analysis. All data excluding emergence percentage were analyzed without the highest three treatments, due to high mortality in those three treatments. Emergence percentage was analyzed both with and without the three highest treatments. Leaf water relations data were also further analyzed comparing Ψ_w , Ψ_s , and Ψ_p to the seedling height. The heights measured on the same date as Ψ_w , Ψ_s , and Ψ_p measurements were used for this comparison.

Results

Growing medium Ψ_w decreased quadratically with increasing PEG concentration in the growing medium, from -0.2 to -1.8 MPa (Fig. 1). The growing media containing the two highest PEG concentrations (72 and 83 g·L⁻¹) had water potentials at or below the permanent wilting point (-1.5 MPa). Volumetric content increased significantly with increasing PEG concentration in the growing medium [volumetric water content = $29 - 6.78 \times 10^{-2}x + 1.71 \times 10^{-3}x^2$, $R^2=0.83$, $P=0.0001$; x = PEG concentration in the growing medium (g·L⁻¹)]. However, in all but the

highest treatments, the average volumetric water content was within 2.5% of 25%. The volumetric water content for the highest treatment was 36%.

The emergence percentage decreased gradually and in a quadratic manner with increasing concentrations of PEG-8000 (Fig. 2). Emergence percentage was below 50% for plants grown in PEG rates from 62-83 g·L⁻¹. For this reason, these treatments are not considered further. Emergence percentage for salvia grown in PEG-8000 rates from 0-50 g·L⁻¹ was not significantly different, although there was a trend for emergence percentage to decrease for salvia grown in PEG-8000 even within the lower rates (Fig. 2). Emergence percentage ranged from 81% in controls to 61% for seedlings treated with 50 g·L⁻¹ of PEG-8000. However, seedling emerged later when plants were grown with increasing concentrations of PEG-8000. Emergence was delayed up to 5 d for plants treated with the most PEG-8000 (16 d versus 11 d for control plants).

The EC was similar throughout the growing medium ($\approx 3.5 \text{ dS}\cdot\text{m}^{-1}$) before PEG-8000 was added. After the growing medium was treated with PEG-8000, EC decreased linearly with increasing PEG-8000 rate (Fig. 3). Initially, the EC of growing medium containing PEG-8000 was up to $2.0 \text{ dS}\cdot\text{m}^{-1}$ lower than the EC of the non-treated growing medium. However, as the experiment progressed, the effect of PEG concentration on the EC decreased. The EC of the pore water of the growing medium still decreased linearly with increasing PEG-8000 concentration in the growing medium 10 and 25 d after seeding. However, the EC in the growing medium treated with $50 \text{ g}\cdot\text{L}^{-1}$ of PEG-8000 was less than $1 \text{ dS}\cdot\text{m}^{-1}$ lower than the EC in the non-treated growing medium on those two days. At 42 and 67 d after seeding, PEG-8000 had no effect on EC.

As for morphology, height at emergence was not affected by the addition of PEG-8000 to the growing medium (data not shown). However, seedling height decreased quadratically with

increasing PEG-8000 concentrations at 21, 35, 56, and 70 d after seeding (Fig. 4). After emergence, height of seedlings treated with the highest PEG-8000 concentration ($50 \cdot \text{L}^{-1}$) was approximately half of non-treated salvia. Hypocotyl height of salvia was similar at PEG concentrations of 0 to $30 \text{ g} \cdot \text{L}^{-1}$, but decreased at higher concentrations. At 21 d after seeding, salvia grown in $50 \text{ g} \cdot \text{L}^{-1}$ of PEG-8000 had hypocotyls that were 35% shorter than non-treated plants. Seedling width also decreased with increasing concentrations of PEG-8000 throughout the experiment (Fig. 5). Plants grown with the highest concentration of PEG-8000 were 53% shorter and 20% narrower than controls at harvest, when seedlings were marketable. At this time, compactness of salvia seedlings increased quadratically, and up to 33%, as they were treated with higher rates of PEG-8000. However, seedlings treated with PEG-8000 had lower stem densities compared to non-treated controls (Fig. 6).

At harvest, leaf area decreased linearly for seedlings treated with increasing rates of PEG-8000, but specific leaf weight was not significantly different (Fig. 7). Salvia treated with the most PEG-8000 had 31% less leaf area than control plants. Shoot dry weight also decreased quadratically for seedlings grown in more PEG-8000. There was a large difference in shoot dry weight (40 mg) between non-treated seedlings and salvia treated with $15 \text{ g} \cdot \text{L}^{-1}$ of PEG-8000. However, all PEG-treated seedlings had similar shoot dry weight ($\sim 50 \text{ mg}$, Fig. 7). Root length and dry weight decreased linearly (dry weight) or quadratically (length) for seedlings treated with more PEG-8000. Seedlings treated with the most PEG-8000 had roots that were approximately 70 mm shorter and weighed 47% less than root of non-treated salvia (Fig. 7).

Leaf Water Relations

Early in the experiment (21 d after seeding), Ψ_w decreased quadratically from -0.6 MPa in control plants to a minimum of -1.4 MPa and Ψ_p decreased linearly as PEG concentration in the

growing medium increased (Fig. 8). Leaf Ψ_p of all seedlings grown in PEG-8000 was below zero 21 d after seeding. At 21 d after seeding, height decreased significantly, as leaf water potential decreased (height=1.41+0.506 Ψ_w , $r^2=0.44$, $P=0.0399$). Osmotic potential was not statistically different from control seedlings at this time.

At 35 d after seeding, Ψ_s decreased linearly as PEG-8000 rate increased. PEG-8000 concentration affected Ψ_s less at 35 d after seeding than Ψ_w at 21 d after seeding. Non-treated salvia had a Ψ_s of -1.02 MPa, 35 d after seeding, and the lowest Ψ_s (-1.19 MPa) was measured in salvia treated with 50 g·L⁻¹ PEG-8000. Height reductions at 35 d after seeding were correlated with reductions in Ψ_s (height=-0.986-0.00439 Ψ_s , $r^2=0.55$, $P=0.0131$). Water potential and Ψ_p were not significantly affected by PEG-8000 concentration in the growing medium at 35 d after seeding.

At 56 d after seeding, Ψ_p of seedlings decreased with increasing PEG rate, but Ψ_w and Ψ_s were not affected by PEG-8000 treatments. For controls, Ψ_p was 0.33 MPa, and the lowest Ψ_p (-0.04 MPa) was measured in salvia treated with 42 g·L⁻¹ of PEG-8000. Salvia treated with 30-50 g·L⁻¹ of PEG-8000 all had Ψ_p close to or below zero. Height of seedlings did not decrease significantly as Ψ_p decreased 56 d after seeding, and Ψ_p was positive for almost all seedlings at 35 and 56 d after seeding.

Anatomy

Salvia hypocotyl diameter and xylem cross-sectional area decreased quadratically to increasing PEG-8000 concentration within the growing medium (Figs. 9 and 10). Seedlings treated with 30 g·L⁻¹ of PEG-8000 had the smallest hypocotyl diameter (1.23 mm) and xylem cross-sectional area (0.22 mm²) compared to non-treated seedlings which had a hypocotyl diameter of 1.62 mm and xylem cross-sectional area of 0.62 mm². The cross-sectional area of

phloem in hypocotyl cross sections also responded quadratically as seedlings were treated with more PEG-8000. Non-treated salvia had phloem cross-sectional areas of approximately 0.23 mm², while salvia treated with the most PEG-8000 had phloem cross-sectional areas of approximately 0.15 mm². Pith cross-sectional area in hypocotyls was not affected by PEG-8000 treatments, however, there appeared to be less pith in seedlings treated with 30 or 50 g·L⁻¹ of PEG-8000, and, in some cases, pith was not present. The ratios of xylem to phloem cross-sectional area and xylem to hypocotyl cross-sectional area decreased with increasing PEG-8000 concentration (xylem:phloem=2.79-0.0127x, $r^2=0.41$, $P=0.0335$; xylem:hypocotyl=0.278-0.002x, $r^2=0.52$, $P=0.0073$, where x=PEG-8000 concentration). The ratio of phloem to hypocotyl cross-sectional area was not affected by PEG-8000.

Xylem element diameter in hypocotyls was not affected by PEG-8000 treatments (Figs. 10 and 11). However, xylem element number decreased rapidly and in a quadratic manner for seedlings grown in increasing PEG-8000 concentrations (Figs. 10 and 11). The hypocotyl xylem element number was different by only up to 200 for all PEG-treated seedlings, but, non-treated salvia had approximately twice the xylem element number of PEG-treated salvia (4800 versus 2600 xylem elements).

For roots, there was a trend for xylem cross-sectional area to decrease with increasing PEG-8000 concentration in the growing medium (data not shown). However, this trend was not significant, and PEG-8000 did not affect the pith cross-sectional area in roots either (data not shown). PEG-8000 concentrations did not affect leaf thickness or the thickness of palisade or spongy mesophyll layers (data not shown).

Post-Transplant Morphology

Fourteen d after seedlings from the border were transplanted, height of PEG-treated salvia was still less for seedlings treated with increasing PEG-8000 concentrations in the growing medium (Fig. 12). Salvia that had been treated with $42 \text{ g}\cdot\text{L}^{-1}$ of PEG-8000 were the shortest, and were approximately 40 mm shorter than non-treated salvia. Twenty-eight d after transplanting, salvia that had been treated with greater quantities of PEG-8000 were not significantly different than non-treated salvia (Fig. 12). Flowering was delayed by PEG-8000, and the delay was greater for seedlings treated with increasing rates of PEG-8000. Salvia treated with PEG-8000 flowered as much as 12 d later than non-treated plants.

Discussion

Water potential of the growing medium decreased with increasing PEG-8000 concentration across a broad range (-0.2 to -1.8 MPa). These concentrations were chosen based on preliminary, unpublished data which showed that salvia would not survive in growing media treated rates of PEG-8000 higher than $83 \text{ g}\cdot\text{L}^{-1}$. Since PEG-8000 reduces the matric potential of substrates, it was expected that the water potential of the growing medium would decrease as more PEG-8000 was added (Steuter et al., 1981).

The volumetric water content of the growing medium increased significantly as more PEG-8000 was added (Table 1). A similar trend was observed in an experiment in which marigolds were treated with varying concentrations of PEG-8000 (Burnett, 2004). However, volumetric water content in treatments ranging from 0 to $45 \text{ g}\cdot\text{L}^{-1}$ of PEG-8000 in the growing medium was similar.

Concentrations higher than $62 \text{ g}\cdot\text{L}^{-1}$ PEG-8000 in the growing medium decreased emergence percentage to 40% at least. In commercial seedling production, attempts are made to maintain emergence percentages close to 100% (Corr, 1998), so the highest three treatments would be unacceptable. Emergence was not close to 100% for salvia treated with $0\text{-}50 \text{ g}\cdot\text{L}^{-1}$ of PEG-8000 in the growing medium. However, since PEG-treated salvia ($15\text{-}50 \text{ g}\cdot\text{L}^{-1}$ of PEG-8000) did not emerge at different rates from non-treated salvia, PEG was not the reason for low emergence percentages. Emergence was also delayed up to 5 d by the addition of PEG-8000 to the growing medium (Fig. 2). However, the expected time for salvia to germinate is 12-15 d (Nau, 1998), and all salvia were within 1 d of the normal time to germinate.

During the first 25 d after seeding, the EC of the growing medium decreased with the addition of higher rates of PEG-8000 to the growing medium (Fig. 3). PEG-8000 appeared to affect EC the most before seedlings had emerged. Afterwards, EC was also affected by seedling uptake of nutrients from the growing medium and fertilization, so the effects of PEG-8000 on EC were smaller or not evident. EC also decreased with the addition of PEG-8000 to Hoagland solution or soilless media (Burnett, 2004), which may have affected nutrient uptake. Impatiens grown in Hoagland solution with varying PEG-8000 concentrations had significantly lower foliar concentrations of nitrogen, calcium, zinc, and copper than impatiens that were not treated with PEG-8000. Impatiens were deficient in zinc and copper, in all treatments, however, zinc and copper concentration decreased with increasing PEG concentration (Burnett, 2004). It would be of interest in future experiments to further explore the relationship between PEG-8000 and nutrient uptake. Also, from a practical standpoint, future experiments should determine if different fertilizer regimes are necessary for commercial greenhouse growers who choose to use PEG-8000 to reduce elongation of seedlings.

Height and width of salvia were reduced throughout the experiment by PEG-8000 treatments, and at 21 d after seeding, hypocotyl height was reduced (Figs. 4 and 5). Similarly, hydroponically grown marigolds and impatiens treated with PEG-8000 were also shorter and more narrow than non-treated marigolds and impatiens (Burnett, 2004). Hypocotyl height was a larger percentage of the overall height of salvia treated with PEG-8000 (average=59%) than for controls (44%) at 21 d after seeding (Fig. 4). However, hypocotyls did not elongate much after 21 d after seeding. At harvest, PEG-treated salvia were 30-50 mm tall, so the hypocotyl, which was an average of 4 mm when measured, contributed approximately 8-13% to the overall height. Salvia treated with 15-42 g·L⁻¹ of PEG-8000 were of equal or greater quality compared to non-stressed salvia when seedlings were harvested. Seedlings treated with 50 g·L⁻¹ of PEG-8000 had some foliar necrosis.

Height is probably the most important morphological factor considered in overall plant quality. Ideally though, seedlings that are both shorter and more compact would be desirable. In this experiment, salvia were both shorter and more compact (leaf area/height) in response to PEG treatments (Fig. 6). Previously, Eakes et al. (1991), also reported that annual salvia exposed to episodic drought (with leaves reaching water potentials of -1.1 to -1.4 MPa on four separate occasions) were more compact. In that experiment, leaf area was reduced less than shoot dry weight, but height was not measured (Eakes et al., 1991). In contrast, van Iersel and Nemali (2004) found that drought-stressed marigolds were less compact than non-stressed marigolds. PEG-treated marigolds were also less compact than non-treated marigolds (Burnett, 2004). The relationship between compactness and drought stress may be species specific. Another measure of compactness is stem density. Stem density decreased for salvia that were grown with

increasing PEG-8000 (Fig. 6), probably because PEG-treated salvia had thinner stems than non-treated salvia (Fig. 9).

Leaf area decreased for salvia treated with increasing rates of PEG-8000 (Fig. 7). However, at lower PEG-8000 concentrations shoot dry weight was affected to a larger extent than leaf area (Fig. 7). Osório et al. (1998) reported that leaf area and shoot dry weight of *Eucalyptus globulus* Labill. are equivalently affected by drought stress, while Eakes et al. (1991) found that shoot dry weight of annual salvia is affected by drought stress to a larger degree than leaf area. Similar to compactness, this relationship is probably species specific and deserves more attention in the future.

Root elongation and dry weight were also reduced by additions of PEG-8000 to the growing medium (Fig. 7). Similarly, drought-stressed pepper seedlings had shorter and lighter roots than non-stressed peppers (Lescovar and Cantliffe, 1992; Watts et al., 1981). Roots are often less affected by drought stress than shoots, and drought-stressed plants often have higher root to shoot ratios than non-stressed plants (Sharp and Davies, 1979; Hsiao and Jing, 1987). In this experiment, root dry weight appeared to be affected by PEG-8000 treatments less than shoot dry weight (Fig. 7). However, root to shoot ratios were not significantly different.

Although roots were shorter and smaller, this would not necessarily decrease the quality of salvia seedlings. All salvia had substantial root systems. In fact, roots of seedlings treated with all concentrations of PEG-8000 except $15 \text{ g}\cdot\text{L}^{-1}$ were not circling the bottoms of cells, while non-treated seedlings had roots that were circling the bottoms of cells. Such root restriction may be detrimental to growth after transplanting and is not desirable (NeSmith and Duval, 1998). Non-treated salvia would not have exhibited root circling if they had been transplanted sooner, however, many commercial greenhouse growers keep seedlings in the greenhouse for many days

or even weeks before transplanting them. So, PEG-treated seedlings could probably be kept in their original containers for longer periods of time before roots circled container bottoms.

Leaf Water Relations

Early in the experiment (21 d after seeding), Ψ_w and Ψ_p were reduced by additions of PEG-8000 to the growing medium, and all seedlings treated with PEG-8000 had Ψ_p below zero (Fig. 8). Height after 21 d was significantly lower as Ψ_w decreased. Since Ψ_p was negative and decreased with the addition of PEG-8000, cell elongation was probably reduced, because Ψ_p is the driving force for cell elongation (Carpita and McCann, 2000; Cosgrove, 1997; van Volkenburgh 1999; Frensch, 1997).

Later in the experiment, the effect of PEG-8000 on the components of leaf Ψ_w was not as great. Water potential was not affected by PEG-treatments, but Ψ_s was reduced at 35 d after seeding, and Ψ_p was reduced at 56 d after seeding by additions of PEG-8000 to the growing medium (Fig. 8). It is possible that salvia seedlings were acclimated to the PEG-8000 concentrations in the growing medium later in the experiment. Or, Ψ_w could have been affected less by treatments at this stage because PEG-8000 could have been washed out of the growing medium. PEG-8000 is highly water soluble, and, despite efforts to keep PEG-8000 in the growing medium, the possibility that PEG-8000 was washed out of the growing medium by irrigations cannot be eliminated.

Anatomy

Salvia seedlings have atypical hypocotyl anatomy. Usually, the vascular system of herbaceous dicotyledons is arranged in vascular bundles separated by interfascicular tissue (Esau, 1976). However, many seedlings in the Lamiaceae family have continuous xylem in stems (Metcalf and Chalk, 1957). This appears to be the case for annual salvia (Fig. 11).

Hypocotyl diameter and xylem and phloem cross-sectional area were reduced by the addition of PEG-8000 to the growing medium (Figs. 9 and 11). Reduced hypocotyl diameter and xylem cross-sectional area are typical effects of drought stress. For example, grape (*Vitis vinifera* L.) grown at lower than optimal soil water potential (-0.07 MPa) had reduced above ground shoot diameter and xylem cross-sectional area than grape grown at optimal soil water potential (-0.01 MPa) (Lovisolo and Schubert, 1998). In this experiment, xylem cross-sectional area decreased preferentially compared to other tissues because the ratio of xylem to phloem and stem cross-sectional areas decreased with increasing PEG-8000 concentration. It appears that the reduction of xylem tissue is one anatomical adaptation of salvia seedlings to the presence of PEG-8000 in the growing medium.

Reductions in xylem cross-sectional area may be due to reductions in xylem element size, xylem element number, or both. Vessel size was reduced in drought stressed grapes (Lovisolo and Schubert, 1998). According to Poiseuille's equation ($F = \Delta P \pi r^4 \cdot (8\eta l)^{-1}$, where F=flow rate, ΔP =pressure difference, r = radius, η =viscosity, and l=length of a tube), plants often increase vessel size when water is not limiting to increase the rate of water flow through xylem elements. Sunflower (*Helianthus annuus* L.) and apple cactus (*Cereus peruvianus* L. (Mill.)) had smaller vessel diameter when grown at low soil water contents (Penfound, 1931; Arnold and Mauseth, 1997). For salvia, xylem element size was not significantly different; xylem cross-sectional area was smaller in PEG-treated salvia due to reduced xylem element number (Fig. 10). It is theorized that vessel diameter is less at low soil water potentials to reduce the incidence of embolism (Lo Gullo et al., 1995; Zimmerman, 1983). By comparison, apple cactus had narrow vessels, but, vessel density (the number of vessels in 1 mm² of axial wood) was not affected (Arnold and Mauseth, 1997). Phloem diameter also decreased in seedlings grown with more PEG-8000. This

was probably not an adaptation to drought, rather it was likely due to the fact that seedlings grown in PEG-8000 were smaller than non-treated controls.

Root anatomy was not significantly affected by PEG-8000 concentration in the growing medium, but, as discussed above, root length and dry weight were reduced by PEG-8000. Root, stele, and cortex diameter of soybean (*Glycine max* (L.) Merr.) and peach (*Prunus persica* (L.) Batsch.) also were not affected by drought stress (Rieger and Litvin, 1999). With regard to elongation, roots are less sensitive to drought stress than shoots (Frensch, 1997). In this experiment, drought stress did not affect the anatomical root features examined.

Post-Transplant Morphology

Salvia treated with PEG-8000 flowered up to 12 d after non-treated salvia (Fig. 12). This may be a disadvantage, especially for the highest three treatments. However, taller cultivars of annual salvia, such as 'Bonfire', are typically shipped before they have flowered (Nau, 1998). Salvia treated with PEG-8000 also were smaller than non-treated salvia at 14 d after transplanting (Fig. 12). Annual salvia grows rapidly, and it is usually recommended to apply growth retardants to reduce elongation after seedlings are transplanted (Nau, 1998). However, salvia seedlings treated with PEG-8000 might need fewer chemical growth retardants, since they were shorter than non-treated salvia 14 d after transplanting. This residual height control was transitory, and PEG-treated seedlings were similar in size to non-treated seedlings when 28 d after transplanting (Fig. 13).

Conclusions

PEG-8000 appears to be a promising alternative to the use of chemical growth retardants in commercial greenhouse production of annual salvia. Shoot and root elongation and hypocotyl height of salvia were reduced by the addition of 15-50 g·L⁻¹ of PEG-8000 to the growing medium. The optimal rates appeared to be 15-42 g·L⁻¹ of PEG-8000. Leaf Ψ_w and Ψ_p were lower for seedlings treated with PEG-8000, and reductions in leaf Ψ_w were significantly correlated with height reductions. Turgor potential is the driving force for cell elongation, so reduced Ψ_w and Ψ_p were probably the main reason that PEG-8000 reduced elongation of annual salvia. Salvia also exhibited typical anatomical changes associated with mild drought stress. Seedlings treated with PEG-8000 had narrower hypocotyls due to an overall reduction in vascular tissues without detectable changes in specific cell types. Stems could have simply been smaller because PEG-treated seedlings were smaller. However, xylem tissue decreased because of PEG-8000, not only because salvia seedlings grown in PEG-8000 were smaller than non-treated salvia. Xylem cross-sectional area was reduced because there were fewer xylem elements, not smaller xylem element diameter in PEG-treated salvia as compared to non-treated salvia. After salvia were transplanted and grown without PEG-8000 in the growing medium, PEG-treated seedlings were shorter than non-treated seedlings 14 d after they were transplanted. This residual effect of PEG-treatments would be desirable for commercial greenhouse growers because they would have to use fewer, if any, chemical growth retardants on PEG-treated salvia seedlings. However, before PEG-treatments would be useful commercially, more research needs to be conducted. This would include economical analysis of the cost of PEG treatments, and it would also be necessary to

develop cultural recommendations for watering seedlings without leaching PEG or measuring volumetric water content daily.

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Figure 1. Water potential of substrates with different PEG-8000 concentrations. Data points are the mean of three measurements with bars representing the standard deviation. The regression curve indicates a significant quadratic trend. Water potential = $0.229 - 5.67 \times 10^{-3} \times x - 1.58 \times 10^{-4} \times x^2$, $R^2 = 0.71$, $P = 0.0001$, where x = PEG-8000 concentration in the growing medium.

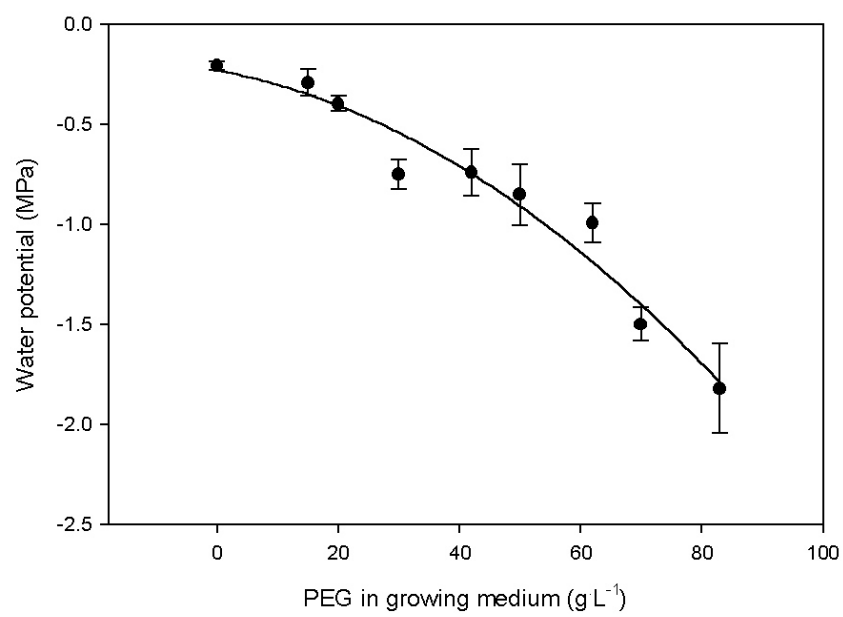


Figure 2. The effects of PEG-8000 in the growing medium on emergence percentage and time to seedling emergence. Data points are the mean of 6 replications with bars representing the standard error. Regression curves indicate significant quadratic effects. Percentage emergence = $79.3+35.7x-10200x^2$, $R^2 = 0.77$, $P = 0.0001$, days to emergence = $11+3.32\times 10^{-2}x+1.36\times 10^{-3}x^2$, $R^2 = 0.75$, $P = 0.0001$, x = PEG-8000 concentration in the growing medium.

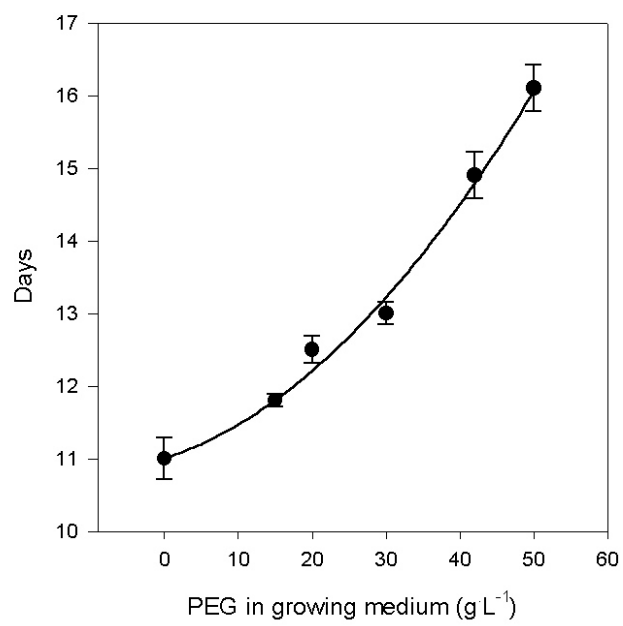
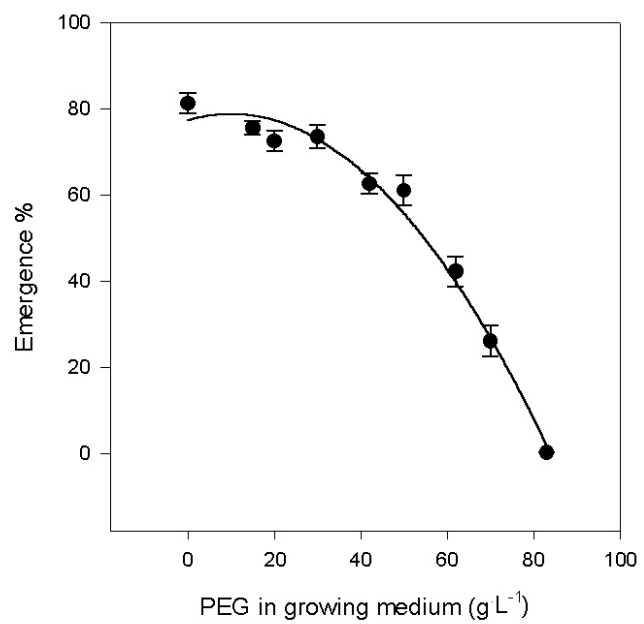


Figure 3. Electrical conductivity of the pore water of the growing medium as affected by PEG-8000 concentrations in the growing medium. Data are the means of 6 replications with bars representing standard error. Regression curves indicate significant linear trends.

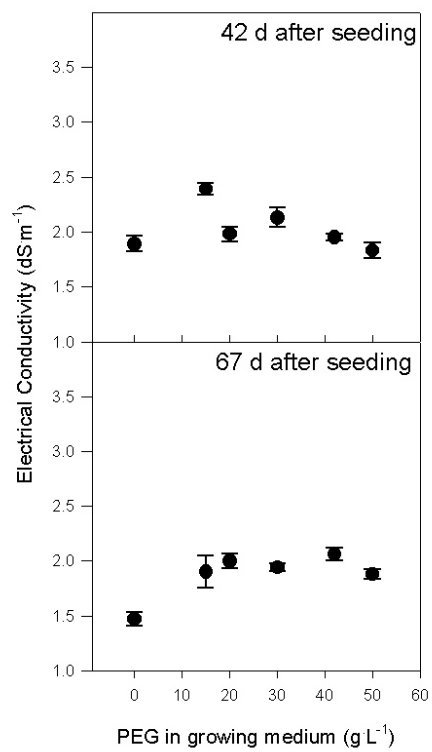
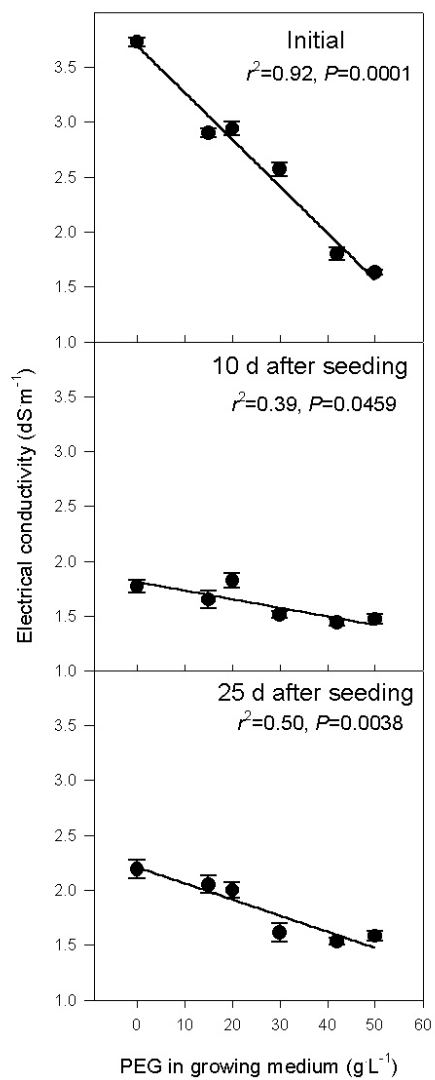


Figure 4. The effects of PEG-8000 in the growing medium on height of salvia at 21, 35, 56, and 70 d after seeding (harvest). Data points are the mean of 6 replications with bars representing standard error, and curves show significant quadratic effects.

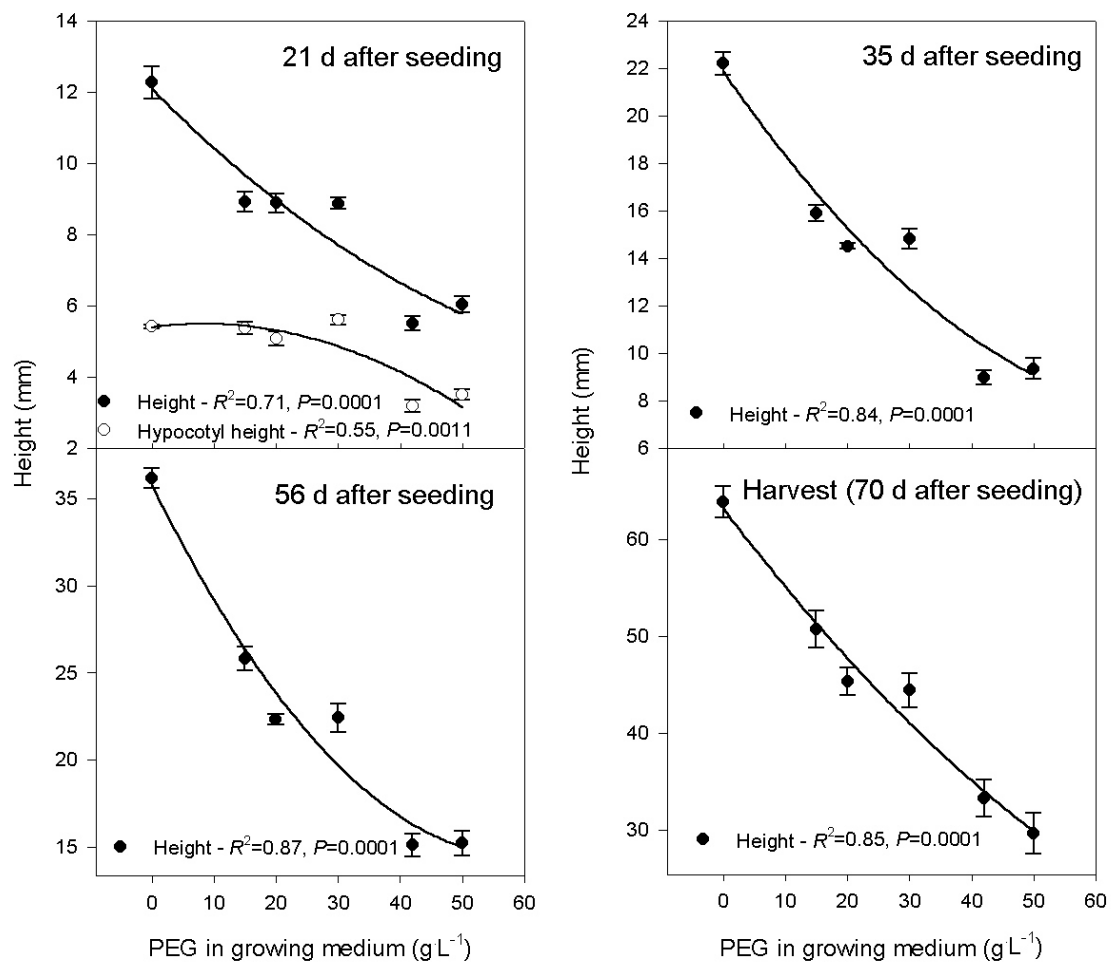


Figure 5. The effects of PEG-8000 in the growing medium on width of salvia 21, 35, 56, and 70 d after seeding (harvest). Data points are the mean of 6 replications with bars representing standard error, and curves show significant quadratic effects.

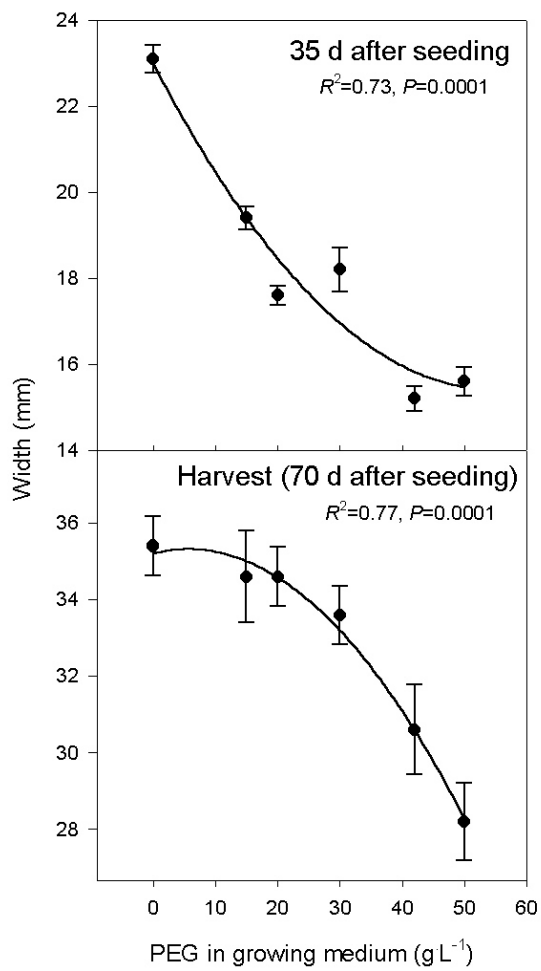
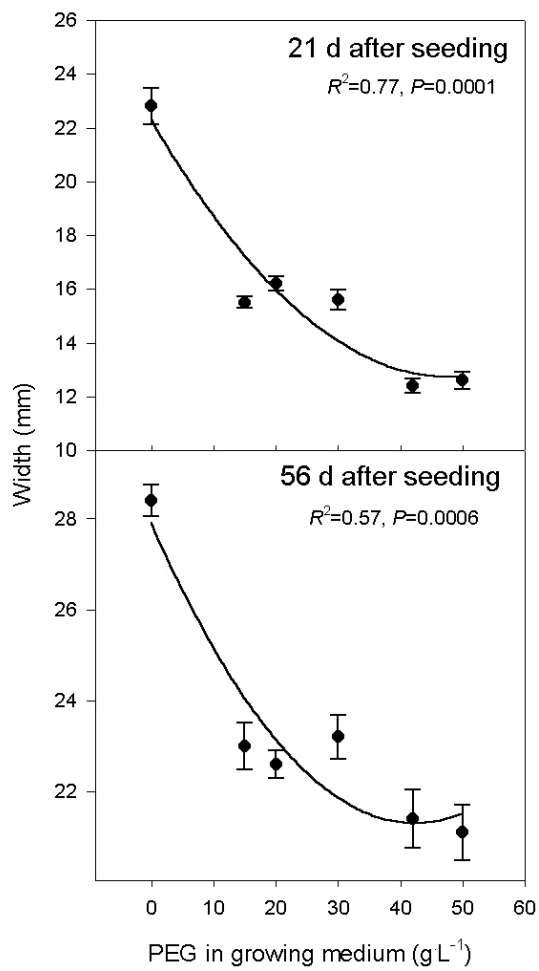


Figure 6. The effects of PEG-8000 in the growing medium on compactness (leaf area/height at harvest) and stem density (stem dry weight/stem length at harvest) of salvia treated with PEG-8000. Salvia were harvested 70 d after seeding. Data points are the mean of 6 replications with bars representing standard error, and curves show significant quadratic effects. Compactness = $2.09+2.0^{-2}x+1.49^{-4}x^2$, $R^2 = 0.55$, $P = 0.0011$; stem density = $4.8-9.39^{-2}x+9.89^{-4}x^2$, $R^2 = 0.64$, $P = 0.0002$, where x = PEG-8000 concentration in the growing medium.

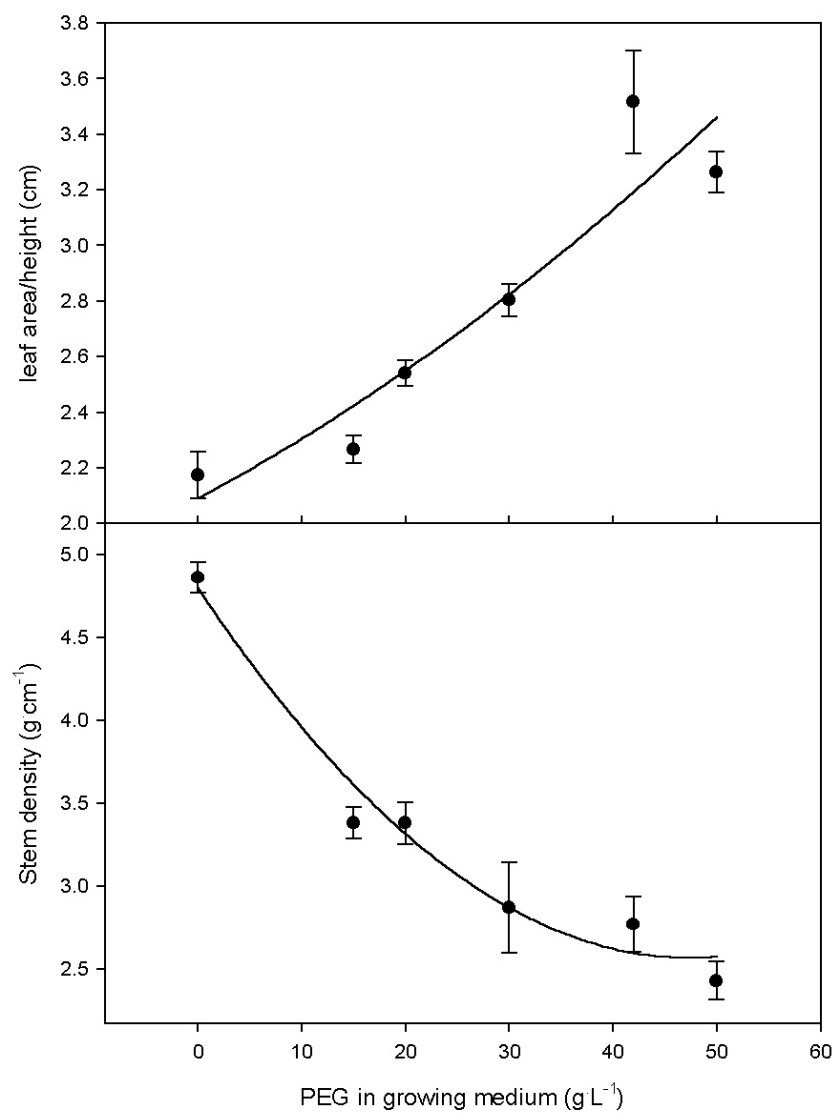


Figure 7. The effects of PEG-8000 in the growing medium on leaf area, specific leaf weight, shoot and root dry weights, and root length per plant for salvia treated with PEG-8000 as measured at harvest (70 d after seeding). Data points are the mean of 6 replications with bars representing standard error, and curves show significant linear or quadratic effects.

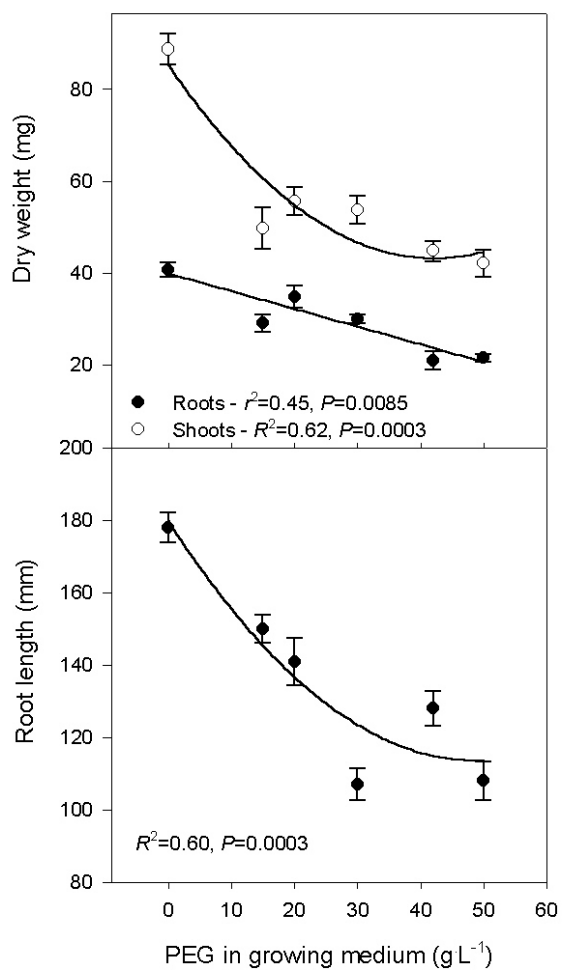
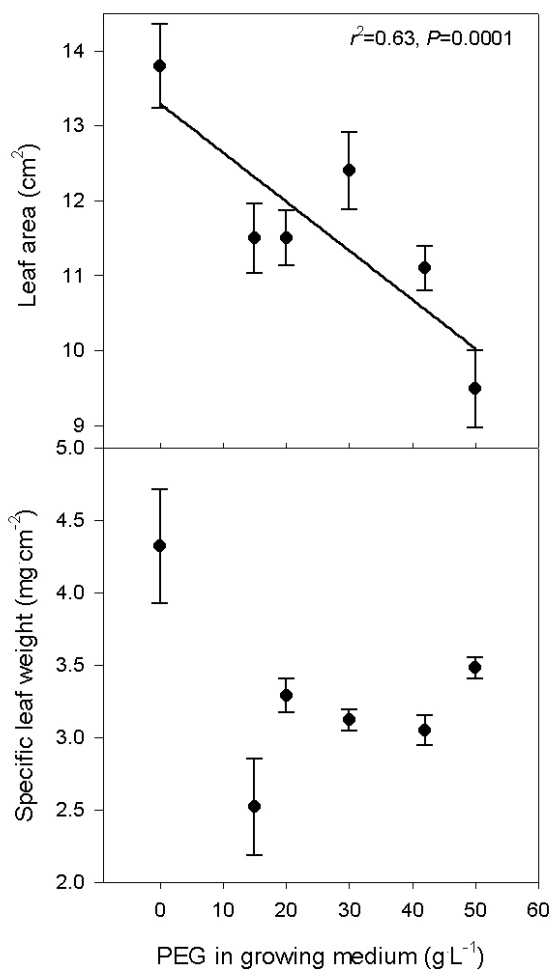


Figure 8. Water, osmotic, and turgor potential of salvia leaves treated with varying concentrations of PEG-8000. Second acropetal pair of salvia leaves were measured midday, 21, 35, and 56 d after seeding. Data points are the mean of three replications with bars representing standard error, and curves show significant linear or quadratic effects.

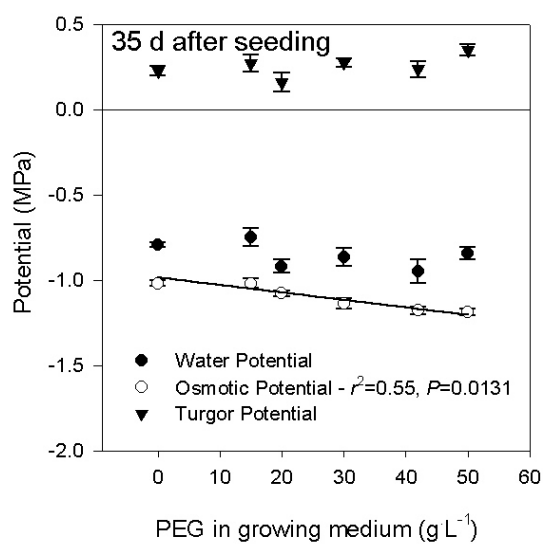
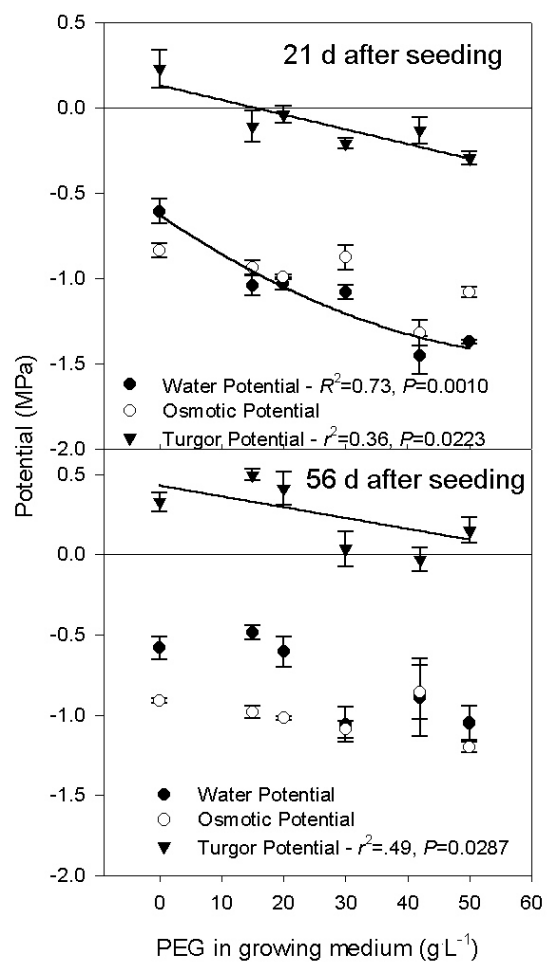


Figure 9. Hypocotyl diameter and xylem, phloem, and pith cross-sectional area of salvia treated with varying concentrations of PEG-8000, as measured at harvest (70 d after seeding). Data points are the mean of 4 replications with bars representing standard error, and curves show significant quadratic effects.

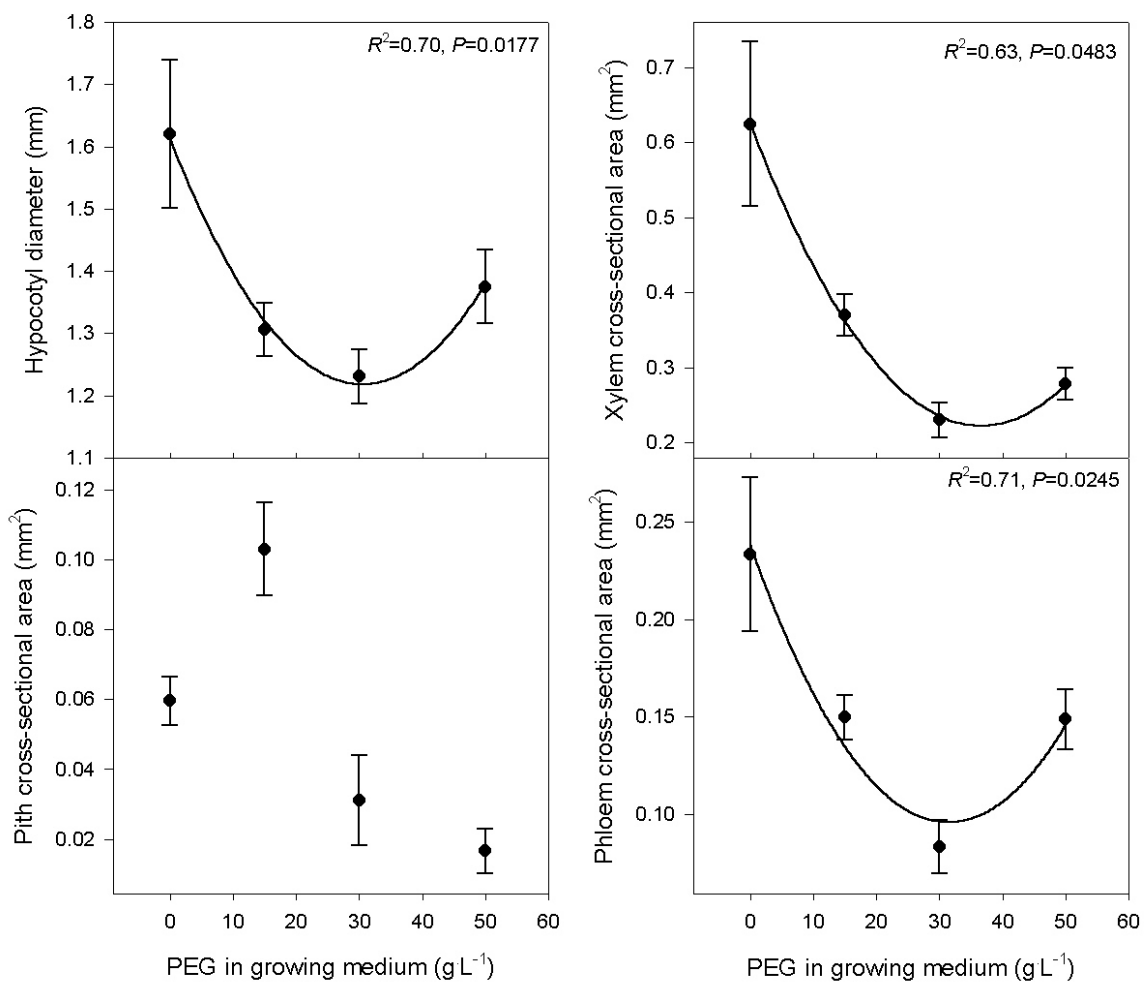
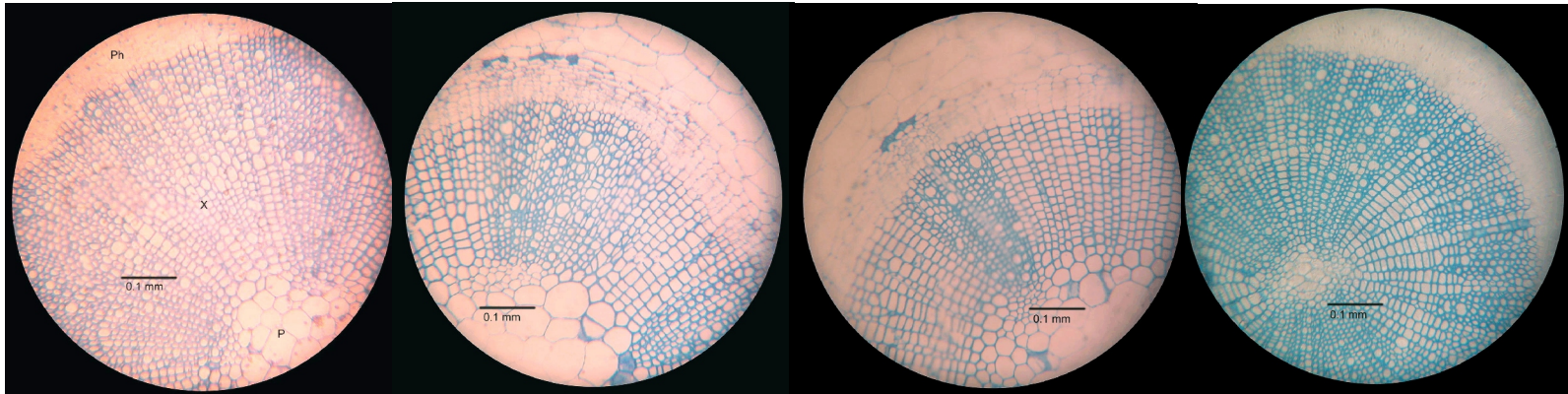
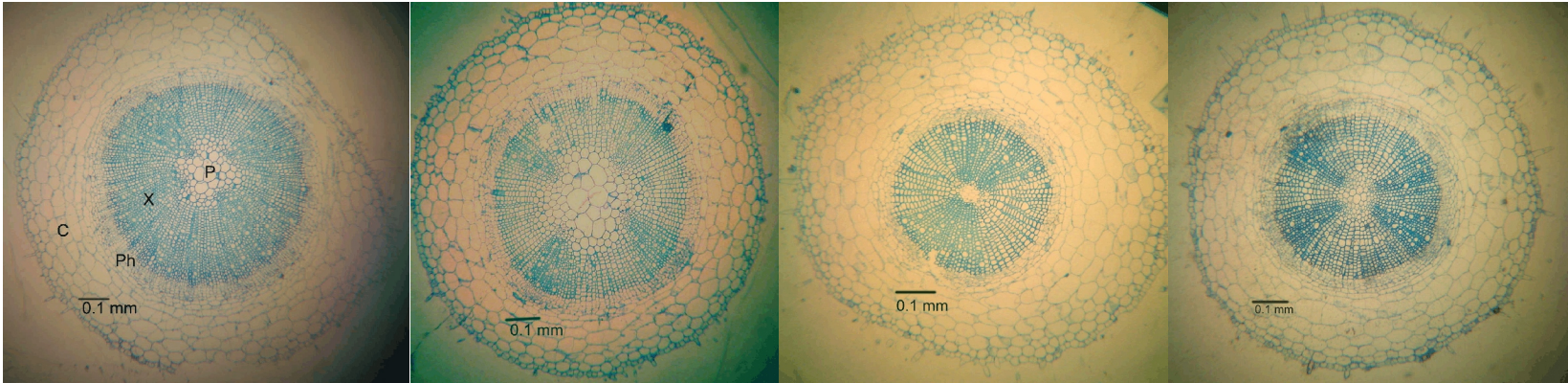


Figure 10. Hypocotyl cross sections from annual salvia treated with 0, 15, 30, or 50 g·L⁻¹ of PEG-8000. Pictures in the upper row are of entire hypocotyls, and the close-ups below are taken of hypocotyls treated with the same concentrations of PEG-8000. Both figures are labeled as follows: P=pith, X=xylem, Ph=phloem, and C=cortex.



Control

15 g·L⁻¹

30 g·L⁻¹

50 g·L⁻¹

Figure 11. Xylem element number and diameter in hypocotyls of salvia treated with varying concentrations of PEG-8000, as measured at harvest (70 d after seeding). Data points are the mean of 4 replications with bars representing standard error, and curves show significant quadratic effects. Xylem element number = $4716 - 150x + 2.25x^2$, $R^2 = 0.77$, $P = 0.0097$, where x = PEG-8000 concentration in the growing medium.

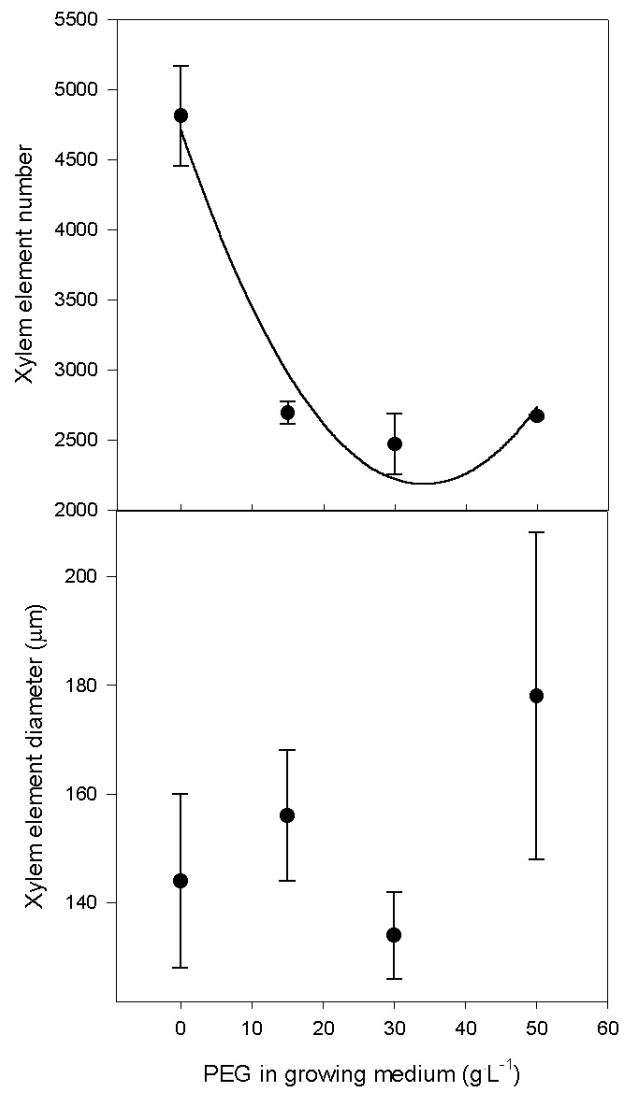
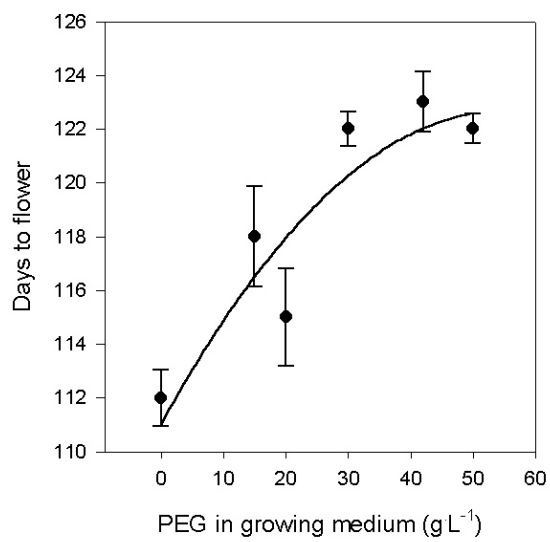
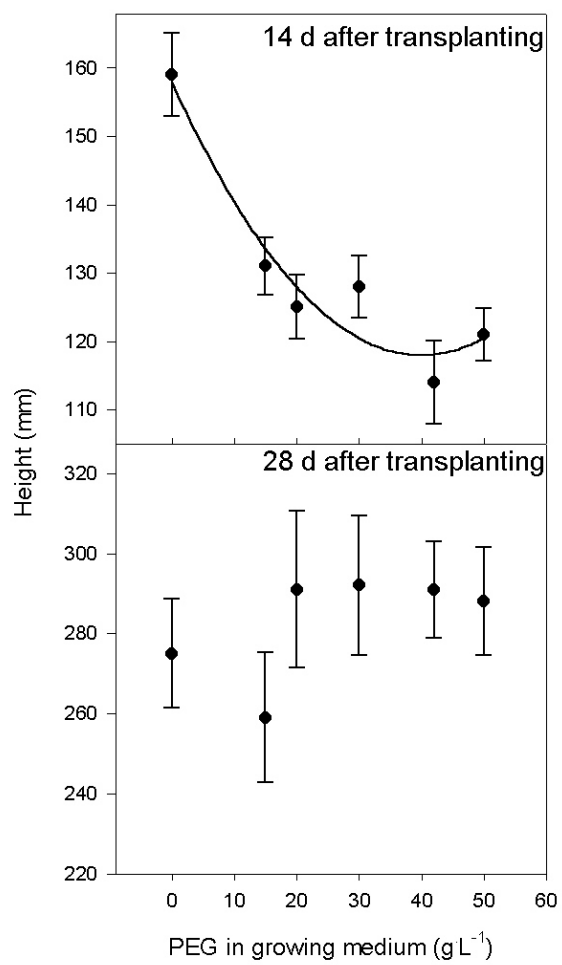


Figure 12. Effects of PEG-8000 concentrations in the growing medium on time to flower and height after transplanting. Height was measured 14 and 28 d after transplanting. Data points are the mean of 6 replications with bars representing standard error, and curves show significant quadratic effects. Days to flower = $111+0.424x-3.85^{-3}x^2$, $R^2=0.47$, $P=0.0072$; height at 14 d after transplanting = $158-2x+0.025x^2$, $R^2 = 0.73$, $P = 0.0001$, where x = PEG-8000 concentration in the growing medium.



CHAPTER 5

EFFECTS OF PEG-8000 ON GROWTH AND WHOLE CANOPY CARBON DIOXIDE

EXCHANGE OF *TARGETES PATULA* 'BOY ORANGE'³

³Burnett, S.E., M.W. van Iersel, and P.A. Thomas. To be submitted in *The Journal of the American Society for Horticultural Scientists*.

Abstract

Height is one major factor determining quality of annual seedlings; short seedlings are preferred over tall seedlings because they are more aesthetically pleasing, cheaper to ship, and easier to manage in greenhouses. Osmotic compounds slow elongation of hydroponically grown seedlings by imposing controlled drought, but it is unknown if osmotic compounds may be used as growth retardants in commercial seedling production. Polyethylene glycol 8000 (PEG-8000) was incorporated into a peat-based growing medium at the following concentrations: 0, 15, 20, 30, 42, 50, 62, 72, or 83 g·L⁻¹. Marigold (*Tagetes patula* L.) was seeded in the growing medium to determine if the controlled drought stress imposed by PEG-8000 treatments would reduce height of marigold seedlings. Marigold hypocotyl height was reduced by PEG-8000, and PEG-treated seedlings were up to 38% shorter than non-treated controls when they were considered marketable. Marigold cotyledon water (Ψ_w), osmotic (Ψ_s), and turgor potential (Ψ_p) were significantly reduced by PEG-8000, and Ψ_p was close to zero for all PEG-treated seedlings. Whole plant photosynthesis, whole plant respiration, and photosynthesis/leaf area were reduced by PEG-8000, while specific respiration increased for seedlings treated with PEG-8000. It appears that marigold seedlings were shorter because of reduced leaf Ψ_p and reductions in photosynthesis. However, PEG-induced drought stress was probably the main cause of reductions in photosynthesis. Fourteen d after transplanting, PEG-treated marigolds were still shorter and flowered up to 5 d later compared to non-treated seedlings. It appears that PEG-8000 reduces height and hypocotyl height of marigold seedlings. Since PEG-treated seedlings are shorter after transplanting, commercial greenhouse growers would have to apply fewer, if any, chemical growth retardants to PEG-treated seedlings.

French marigold (*Tagetes patula* L.) is a classic landscape bedding plant that flowers throughout the entire summer (Armitage, 2001). However, marigolds grow quite rapidly and tend to stretch when grown in greenhouses. Seedling transplants, especially, tend to out-grow their small substrate volumes quickly and become leggy (Styer and Koranski, 1997). Overgrown transplants are difficult to grow, more expensive to ship, and aesthetically less pleasing than compact transplants. This is a serious problem for seedling producers who sell their products to other growers. Purchasers of seedlings have more exacting expectations of plant quality than the average consumer.

A particular problem for marigold seedling growers is that most of the elongation of marigold seedlings is in the hypocotyl, which may account for as much as 50% of the overall seedling height. So, to successfully control height of marigolds, one must reduce hypocotyl height. Plant growth retardants are one option for reducing seedling growth; however, they are typically applied after plants have their first true leaves (Uniroyal Chemical, 2000), when much of the hypocotyl elongation has already occurred. Recent research has shown that soaking seeds in chemical growth retardants will reduce hypocotyl elongation of seedlings (Fletcher and Kraus, 1995; Pasian and Bennett, 1999; Pill and Gunter, 2001; Souza-Machado et al., 1997). Even with these recent expansions of the use of growth retardants, many growers still remain hesitant to use chemical growth retardants on young seedlings, since they may stunt plant growth. This sort of mistake could delay a seedling crop and even stunt growth after transplant (Ball, 1991). In addition, stunted seedlings would be difficult to sell.

Many seedling growers reduce marigold growth and hypocotyl elongation by limiting the amount of water applied to young seedlings. When plants are grown in a relatively dry substrate, they are smaller due to decreased cell number and cell length (Sommer et al., 1999). This is partly

because plants grown under dry conditions usually have lower Ψ_w , Ψ_s , and Ψ_p . Turgor potential, in excess of a minimum 'yield threshold', is the driving force for cell elongation (van Volkenburgh, 1999).

Low levels of drought stress result in physiological effects that could be seen as beneficial for seedling production, including decreased extension growth (Mohr and Schopfer, 1995). Drought stress has been observed to reduce growth of broccoli (*Brassica oleracea* L. var. *Italica*) transplants, annual salvia (*Salvia splendens* F. Sellow. ex Roem & Shult.), and marigolds (Latimer and Severson, 1997; Eakes et al., 1991; van Iersel and Nemali, 2004). Plants grown with minimal water also tend to resist mechanical stress and may survive transplanting more readily (Liptay et al., 1998).

However, it is difficult to determine how much drought stress is appropriate. Seedling growers often grow plants in substrate volumes smaller than 10 mL. So, in high light and temperature typical in greenhouses, seedlings can deplete their water supply and become severely drought-stressed rapidly. Detrimental effects of drought stress include decreased photosynthesis and even plant death (Mohr and Schopfer, 1995).

It would be desirable for greenhouse growers to have a method of reducing substrate Ψ_w to control plant growth and hypocotyl elongation without risking plant damage or death. One option is to use osmotic compounds, such as polyethylene glycol-8000 (PEG-8000), to reduce plant growth. Polyethylene glycol is an inert molecule that readily bonds with water (Kjellander and Florin, 1981). In hydroponics, PEG-8000 reduced growth of impatiens (*Impatiens walleriana* Hook. F.) and marigolds, and at low rates did not appear to damage plants (Burnett et al., 2004). In addition, the presence of continuous, low-level drought stress, where plants are not

allowed to visibly wilt, would be more practical in commercial settings than episodic drought (Liptay et al., 1998).

Since one of the negative effects of severe drought is decreased photosynthesis, it would be of interest to determine how photosynthesis and respiration are affected by PEG-8000. Annual salvia exposed to episodic drought, where plants are drought-stressed to the wilting point (with leaves reaching Ψ_w of -1.1 to -1.4 MPa on four separate occasions) photosynthesize less than non-stressed plants (Eakes et al., 1991). This could be seen as detrimental; however, plants also recovered from drought to exhibit normal photosynthesis and increased tolerance to subsequent stress (Eakes et al., 1991).

Therefore, the primary purpose of this experiment was to determine whether PEG-8000 may be used to reduce elongation of marigold seedlings. Secondly, we attempted to determine appropriate rates for effective height control. Finally, whole-plant photosynthesis and respiration of marigolds grown in PEG-8000-treated growing media were compared to photosynthesis and respiration of non-treated plants to get a better understanding of the physiological effects of continuous drought.

Materials and Methods

Polyethylene glycol 8000 was mixed with water and added to a peat-based growing medium specifically formulated for germinating seedlings (a mixture of sphagnum peat, perlite, and vermiculite; Germinating mix, Fafard, Anderson, S.C.) to obtain the following concentrations in the growing medium: 0, 15, 20, 30, 42, 50, 62, 70, and 83 gL⁻¹. Each PEG-8000 and growing medium combination was then shaken vigorously in a large plastic container for ten minutes to

produce a homogenous mix. Treated growing medium was placed in 6×6 cell sections cut from trays with 288 cells (cell volume = 8.5 mL). Each 6×6 section was an experimental unit.

Substrate Ψ_w of three samples from each treatment was measured using a vapor pressure osmometer (Model 5520 Vapro Vapor Pressure Osmometer, Wescor, Inc., Logan, Utah). The volumetric water content of the growing medium during these measurements was approximately 29.3%, which was the target water content for the duration of the experiment.

Two marigold ‘Boy Orange’ (Pan American Seed Company, West Chicago, Ill.) seeds were planted in each cell on June 6, 2003. Marigold seeds were grown until emergence in a growth chamber (E-15, Conviron, Winnipeg, Canada, temperature = 20 °C). While in the growth chamber, plants were misted overhead by hand as needed to maintain a constant volumetric water content (29%±3%) using a mist nozzle hose attachment. After emergence (11 d after seeding), seedlings were grown on a mist bench in a glasshouse (temperature = 24.6±4.2 °C) and irrigated approximately 20 s every 30 min from 8:00 am to 5:00 pm (EST). To obtain appropriate irrigation levels, mist irrigation was changed to 20 s every 20 min from 6:00 am until 6:00 pm 15 d after seeding, then changed again to 20 s every 30 min from 6:00 am until 6:00 pm on 19 d after seeding.

The smallest seedling was removed from cells containing more than one plant 22 d after seeding. All seedlings were fertilized twice weekly with a 20N:8.7P:16.6K fertilizer (20-20-20 General Purpose, Scotts Company, Marysville, Ohio) solution with a N-concentration of 200 mg·L⁻¹, beginning when the first true leaves were visible (23 d after seeding). When seedlings were fertilized, they were removed from the mist bench and hand-misted overhead with a fertilizer solution using a mist nozzle for 20 s. Electrical conductivity (EC) of the pore water in the growing medium of one cell/replication was measured at the beginning of the experiment (before

and after PEG-8000 was added to the growing medium) and bi-weekly afterwards using an ECK-1 Basic SigmaProbe (Delta-T Devices, Ltd., Cambridge, UK), which is inserted directly into the medium. Granular Marathon (active ingredient: 1% imidacloprid, Olympic Horticultural Products, Mainland, Pa.) was applied to seedlings at a rate of 5 mL for each experimental unit.

Experimental unit weight was recorded twice daily for the duration of the experiment and plants were removed from mist or not watered in the growth chamber when necessary to maintain constant volumetric water contents ($29\% \pm 3\%$). This also prevented leaching which would result in loss of PEG and thus change treatment levels. Empty tray weights were noted at the beginning of the experiment. After emergence, one seedling from the outermost row was harvested bi-weekly. The weight of this seedling was multiplied by the number of plants in each tray to account for changes in plant weight throughout the experiment. Each tray contained approximately 307 mL of growing medium, and the same volume of excess treated growing medium was dried in a drying oven at approximately 80 °C. So, the amount of water in each tray was calculated by subtracting the tray, seedling, and dry growing medium weight from the experimental unit weight. The drying oven did not completely remove all the water bonded to PEG-8000 from the growing medium (unpublished results). Thus, the estimated dry weight of the PEG-treated growing media included some water, which apparently was permanently bound to the PEG-8000. This water was not available for plant uptake.

Data collected include the number of days to seedling emergence (when cotyledons were perpendicular to the hypocotyl), percentage emergence (22 d after seeding), and height and width at emergence. Plant width was the maximum horizontal distance between any two leaf tips. Plants in the outside rows of the 6×6 cell sections were not used for any measurements (except those made after transplant as discussed below) to prevent edge effects. After plants were moved

to the greenhouse, the establishment percentage after emergence (the number of emerged seedlings that were established divided by total the number of emerged seedlings \times 100%) was noted (22 d after seeding). Height, hypocotyl height, and width were measured bi-weekly until the termination of the experiment (18, 32, and 49 d after seeding). At harvest (49 d after seeding), leaf area (LI-3100 Leaf Area Meter, Li-Cor, Inc., Lincoln, Nebr.) and longest root length were measured. Shoot (stems and leaves), inflorescence, and root tissues were dried in an oven at 80 °C for at least three days; then dry weights were measured. Stem density (stem dry weight/stem length), compactness (shoot dry weight/height at harvest), compactness (shoot dry weight/height at harvest) and specific leaf weight (leaf dry weight/leaf area) were calculated from these data. All living seedlings except those from the border were harvested for shoot data, but for roots, only four representative plants were harvested.

Leaf Water Relations

Midday leaf Ψ_w and Ψ_s of marigold cotyledons at 18 d after seeding and second acropetal leaves at 32 and 49 d after seeding were measured using thermocouple psychrometers (leaf cutter psychrometers, J.R.D. Merrill Specialty Equipment, Logan, Utah). Cotyledons were measured early in the experiment. Plants from three representative blocks were selected for psychrometer measurements. Leaf samples enclosed in the psychrometer chambers were equilibrated in a water bath at 25 °C for four hours before measurement. Water potential of intact leaves was measured first using a microvoltmeter. Then, leaf samples were frozen to disrupt cell membranes and remove Ψ_p . Samples were then re-equilibrated as described above and Ψ_s was measured. Ψ_p was calculated by subtracting Ψ_s from Ψ_w .

Whole Plant Carbon Exchange

In addition to morphological measurements, whole-plant photosynthesis of four blocks was measured at the termination of the experiment. Since few plants were alive in the growing medium containing the $83 \text{ g}\cdot\text{L}^{-1}$ growing medium, photosynthesis and respiration of this treatment were not measured. All plants and growing medium from the outermost rows and dead plants were removed prior to carbon exchange measurements.

Whole-plant carbon exchange rates were measured in the light (photosynthesis, *PPF* was approximately $240 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and dark (respiration). Carbon exchange was measured at 20 min intervals in multiple acrylic chambers as described by van Iersel and Bugbee (2000). Ambient airflow into the system was measured using mass flow meters (GFM37-32, Aalborg Instruments and Controls, Monsey, N.Y.). Carbon dioxide concentration was measured before and after ambient air entered the chamber using an infrared CO_2 analyzer (LI-6262; LI-COR, Lincoln, Neb.) in differential mode. Respiration was measured outside of growth chambers in acrylic chambers covered with opaque cloths (*PPF* = $0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Each group of plants was measured at $22 \text{ }^\circ\text{C}$ until photosynthesis or respiration rates had stabilized. Any data collected before rates had stabilized were excluded from the analysis. Plants were harvested as described above immediately after photosynthesis and respiration measurements had ceased. To correct for differences in plant size among treatments, photosynthesis was divided by leaf area and respiration was corrected for total plant dry weight.

Post-transplant Morphology

When the seedlings were harvested, six representative plants from outermost rows were transplanted into a six-cell pack (cell volume = 380 mL) containing a peat-based growing medium (Fafard 3M, Fafard, Anderson, S.C.). Plants were grown on ebb and flow benches irrigated daily

with a 20N:8.7P:16.6K fertilizer (20-20-20 General Purpose, Scotts Company, Marysville, Ohio) solution with a N concentration of 150 mg·L⁻¹. After transplanting, no effort was made to keep PEG-8000 in the growing medium. Height was measured bi-weekly after transplanting until marigolds were considered marketable (28 d after transplanting). The number of days to the appearance of the first flower (the first basipetal ray flower was bright orange and perpendicular to the peduncle) was noted.

The experimental design was a complete randomized block design with six replications/treatment, 16 subsamples/treatment before seedling harvest, and 6 subsamples/treatment after transplanting. For root harvest data, there were 4 subsamples/treatment. Data were analyzed using regression analysis to test for significant ($P < 0.05$) linear and quadratic effects of PEG-8000 concentrations (Proc GLM, Statistical Analysis Systems, Cary, N.C.). Volumetric water contents were averaged over all measurements before analysis. All data, excluding growing medium Ψ_w and water content, days to emergence, emergence percentage, and percentage establishment were analyzed without the highest three treatments due to the high mortality in those three treatments. Leaf water relations data were also further analyzed comparing Ψ_w , Ψ_s , and Ψ_p to the seedling height when leaf water relations were measured.

Results

Growing medium Ψ_w decreased significantly with increasing PEG-8000 concentration in the growing medium (Fig. 1). The growing media containing PEG-8000 concentrations of 70 and 83 g·L⁻¹ had a substrate Ψ_w near or below the permanent wilting point (-1.5 MPa) (Fig. 1). For

the remaining treatments, the range of Ψ_w was -0.21 to -1.00 MPa. The volumetric water content of the growing media increased significantly with increasing PEG concentrations, but was similar for the growing media with PEG-8000 concentrations of 0 to 42 g·L⁻¹ were similar (Table 1).

Marigold seedlings treated with PEG-8000 emerged up to 5 d later than non-treated controls and the emergence percentage decreased with increasing PEG-8000 rate (Fig. 2). The lowest emergence percentage was 83%, for plants grown in a PEG-8000 rate of 83 g·L⁻¹ (Fig. 2). The establishment percentage after emergence also decreased with increasing PEG concentration (Fig. 2). Emergence date and percentage establishment were affected most for the three highest PEG treatments.

Only plants grown in PEG-8000 concentrations from 0-50 g·L⁻¹ were included in the analysis for the remaining data, since percentage establishment was quite low for the three highest treatments. Before PEG-8000 was added, the EC of the growing medium was uniform (≈ 3.5 dS·m⁻¹), but afterwards, the EC of the growing medium decreased with increasing PEG-8000 rate (Fig. 3). Initially, the EC of the non-treated growing medium was 3.6 dS·m⁻¹, and the lowest EC (1.5 dS·m⁻¹) was in the growing medium treated with 50 g·L⁻¹ of PEG-8000. As the experiment progressed, the effects of PEG on EC were more difficult to interpret (Fig. 3). There was no significant difference in EC at 10 and 34 d after seeding, but at 21 and 49 d after seedling, EC was significantly different when PEG-8000 was in the growing medium (Fig. 3). However, at these times the effect was quadratic with the highest ECs occurring at intermediate PEG concentrations (15 - 30 g·L⁻¹).

Seedlings were shorter with increasing PEG-8000 rates in the growing medium (Fig. 4). The shortest plants at emergence were those treated with the highest concentration of PEG-8000, and they were 13% shorter than non-treated marigolds (Fig. 4). Throughout the experiment,

seedling height was less with increasing PEG-8000 rate in the growing medium. At 18, 32, and 49 d after seeding, seedlings treated with 50 g·L⁻¹ of PEG-8000 were 33%, 38%, and 38% shorter than non-treated seedlings, respectively (Fig. 4). Also, at 18 d after seeding, hypocotyl height decreased with increasing amounts of PEG-8000 (Fig. 4). Marigold width also decreased with increasing PEG-8000 rate for the duration of the experiment. The most narrow plants were those treated with 50 g·L⁻¹ of PEG-8000 and they were 8%, 33%, 36%, or 15% less wide than non-treated seedlings when measured at emergence and 18, 32, or 49 d after seeding, respectively (Fig. 5). Compactness decreased with increasing PEG-8000 rate, and all PEG-treated seedlings were approximately 20% less compact than the control seedlings (Fig. 6).

Leaf areas and root lengths of PEG-treated marigolds decreased as plants were treated with increasing quantities of PEG-8000. Again, the greatest reduction was for seedlings treated with 50 g·L⁻¹ of PEG-8000, which had 43% smaller leaf areas and 53% shorter roots. Specific leaf weight decreased quadratically with increasing PEG-8000 rate [specific leaf weight (g·m⁻²) = $22.2+9\times 10^{-4}x-3.4\times 10^{-3}x^2$, where x = PEG-8000 concentration, $R^2=0.72$, $P=0.0001$; results not shown]. The specific leaf weight for control seedlings was 22 g·m⁻², compared to a specific leaf weight of 14 g·m⁻² for plants treated with 50 g·L⁻¹ of PEG-8000. Root, shoot, and inflorescence dry weights decreased with increasing PEG-8000 rate; the dry weight was reduced up to 57%, 79%, and 57% for root, shoot, and inflorescence dry weights, respectively. (Fig. 7). Stem density decreased linearly with increasing PEG-8000 concentration as well (up to 40%) (Fig. 7).

Leaf Water Relations

At 18 d after seeding, leaf Ψ_w , Ψ_s , and Ψ_p decreased with increasing PEG-8000 (Fig. 8). Plants grown with 20-50 g·L⁻¹ of PEG-8000 had a Ψ_p close to zero, compared to the control which had a Ψ_p of 0.28 MPa 18 d after seeding. Leaf Ψ_w decreased with increasing PEG-8000

concentration at 32 d after seeding (Fig. 8). For controls, Ψ_w was -0.75 MPa, and 50 g·L⁻¹ of PEG-8000 reduced Ψ_w to -1.65 MPa. Leaf Ψ_s was not affected 32 d after seeding, but decreased with increasing PEG-8000 rate at 49 d after seeding (Fig. 8). Turgor potential was not affected on either of the last two measurement dates, and was positive for all PEG concentrations.

Eighteen d after seeding, seedling height was positively correlated with Ψ_w and Ψ_s (height = $3.36+0.529\times\Psi_w$, $r^2=0.48$, $P=0.0304$; height = $3.64+0.763\times\Psi_s$, $r^2=0.46$, $P=0.0394$). There was a trend for seedlings with lower Ψ_p to be shorter 18 d after seeding, however, this trend was not significant ($P = 0.09$). Seedling height at 32 d after seeding was significantly lower in seedlings with decreasing Ψ_w , Ψ_s , and Ψ_p (height = $7.18+1.76\times\Psi_w$, $r^2=0.62$, $P=0.0029$; height = $6.5+1.03\times\Psi_s$, $r^2=0.43$, $P=0.0429$; height = $4.91+1.04\times\Psi_p$, $r^2=0.42$, $P=0.0462$). Only Ψ_s was correlated with seedling height at 49 d after seeding (height = $9.42+2.94\times\Psi_s$, $r^2=0.71$, $P=0.0005$).

Whole-Plant Carbon Exchange

Whole-plant photosynthesis decreased with increasing PEG-8000 rate (Fig. 9). When photosynthesis was corrected for differences in leaf area among treatments, marigolds still photosynthesized less when treated with increasing rates of PEG-8000 (Fig. 9). Whole-plant respiration decreased with increasing PEG-8000 rate. However, when respiration was corrected for dry weight, respiration rose with increasing PEG concentration (Fig. 9).

Post-Transplant Morphology

The time to flowering increased with increasing amounts of PEG-8000 in the growing medium (Fig. 10). Plants grown with 42 or 50 g·L⁻¹ of PEG-8000 flowered approximately 5 d after non-treated plants. Plant height at two weeks after transplanting decreased gradually, and up to 20%, with increasing PEG concentrations in the growing medium. Four weeks after

transplanting, plant height in all PEG treatments was approximately 275 to 280 mm, 12 mm shorter than the control plants (Fig. 11).

Discussion

The Ψ_w of the growing medium decreased as more PEG was added (Fig. 1). This was the expected trend, since PEG-8000 reduces the matric potential of media (Steuter et al., 1981). The range of Ψ_w was broad, and growing media containing the highest treatments were near the permanent wilting point (-1.5 MPa). Since the effects of PEG-8000 on growth and morphology of marigolds have never been reported, a broad range was desirable.

Volumetric water content was actually greater for growing media containing the most PEG-8000 (Table 1). Since the Ψ_w of the growing media was measured at the same volumetric water content, the Ψ_w in Fig. 1 may not have been entirely representative for Ψ_w values throughout the experiment. The higher volumetric water content in treatments with high PEG concentrations likely resulted in an increase in Ψ_w as well, and actual differences in substrate Ψ_w may have been smaller than those in Fig. 1.

One of the potential negative effects of drought is a decrease in emergence percentage and plant establishment (Mohr and Schopfer, 1995). In this experiment, emergence was delayed and suppressed in the three highest PEG concentrations (62-83 g·L⁻¹) (Fig. 3). Seeds in all three treatments emerged at approximately 80-90%. It was surprising that marigolds emerged at such high percentages in growing media that had Ψ_w near the permanent wilting point. However, *Colophospermum mopane* seedlings that had been imbibed for 24 h in CaCl₂ solutions at -0.03 MPa grew in PEG solutions in a vermiculite matrix at Ψ_w as low as -2.1 MPa (Johnson et al.,

1996). After emergence, marigold seedlings were transferred to the greenhouse. At this time, approximately 40-60% of the plants that had emerged in the three highest treatments died when exposed to the higher light levels and variable temperatures and relative humidity levels typical in greenhouses. Because of this low establishment rate, those treatments are unacceptable for practical applications and will not be discussed further.

The seeds in the remaining treatments emerged at percentages above 90% and seedling emergence was not delayed more than 2 d compared to control seedlings. Marigolds grown in the three lowest PEG concentrations (15 - 30 g·L⁻¹) had similar establishment percentages as controls (near 100%). However, when marigolds were grown with PEG concentrations in the 42-50 g·L⁻¹ range, the establishment percentage was between 80-90%. In commercial greenhouse seedling production, growers aim to have nearly 100% of plants emerge and become established (Corr, 1998). If this higher concentration range is used, empty cells where seeds did not emerge or died in the greenhouse would have to be 're-plugged' with living seedlings, which would be a significant cost to plug growers.

Before seedlings emerged, the EC of the growing medium decreased as PEG-8000 concentration increased. A similar trend was observed for hydroponic marigolds and impatiens treated with PEG-8000 (Burnett et al., 2004). In that experiment, nutritional analyses of impatiens indicated that foliar concentrations of nitrogen, calcium, zinc, and copper decreased, while nickel and phosphorus increased with increasing PEG-8000 concentrations in the solution (Burnett et al., 2004). The impatiens were deficient in zinc and copper in all treatments (according to guidelines in Mills and Jones, 1996), and the foliar concentration of zinc and copper decreased with increasing PEG-8000 concentration. So, these micronutrients should be monitored if PEG-8000 is used in a commercial setting (Burnett et al., 2004). After seedlings

emerged, the effects of PEG-8000 on EC were less clear, possibly due to the addition of fertilizer to and plant uptake of nutrients from the growing medium. Thus, the low EC in treatments with low PEG concentrations may be related to increased growth and nutrient uptake in those treatments.

Increasing PEG concentrations resulted in seedlings that were shorter, narrower, had shorter hypocotyls, and had smaller leaf areas and shoot dry weights. Similar results were observed when marigolds were grown in Hoagland solutions containing PEG-8000 (Burnett et al., 2004). Marigolds treated with PEG concentrations of 15-30 g·L⁻¹ were of similar or superior quality to non-treated controls, and, since they were shorter, would be cheaper to ship and easier to sell. Use of osmotic compounds, such as PEG-8000, may be an alternative to the use of chemical growth retardants or traditional non-chemical growth retardant methods. In addition, PEG can be applied early (before or shortly after emergence) to reduce hypocotyl elongation. In plants such as marigold, hypocotyl height can account for much of a plant's height. For example, in this experiment, hypocotyl height was 60% of the overall height of control seedlings 18 d after seeding. So, height control must reduce hypocotyl height to be effective. Although the hypocotyl height decreased when marigolds were treated with PEG-8000, hypocotyl height was 60-65% of the overall height in all treatments.

At harvest, compactness and stem density of marigold seedlings decreased with increasing PEG-8000 concentrations. Similarly, the compactness of hydroponic marigolds grown with PEG-8000 in the solution (Burnett et al., 2004) and drought-stressed marigolds (van Iersel and Nemali, 2004) decreased with increasing drought stress. However, height, and particularly hypocotyl height, are more important indicators of marigold seedling quality than compactness. Tall, but compact, seedlings would also be more expensive to ship than short seedlings that are

less compact. So, even though control plants were more compact, the shorter PEG-treated plants would be more desirable to commercial greenhouse growers.

Root length and leaf area decreased as marigolds were treated with higher PEG-8000 concentrations. Although this may seem like a disadvantage, control marigolds were pot-bound and those plants' roots were circling around the bottoms of cells. In most plants treated with PEG-8000, roots extended to the bottom of cells, but did not exhibit circling (some root circling was observed in plants treated with $15 \text{ g}\cdot\text{L}^{-1}$). Root restriction may be detrimental to plant vigor after transplant (Nesmith and Duval, 1998), so reductions in root weight and length are desirable in some cases. Roots probably would not have been circling the bottoms of containers of control seedlings if they had been transplanted earlier. However, seedlings are often held in the greenhouse for weeks before transplanting, so roots that show delayed root circling would be desirable. After transplanting, roots in all treatments appeared similar.

Leaf Water Relations

Early in the experiment, leaf Ψ_w , Ψ_s , and Ψ_p decreased with increasing PEG-8000 concentration. Reductions in Ψ_w , and especially in Ψ_p , are associated with decreased cell elongation (Cosgrove, 1997). So, it appears that PEG-8000-induced changes in plant water status were important for observed reductions in plant growth.

At the last two measuring days, this trend continued, but was not always significant. It is possible that marigolds acclimated to low substrate Ψ_w . Or, since cotyledons were measured at two weeks after transplanting, while sections of regular leaves were measured at 4 and 6 weeks after transplanting, it is possible that differences in leaf Ψ_w were less consistent in a section of a leaf compared to a whole cotyledon. It is also possible that some of the PEG-8000, which is highly water soluble, was leached out of the growing medium.

Whole Plant Carbon Exchange

Whole-plant photosynthesis decreased as plants were grown with increasing concentrations of PEG-8000 in the growing medium (Fig. 9). However, since the presence of PEG-8000 in growing media was associated with decreased leaf areas, it was vital to correct photosynthesis rates for differences in plant size. Photosynthesis per unit leaf area also decreased with increasing PEG-8000 concentrations (Fig. 9), so it is evident that changes in photosynthesis were caused at least partly by adjustments in leaf physiology. Rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavendula stoechas* L.) grown without water for ten d ($\Psi_w = -3.0$ MPa) also decreased whole plant photosynthesis and photosynthesis per unit leaf area (Nogués et al., 2001).

On a leaf level, photosynthetic reductions associated with drought may be due to decreases in stomatal conductance, mesophyll conductance, Rubisco activity, or decreases in electron transport and phosphorylation within the chloroplast (Jones, 1992; Kaiser, 1987). In this experiment, it is likely that changes in stomatal conductance were at least partly responsible. As the Ψ_w of the growing medium decreases, stomata typically close (Jones, 1992). Conversely, changes in mesophyll conductance usually involve anatomical changes either through adaptation or leaf damage. When annual salvia were exposed to episodic drought, stomatal conductance was the first factor limiting photosynthesis. However, as drought stress continued, mesophyll conductance started limiting photosynthesis as well (Eakes et al., 1991). Since marigolds grown in PEG-8000 were exposed to mild drought over a long period of time, it is possible that leaves from PEG-8000-treated marigolds were thicker, and airspaces within the mesophyll layer were smaller. In addition, specific leaf weight decreased as marigolds were treated with more PEG-8000. Unfortunately, it was impossible to measure mesophyll and stomatal conductance in this experiment due the small size of the leaves.

Lower photosynthesis rates with increasing PEG-concentrations undoubtedly contributed to reductions in plant growth. In the two highest PEG concentrations discussed (42 and 50 g·L⁻¹), whole-plant photosynthesis and photosynthesis per unit leaf area were close to zero. Reductions in photosynthesis may be a disadvantage. However, previously drought-stressed plants also are less likely to wilt after transplanting than non-stressed plants, when exposed to a subsequent drought (Eakes et al., 1991). This may be an advantage for greenhouse growers who could market their plants as ‘drought-resistant’. Even so, photosynthesis rates for seedlings treated with 42 and 50 g·L⁻¹ of PEG-8000 were so low that these treatments would be undesirable.

Since PEG-8000 may be an alternative to chemical growth retardants in the greenhouse, it is also of interest to compare PEG-8000 treatment effects to those observed in growth retardant experiments. Leaf photosynthesis of grapes and strawberries treated with paclobutrazol or flurprimidol were also lower than those of non-treated plants (Hunter and Proctor, 1994; Archbold and Houtz, 1988). Photosynthesis of strawberries treated with paclobutrazol has also been reported to increase; however, this was probably due to delays in fruit set in paclobutrazol-treated plants (Deyton et al., 1991).

Whole-plant respiration decreased with increasing concentrations of PEG-8000. When data were corrected for differences in plant dry weight, there was an increase in respiration with increasing PEG-concentrations (Fig. 9). Although stomata typically close when soil Ψ_w is low, this does not affect respiration as much as photosynthesis because oxygen is readily available in the atmosphere and can enter the leaf in sufficient quantities through diffusion (Kramer and Boyer, 1995). In fact, whole-plant respiration actually exceeded photosynthesis in plants grown with PEG concentrations from 42-50 g·L⁻¹. However, this could have been due partly to

respiration by soil microbes, which may have affect photosynthesis and respiration measurements substantially when small seedlings are measured (van Iersel and Bugbee, 2000).

Similarly, drought stressed rosemary and lavender had lower whole shoot respiration than non-stressed plants (Nogués et al., 2001). Respiration per unit dry matter was not reported in their experiment. According to McCree (1986), drought stressed plants have been reported to decrease and increase whole plant maintenance respiration compared to non-stressed plants. This discrepancy could be due to the fact that plants exposed to extremely low soil Ψ_w quickly or exposed to drought for more than 14 d tend to have higher or equivalent respiration rates compared to non-stressed plants (McCree, 1986). Marigolds were grown in growing media containing PEG-8000 for 49 d before respiration rates were measured. The duration of drought stress in this experiment may be one cause of increased respiration per unit dry weight.

Post-Transplant Morphology

Flowering was gradually delayed for seedlings that were treated with increasing PEG-8000 concentrations (Fig. 10). A delay in flowering would not seem desirable. However, marigolds transplants are usually grown for 3-4 weeks before they are sold (Kessler, 1998), and all marigolds in this experiment were flowering approximately 2 weeks after transplanting. So, a flowering delay of 5 d should not delay the sale of marigolds. In this experiment, height of marigolds treated with PEG-8000 was reduced after transplanting with increasing concentrations of PEG-8000 (Fig. 11). There was no attempt to keep PEG-8000 in the growing medium after transplanting, so height reductions after transplanting were due to residual effects of the PEG treatments.

Although reduced height after transplanting may be seen as a disadvantage, growers likely would consider it advantageous. Height of plants that stretch in the seedling stage, like

marigolds, is also difficult to control after transplanting. Height reduction strategies such as restricting water or nutrients after transplant are recommended for marigolds, and growth retardant applications may be necessary (Kessler, 1998). Since PEG-treated plants were shorter after transplanting, commercial greenhouse growers would be able to spray chemical growth retardants later, if at all. Or, growers who apply growth retardants more than once may be able to skip one growth retardant application, which would reduce production costs.

Conclusions

Growing medium concentrations of PEG-8000 from 15-50 g·L⁻¹ reduced growth and hypocotyl height of French marigold. Thus, PEG-8000 may be a useful alternative to the use of chemical growth retardants or traditional non-chemical methods of reducing height. Higher concentrations of PEG-8000 (62-83 g·L⁻¹) reduced emergence and establishment of seedlings and should be avoided. Since plants grown with PEG-8000 concentrations from 42-50 g·L⁻¹ had very low photosynthetic rates, and less than optimal emergence and establishment percentages, these treatments should probably be avoided as well. Marigold plugs were shorter after transplanting when treated with increasing amounts PEG-8000. This would be of interest to growers who purchase marigold plugs, since slower growth after transplanting may reduce the number of growth retardant applications necessary. Drought-induced reductions in leaf Ψ_w were correlated to reductions in growth. Reductions in photosynthesis also appeared to contribute to overall slower growth in plants treated with increasing concentrations of PEG-8000. However, reduced photosynthesis was probably influenced by low substrate Ψ_w , which may have decreased stomatal conductance. Future research should focus on improving the commercial applicability of using

osmotic compounds, such as PEG-8000, to reduce elongation of seedlings. It is important to develop watering regimes for growing plants under osmotic stress, since PEG-8000 is highly water-soluble and easily leaches out of growing media. In addition, simple methods of applying osmotic compounds, such as pre-mixing osmotica in commercial growing media, should be explored.

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Table 1. Mean and standard deviation of volumetric water content of the soilless growing medium in each PEG-treatment. Each experimental unit was weighed twice daily. All water content measurements for the duration of the experiment were averaged over treatments.

Volumetric water content = $28.1 - 61.7x + 1780x^2$, $R^2 = 0.82$, $P = 0.0001$.

<u>[PEG-8000] (g·L⁻¹)</u>	<u>Mean Volumetric Water Content</u>
0	28±8%
15	28±8%
20	28±7%
30	27±8%
42	28±7%
50	30±7%
62	32±7%
70	32±7%
83	36±7%

Figure 1. Water potential of the growing medium as affected by PEG-8000 treatments. Data points are the mean of three measurements with bars representing the standard deviation.

Regression curve indicates a significant quadratic trend. Water potential = $0.229 - 5.67 \times 10^{-3}x - 0.000158x^2$, $R^2 = 0.71$, $P = 0.0001$, where x = PEG-8000 concentration in the growing medium.

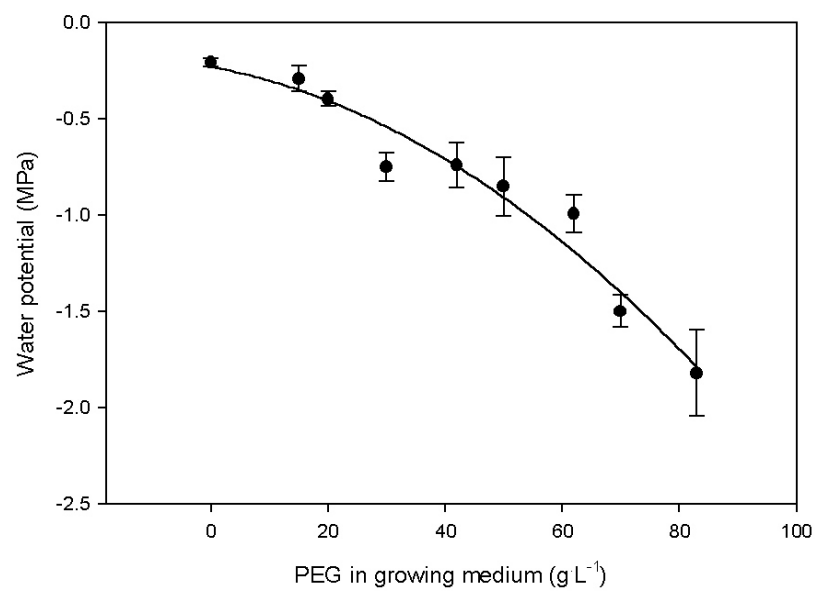


Figure 2. Effects of PEG-8000 application on seedling emergence percentage, days to emergence, and percentage establishment of marigold. Data points are the mean of six replications with bars representing the standard deviation. Regression curves indicate significant linear or quadratic effects. Days to emergence = $4.56+0.065x-0.000509x^2$, $r^2 = 0.76$, $P = 0.0001$; emergence percentage = $0.98+0.57x+28.3x^2$, $R^2=0.46$, $P = 0.0001$; establishment percentage = $99.2+0.259x-0.0127x^2$, $R^2=0.91$, $P=0.0001$, where x = PEG-8000 concentration in the growing medium.

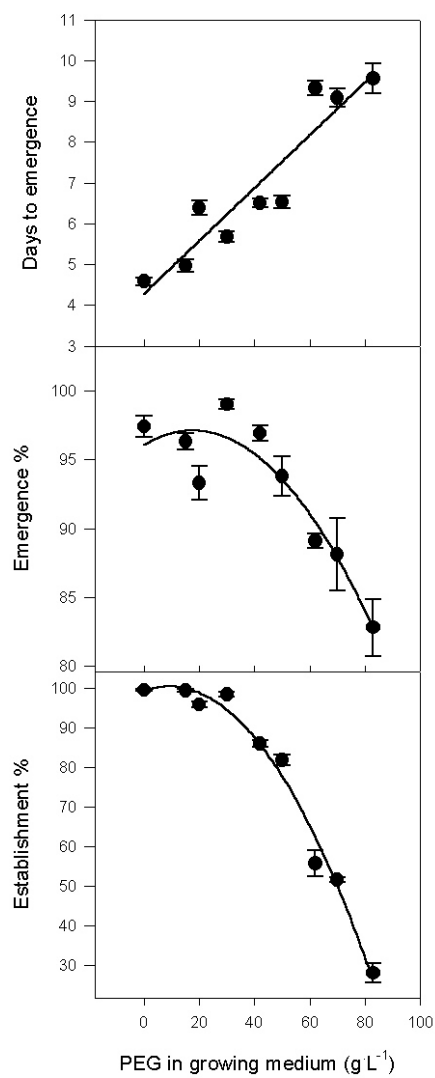


Figure 3. Electrical conductivity of the pore water of the growing medium as affected by PEG-concentrations in the growing medium, measured bi-weekly throughout the experiment. Data are the means of six replications with bars representing the standard deviation. Regression curves indicate significant quadratic trends.

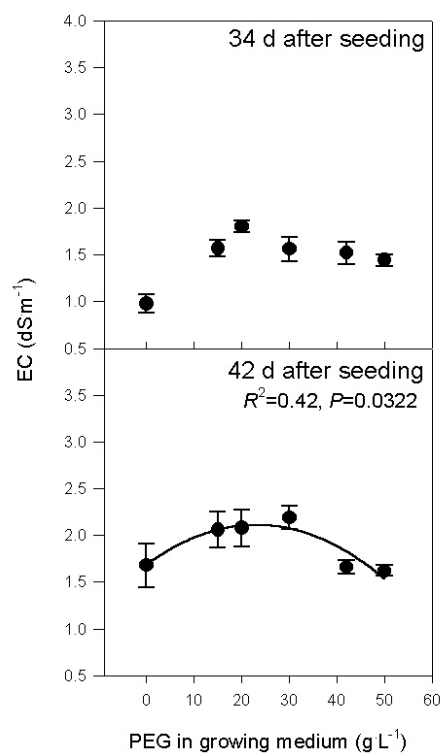
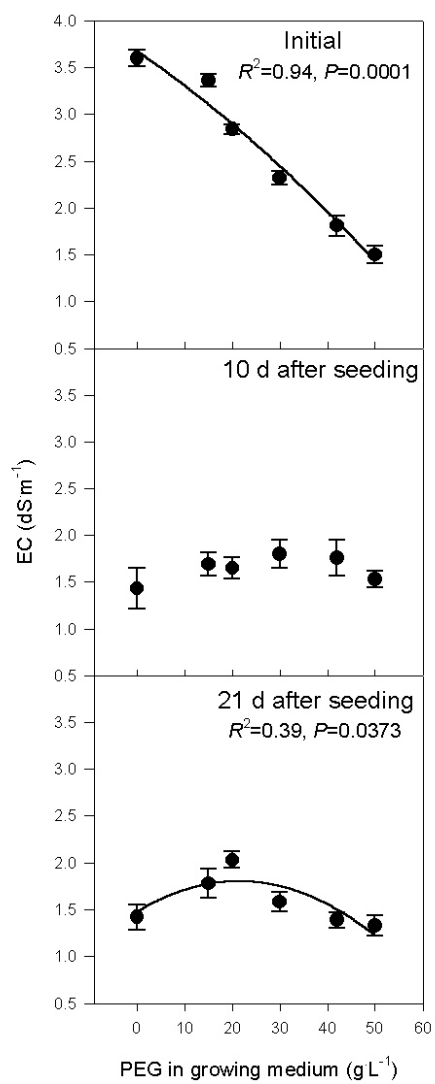


Figure 4. The effects of PEG-8000 in the growing medium on the height of marigold seedlings, as measured at emergence and 18, 32, and 49 d after seeding. Hypocotyl height was measured 18 d after seeding. Data points are the mean of six replications with bars representing the standard error. Regression curves indicate significant linear or quadratic effects.

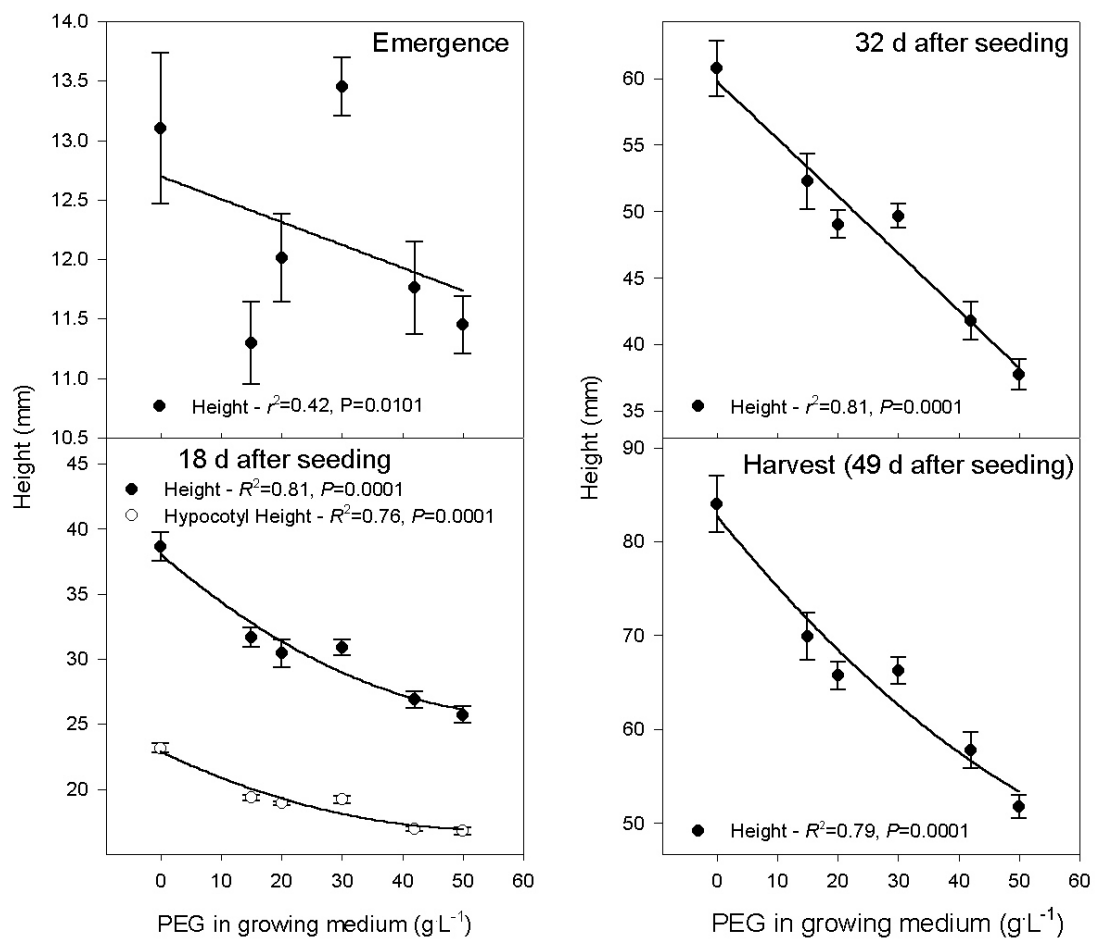


Figure 5. The effects of PEG-8000 in the growing medium on the width of marigold seedlings measured at emergence (cotyledons perpendicular to hypocotyl) and 18, 32, and 49 d after seeding. Data points are the mean of six replications with bars representing the standard error. Regression curves indicate significant linear or quadratic effects.

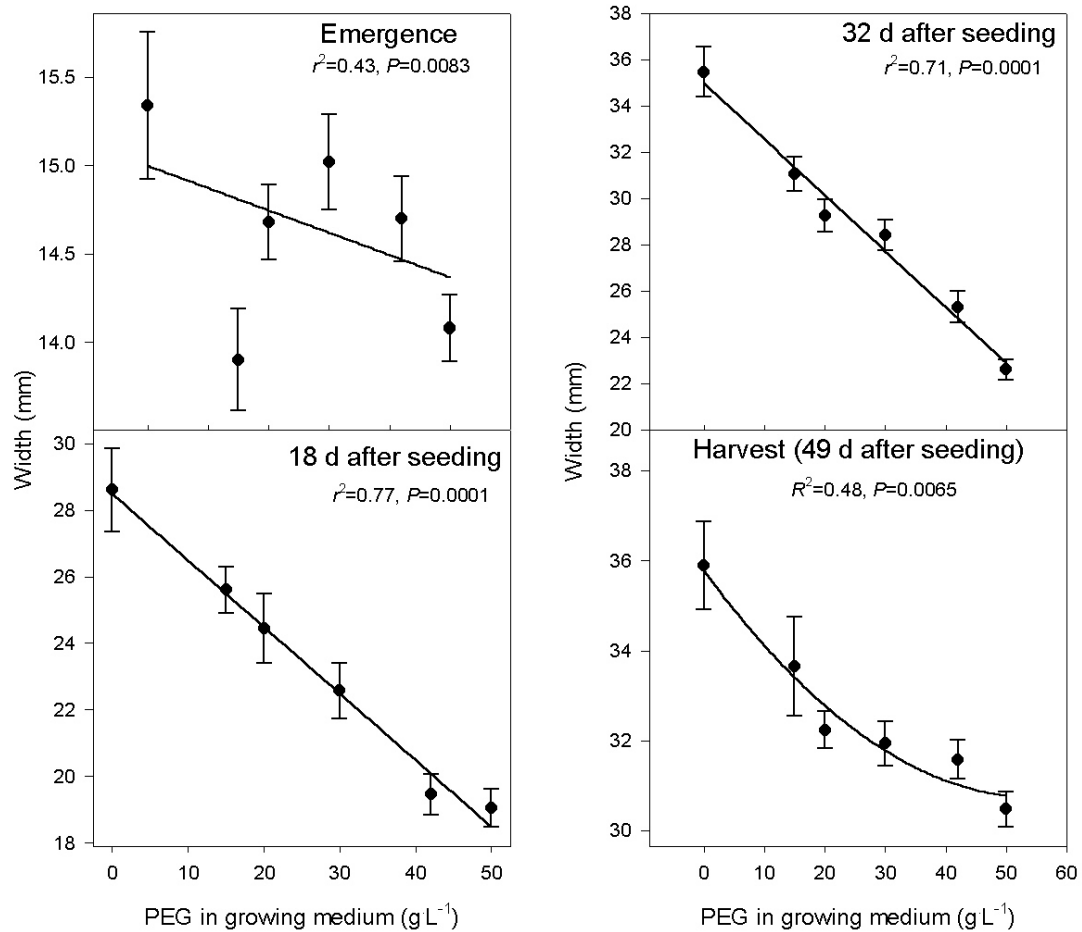


Figure 6. The effects of PEG-8000 in the growing medium on the compactness (shoot dry weight/height at harvest) of marigold. Data points are the mean of six replications with bars representing the standard error. Regression curves indicate significant quadratic effects.

Compactness = $6.11 \cdot 10^{-3} - 5.7 \cdot 10^{-5}x + 6.1 \cdot 10^{-7}x^2$, $R^2 = 0.65$, $P = 0.0001$, where x = PEG-8000 concentration in the growing medium.

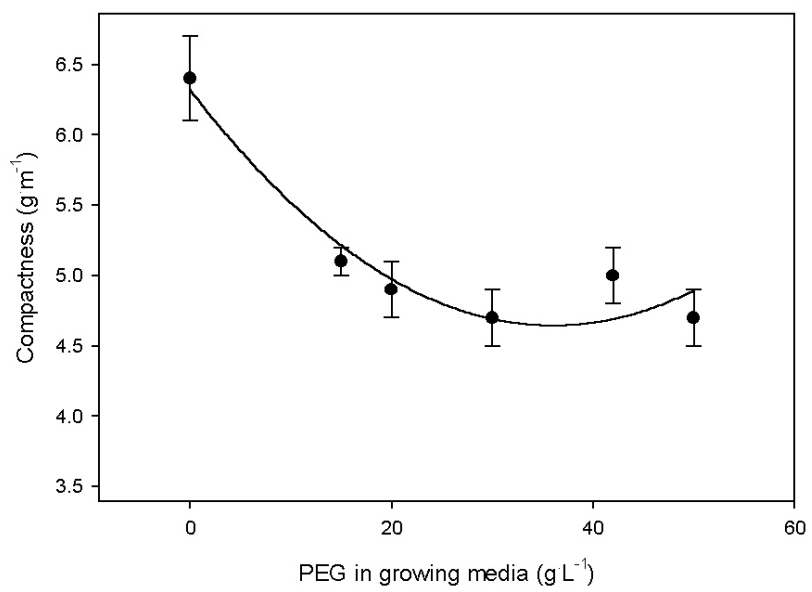


Figure 7. The effects of PEG-8000 in the growing medium on the leaf area , root length, stem density (stem dry weight/stem length), and shoot, root, and inflorescence dry weight of marigold at harvest (49 d after seeding). Data points are the mean of six replications with bars representing the standard error. Regression curves indicate significant linear or quadratic effects.

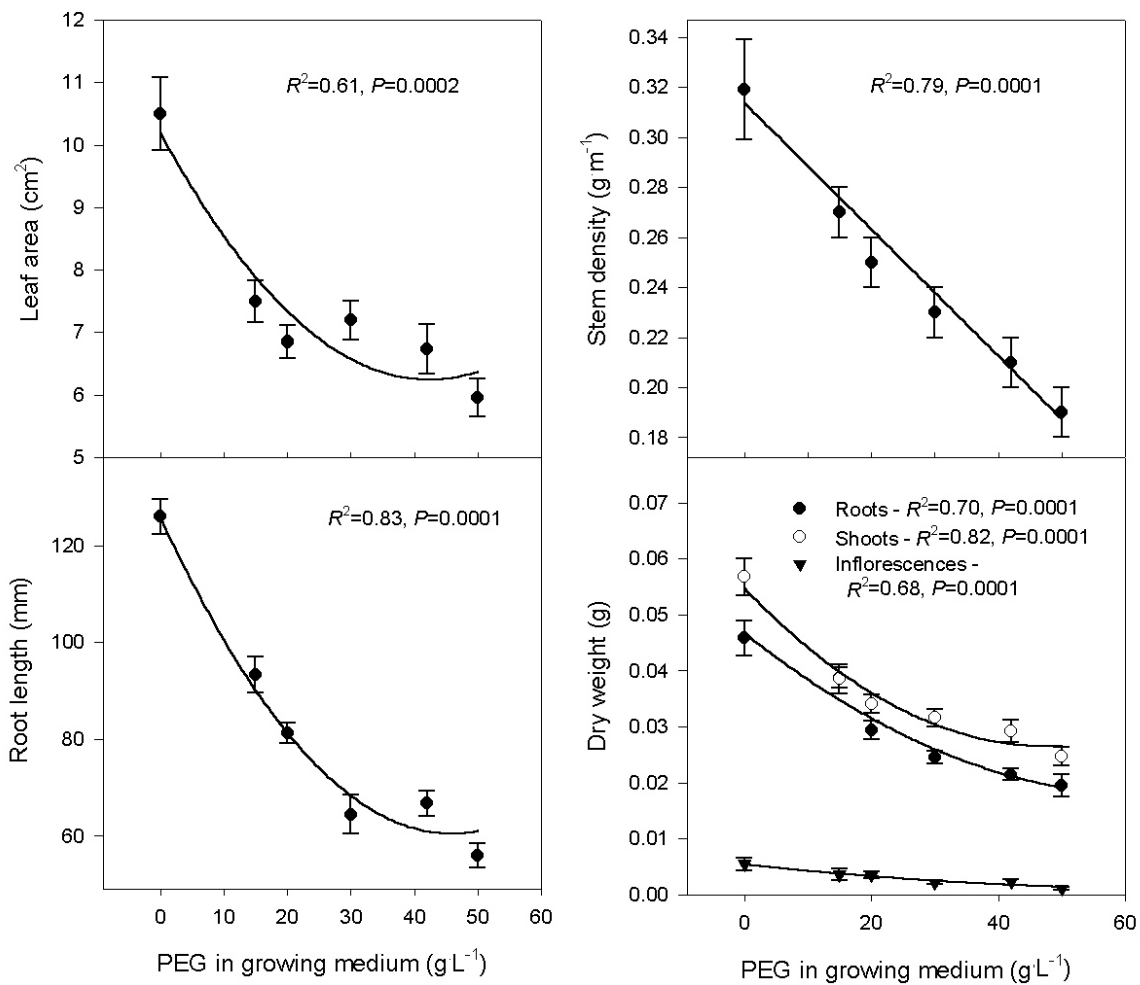


Figure 8. Water, osmotic, and turgor potential of marigold leaves treated with varying concentrations of PEG-8000. Measurements were taken midday 18, 32, and 49 d after seeding on whole cotyledons for the first measurement and on leaf tips of the second acropetal leaf on subsequent measurement days. Data points are the mean of three replications with bars representing the standard deviation. Regression curves indicate significant linear or quadratic effects. Eighteen d after seeding, $\Psi_w = -0.473 - 0.0176x$, $r^2 = 0.69$, $P = 0.0013$; $\Psi_s = -0.812 - 1.02e^{-3}x - 1.88e^{-4}x^2$, $R^2 = 0.66$, $P = 0.0080$; $\Psi_p = 0.248 - 6.39e^{-3}x$, $r^2 = 0.46$, $P = 0.0397$; thirty-two d after seeding, $\Psi_w = -0.787 - 0.0149x$, $r^2 = 0.60$, $P = 0.0041$; forty-two d after seeding, $\Psi_s = -0.834 - 1.27e^{-2}x - 1.77e^{-5}x^2$, $R^2 = 0.63$, $P = 0.0084$, where x = PEG-8000 concentration in the growing medium.

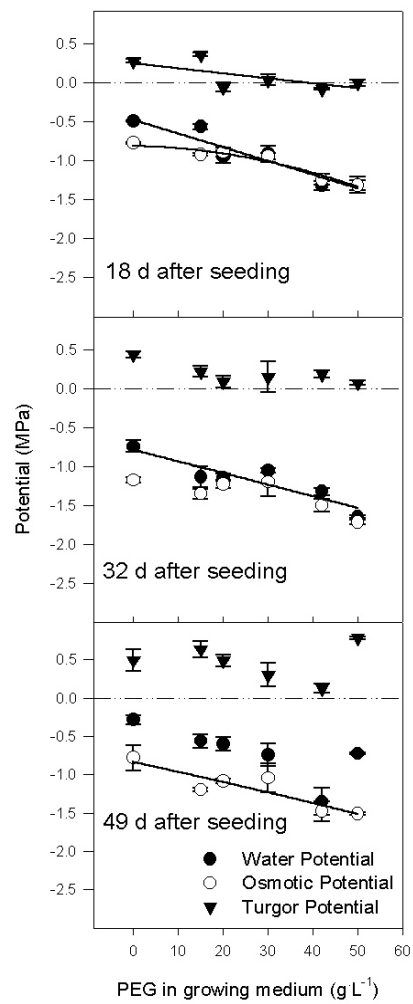


Figure 9. Photosynthesis and respiration of marigolds treated with PEG-8000 (measured 49 d after seeding). Photosynthesis and respiration are shown on a whole plant basis (upper panels) and are corrected for leaf area (photosynthesis) or shoot dry weight (respiration) in the lower panels. Data points are the mean of four replications with bars representing the standard error. Regression curves indicate significant linear or quadratic effects. Photosynthesis = $1.62 - 5.73x^2 + 5.28x^4$, $R^2 = 0.62$, $P = 0.0021$; photosynthesis/leaf area = $1.55 - 1.93x^2 - 2.72x^4$, $R^2 = 0.49$, $P = 0.0242$; respiration = $1.26 - 7.47x^3$, $r^2 = 0.65$, $P = 0.0004$; respiration/dry weight = $12.2 + 0.246x$, $r^2 = 0.73$, $P = 0.0001$, where x = PEG-8000 concentration in the growing medium.

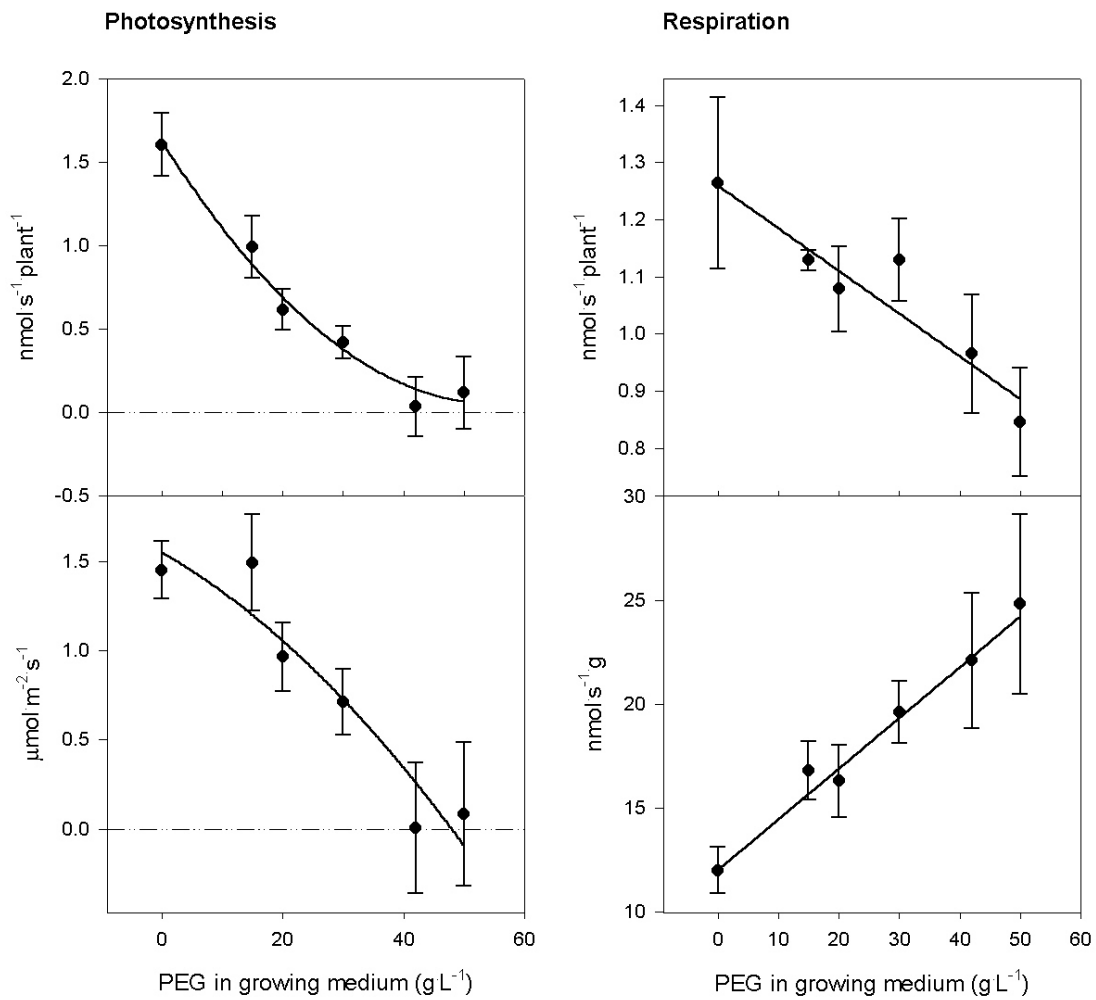


Figure 10. Time to flowering of marigolds as affected by varying PEG-8000 concentrations in the growing medium. Seedlings were transplanted 49 d after seeding. Data points are the mean of 6 replications with bars representing the standard error. Regression curve indicates a significant linear effect. Days to flowering = $61+0.12x$, $r^2=0.74$, $P=0.0001$, where x = PEG-8000 concentration in the growing medium.

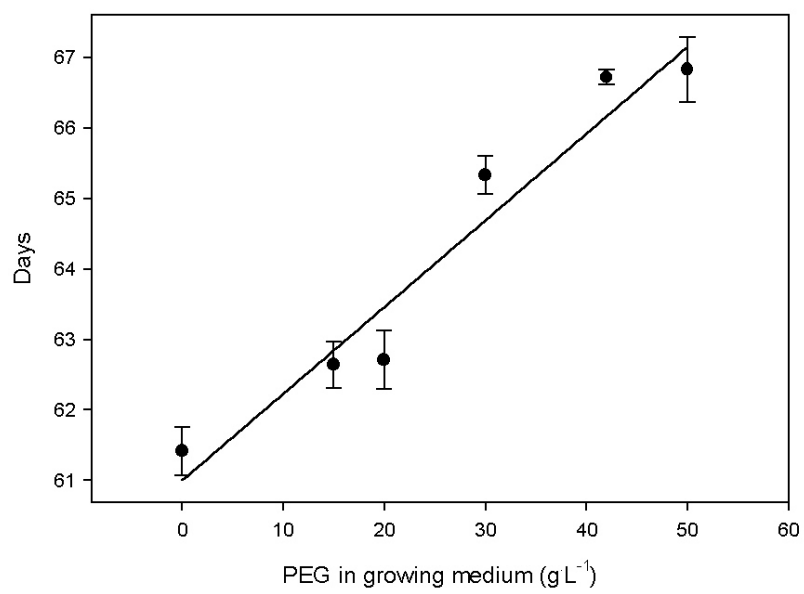
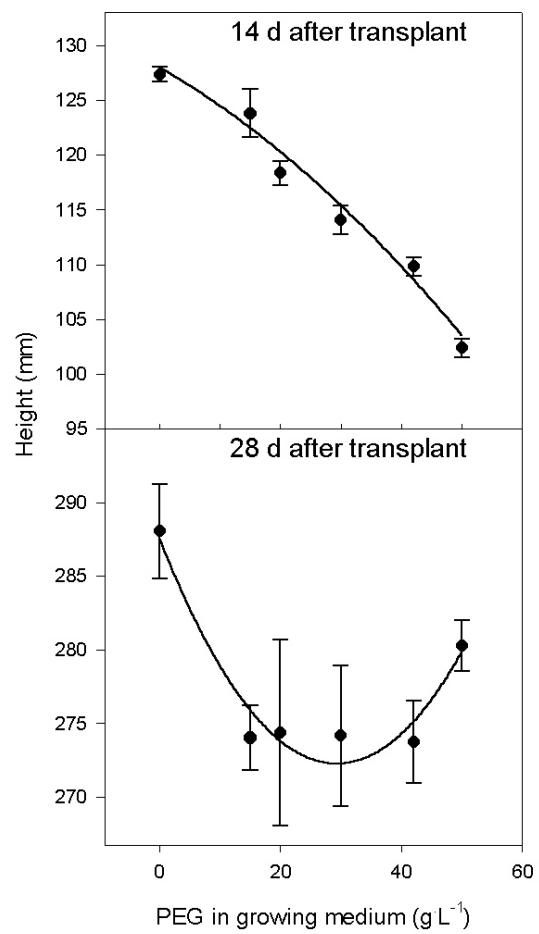


Figure 11. Height of transplanted marigolds after seedlings were grown with different concentrations of PEG-8000 in the growing medium. After transplanting, no effort was made to keep PEG-8000 in the growing medium. Data points are the mean of six replications with bars representing the standard error. Regression curves indicate significant quadratic effects. Height at 14 d after transplanting = $128 - 0.314x - 0.00349x^2$, $R^2 = 0.78$, $P = 0.0001$; Height at 28 d after transplanting = $288 - 1.04x + 0.0178x^2$, $R^2 = 0.42$, $P = 0.0210$, where x = PEG-8000 concentration in the growing medium.



CHAPTER 6
POST-EMERGENCE DRENCHES WITH PEG-8000 REDUCE GROWTH OF SALVIA AND
MARIGOLDS⁴

⁴Burnett, S.E., P.A. Thomas, and M.W. van Iersel. To be submitted in *HortTechnology*.

Abstract.

Annual seedlings are sold to commercial greenhouse growers who value quality. Short seedlings are considered to be of high quality because they are less expensive to ship, easier to manage, and more aesthetically pleasing than tall seedlings. We determined whether previously observed height reductions caused by PEG-8000 incorporated into the growing medium were caused by a delay in maturation or by actual reductions in elongation. Annual salvia (*Salvia splendens* F. Sellow. ex Roem. & Shult. 'Bonfire') and French marigold (*Tagetes patula* L.) seedlings were treated with drenches of PEG-8000: 0, 15, 20, 30, 42, 50, 62, 72, or 83 g·L⁻¹. At least 20% or more of seedlings treated with 62 to 83 g·L⁻¹ of PEG-8000 were dead 14 d after treatments were applied. For this reason, these PEG-8000 concentrations should be avoided. Salvia and marigolds treated with the remaining PEG-8000 concentrations were up to 34 and 14% shorter, respectively. Leaf water (Ψ_w) and turgor potential (Ψ_p) also decreased for salvia which were grown with greater concentrations of PEG-8000, and this is likely one cause of the observed reduction in elongation. So, it appears that PEG-8000 actually slows seedling growth by reducing elongation rates, and not by delaying seedling development.

Production of annual bedding plant seedlings in the form of ‘plugs’ is rapidly becoming an important aspect of floriculture. Until the mid-1960s, most growers propagated seedlings in large trays without individual cells (Thomas, 1993). However, in the past 15 to 20 years, seedlings have been grown in trays containing small individual cells or ‘plugs’ for each plant. Plug production is advantageous because root hairs are damaged less during transplant, and plugs may be held in greenhouses for longer periods than bare-root seedlings (Styer and Koranski, 1997). Plants grown from plugs also tend to be more uniform and survive transplanting better than bare-root seedlings (Thomas, 1993). Plug production has become popular and in 1998, approximately \$107 million was in this form of production (National Agricultural Statistics Service, 1998).

Despite the growing popularity of plugs, this aspect of floriculture is still in its infancy, and there are several production problems that must be resolved. One problem with transplant production is that plants tend to out-grow their small substrate volumes quickly and become leggy (Styer and Koranski, 1997). Elongated transplants are more difficult to grow, more expensive to ship, and less aesthetically pleasing than compact transplants. Purchasers of plugs have more exacting expectations of plant quality than the average consumer.

One promising new option for controlling growth of annual seedlings is imposing controlled drought stress through the incorporation of osmotic compounds, such as polyethylene glycol or PEG-8000 to growing media. PEG-8000 forms hydrogen bonds with water (Kjellander and Florin, 1981) in the growing medium, making it unavailable for plant uptake. This osmotic compound has reduced growth of marigolds (*Tagetes patula* L.) and annual salvia (*Salvia splendens* F. Sellow. ex Roem. & Shult. ‘Bonfire’) when incorporated into growing media before seeding (Burnett, 2004). One advantage of PEG-8000 over other methods of reducing plant growth is that it also reduces hypocotyl height of seedlings (Burnett, 2004). Many commercial

growers avoid using chemical plant growth retardants for seedlings, because chemical growth retardants are applied according to the label restrictions (i.e. after the first true leaves have emerged) hypocotyl height will not be reduced. Since hypocotyl height can account for 60% of the height of marigold seedlings (Burnett, 2004), chemical growth retardants may not be the best option.

When PEG-8000 was incorporated into the growing medium, height and hypocotyl height of salvia and marigold decreased with increasing PEG-8000 concentration (Burnett, 2004). Height reductions were significantly correlated with reductions in leaf Ψ_w (Burnett, 2004). However, in that experiment, seeds were planted into a growing medium that already contained PEG-8000, and seedlings emerged later when grown with increasing PEG-8000 concentrations (Burnett, 2004). This raises the question whether PEG-8000 additions to the growing medium simply cause a delay in plant development, or if they truly inhibit elongation.

In this experiment, PEG-8000 was applied as post-emergence growing media drenches to salvia and marigold seedlings to determine whether PEG-8000 can regulate growth if it is applied after seed emergence, and to determine if PEG-8000 may be applied as a drench to reduce seedling elongation.

Materials and Methods

Two annual salvia 'Bonfire' (Ball Seed, West Chicago, Ill.) or marigold 'Boy Orange' (PanAmerican Seed, West Chicago, Ill.) seeds were planted in each cell of 6×6 cell sections (the experimental unit) cut from 288 trays (cell volume = 8.5 mL) containing a peat-based germinating mix (Germinating mix, Fafard, Anderson, S.C.) on June 23 and October 9, 2003 for salvia and

marigolds, respectively. Seedlings were grown on a mist bench in a glass greenhouse (Maximum *PPF* levels (marigold) = $809 \pm 212 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature (salvia): $25.2 \pm 4.4 \text{ }^\circ\text{C}$, temperature (marigold): $20.6 \pm 4.6 \text{ }^\circ\text{C}$) and misted 5 s every 5 min from 8:00 am to 5:00 pm (EST) until seedlings had emerged and their cotyledons were perpendicular to the hypocotyl. Then, seedlings were removed from the mist bench for 2-4 d until the growing medium was moist, but not at container capacity. One of the two seedlings from each cell was removed, so that all remaining plants were of uniform size.

Polyethylene glycol 8000 (PEG-8000) was mixed with de-ionized water, and 1 mL of PEG-8000 solution was applied to the growing medium using a pipettor (Pipetman, Rainin Instrument Co., Inc., Woburn, Mass) to obtain the following concentrations in the growing medium: 0, 15, 20, 30, 42, 50, 62, 70, and $83 \text{ g}\cdot\text{L}^{-1}$, 18 and 12 d after seeding for salvia and marigolds, respectively. After the growing medium was treated with PEG-8000, seedlings were grown on a mist bench and irrigated 20 s every 30 min from 6:00 a.m to 6:00 p.m for the duration of the experiment. The weight of each experimental unit was recorded twice daily and plants were removed from mist when necessary to maintain constant volumetric water contents in the growing medium (target range= $29.3\% \pm 3\%$). This also prevented leaching which would result in loss of PEG and thus change treatment levels.

To calculate water content, tray weight was estimated as in previous experiments (Burnett et al., 2004). Also, one seedling from the border was harvested once monthly (salvia) or bi-weekly (marigolds). The weight of this seedling was multiplied by the plant number in each tray to account for plant size differences throughout the experiment. The weight added to each experimental unit by PEG-8000 was calculated for each tray. Since each tray contained approximately 307 mL of growing medium, this same volume of non-treated growing mix was

reserved and dried in an oven at approximately 80 °C. The amount of water in each tray was calculated by subtracting the weights of the dry growing medium, the tray, PEG-8000, and the plants from the total weight of each experimental unit. Plants were fertilized using a mist nozzle at least twice a week using a 20N:8.7P:16.6K fertilizer (20-20-20 General Purpose, Scotts Company, Marysville, Ohio) with a N concentration of 200 mg·L⁻¹, starting after treatments with PEG-8000 were applied.

Unless otherwise stated, only plants growing in the 16 innermost cells were measured to avoid edge effects. Survival percentage was calculated as the percentage of seedlings that were alive 14 d after treatment, assuming that there were 16 seedlings initially. Height and width were measured bi-weekly after PEG treatment and hypocotyl height was measured 32 and 26 d after seeding for salvia and marigold, respectively. Plant width was the maximum horizontal distance between any two leaf tips. Salvia and marigold were harvested 88 or 40 d after seeding. At that time, leaf area was measured using a leaf area meter (LI-3100 Leaf Area Meter, Li-Cor, Inc., Lincoln, Nebr.). In addition, shoot (stems and leaves) and root tissues were dried in an oven at 80 °C for at least three days; then dry weights were measured. For shoot dry weight, all 16 plants growing in the innermost cells were harvested. However, roots were only harvested from four representative plants. Compactness was calculated from these data as the ratio of leaf area to shoot height at harvest.

Leaf Water Relations

Midday leaf Ψ_w of salvia leaves was measured 32 d after seeding using individually-calibrated leaf-cutter thermocouple psychrometers (J.R.D. Merrill Specialty Equipment, Logan, Utah). Leaf samples enclosed in psychrometer chambers were equilibrated in a water bath at 25 °C for 4 h before measurement. Water potential of intact leaves was measured first using a

microvoltmeter (J.R.D. Specialty Equipment). Then, leaf samples were frozen to disrupt cell membranes and remove Ψ_p . Samples were then re-equilibrated as described above and osmotic potential (Ψ_s) was measured. Finally, Ψ_p was calculated by subtracting Ψ_s from Ψ_w .

The experimental design was a randomized complete block design with six blocks and 16 subsamples/treatment before seedling harvest. For root harvest data, there were 4 subsamples/treatment. The means of data collected for subsamples within an experimental unit were calculated and data were then analyzed using general linear models (Statistical Analysis Systems, Cary, N.C.). Substrate volumetric water contents were averaged over all measurements before analysis. Data were tested for significant ($P < 0.05$) linear and quadratic effects of PEG-8000 concentrations.

Results

The calculated substrate water content was not significantly different among the treatments for salvia, but increased significantly with increasing PEG concentrations for marigolds (Table 1). For salvia, the mean volumetric water content was 29.6%. Marigold and salvia survival percentage decreased when seedlings were treated with more than 50 g·L⁻¹ of PEG-8000 in the growing medium (Fig. 1). Plants treated with 15 to 50 g·L⁻¹ of PEG-8000 all had nearly 100% survival, but for seedlings treated with 62 to 83 g·L⁻¹ of PEG-8000, survival was close to or below 80%. Since survival percentages below 80% would be unacceptable for commercial applications, morphological data were analyzed without these three treatments.

For the remaining six treatments, marigolds and salvias became shorter as they were treated with increasing concentrations of PEG-8000 (Figs. 2 and 3). At the first measurement,

heights of PEG-treated plants were up to 28% less for salvia and 15% less for marigolds, as compared to the height of control plants. When plants were considered marketable (at harvest), heights were reduced up to 39% (salvia) and 14% (marigold). Salvia treated with 15 to 30 g·L⁻¹ of PEG-8000 were all of approximately the same height throughout the experiment. Hypocotyl elongation decreased as marigolds and salvia were grown in more PEG-8000 (Figs. 2 and 3). Hypocotyl height was reduced up to 15 % or 12% for salvias and marigolds, respectively, after treatment with the highest PEG rate (50 g·L⁻¹) (Figs. 2 and 3). Salvia width also decreased quadratically (60 and 74 d after seeding) or linearly (88 d after seeding) with increasing amounts of PEG-8000 in the growing medium (Fig. 4). Marigold width decreased quadratically as seedlings were treated with increasing quantities of PEG-8000 throughout the experiment (Fig. 3). Compactness, calculated as the ratio of leaf area to shoot height, was not significantly different for marigolds and increased quadratically up to 16% for salvias with increasing PEG concentrations (Fig. 5).

Increasing rates of PEG added to the growing medium reduced shoot dry weight at harvest quadratically by up to 32% and 23% for salvia and marigolds, respectively, compared to non-treated control plants (Figs. 6 and 7). Leaf area of salvia at harvest was significantly reduced when seedlings were grown with increasing quantities of PEG-8000 in the growing medium (Fig. 6). Root dry weight was also reduced up to 23% (salvia) or 39% (marigold), and root length of both species decreased with increasing concentrations of PEG in the growing medium. Root length of salvias decreased linearly, and root length of marigolds decreased quadratically.

Leaf Water Relations

Salvia leaf Ψ_w decreased quadratically, and Ψ_p decreased linearly with increasing PEG-8000 concentration ($\Psi_w = -0.815 - 0.0227x + 0.000263x^2$, $\Psi_p = 0.46 - 0.00996x$, Fig. 8). Salvia treated

with 42 or 50 g·L⁻¹ of PEG-8000 had Ψ_p that was almost zero. Salvia height at 32 d after seeding was less as Ψ_w decreased (height=2.08+0.692 Ψ_w , $r^2=0.58$, $P=0.0054$). Ψ_s was not significantly affected by PEG-8000 treatments.

Discussion

The highest three PEG- treatments (62-83 g·L⁻¹) were fatal for many salvia seedlings, while marigolds did not survive well in the highest two PEG-8000 rates (Fig. 1). In earlier experiments, PEG-8000 rates of 62-83 g·L⁻¹ had substrate Ψ_w of -1.0 to -1.8, which is near or below the permanent wilting point (Burnett, 2004). The resulting drought stress is likely the cause of reduced survival of salvia and marigolds at these high PEG-concentrations. In this experiment, most of the seedlings treated with 70-83 g·L⁻¹ of PEG-8000 died within a few days after the growing medium was treated with PEG-8000. Seedlings treated with 62 g·L⁻¹ of PEG-8000 survived longer. When survival percentage was measured, (14 d after PEG treatments were applied), 80% or less of the seedlings treated with 62 g·L⁻¹ of PEG-8000 were alive. Since such high mortality rates would be unacceptable for commercial greenhouse growers, the highest three treatments will not be further discussed, and were not included in the analysis of morphological data.

Both salvia and marigolds were shorter and narrower throughout the experiment, and had shorter hypocotyls than non-treated controls. In previous experiments, PEG-8000 also reduced growth of marigolds and salvia when incorporated in the growing medium prior to seeding (Burnett, 2004). However, the time to emergence was delayed for seeds planted in growing medium treated with PEG-8000, so PEG-treated plants were younger than non-treated plants,

complicating the interpretation of the results. In the current experiment, seedlings of the same age were treated with PEG-8000, and plant size was reduced with increasing PEG concentrations.

Thus, it appears that PEG treatments actually reduced growth and did not just delay emergence.

Salvia compactness increased with increasing PEG-8000 concentrations, while marigold compactness was not affected by PEG-8000, although there was a general trend for marigolds to be less compact when grown in PEG-8000. In previous research, van Iersel and Nemali (2004) observed that drought-stressed marigolds were shorter, but less compact than well-watered plants. This would be expected, since leaf area is typically more affected by drought than stem elongation (Osório et al., 1998). However, species seem to vary in some morphological responses to drought, since salvia were more compact when grown in PEG-8000. Annual salvia was also more compact when exposed to moisture-stress conditioning (Eakes et al., 1991). However, Eakes et al. (1991) defined compactness as the relationship between shoot dry weight and leaf area. Height was not reported in their study, but salvia shoot dry weight was reduced more than leaf area (Eakes et al., 1991). In previous experiments, marigolds treated with PEG-8000 were less compact and salvia were more compact than non-treated plants (Burnett, 2004).

Compactness is not often quantified in growth retardant studies, because height is the most important factor determining whether growth control has been successful. Even though marigolds were not more compact when treated with PEG-8000, their quality was increased, since they were shorter and had shorter hypocotyls. Salvia and marigold seedlings grown in PEG-8000 rates of 15 - 42 g·L⁻¹ were of equal or greater quality than non-treated plants. However, plants grown in 50 g·L⁻¹ of PEG-8000 had foliar necrosis in some cases, which decreased quality.

As for other morphological factors, observed reductions in root length and dry weight may at first glance be seen as a disadvantage. However, rapidly growing plants, such as marigolds and

salvia often have roots that circle the bottoms of plug cells. In fact, root circling was observed in some controls, and root restriction tends to delay recovery after transplanting (NeSmith and Duval, 1992). Salvia and marigolds grown in PEG-8000 from 20-42 g·L⁻¹ had healthy, substantial roots that filled out plug cells, but that were not circling the bottoms of containers.

Salvia had lower leaf Ψ_w and Ψ_p that correlated with the observed growth reductions. Turgor potential in excess of a minimum 'yield threshold' results in cell elongation (Carpita and McCann, 2000; Cosgrove, 1997; van Volkenburgh 1999; Frensch, 1997) and thus plays an important role in the height increase of plants. Roots are less sensitive to water stress than leaves, but also elongate less in soils at low Ψ_w (Jones, 1992; Kramer and Boyer, 1995). It appears that reductions in Ψ_w and Ψ_p were one factor associated with reduced growth in PEG-treated plants.

PEG-8000 seems to reduce elongation by reducing leaf Ψ_w , not by delaying emergence, and would be an alternative to the use of chemical growth retardants in seedling production. Future research should focus on developing cultural recommendations for the use of PEG-8000. These cultural recommendations should include fertilizer recommendations because in previous experiments, PEG-8000 reduced foliar nitrogen, calcium, zinc, and copper levels in *Impatiens wallerana* Hook.f. (impatiens) (Burnett, 2004). Specific misting schedules should also be recommended so growers using PEG-8000 as a growth retardant would not have to directly measure water content daily.

Conclusions

Marigolds and salvia treated with PEG-8000 drenches from 15-50 g·L⁻¹ were shorter and had shorter hypocotyls than non-treated controls. Salvias treated with PEG were more compact

as well. Seedlings treated with 15-42 g·L⁻¹ of PEG-8000 in the growing medium were of equivalent quality or greater quality, compared to non-treated seedlings. PEG-treated seedlings would be cheaper to ship, and were easier to maintain in the greenhouse than non-treated controls. Since PEG-8000 was applied when seedlings were the same age in this study, plants were smaller, not just younger after treatment with PEG-8000. Reduced leaf Ψ_w and Ψ_p were correlated with reduced height, and likely are one cause of reduced growth in PEG-treated seedlings. Osmotic compounds, such as PEG-8000, would be an alternative to the use of traditional growth retardants or non-chemical growth control in seedling production of salvia and marigold. It would be of interest to develop other, more convenient methods of applying PEG, such as adding it to commercial soilless mixes before they are sold to growers.

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Table 1. Mean and standard deviation of the volumetric water content of growing media treated with different concentrations of PEG-8000 for marigolds. Water content was measured twice daily throughout the experiment. Data points are the means \pm standard deviation of volumetric water contents across all six blocks and times of measurement.

<u>[PEG-8000] (g·L⁻¹)</u>	<u>Volumetric Water Content</u>
0	27.7±6%
15	27.4±6%
20	27.9±6%
30	28.0±6%
42	28.7±7%
50	29.0±6%
62	29.6±6%
70	30.0±6%
83	29.6±6%

Figure 1. Survival percentage of salvia and marigold, 14 d after plants were treated with PEG-8000 drenches. Data points are the average across six blocks with one bar representing the standard error, and curves show significant quadratic effects.

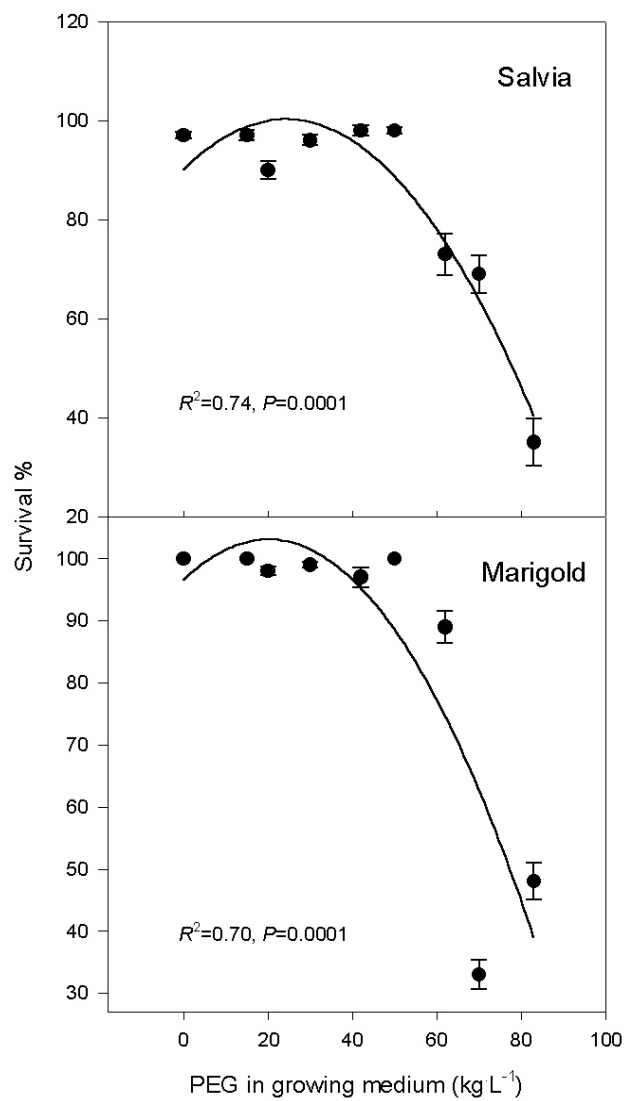


Figure 2. Height of salvia treated with PEG-8000 soil drenches. Seedlings were measured 32, 46, 60, and 88 d after seeding (harvest). Data points are the mean of six replications with the standard error represented by a bar, and curves show significant linear or quadratic effects.

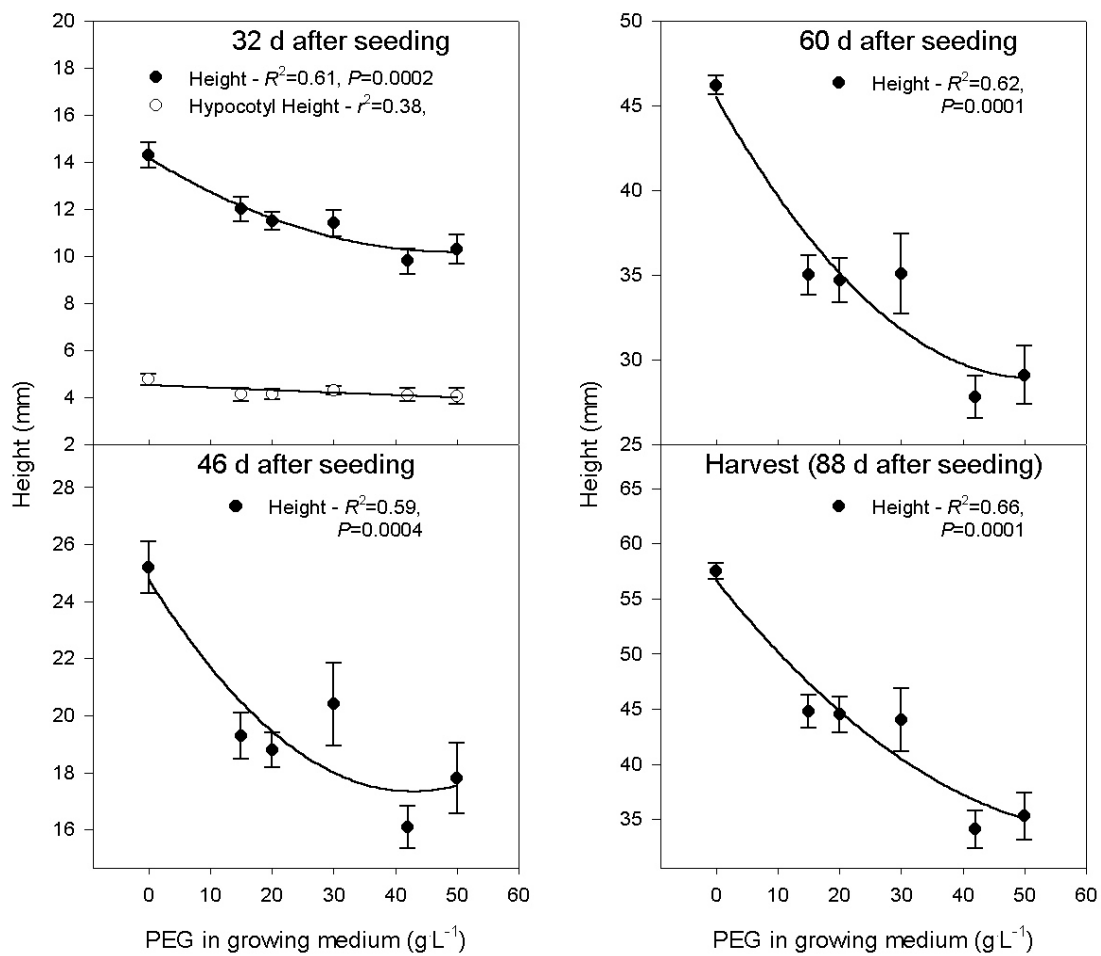


Figure 3. Height and width of marigolds treated with PEG-8000 soil drenches. Seedlings were measured 22 and 40 d after seeding (harvest). Data points are the mean of six replications with the standard error represented by a bar, and curves show significant linear or quadratic effects.

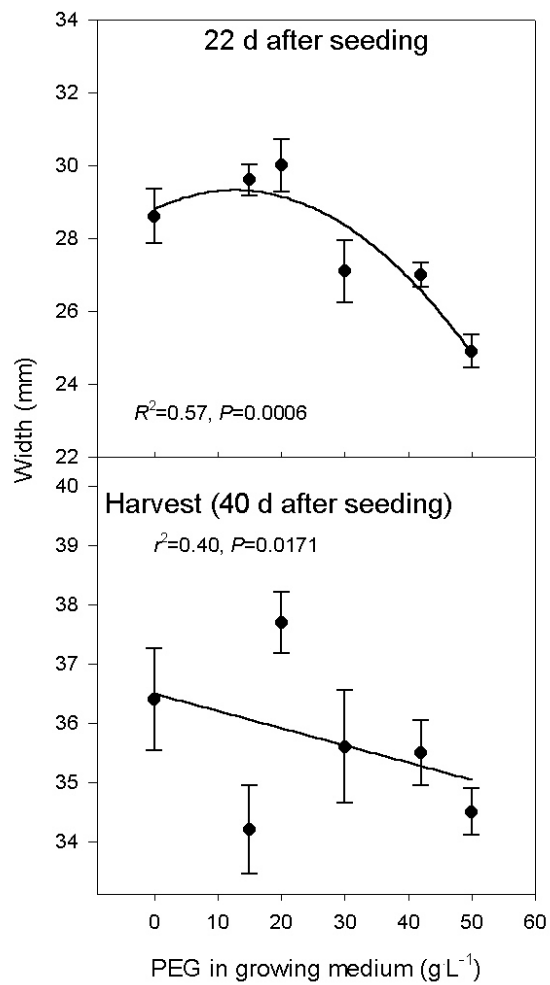
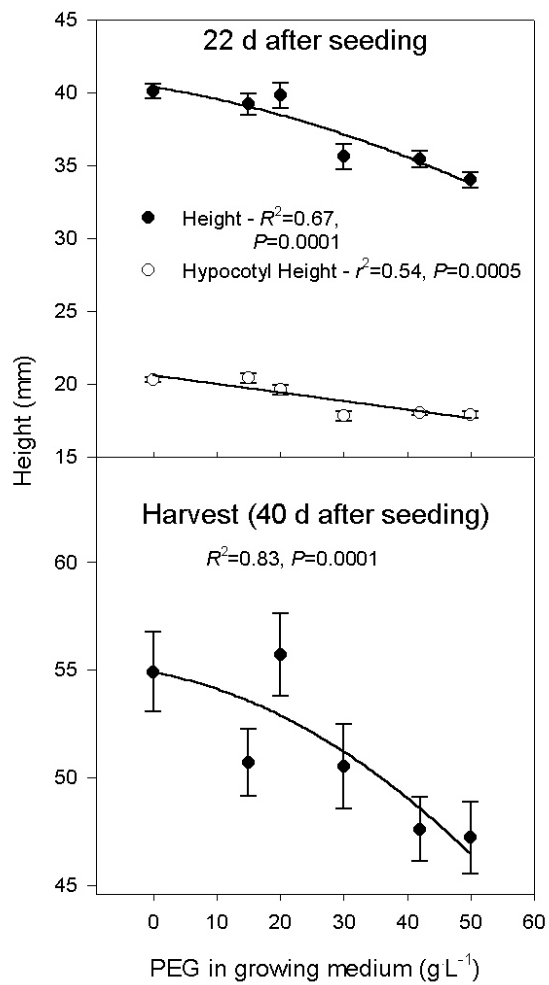


Figure 4. Widths of salvia treated with PEG-8000 soil drenches. Seedlings were measured 32, 46, 60, and 88 d after seeding (harvest). Data points are the mean of six replications with the standard error represented by a bar, and curves show significant linear or quadratic effects.

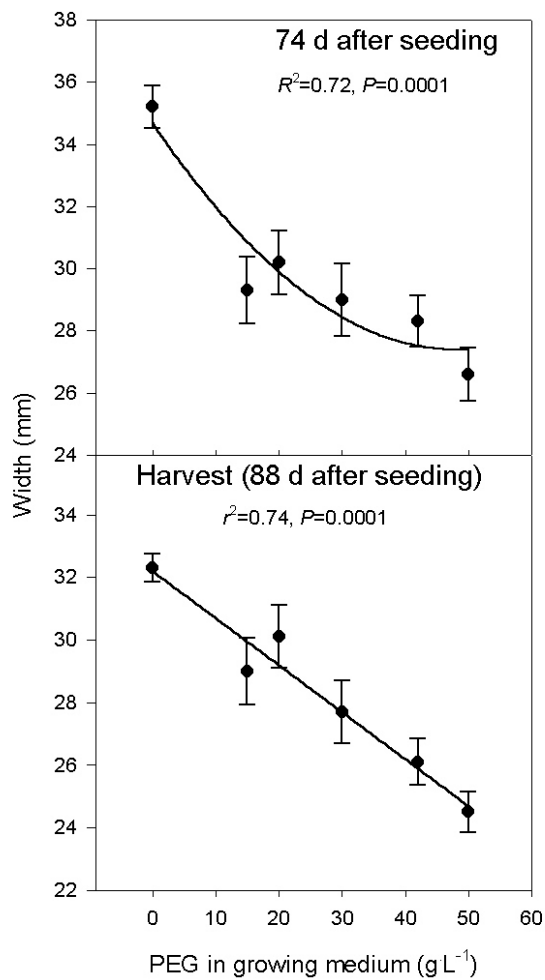
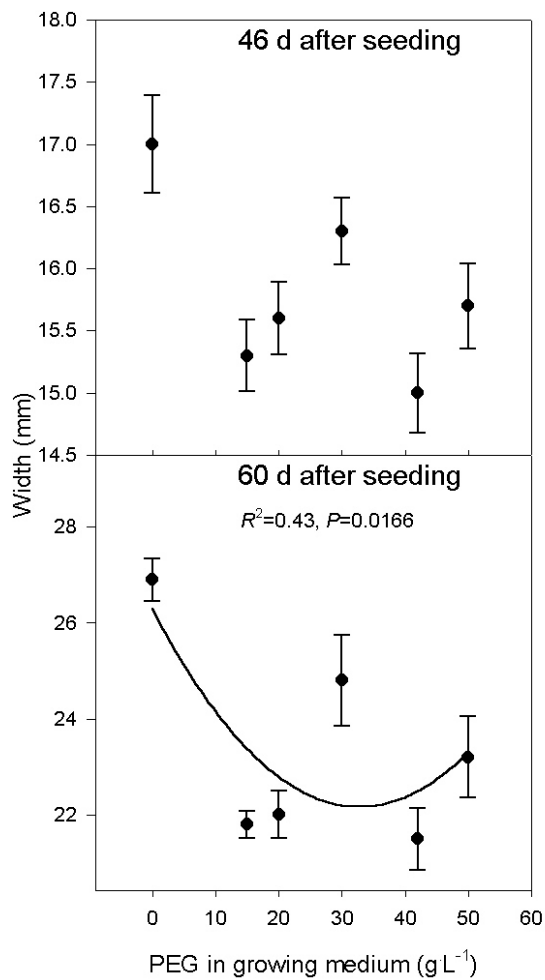


Figure 5. Compactness (leaf area/height at harvest) of salvia and marigolds treated with PEG-8000 soil drenches. Salvia were harvested 88 d after seeding, and marigolds were harvested 40 d after seeding. Data points are the mean of six replications with the standard error represented by a bar, and curves show significant quadratic effects. Salvia compactness = $2.07 + 0.0193x - 0.000217x^2$, where x = PEG-8000 concentration in the growing medium.

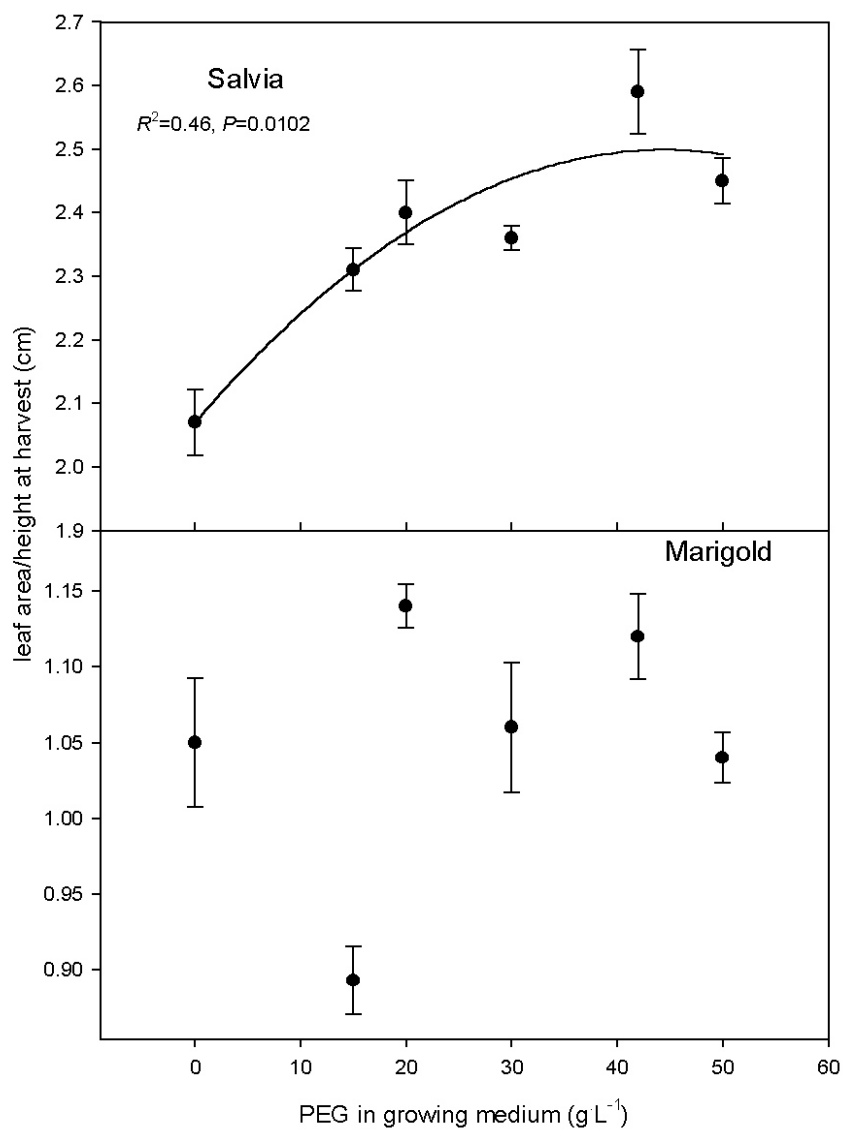


Figure 6. Shoot and root dry weights, root length, and leaf area at harvest (88 d after seeding) for salvia treated with PEG-8000 soil drenches. Data points are the mean of six replications with the standard error represented by a bar, and curves show significant linear or quadratic effects.

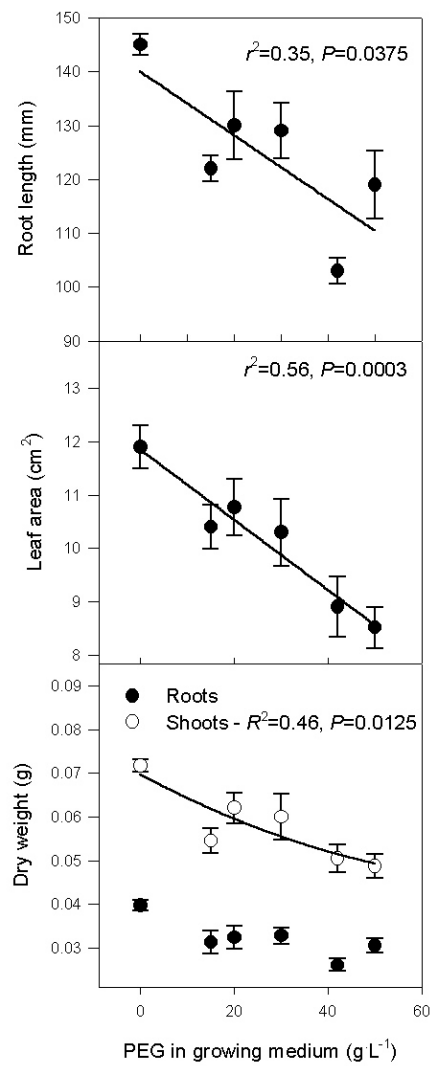


Figure 7. Shoot and root dry weights, root length, and leaf area at harvest (40 d after seeding) for marigolds treated with PEG-8000 soil drenches. Data points are the mean of six replications with the standard error represented by a bar, and curves show significant linear or quadratic effects.

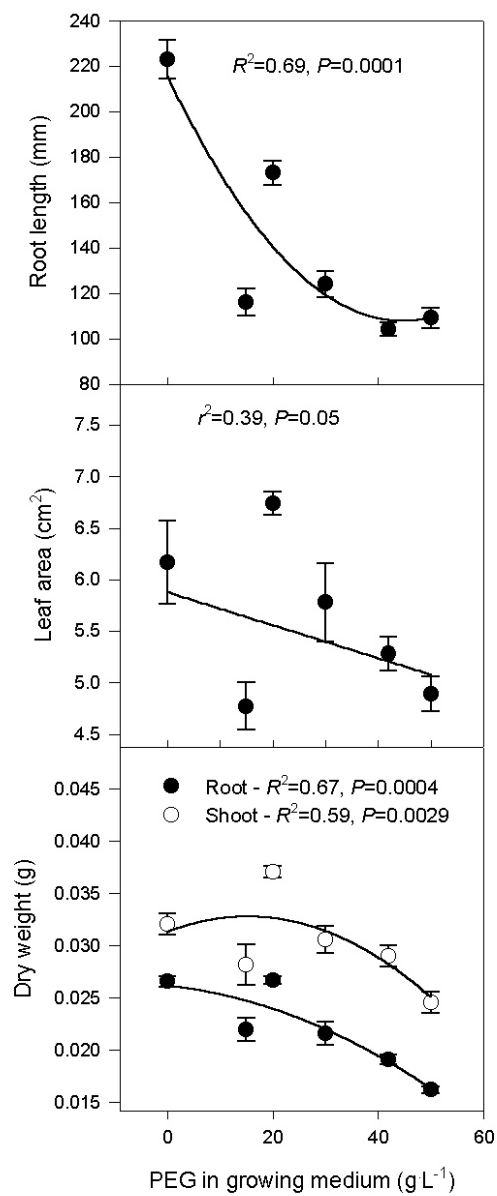
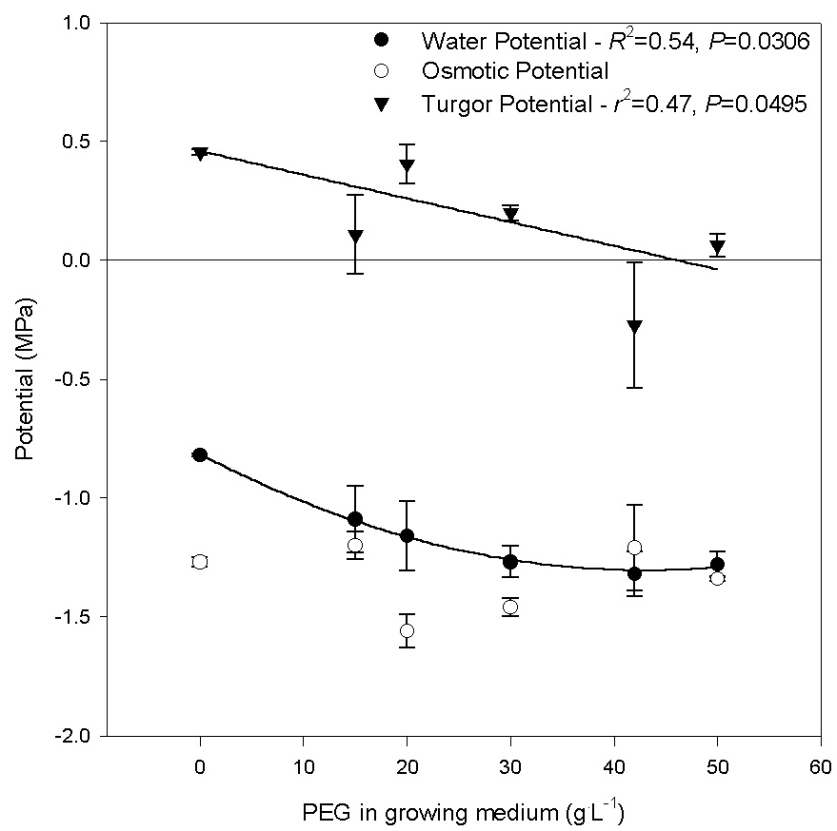


Figure 8. Leaf Ψ_w , Ψ_s , and Ψ_w of salvia treated with PEG-8000 soil drenches. Salvia leaves (second from the top) were measured 32 d after seeding. Data points are the mean of three replications with the standard error represented by a bar, and curves show significant linear or quadratic effects. Water potential = $-0.815-0.0227x+0.000263x^2$, turgor potential = $0.46-0.00996x$, where x = PEG-8000 concentration in the growing medium.



CHAPTER 7

CONCLUSIONS

The osmotic compound, polyethylene glycol 8000 (PEG-8000) reduced growth of *Tagetes patula* (marigold) and *Impatiens wallerana* (impatiens) grown in Hoagland solution. Plants treated with 10 or 25 g·L⁻¹ PEG-8000, for marigolds or impatiens, respectively, were shorter and of greater or equivalent quality to non-treated plants. However, leaves of impatiens treated with PEG-8000 contained less nitrogen, calcium, copper, and zinc than leaves from non-treated impatiens. All impatiens were deficient in copper and zinc, but not nitrogen and calcium. PEG-8000 also decreased the electrical conductivity of Hoagland solution, so it is possible that since PEG-8000 is a polar compound, it forms bonds with nutrient ions.

When use of PEG-8000 was expanded to soilless growing medium, which is typically used by commercial greenhouse growers to grow ornamental seedlings, seedlings treated with 62-83 g·L⁻¹ of PEG-8000 had emergence and establishment percentages that would not be commercially acceptable. However, both salvia and marigolds treated with 15-50 g·L⁻¹ of PEG-8000 were shorter than non-treated seedlings. Water, osmotic, and turgor potential of marigolds and salvia decreased for seedlings treated with increasing concentrations of PEG-8000. Height was correlated with decreasing turgor potential, which is the driving force behind cell elongation. Salvia treated with PEG-8000 also had smaller stem diameters and smaller xylem and phloem cross-sectional area.

In addition, marigolds treated with PEG-8000 photosynthesized less than non-treated marigolds on a whole plant and leaf area basis. Marigold respiration decreased with increasing PEG-8000 concentration on a whole plant basis, but increased on a dry matter basis. Reductions in photosynthesis could be another cause for the reduced elongation observed in marigolds, but is commonly associated with drought stress, such as that imposed by PEG-8000.

Although PEG-8000 reduced elongation of salvia and marigolds, it also delayed emergence. For this reason, it was necessary to determine whether PEG-8000 decreased elongation by imposing physiological drought, or if seedlings treated with PEG-8000 were smaller because they were developmentally delayed. When salvia and marigolds of the same age were treated with PEG-8000 soil drenches from 0 to 83 g·L⁻¹ of PEG-8000 in the growing medium, PEG-treated seedlings were still shorter than non-treated seedlings.

In conclusion, the osmotic compound, PEG-8000 may be used as an alternative to chemical plant growth retardants to reduce elongation of ornamental seedlings. Since PEG-8000 also decreased the foliar concentration of essential plant macro- and micro-nutrients in impatiens, it would be of interest to further explore how PEG-8000 affects the nutrient balance in hydroponic culture and in soilless growing media. In addition, PEG-8000 is a highly water soluble compound, so it would be important to develop watering regimes for growers who use PEG-8000.

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