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Ethylene Sensitivity in *Mandevilla* and the Effect of 1-Methylcyclopropene (1-MCP)

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Abstract – **Ethylene is a gaseous phytohormone that mediates plant adaptive responses at a physiological and molecular level. It is a simple alkene hydrocarbon (C2H4) that is both biogenic and anthropogenic in origin. As a by product of the burning of fossil fuels, ethylene is the most abundantly produced organic molecule on earth. In the horticulture industry, the regulation of endogenous ethylene production and perception of exogenous ethylene in sensitive plant species has considerable economic implications, as ethylene damage causes an estimated annual floriculture crop loss of up to 30% (Agro Fresh 2021). Ethylene biosynthesis and its associated effect on plant defences and developmental processes have been well studied, but there is limited information available on the effect of ethylene on members of the genus *Mandevilla* (Dipladenia). This study was designed to investigate the relative sensitivity of several cultivars of *Mandevilla* sanderi to ethylene gas exposure and to test whether the symptoms of ethylene damage are mitigated by the application 1-methylcyclopropene (1-MCP), commercially available as EthylBloc™.**

Keywords – **Ethylene, Abscission, 1-Methylcyclopropene (1-MCP), Horticulture, *Mandevilla*, Floriculture, Ethephon.**

I. Introduction

In plants, ethylene plays a role in flower production, fruit ripening, gas exchange, response to biotic and abiotic stress, sex determination, root morphogenesis, and abscission of flowers, fruits, and leaves (Naing et al. 2022). In combination with other plant signaling hormones such as auxin, jasmonic acid, and cytokinin, ethylene influences the relative investment of plant resources into growth, reproduction, and defense (Leon-Reyes et al. 2009). Upregulation of ethylene production triggers plant defense mechanisms, allowing plants to respond to stress and maximize fitness under suboptimal growing conditions (Iqbal et al. 2017).

Production of ethylene gas is metabolic in origin and is accomplished by living cells in response to environmental and chemical stimuli. Biogenic ethylene production requires the synthesis of its precursor 1-aminocyclopropane-1 carboxylic acid (ACC), which is mediated by the enzyme ACC synthase (IARC 1994). The extent of ethylene production is a function of the intensity and duration of stress periods (Ravanbakhsh et al. 2018). Ethylene is translocated within the plant by diffusion and is thought to be synthesized at or near its site of action (Chang 2016).

Ethylene response varies considerably between and within species. Enzymes involved in ethylene signal transduction and ethylene-related stress perception are encoded by large multigene families, and ethylene response is dependent upon a number of complex factors, such as receptor affinity, expression pattern, and turnover (i.e., the rate at which an enzyme converts its substrate) (Ravanbakhsh et al. 2018). Additionally, ethylene sensitivity for a particular crop may vary across the growing season. In a 2011 experiment by Macnish et al., *Anthurium scherzerianum* 'Red Hot' and *Codiaeum variegatum pictum* 'Petra' *plants* harvested in August, September, and/or February were insensitive to ethylene, whereas those harvested in May were found to be responsive.

Plant-associated microbiota mitigate the damaging effects of ethylene by reducing the intensity of stressors, detoxifying harmful substances, and producing plant-protective substances (Ravanbakhsh et al. 2018). Conversely, plant-pathogenic bacteria and fungi, such as *Fusarium oxysporum*, *Ralstonia solanacearum* and *Pseudomonas syringae*, are known to synthesize ethylene directly (Weingart et al. 1999).

Commercially available products for limiting ethylene damage include adsorbent chemicals and scrubbers, which remove ethylene gas from the environment, and chemical inhibitors, which prevent ethylene biosynthesis or signal transduction. The most commonly used chemical inhibitors are 2-aminoethoxyvinyl glycine (AVG), silver thiosulfate (STS), and 1-methylcyclopropene (1-MCP). AVG is an inhibitor of ACC synthase, which is required for ethylene synthesis. Silver thiosulfate and 1-MCP are both ethylene action inhibitors, which prevent ethylene-dependent responses by irreversibly binding to ethylene receptors (Schaller and Binder 2017).

A. *Objectives*

(i) To identify the symptoms of ethylene-induced stress response in *Mandevilla sanderi*.

(ii) To determine the extent to which several cultivars of *Mandevilla sanderi* vary in their response to ethylene gas exposure.

(ii) To test whether ethylene-induced symptoms could be reduced or eliminated by treatment with *1-methylcyclopropene (1-MCP)*.

II. Materials and Methods

A. *Experiment 1*

(i) *Objectives*

The objectives of this experiment were to identify the symptoms of ethylene damage and to determine the relative ethylene sensitivity of three cultivars of container-grown *Mandevilla*: ‘Rio White’, ‘Grande Red’, and ‘Rio Pink’.

(ii) *Methodology*

Four replications of this experiment were done between May and October. At the time of the experiment, all plants were approximately 20 weeks old with well-established roots growing in quart-sized pots (43 in^3). Plants were watered and fertilized several days prior to the experiment so soil was damp but not saturated. Senescent flowers and buds were removed, as were yellowed or damaged leaves. The number of flower buds and open flowers on each plant was recorded before the start of the experiment. Plants were then divided randomly into three treatment groups and transferred to plastic containers with the approximate dimensions 24”L x 16”W x 14”H (3.11 ft^3). Pure ethylene gas was not available, so two known sources of ethylene were used: ripe bananas and FLOREL® brand growth regulator, manufactured by Monterey Lawn and Garden, Fresno, CA. The intention was that if both treatments induced similar symptoms, it could be reasonably surmised that effect was due to ethylene, rather than some extraneous variable. FLOREL® (Ethephon, [(2-chloroethyl) phosphonic acid] 3.9%) was applied as a foliar spray at a rate of 500 ppm to one treatment group. Approximately six ripe bananas were placed inside the container of another treatment group. Containers were immediately sealed after the application of the FLOREL® and bananas. The third group consisted of experimental control (no treatment). Containers remained sealed for 72 hours and were stored in the dark at room temperature (approximately 75 °F). After 72 hours, plants were removed from the containers, and buds and flowers remaining on the plant were counted.

B. *Experiment 2*

(i) *Objective*

The objective of this experiment was to determine whether ethylene-induced symptoms could be reduced or prevented using EthylBloc™ *1-methylcyclopropene (1-MCP).*

(ii) *Methodology*

Three replications of this experiment were done in December using three cultivars of *Mandevilla*: 'Calypso White', 'Raspberry Splash', and 'Grande Red'. Plants were prepared as described in *Experiment 1* and randomly divided into two experimental groups. FLOREL® was applied to all plants as a foliar spray at a rate of 500 ppm. EthylBloc™ was applied to one group at the label rate of 0.15 mL/ ft^3. After activating with the buffer solution, the open plastic cup containing the EthylBloc™ (FloraLife, Kent, OH) was placed on top of the soil of one of the plants near the center of the group. Plants were placed in sealed containers for 72 hours and data was collected as described in *Experiment 1*.

III. Results and Discussion

A. *Experiment 1*

All three cultivars showed an increase in the amount of dropped flower buds and open flowers in response to exposure to ethylene. Ethylene response resulting from the inclusion of ripe bananas and the application of FLOREL® produced similar results, so the data are shown grouped together as "ethylene". Overall, exposure to ethylene resulted in a 132.41% increase in bud abscission and 106.68% in open flower abscission (Fig. 1).

Fig. 1. Control 'Grande Red' on left and ethylene-treated 'Grande Red' on right.

The data below (Fig. 2) suggest that of the three cultivars tested, 'Rio Pink' is the most sensitive to ethylene, as ethylene exposure resulted in the greatest increase in the percentage of dropped buds and flowers. However, 'Rio White' had the highest percentage of dropped buds and flowers in the *control* group, which suggests that for this cultivar a heightened stress response was induced solely from the conditions of the experiment.

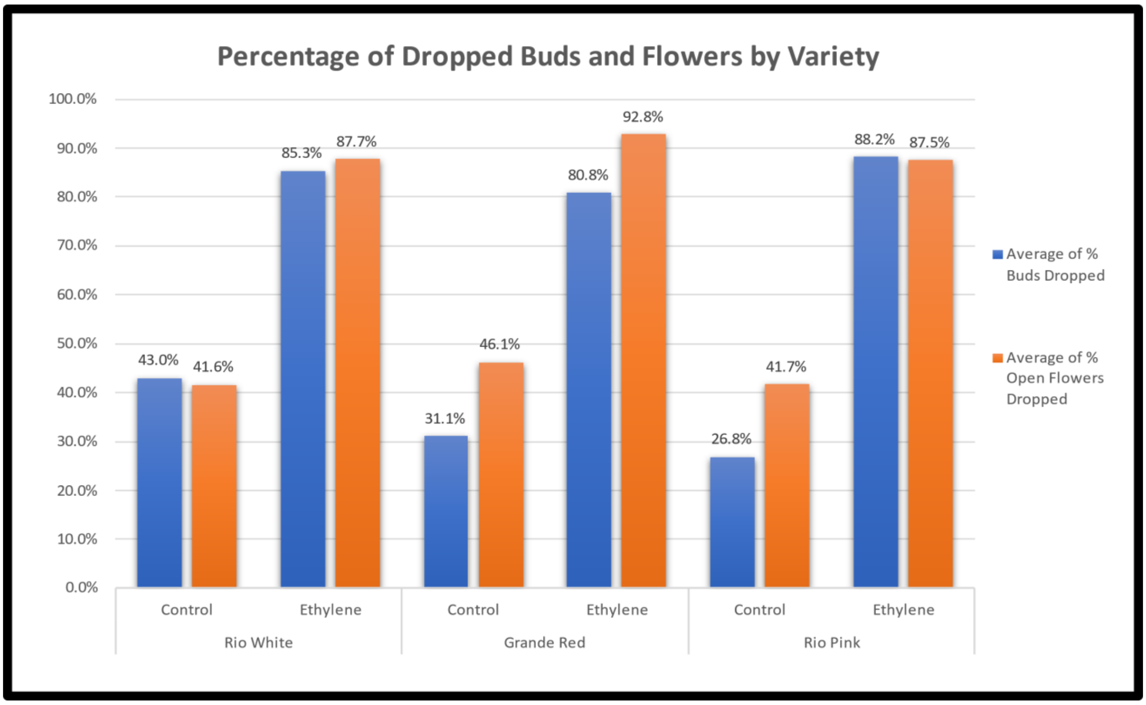

Fig. 2. In 'Rio White', ethylene exposure resulted in a 98.37% increase in bud drop and 110.82% increase in flower drop compared with control group. In 'Grande Red, there was a 159.81% increase in bud drop and 101.30% increase in flower drop. In 'Rio Pink', there was a 229% increase in bud drop and a 109.83% increase in flower drop compared with control group.

Table 1. The total number of open flowers and buds for each cultivar before and after ethylene treatment.

	Total Number of Open Flowers Before Treatment	**Total Number of Open Flowers After Treatment**	**Total Number of Open Flowers Dropped**	**Total Number of Buds Before Treatment**	**Total Number of Buds After Treatment**	**Total Number of Buds Dropped**
'Grande Red'	53	12	41	103	46	57
Control	18	9	9	49	33	16
Ethylene	35	3	32	54	13	41
'Rio Pink'	15	4	11	33	11	22
Control	5	3	2	11	8	3
Ethylene	10	1	9	22	3	19
'Rio White'	95	27	68	140	55	85
Control	29	19	10	42	30	12
Ethylene	66	8	58	98	25	73

Fig. 3. Veinal Chlorosis of 'Rio White' leaves.

Yellowing of leaves beginning as veinal chlorosis was observed in all ethylene-exposed plants (Fig. 3 & 4). Leaf yellowing did not appear to be related to the age of the tissue, as leaves from all areas of the canopy were affected. This is contradictory to the findings of a 2005 research study by Jing et al., which found that chlorophyll degradation in *Arabidopsis thaliana* was strongly correlated with leaf age. Ethylene-related chlorophyll degradation is a result of organized cell dismantling and the recycling of nutrients from senescent leaves to other organs. Ethylene has been shown to induce or accelerate the biochemical processes involved in leaf senescence, including nucleic acid reduction, protein degradation, turnover degradation, membrane disruption, lipid degradation, peroxidation, and leaf pigment breakdown. (Iqbal et al. 2017). In floral species such as *Matthiola incana* and *Dendranthema grandiflora*, ethylene-induced leaf senescence manifests as thickening and malformation of leaves, poor growth, and leaf epinasty (Reyes-Arribas et al. 2001).

Leaf abscission is a coordinated process involving structural changes in the cells near the abscission zone and occurs during normal development as well as in response to environmental signals (Taylor and Whitelaw 2001). Abscission can be induced by physical and environmental factors, such as photoperiod, water stress, mechanical wounding. Ethylene is believed to be involved in the transmission of signals related to wounding and pathogen attack, as endogenous ethylene levels increase in response to injury as a result of increased synthesis of ACC (O'Donnell et al. 1996). In this experiment, the number of abscised leaves was comparable for all cultivars and was assessed only qualitatively.

Fig. 4. Yellowing of leaves in ethylene-treated plants irrespective of age and position in 'Rio White'.

B. *Experiment 2*

EthylBloc™ significantly reduced the number of abscised leaves, flowers, and buds in all three cultivars (Fig. 5 & 6). The effect was most pronounced in 'Calypso White', which also showed the greatest extent of defoliation as a result of ethylene exposure.

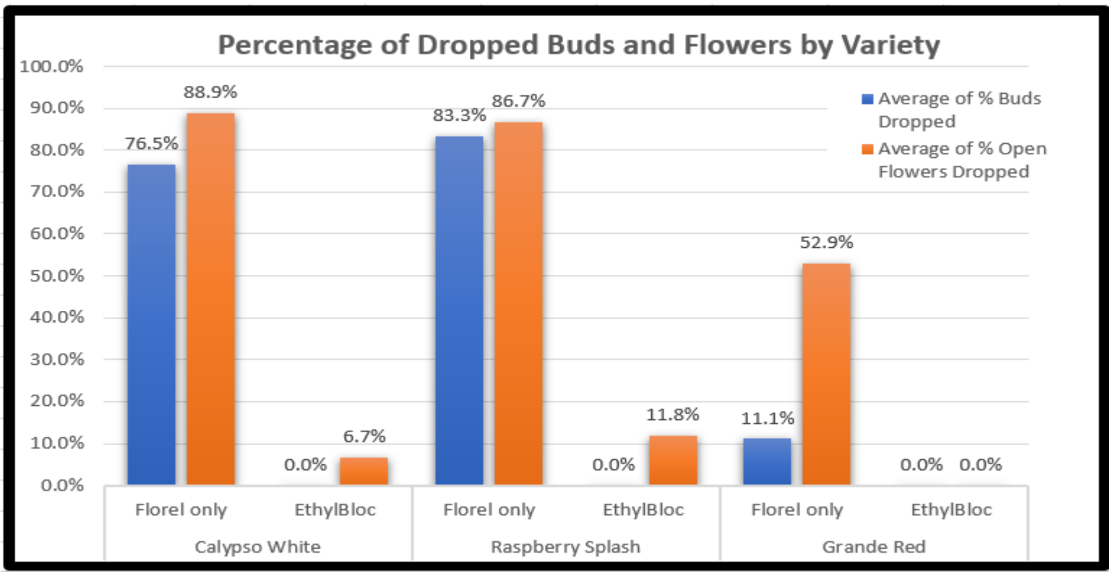

Fig. 5. In 'Calypso White', EthylBloc™ reduced bud and flower abscission by 100% and 92.46%, respectively. In 'Raspberry Splash', bud and flower abscission were reduced by 100% and 86.39%.

Interestingly, the 'Grande Red' plants used in this experiment were far more tolerant to ethylene than those in Experiment 1, despite treatment conditions being the same. Experiment 1 was conducted during the hot summer months and Experiment 2 was done in winter; it is possible that the plants used in Experiment 1 were already experiencing stress due to the high temperatures, and either produced ethylene in larger quantities or experienced a heightened response to the levels present. This reinforces the point that hormone signaling in plants is the result of complex and dynamic interactions of numerous physical, chemical, biological, and environmental factors.

1-MCP is believed to influence a number of plant physiological processes, such as respiration, biosynthesis of ethylene and other volatiles, chlorophyll degradation, protein and membrane regulation, and cell metabolism (Blankenship and Dole 2003). In a 2010 study by Kawakami et al., treatment of water-stressed plants with 1-MCP resulted in an increase in both stomatal resistance and the activity of antioxidant enzymes, as well as a less negative water potential and better maintenance of membrane activity, though transpiration and water-use efficiency were not significantly enhanced. 1-MCP has also been shown to mitigate the effects of salt (osmotic) stress in rice (Hussein et al. 2019).

The 1-MCP molecule is structurally similar to ethylene and is believed to occupy the ethylene receptors of plants, thus preventing ethylene from binding to receptor sites. The affinity of 1-MCP for the receptor is approximately 10x greater than that of ethylene, and 1-MCP is active at much lower concentrations (Sisler and Serek 1997). When 1-MCP is combined with a specific amount of water or another activating solution, it is released into the air as a volatile gas. Plants must be exposed to 1-MCP in an enclosed space for several hours for the treatment to be effective. A treatment duration of 12-24 hours is generally sufficient, though lower concentrations applied over a longer period of time may be as effective as higher concentrations (Blankenship and Dole 2003).

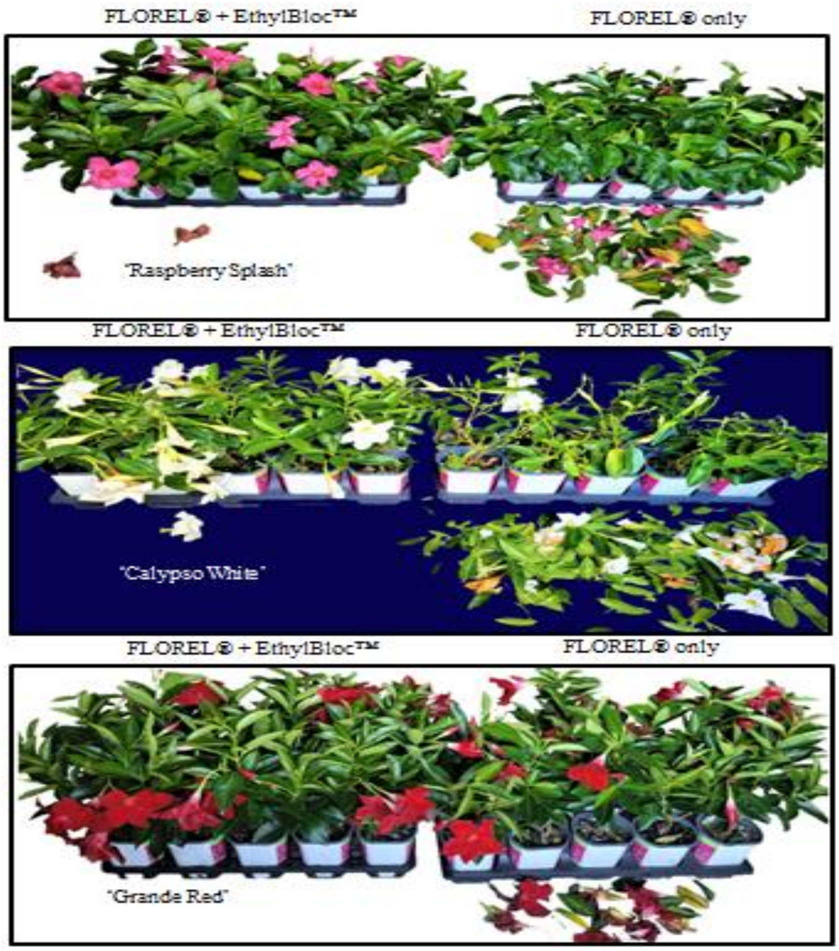

Fig. 6. The effect of FLOREL® and EthylBloc™ individually and in combination on 'Calypso White', 'Raspberry Splash', and 'Grande Red'.

IV. Conclusion

All cultivars tested in this experiment showed a significant increase in the number of abscised leaves, buds, and flowers as a result of exposure to ethylene. Symptoms of ethylene stress were drastically reduced or eliminated by the application 1-methylcyclopropene (EthylBloc™). However, the decision to use EthylBloc™ during plant transport and shipment should be evaluated from a practical and economic standpoint; the cost of using EthylBloc™ should reasonably not exceed the financial impact of ethylene damage. Also, it is important to note that this experiment was intended to maximize ethylene synthesis, not to replicate conditions experienced by plants during transport. Cultural practices that minimize ethylene production, such as reduced temperatures and avoidance of soil oversaturation prior to shipping, may outweigh the potential benefits of using EthylBloc™.

References

[1] Abeles, F.B., Morgan, P.W., and Saltveit, M. E. Jr. (1992). Ethylene in Plant Biology, 2nd Edn. San Diego, CA: Academic Press.
[2] Aharoni, N., and Lieberman, M. (1979). Ethylene as a regulator of senescence in tobacco leaf discs. Plant Physiol. 64, 801–804. doi: 10.1104/pp.64.5.801
[3] Alexander, L., and Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J. Exp. Bot. 53, 2039–2055. doi: 10.1093/jxb/erf072

[4] Barry, C.S., and Giovannoni, J.J. (2007). Ethylene and fruit ripening. J. Plant Growth Regul. 26, 143. doi: 10.1007/s00344-007-9002-y
[5] Beyer, E. M. (1976). A potent inhibitor of ethylene action in plants. Plant Physiol. 58, 268–271. doi: 10.1104/pp.58.3.268
[6] Blankenship, S.M. and Dole, J.M. (2003). 1-methylcyclopropene: A Review. Postharvest Biology and Technology, 28(1), pp. 1–25.
[7] Chang C. 2016, Q&A: How do plants respond to ethylene and what is its importance. *BMC Biol* 14: 7.
[8] Hansen, H., and Grossmann, K. (2000). Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiol. 124, 1437–1448. doi: 10.1104/pp.124.3.1437
[9] Hussain, S., Bai, Z., Huang, J., Cao, X., Zhu, L., Zhu, C., Khaskheli, M.A., Zhong, C., Jin, Q., & Zhang, J. (2019). 1-Methylcyclopro--pene Modulates Physiological, Biochemical, and antioxidant responses of rice to different salt stress levels. Frontiers in plant science, 10. https://doi.org/10.3389/fpls.2019.00124
[10] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 60. Lyon (FR): International Agency for Research on Cancer; 1994.
[11] Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR. 2017, Ethylene role in plant growth, Development and Senescence: Interaction with Other Phytohormones. *Front Plant Sci*. 8:475.
[12] Jing, H. C., Schippers, J. H., Hille, J., and Dijkwel, P. P. (2005). Ethylene-induced leaf senescence depends on age-related changes and OLD genes in Arabidopsis. J. Exp. Bot. 56, 2915–2923. doi: 10.1093/jxb/eri287
[13] Kawakami, E.M., Oosterhuis, D.M. & Snider, J.L. Physiological effects of 1-Methylcyclopropene on well-watered and water-stressed cotton plants. J Plant Growth Regul 29, 280–288 (2010).
[14] Kevany, B. M., Tieman, D. M., Taylor, M. G., Cin, V. D., and Klee, H. J. (2007). Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J. 51, 458–467. doi: 10.1111/j.1365-313X.2007.03170.x
[15] Khan, N. A. (2005). The influence of exogenous ethylene on growth and photosynthesis of mustard (Brassica juncea) following defoliation. Sci. Hortic. 105, 499–505. doi: 10.1016/j.scienta.2005.02.004
[16] Khan, N.A., Mir, M.R., Nazar, R., and Singh, S. (2008). The application of ethephon (an ethylene releaser) increases growth, photosynthesis and nitrogen accumulation in mustard (Brassica juncea L.) under high nitrogen levels. Plant Biol. 10, 534–538. doi: 10.1111/j.1438-8677.2008.00054.x
[17] Leon-Reyes A, Spoel S.H, De Lange E. S, Abe H, Kobayashi M, Tsuda S, Millenaar F.F, Welschen R.A, Ritsema T, & Pieterse C.M. 2009, Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. *Plant Physiol.* 149(4):1797-809.
[18] Lin, Z., Zhong, S., and Grierson, D. (2009). Recent advances in ethylene research. J. Exp. Bot. 60, 3311–3336. doi: 10.1093/jxb/erp204
[19] Macnish, A.J., Leonard, R.T., & Nell, T.A. 2011, Sensitivity of potted foliage plant genotypes to Ethylene and 1-Methylcyclopropene, *Hort Science horts*, 46(8), 1127-1131.
[20] Métraux, J. P., and Kende, H. (1983). The role of ethylene in the growth response of submerged deep water rice. Plant Physiol. 72, 441–446. doi: 10.1104/pp.72.2.441
[21] Naing A. H, Win N.M, Kyu S. Y, Kang I.K, & Kim C, K. 2022, Current progress in application of 1-Methylcyclopropene to improve postharvest quality of cut flowers. *Horticultural Plant Journal*, 8(6):676-688.
[22] O'Donnell PJ, Calvert CM, Atzorn R, Wasternack C, Leyser HMO, Bowles DJ. 1996. Ethylene as a signal mediating the wound response of tomato plants. Science 274: 1914– 1917.
[23] Ravanbakhsh, M, Sasidharan, R, Voesenek, L.A.C.J, Kowalchuk G, A, & Jousset, A. 2018, Microbial modulation of plant ethylene signaling ecological and evolutionary consequences. *Microbiome.* 6:52.
[24] Reid, M.S. (1995). “Ethylene in plant growth, development, and senescence,” in Plant Hormones, ed. P. J. Davis (Dordrecht: Springer). doi: 10.1007/978-94-011-0473-9_23.
[25] Riov, J., and Goren, R. (1979). Effect of ethylene on auxin transport and metabolism in midrib sections in relation to leaf abscission of woody plants. Plant Cell Environ. 2, 83–89. doi: 10.1111/j.1365-3040.1979.tb00778.x
[26] Reyes-Arribas, T., Barrett, J.E., Huber, D.J., Nell, T.A., and Clark, D.G. (2001). Leaf senescence in a non-yellowing cultivar of chrysanthemum (Dendranthema grandiflora). Physiol. Plant. 111, 540–544. doi: 10.1034/j.1399-3054.2001.1110415.x
[27] Sisler, E.C. and Serek, M. (1997) Inhibitors of Ethylene Responses in Plants at the Receptor Level: Recent Developments. Physiologia Plantarum, 100, 577-582.
[28] Schaller G.E & Binder B.M. 2017, Inhibitors of Ethylene Biosynthesis and Signaling. *Methods Mol Biol*. 1573:223-235.
[29] Taylor, J. E., and Whitelaw, C. A. (2001). Signals in abscission. New Phytol. 151, 323–340. doi: 10.1046/j.0028-646x.2001.00194.
[30] Wang, K. L., Li, H., and Ecker, J. R. (2002). Ethylene biosynthesis and signaling networks. Plant Cell 14, 131–151.
[31] Weingart, H., Völksch, B., & Ullrich, M. S. 1999, Comparison of ethylene production by *Pseudomonas syringae* and *Ralstonia solanacearum*. *Phytopathology.* 89:360-365.

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Brianna Humphreys, is a dedicated horticulturist with a diverse professional background in research, technical writing, and integrated pest management. She received a Bachelor of Science degree in Biology in 2014 from the College of Charleston, where much of her coursework focused on plant physiology, ecology, and taxonomy. She assisted with a research project intended to provide insight into gene function in the model organism *Arabidopsis thaliana* by quantifying performance-related traits in a standardized growth environment to reveal knockout phenotypes and support the development of a Gene Ontology knowledgebase. She is currently working as the IPM Manager at Fernlea Nurseries in Florida, where she designs and implements research trials to improve crop resistance to biotic and abiotic stressors, maximize nutrient use efficiency, and optimize plant physiological processes that favor crop yield and vigor.

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