Special Research Report # 458: Evaluating the Use of the Seedling Hypocotyl Elongation Assay to Predict Mature Plant Ethylene Sensitivity.

Funding Generations of Progress Through Research and Scholarships

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BACKGROUND

Exposure to ethylene during greenhouse production, shipping, and retailing reduces the shelf life and consumer performance of many ornamental plants. Ethylene can accelerate petal senescence, flower abscission, epinasty, leaf chlorosis, and leaf abscission. These symptoms reduce plant quality and lead to increased postproduction/ postharvest shrink by making crops unmarketable (Jones and Edelman, 2013).

Seedlings that are exposed to ethylene exhibit the triple response, which is characterized by an exaggerated apical hook, hypocotyl thickening, and reduced hypocotyl elongation. Hypocotyl length in dark grown seedlings decreases with increasing concentrations of ethylene. The hypocotyl elongation response can be used to determine if a plant at the seedling stage is sensitive to ethylene, but it is not clear whether this consistently predicts mature plant ethylene sensitivity.

MATERIALS & METHODS

Seedling hypocotyl elongation assay. Eighteen bedding plant species (Table 1) were screened at the seedling stage using the hypocotyl elongation assay. Seeds were sown on Petrie dishes containing sterile filter paper saturated with 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor to ethylene biosynthesis in plants. Concentrations included 0 (controls), 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 or 100 µM. Seedlings were held in the dark at 23° C for 7 to 14 days, after which hypocotyl length was measured as a percentage of the control (0 µM ACC). This was referred to as the relative hypocotyl length. Seedling ethylene sensitivity was classified as high (relative hypocotyl length below 50%), medium (50-79%), low (80-99%) or no response (100%).

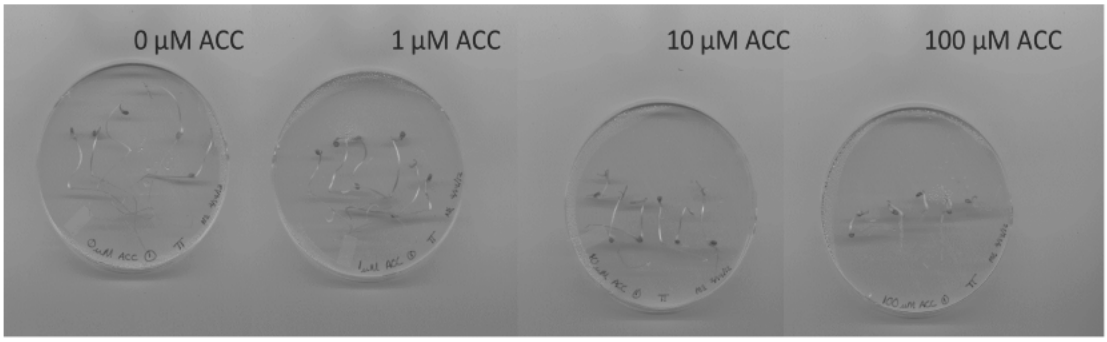

Figure 1. Hypocotyl shortening in tomato 'Tumbler' at higher concentrations of ACC.

M species were grown from seed in the OARDC greenhouses in Wooster, OH. When plants had at least 4 open flowers, mature plants were enclosed in chambers and treated with 0, 0.01, 0.1, 1, or 10 ppm ethylene gas (in the dark at 23° C). Plants were removed from the chambers after treatment with the various concentrations of ethylene for 24 hours. Visual observations of symptoms and photographs were taken at 0 (immediately upon removal from the ethylene), 1, 2, 5 and 7 days. The severity of flower senescence, flower abscission, leaf senescence (i.e., yellowing), leaf abscission, and leaf epinasty was rated on a scale of 0 to 5. A rating of 5= 0% of the plant showing leaf damage or 0% of the flowers showing symptoms; 4= 1 - 20%; 3= 21- 40%; 2= 41- 60%; 1= 61- 80%; and 0= 81- 100%. For additional details on methods and statistical analyses see Edelman et al., 2014.

RESULTS

Thirteen of the bedding plant species exhibited a decrease in hypocotyl length with increasing ACC concentrations, and their relative hypocotyl lengths resulted in classifications from medium to high ethylene sensitivity at the seedling stage (Figure 1, Table 1 and Edelman et al., 2014). Five species (angelonia, dahlia, lobelia, salvia, and zinnia) had relative hypocotyl lengths of 80 to 100% of the control at the highest ACC concentration of 100 µM. These plants were given a seedling classification of low ethylene sensitivity or no response (zinnia).

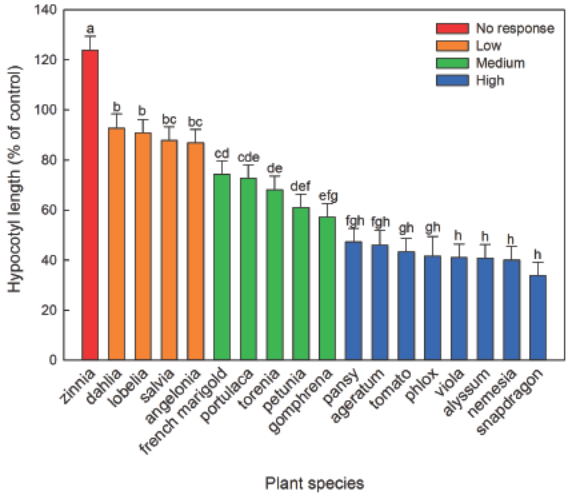

Figure 2. relative hypocotyl lengths at 100 µM ACC.

Differences in ethylene symptoms and the severity of the responses were observed among mature plants of the 18 bedding plant species. Flower abscission or senescence was observed in 15 of the 18 species after treatment with ethylene (Table 1). Torenia abscised some flowers at ethylene concentrations as low as 0.01 ppm, while flower loss was first observed in nemesia, phlox, salvia, and tomato plants treated with 0.1 ppm ethylene (Edelman et al., 2014). Flower senescence was observed in 10 of the 18 species after exposure to ethylene (Table 1). At 10 ppm, petunia received a rating of 0, with all open flowers senescent (i.e., wilting) immediately after the ethylene treatment. Petal wilting in petunia was observed at concentrations as low as 0.01 ppm. Flower senescence was observed in dahlia, portulaca, and lobelia plants treated with 0.1 ppm ethylene for 24 hours. Vegetative symptoms of ethylene damage included leaf

chlorosis, abscission, and epinasty (Table 1). Ageratum, dahlia, French marigold, tomato, and salvia exhibited epinasty, or the downward curvature of the leaf petiole. The most severe epinastic response at the lowest ethylene concentrations was observed in tomato (Figure 3). Marigold, which is often considered to be an ethylene insensitive crop, showed mild symptoms of leaf epinasty, but all plants had completely recovered by the 1 day observation (Edelman et al., 2014). Extensive leaf abscission was observed in portulaca and moderate leaf yellowing was observed in alyssum. The most severe symptom in mature plants was used to determine its ethylene sensitivity classification, so that ethylene sensitivity responses in seedlings could be compared to mature plants.

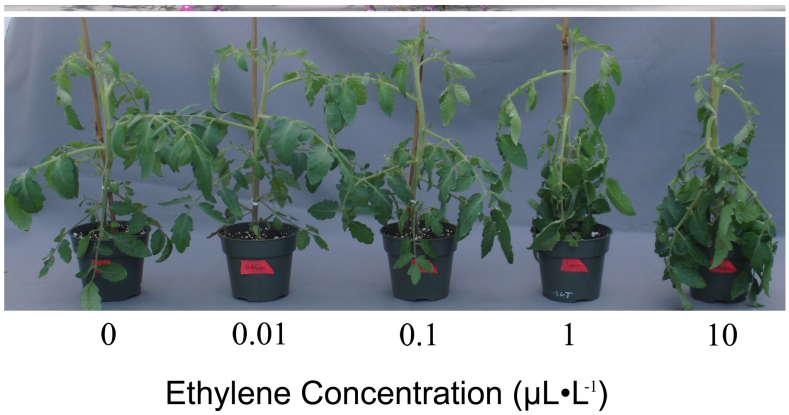

Figure 3. Epinasty in tomato 'Tumbler' treated with different concentrations of ethylene for 24 hours.

For 14 of the 18 species, ethylene sensitivity in seedlings was similar to that in mature plants. The most striking differences were observed in lobelia, salvia, viola, and pansy. The hypocotyl elongation screen showed that lobelia and salvia seedlings had low sensitivity to ethylene, while mature plant screens indicated high sensitivity. Both viola and pansy had the opposite response, with high responses at the seedling stage and very low responses to ethylene at the mature plant stage.

CONCLUSION

Ethylene responsiveness or sensitivity is affected by developmental stage. Seedlings or young plant responses may, therefore, not be a reliable means of predicting mature plant sensitivity. In species where the responses have been shown to be similar between seedlings and mature plants, the seedling hypocotyl elongation assay may be a useful tool for evaluating differences in ethylene sensitivity between cultivars.

IMPACT TO THE INDUSTRY

It is important for floriculture producers, retailers, and plant breeders to know which plants are sensitive to ethylene. This information can be used by producers to identify the cultivars that will withstand long-distance shipping and have the longest shelf life. They can also determine which plants will benefit from treatment with ethylene inhibitors like 1-methylcyclopropene (1-MCP). Plant breeders could use a rapid seedling screen to determine the ethylene sensitivity of new plant introductions and to identify less sensitive germplasm for breeding.

Table 1. Ethylene classifications from seedling hypocotyl elongation and mature plant screens

Plants	Seedling sensitivity	Mature plant sensitivity	Mature plant symptoms
Ageratum houstonianum **'High Tide Blue'**	high	high	leaf epinasty
Angelonia angustifolia **'Serena Lavender Pink'**	low	low	flower abscission, flower senescence
Antirrhinum majus **(snapdragon) 'Bedding Rocket Lemon'**	high	medium	flower abscission
Dahlia × hybrida **'Figaro White'**	low	medium	flower abscission, flower senescence, leaf epinasty
Gomphrena globosa **'Fireworks'**	medium	no response	none
Lobelia erinus **'Regatta Rose'**	low	high	flower senescence
Lobularia maritima **(alyssum) 'Snow Crystals'**	high	medium	flower abscission, leaf chlorosis
Nemesia fruticans **'Poetry Blue'**	high	high	flower abscission, flower senescence
Petunia × hybrida **'Carpet White'**	medium	high	flower senescence
Phlox drummondii **'Palona Light Salmon'**	high	medium	flower abscission, flower senescence
Portulaca grandiflora **'Margarita Scarlet'**	medium	high	flower senescence, leaf abscission
Salvia splendens **'Red Hot Sally'**	low	high	flower abscission, leaf epinasty
Solanum lycopersicum **(tomato) 'Tumbler'**	high	high	flower abscission, leaf epinasty
Tagetes patula **(french marigold) 'Durango Bee'**	medium	medium	leaf epinasty
Torenia fournieri **'Clown Blue'**	medium	high	flower abscission
Viola cornuta **'Sorbet XP Orange'**	high	low	flower senescence
Viola × witrockiana **(pansy) 'Matrix Lemon'**	high	low	flower senescence
Zinnia marylandica **'Double Zahara Fire'**	no response	low	flower senescence

PUBLICATIONS

Edelman N.F, Kaufman B.A. and M.L. Jones (2014) Comparative evaluation of seedling hypocotyl elongation and mature plant assays for determining ethylene sensitivity in bedding plants. HortScience 49:472-480.

Jones M.L. and N. Edelman (2013) Preventing ethylene-related losses during the postproduction care and handling of greenhouse crops. Greenhouse Management 32 (1; Jan) 38-44.

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