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A STUDY OF SIMULATED ENVIRONMENTAL SHIPPING CONDITIONS
ON SENESCENCE AND TRANSPLANT RECOVERY OF SEEDLING GERANIUM
(PELARGONIUM X HORTORUM BAILEY)

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Marjorie Susan Dean

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William H. Carlson
Major professor

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A STUDY OF SIMULATED ENVIRONMENTAL SHIPPING CONDITIONS ON SENESCENCE AND TRANSPLANT RECOVERY OF SEEDLING GERANIUM (*PELARGONIUM* X *HORTORUM* BAILEY)

By

Marjorie Susan Dean

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ABSTRACT

A STUDY OF SIMULATED ENVIRONMENTAL SHIPPING CONDITIONS OF SENESCENCE AND TRANSPLANT RECOVERY OF SEEDLING GERANIUM (PELARGONIUM X HORTORUM BAILEY)

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The effect of ethylene, benzyladenine, and environmental factors were investigated in simulated shipping conditions for up to 96 hours for seedlings of Pelargonium x hortorum Bailey (cv. Sprinter Scarlet). Leaf yellowing decreased when plants were shipped at cool temperatures (e.g. 2 to 10°C) except for plants at 10°C in waxed cardboard boxes. Increase in duration of darkness caused an increase in leaf mortality and the number of days to flower after potting, while decreasing the ability of plants to recover after planting. A dose of ethylene 0.1 µl/liter for 48 hours increased leaf yellowing, while higher concentrations (1-10 µl/liter) caused leaf yellowing when applied for 12 to 48 hours. Delay in flowering was caused by as little as 0.1 µl/liter of ethylene and 1 and 10 µl/liter produced the

same results. Leaf abscission increased after 96 hours of treatment with 1 or 10 µl/liter. CO_2 at 5 percent v/v in air did not inhibit the ethylene effects noted. Ethylene synthesis decreased after 24 hours of darkness to a steady state in drought stressed plants, while normally watered or heavily watered plants decreased to a low steady state of ethylene production at 24 hours. Leaf yellowing was not affected by watering regimes. Drought stressed plants showed greater leaf mortality and resulted in shorter plants.

BA (10^{-4}M) applied as a foliar spray when applied to geraniums decreased yellowing of plants held in the dark and reduced plant height, but 48 percent of the leaves on these plants were damaged by BA.

DEDICATION

To my parents and grandparents

who gave me my agricultural heritage and appreciation.

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INTRODUCTION

Pelargonium x hortorum Bailey, Geranium, is one of the largest growing floricultural crops in the U.S. (Voigt, 1971). The introduction of cv. Nittany Lion Red (a seedling geranium) started a breeding selection program for earliness of flowering and reduced plant height. This improved the cultural practices and production efficiency for this crop which was traditionally propagated by cuttings (Craig, 1971).

As seed lines were introduced and seed production increased, a market developed for seedlings to be "finished off" by growers as a potted crop. Along with this came problems in shipping the seedlings to the growers. Plants (80 days old) shipped to producers have shown severe leaf yellowing and stunting (Carlson, 1977). The present study investigates the effects of environmental factors such as duration of darkness, temperature as a pretreatment and in simulated transit, and handling of plants on leaf yellowing, leaf death, replant survival, and days to flower. Ethylene was studied as a deteriorative factor in the environment during shipping contributing to problems after potting. Benzladenine (BA)

was investigated as a supplement to endogenous cytokinins to attenuate development of senescence.

LITERATURE REVIEW

Physiology of Senescence

Classification

Senescence is the natural deterioration of a partial or whole unit of life which leads to death (Leopold, 1975). The external expressions of physiological signals of senescence can be classified into four basic categories. First is the "overall" death of annual plants, bienneal and other monocarpic. Second, "top senescence" is the death of the above ground parts of biennial plant. Third, is "deciduous senescence" in which leaves of trees and shrubs abscise annually but the main stems and roots remain viable. Finally, there is "progressive senescence" which is the gradual progression of leaf death from the base to the top of the plant and is the topic of main consideration in this thesis.

Physiological Changes

The process of leaf senescence appears to be the initiation of the collapse of individual cells which develop into a large region of leaf yellowing. Examination of the ultrastructure reveals that various organelles break down at different rates (Woolhouse, 1967). Membrane degradation, causing an increase in cell

permeability has been shown in senescing tissue (Sacher, 1957; Eilam, 1965). Shaw and Manocha (1965) examined senescing wheat leaves by electron microscopy and observed that after two days of detachment from the plant, the endoplasmic reticulum of the mesophyll cell began to change and disappeared after four to five days along with the cytoplasmic ribosomes. Chloroplast grana disappeared, mitochondria became swollen with loss of fine structure and nuclear damage was evident after three days. By the eighth day the plasma membrane showed damage and the mitochondria had disappeared along with most of the nuclei and chloroplasts, resulting in yellowing (Shaw and Manocha, 1965).

Metabolic Changes

Coinciding with these structural changes are changes in metabolic activities. Lewington, et al. (1967) illustrated a decrease in protein as well as chlorophyll as attached cucumber cotyledons senesced. The reduction of protein is considered a part of senescence as is reduction of chlorophyll (Leopold, 1975).

Two processes may explain the decline in protein content: an interruption of protein synthesis or an increase in the degradation of protein (Wareing and Phillips, 1973). Parups (1971) has shown from electrophoresis of proteins that with aging there is a decrease

in proteins of leaves of Rosa hybrida L. cv. Forever Yours, x Anthirrhinum majus L. cv. Thite Leton, Dianthus caryophyllus L. cv. Sim, and Chrysanthemum morifolium Ram cv. Fred Shoesmith. Also, with aging, proteins seemed to become more heterogeneous.

The reduction of chlorophyll and protein may be structurally associated. Woolhouse (1967) found chloroplast fraction I declined most rapidly and this protein makes up almost 50 percent of the leaf protein. Chloroplasts rather than the cytoplasma of Oenothera (evening primrose) were found to be a major source of proteins (Zucker and Stenson, 1962).

Leaf senescence is also characterized by the decline in levels of RNA. Osborne (1962) found this decrease in Xanthium pennsylvanicum Wall. She concluded that materials which retard senescence maintain synthesis of RNA. These materials caused an increase in incorporation of ^{14}C-Gorotic acid to RNA in intact leaves. Similar results were found by Fletcher (1969) in attached leaves of Phaseolus vulgaris L. (red kidney bean). However, the senescence retarding materials plus actinomycin-D, a potent inhibitor of RNA synthesis on excised broccoli (Brassica oleracea L. cv. italica, cv. Coastal) leaves, stabilized the chlorophyll and protein content while RNA level was reduced. Therefore, continued RNA synthesis does not seem to be obligatory in regard to maintaining

protein and chlorophyll in this role of reduction of senescence (Von Abrams and Pratt, 1968).

In peas (Pisum sativum L., cv. Alaska), a decrease in tRNA $^{Leu}_{1,2,5\&6}$ was found (Wright, et al., 1972) with yellowing of leaves after flowering. Chloroplast preparations contained a smaller ratio of tRNA $^{Leu}_{1\&2}$ to tRNA $^{Leu}_{5}$ than an intact leaf indicating general tRNA in the leaf may be due to loss in the chloroplast.

Photosynthesis and respiration decline as part of the general degradation process. Photosynthesis has been shown to reach a maximum in pea leaves (Pisum sativum L. cv. Laxton's Progress) before the leaf reaches full size and then a slow fall was detected (Smillie, 1962). In Nicotiana, the decline in photosynthesis was more pronounced in the younger leaves than in the older basal leaves (Sestak and Catsky, 1962). Smillie (1962) found respiration of pea leaves declined continuously as the leaf matured. However, in other species such as strawberries a temporary rise in respiration was found which is similar to the climatric found in fruits (Arney, 1947; Yemm, 1956).

Environmental Regulation

Environmental factors such as temperature, nutrients, darkness, water stress, and heat stress have been shown to effect the rate of leaf senescence.

Temperature

Mothes and Engelbrecht (1963) found detached tobacco leaves senesced faster when exposed to a high temperature stress of 49°C for two minutes. Areas which were exposed to high temperatures yellowed while green areas acted as sinks for metabolites from the yellowed regions. A general deterioration of proteins in association with yellowing has also been demonstrated (Mothes and Baudisch, 1958). Kaltaler (1962) found with Pelargonuim x hortorum Bailey grown from cuttings, plants maintained at low temperatures (40-42°F) in darkness yellowed or senesced slower. This may be due to a general lowering of metabolism at the low temperature. Chilling may also hasten senescence as evidence by the acceleration of geranium leaf senescence (Yarwood, 1977).

Nutrients

In Algerian oats (Avena sativa, Linn.) plants receiving low phosphorus (P) senesced more slowly than plants with adequate P. Supplemental nitrogen delayed senescence. High phosphorus levels were later

found to prevent senescence by delaying the net export of nitrogen from the foliage (Williams, 1955).

Darkness

Vickery, et al. (1937) exposed excised tobacco leaves to continuous dark or light and found that darkness speeds senescence. Organic solids were decomposed into volatile products. For 72 hours, protein was broken down at the same rate in light as in the dark. Subsequently, the dark cultured leaves decreased in proteins at a faster rate than those in the light with an increase of water soluble nitrogen including an increase in ammonia.

Excised Bryophyllum calycinum leaves exposed to prolonged periods of light increased in starch while malic and citric acid decreased (Vickery, 1956). The reverse was observed in the dark. When transferred from dark to light the starch content increased and the organic acids decreased. However, leaves exposed to light for long periods and returned to the dark could no longer convert acids to starch efficiently. This was observed by comparing the carbon ratio of starch to organic acids after each transfer.

Frank and Kenny (1955), using one-week old corn (Zea mays L.) seedlings, followed the destruction of chlorophyll and carotenoids. After 120 hours at 27^{o}C in

the dark all chlorophyll disappeared while only 40% of the carotenoids were destroyed (72% and 30%). The destruction of chlorophyll and carotenoids was decreased respectively, by adding sucrose during 48 hours of dark treatment following 12 hours of light.

Phaseolus vulgaris, cv. Red Kidney, leaf discs lose protein and chlorophyll in the dark faster than discs remaining in light at 12, 16, and 36 days old. Rate of loss of both constituents increased as the plants grew older (Goldthwaite and Leatch, 1967).

In an effort to determine if chloroplast formation, degradation, or a combination of both occur in Triticum vulgare, cv. Lemhi leaf tips in darkness; sections were treated with aminotriazole, an inhibitor of chloroplast formation. Light retarded the loss of chlorophyll, maintained photosynthesis, and slowed the breakdown of the chloroplast ultrastructure. When a photosynthetic inhibitor, 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea was added, it did not affect the above measures. Also, when sugar was added to nonphotosynthesizing tissues, chloroplast destruction was not slowed. From this, it was concluded that chloroplast destruction must be delayed by light (Haber, et al., 1969).

Water Stress

Morton and Watson (1948) found in sugar beets (Hilleshög) that leaf senescence was not significantly affected by continuous water regimes except when high and low regimes were alternated. Senescence was hastened when high water regime plants were subjected to a dry regime. Plants under a low water situation when transferred to high water conditions had less leaf death than any other treatment.

A decrease in protein was reported by Petrie and Arthur (1943) when tobacco (Nicotiana tobacum L. Hickory Prior) plants were subjected to drought. If plants were watered, the protein assimilation rate returned to a rate similar to plants maintained continuously on a high water regime. In plants under dry a condition, the maximum protein content of the leaves was reached later than for leaves from fully watered leaves. This resulted in a slower growth rate and a delay in whole plant senescence.

Gates (1955) found a similar delay of whole plant senescence in tomatoes (Lycopersicon esculentum, Mill.). Dry weight of lamina decreased when plants wilted from exposure to drought and returned to normal growth rate when watered. The conclusion was drawn that the plants stressed were physiologically younger upon

recovery, but no data were presented to support this statement.

Shah and Loomis (1965) reported an increase in soluble RNA and a decrease in other RNA fractions in detached sugar beet leaves (Beta vulgaris L., cv. MS NBIXNB4 hybrid). Protein decreased under dry conditions.

Hormonal Regulation

The internal regulation of senescence has been investigated by applying natural and synthetic plant hormones: cytokinins, auxins, gibberellic acids, and ethylene. Senescence has been delayed or influenced by the various regulators.

Cytokinins

Richmond and Lang (1957) were first to report a delay in chlorophyll and protein loss in detached Xanthium pennsylvanicum (cocklebur) leaves treated with cytokinins. Primary leaves were removed and the petioles were placed in solutions of water containing 1 or 5 mg of kinetin per liter at 22° to 25°C. After 12 days, the explants in water had lost 60% of the initial protein. While those in 1 mg/l kinetin solution lost 50% and only 15% of treated with 5 mg/l.

Similar results have been found in several species. In two species of tobacco plants (Nicotiana tabacum var. Bright Yellow and N. rustica), detached leaf

discs were floated in a solution of 10^{-5}M of kinetin in dark or light for two or three days at 30°C. In N. tabacum the decrease in protein was suppressed fully in nuclear, mitochondrial, microsomal, and final supernatant fractions. Kinetin caused nucleic acid accumulation in the microsomal and supernatant fractions. N. rustica showed no response to kinetin unless light or sugar were present when protein and nucleic acid rose compared to discs not treated (Suguira, et al., 1962).

In Red Kidney beans, leaf discs from plants 12 to 15 days old were floated on solutions or water in the dark for eight days at 30°C. Kinetin and 6-benzylaminopurin (BAP) reduced protein and chlorophyll loss. Aging of leaf discs did not increase this effect (Leopold and Kawase, 1964).

Three cytokinins have been shown to reduce chlorophyll loss in Maianthemum canadence leaf discs at 25°C for three days of darkness. Kinetin and zeatin were used at 2.5 mg/l and benzyladenine (BA) was used at 5 mg/l. When using BA the loss of chlorophyll was greater in leaves sampled in September versus July samples (Horton, 1977).

Similar effects have been shown in intact seven-day old wheat seedlings (Triticum aiestivum L.) exposed to darkness by Wittinbach (1977). BA at 1 µ/l to 100 µl/l delayed chlorophyll degradation progressively

as a function of BA concentration. Zeatin (0.1 mM) stimulated partial recovery of protein and chlorophyll content during 12 days in the dark. Recovery of photosynthesis capacity was shown to follow a similar pattern.

Wright, et al. (1973) has shown t-RNA is higher in intact young peas (Pisum sativum L. cv. Alaska) and soybeans (Glycine max L., cv. Wayne) than at later stages of natural senescence when sprayed with 10^{-4} zeatin. Also, peas sprayed with zeatin remained green longer than controls.

The role of cytokinins in reducing the loss of RNA and protein is uncertain as to whether it works via promoting synthesis or decreasing degradation. The investigation of incorporation of radioactive percursors of protein and RNA during senescence has been investigated in several plants to determine if stimulation of snythesis is the mechanism. Osborne (1962) floated Xanthium pennsylvanicum leaf discs on solutions of kinetin (40 mg/l) with ^{14}C-l-leucine (t x 10^{-4}M, 10^6 counts/ml) or ^{14}C-orotic acid (2.5 x 10^{-4}M, 10^6 counts/ml) at 24^{o}C in darkness. Also, whole leaves were treated one-half with kinetin and the other with water. Leaf discs were then cut from the two halves and incubated for 1, 2, or 4 hours in either labeled leucine or orotic acid. In both cases, protein and RNA content was greater

in kinetin treated leaves. Also, incorporation of radioactive material was higher in the kinetin disc.

Maaß and Klämbt (1977) have shown cytokinin to have a direct effect on protein synthesis independent of an increase in RNA. ^{14}C-amino acids were applied to tobacco (Nicotiana tabacum L. cv. Wisconsin 38) pith tissue in the presence of actinomycin D (100 µg/ml) to block RNA synthesis. Kinetin (0.4 mg/l) in four hours of darkness at 27°C caused an average increase of 35 percent in protein content over the controls.

In intact bean plants, Pozsar, et al. (1967) have shown an increase in incorporation of labeled glycine into protein by painting kinetin (50 ppm) and BA (30 ppm) onto half of leaves. BA was more effective. Leaf discs from treated leaves were incubated in ^{14}C-glycine for four hours.

Similar results were reported by Fletcher (1969) with RNA and protein on intact red kidney bean (Phaseolus vulgaris L.) plants. Higher levels of RNA, protein and chlorophyll were found in BA (30 mg/l) painted leaves. Primary leaves were painted weekly with kinetin, water or urea until plants had set fruit. BA treatments not only caused higher RNA content but also showed a high level of RNAase activity. The effect of kinetin was observed at two weeks with its optimum effect at six weeks. Urea served as a nitrogen source and was used to show BA was not just supplying N to the senescing plant. No

difference was found between the urea and control. Throughout the life of the plant a higher rate of incorporation of radioactive leucine and arotic acid into protein and RNA was observed in BA treated leaves. However, when plants flowered incorporation of ^{14}C-leucine was found in protein.

Martin and Thimann (1971) theorized that protein degradation is the active role of cytokinins in delaying senescence. Detached oat leaves were placed in darkness and treated with l-serine, kinetin, and cycloheximide (CYC) alone and in combinations to test the theory that proteases were functional in senescence. If this theory were true, then kinetin should prevent protease increase because it delays senescence. Also, serine which was shown to enhance senescence with or without kinetin should increase protease activity. Finally, CYC, a protein inhibitor which was shown to delay senescence and prevent serine enhancement, should prevent protease increases. Two proteolytic enzymes which were isolated did react to the various treatments as expected. Activity of the proteases were decreased by kinetin and CYC and increased by serine.

Further support for the theory of protein degradation was shown by Kuraisbi (1968). Leaf disc of Brassica rapa L. were floated on a solution of ^{14}C-leucine at 25^{o}C in the dark to label existing proteins. After 24 hours,

the discs were transferred to solutions of kinetin (5×10^{-5}M) or water. Kinetin treated tissue retained more radioactivity in the protein fraction than the control tissue after 24 and 48 hours.

The above experiment does not rule out the possibility of cytokinins stimulating bulk protein synthesis (Travares and Kende, 1970). To investigate this possibility, Travares and Kende prelabeled corn leaf disc protein and measured the rate of degradation and the specific activity of the protein. After incubation four to five hours in ^{14}C-leucine (1.5×10^{-6}M), discs were placed in solutions of ^{12}C-leucine (10^{-4}M) with or without BA for 48 hours. Protein breakdown, both labeled and bulk, was decreased by BA. If protein was actively being synthesized during this 48 hours, it would be unlabeled and the specific activity of the total protein would have been lower. However, this was not the case and the authors concluded that the primary action of cytokinin in retarding senescence is mainly through the inhibition of protein degradation.

The mobilization of metabolites to areas treated with kinetin was reported by Mothes, et al. (1959). Gunning and Barkley (1963) reported similar results with detached oat leaves. Labeled carbon and phosphorus (P^{32}) accumulated where kinetin was applied. A polarity was established as acropital. Müller and

Leopold (1966) found similar results in detached corn leaves. Transport of P^{32} was axial and blockage was caused by metabolic inhibitors and steam-killed zones. Kinetin attracted P^{32} and Na^{22} and not Rb^{86}, Cl^{36} or I^{131}. P^{32} was the only material which accumulated. This transport was found to take place in the phloem. BA (4.4 x $\bar{1}0M)^3$ was shown to attract ^{14}C following assimilation of $^{14}CO_2$ by leaves of intact Vitis vinifera L. Older leaves responded very little to BA treatment unless they were placed in darkness (Quinlan and Weaver, 1969). However, Seth and Wareing (1967) found when kinetin was placed on defruited peduncles there was no accumulation of P^{32}. Also, Osborne (1962) has shown in Zanthium leaves with discs treated with kinetin and connected by a strip to another disc treated with water or kinetin there was no difference in senescence when compared to isolated discs treated the same. The delay in senescence was due to the kinetin and not metabolite movement. Therefore it can be concluded that mobilization of metabolites by kinetin cannot explain its retardation of senescence.

Protein decline as a result of water stress as discussed earlier may also be kinetin related. Root factors involved in growth and metabolic activity of shoots have been postulated for years (Chibnall, 1939, and Kulaeva, 1962). Later, kinetin-like factors were

isolated from root exudates of sunflowers (Kende, 1965) and was linked to the reduction of leaf senescence (Sitton, et al., 1967). Itai, et al. (1968) reported in sunflowers, bean, and tobacco when roots were water stressed that cytokinin translocation decreased from their roots. The decrease was found to be reversible.

Cytokinins present in the shoot may also be affected by water stress. Itai and Vaadia (1971) have shown in excised tobacco shoots a reduction of cytokinin activity similar to activity caused by water stress applied to roots. A chemical transformation was shown by use of ^{14}C-kinetin in wilting plants.

Gibberellic Acid, Auxin and Abscisic Acid

Brian, et al. (1959) reported that fall color of several tree species are affected by gibberellin (GA). The delay of senescence has been shown in leaf discs of several species (Beevers, 1966; Fletcher and Osborne, 1965; Whyte and Luckwill, 1966; Horton, 1977). However, cytokinins have been shown to be more effective (Parups, 1970).

Auxins reportedly reduce protein and chlorophyll loss (Parups, 1970; Richmond and Lang, 1957). However, in Xanthium or broccoli leaves α-naphthaleneacetic acid (NAA) did not influence senescence except by depressing the effects of kinetin applications. However, Maaβ and

Klämbot (1977) found in tobacco that auxin has no effect on protein synthesis.

Abscisic acid has also been reported to affect the degradation of chlorophyll very slightly (Wittenbach, 1977). Horton (1977) reported enhanced senescence by retarding the effects of cytokinins and GA.

Mobilization of assimilates has also been shown to be affected by GA and auxins. 3-Indolyl acetic acid (IAA) when applied to defruited peduncles of French beans enhanced the movement of ^{32}P. GA and kinetin when applied alone had no significant effect. However, the greatest accumulation of ^{32}P occurred when the three hormones were used together (Seth and Wareing, 1966). Gunning and Barkley (1963) found conflicting results in detached oat leaves where no movement of radioactive substrates was observed when GA and IAA were applied. In geraniums (Pelargonium zonale L.) Auxin, 2,4-D, NAA, BA, and GA^3 was reported to attract movement through the phloem of ^{32}P, ^{35}S, $^{86}Rb(K)$, but had no effect on ^{45}Ca or ^{36}Cl (Penot and Beraud, 1977). GA_3 was found to be the most effective hormone (Penot and Beraud, 1977).

Ethylene

The economic importance of ethylene as air pollutant affecting greenhouse crop production is a well recognized fact. Carroll and Jansma (1972) surveyed five

counties in southeastern Pennsylvania and found ethylene was responsible for 48.1 percent of a total crop loss of $17,564. Abeles (1971) reported ethylene levels as high as 0.387 µl/liter in atmosphere around Washington, D.C., and most plants are sensitive to ethylene at a level of 0.1 µl/liter.

Krone (1937) reported damage from natural gas (6.1 percent ethylene) on several species of plants. The symptoms of injury were yellowing, premature abscission, epinasty, increased growth of weakened side shoots. Similar results were found (Heck and Pires, 1962) when 89 species from 39 different families were fumigated with ethylene (2,5 and 100 µl/liter) for ten days. Symptoms were death, stunting, inhibition of leaf expansion, injury or death of floral parts, increased abscission, epinasty, chlorosis, and necrosis. Stunting may be the effect of ethylene inhibiting cell elongation. Ethylene inhibited shoot elongation of pea seedlings (Harvey, 1915) and wheat seedlings (Roberts, 1951). Flower inhibition has been shown in Xanthium pennsylvanicum Walln. (Abeles, 1967) when exposed to 10 and 100 ppm of ethylene. Lower concentrations of 1-4 µl/liter of ethylene caused failure of flowering in Chrysanthemum morifolium Ramat under short day conditions. Exposure to alternating concentration showed similar results (Tjia, et al., 1969).

Geraniums have been reported (Reed, 1975) to respond to Florel, an ethylene releasing plant growth regulator, by increased number of branches and flower bud abortion. Krone (1937) reported yellowing and slight leaf abscission when several species of plants were exposed to "Michigan gas" at 2 percent in air for 60 hours.

Chlorophyll degradation by ethylene in celery was reported a practical way for blanching celery (Harvey, 1925). Also, curing tobacco was hastened by ethylene (Pratt and Goeschl, 1969). However, Wittenbach (1977) has shown on intact wheat seedlings ethephon, an ethylene producing compound, has no effect on chlorophyll.

Conflicting reports on other parameters used to measure senescence, RNA and protein, are known. Abeles and Holm (1966) reported an increase in RNA and protein in bean explants in response to ethylene. In wheat seedlings ethylene stimulated protein synthesis in the embryo (Roberts, 1951). However, in pea seedlings ethylene was shown to reduce protein content (Harvey, 1915). Whittenbach (1977) reported not effect on protein content when wheat seedlings were exposed to ethylene. In Rhoeo discolor leaf sections, auxin stimulated protein and RNA synthesis, but ethylene inhibited this stimulation (Sacher and Salminen, 1969).

Ethylene has been shown to influence leaf yellowing by inducing leaf abscission (Abeles, 1971). In this case, leaves would senesce as in detached tissue. Ethylene has been shown to cause abscission of the fruit (McAfee and Morgan, 1972), and this action is widely used in commercial horticulture.

Most of the work in leaf abscission has been on explants since the work of Kendell (1918). Ethylene, auxin, and ABA have all been shown to play an important role in leaf abscission of explants. Auxin has been shown to inhibit abscission (LaRue, 1936). Naturally, the leaf blade has been shown to be inhibitory in petiole abscission, and this was explained as the effect of the auxin content of the blade (Myers, 1940).

Rubinstein and Leopold (1963) reported auxin to have two effects on bean explant abscission called stage I, auxin prevented abscission, and stage II, auxin promoted abscission. This is reflected by the aging of the leaf where younger leaves have a longer inhibition stage (I) than older leaves (Chaterjee and Leopold, 1964).

Ethylene has also been shown to be involved with auxin in this two-stage process. During stage I explants when exposed to ethylene have been shown to be relatively insensitive to the promotive effect of

ethylene on abscission (Abeles, et al., 1967; Jackson and Osborne, 1970). In stage II, ethylene accelerated abscission (Abeles and Rubinstein, 1964).

Jackson and Osborne (1970) found that bean explants produce a surge of ethylene as a response to wounding and then the rate subsides until just before abscission occurred. Also, ethylene applied within 24 hours of leaf blade removal had no effect on abscission, supporting the two-stage theory.

For ethylene to be a factor in seedling damage in transport, a source of ethylene production would have to be available for concentration to reach toxic levels in the shipping containers. Healthy plants produce ethylene at low rates and diseased or injured plants produce much more. The seedlings can also be "gassed" with ethylene as an air pollutant. The internal combustion engine produces large amounts of ethylene as well as carbon monoxide which mimics ethylene action (Abeles, 1971). Gasoline engines (1957 to 1966) produced an average of 211.3 µl/liter. A idling diesel-driven truck produced 64 µl/liter. This could be an important deleterious factor in loading areas if trucks are left running or fork lifts are in active use (Hasek, et al., 1969).

Plants in stress have been shown to produce ethylene. Virus infection in several species of plants caused an increase in ethylene production (Williamson,

1949; Ross and Williamson, 1951). Jackson and Osborne (1970) reported an increase in ethylene production from the cutting of leaf tissue of peas.

Water stressed cotton plants have also been shown to produce ethylene around the petiole. McMichael, et al. (1972) reported that excised or intact cotton petioles, when water deficient, produced more ethylene. Also 12th node petioles (excised) produced almost twice as much ethylene as those from the 5th node. Similar results were found by McAfee and Morgan (1971). The possible difference could be due to leaf age. Intact plants did not show this node difference. After the stressed plants were watered, ethylene production dropped to normal (McMichael, et al., 1972).

Lime plants enclosed in containers for four days of darkness in various ethylene concentrations (0.1 to 10 µl/liter) responded with increased abscission as ethylene increased (Cunningham and Staby, 1975). In every case, the ethylene concentration decreased slightly with time. Samples of air from unopened shipping cartons from Florida were lower than 0.01 µl/liter ethylene. However, leaves still abscised. Kays, et al. (1976) has shown pepper transplants (Capsicium annuum L.) produced more ethylene in darkness. Plants exposed to ethylene at levels greater than 0.1 µl/liter were slower to recover when planted in the field than plants receiving

less ethylene. Leaf abscission was greater as the ethylene concentration increased. Carbon dioxide showed a general trend of inhibiting abscission but was not significantly different from ambient ethylene maintained at low levels.

Atmospheric conditions also influence ethylene production. Oxygen is required for ethylene production (Burg, 1962; Burg and Burg, 1967). In cut flowers CO_2 (carbon dioxide) reversed all known effects of ethylene (Parsons, et al., 1967; Smith and Parker, 1966; Uota, 1969).

Low pressure storage (hypobaric, LPS) has been shown to reduce the effects of ethylene. A low 0_2 level is automatically obtained as the total air pressure is reduced and gases produced by the crop are flushed away by a flow of water saturated air in LPS (Burg, 1973), preventing desication.

LPS has been shown to extend the storage time of ornamental cuttings and plants with no effect on shelf life. Burg (1973) found potted chrysanthemums could be stored two to three weeks longer in LPS than cold storage with no damage. Easter lilies stored for six weeks with color showing responded as normal plants when removed.

Chrysanthemum cuttings have also been shown to have extended storage life in LPS (Burg, 1973; Eisenberg, et al., 1977). Rooted cuttings from conventional cold storage rooted readily on out planting but developed

slowly while cuttings from LPS storage rooted and developed normally when placed in propagation beds (Burg, 1973). Eisenberg, et al. (1977) reported cold storage of 15 genera declined more rapidly than LPS over time. A reduction of pathological invasion, defoliation, and yellowing was observed.

LPS of geranium cuttings was shown to be successful (Eisenberg, et al., 1977). Experiments were conducted on unrooted and rooted cuttings of geraniums, LPS storage in chambers maintained at 1/30 atm with 1 air exchange per hour at 95-98 percent relative humidity (2.2^{o}C), and cold storage in chambers at 2.2^{o}C and 95-98 percent relative humidity. LPS storage produced acceptable quality plants after 4 to 6 weeks while plants in cold storage developed damage after 2 weeks. Rooting response was maintained longer in LPS. Rooted cuttings were stored 2, 4 and 6 weeks in LPS. Those at four weeks showed yellowing when transplanted, and after six weeks developed chlorotic and necrotic areas when transplanted. Cold storage, however, showed complete yellowing at 4 to 6 weeks of storage. Therefore, the removal of ethylene during LPS and the reduced O_2 (necessary for ethylene synthesis) may be responsible for extending the storage life of geranium cuttings.

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SECTION ONE

THE EFFECT OF ETHYLENE AND ENVIRONMENTAL FACTORS ON *PELARGONIUM* X *HORTORUM* BAILEY

THE EFFECT OF ETHYLENE AND ENVIRONMENTAL FACTORS ON PELARGONIUM X HORTORUM BAILEY.

Abstract. The effect of ethylene, benzyladenine, and environmental factors were investigated in simulated shipping conditions for up to 96 hours for seedlings of Pelargonium x hortorum Bailey (cv. Sprinter Scarlet). Leaf yellowing decreased when plants were shipped at cool temperatures (e.g. 2 to 10°C) except for plants at 10°C in waxed cardboard boxes. Increase in duration of darkness caused an increase in leaf mortality and the number of days to flower after potting, while decreasing the ability of plants to recover after planting. A dose of ethylene 0.1 µl/liter for 48 hours increased leaf yellowing, while higher concentrations (1-10 µl/liter) caused leaf yellowing when applied for 12 to 48 hours. Delay in flowering was caused by as little as 0.1 µl/liter of ethylene and 1 and 10 µl/liter produced the same results. Leaf abscission increased after 96 hours of treatment with 1 or 10 µl/liter. CO_2 at 5 percent v/v in air did not inhibit the ethylene effects noted. Ethylene synthesis decreased after 24 hours of darkness to a steady state in drought stressed plants, while

normally watered or heavily watered plants decreased to a low steady state of ethylene production at 24 hours. Leaf yellowing was not affected by watering regimes. Drought stressed plants showed greater leaf mortality and resulted in shorter plants.

Transit of commercial floricultural seedlings is a relatively unexplored area. Marketing of seedling geraniums to commercial growers has raised some problems in leaf yellowing, plant mortality, leaf abscission and stunting of plants in transit or soon after transplanting.

High temperature induction of senescence is well known (Mothes, et al., 1961). Storage of rooted and unrooted cuttings of nursery stock and floricultural crops is improved with low temperature with little or no adverse effects on quality (Snyder and Hess, 1955; Pryor and Stewart, 1962; Flint and McGuire, 1962; Schneider, 1964). Kaltaler (1962) found less damage to geranium cuttings during simulated transit at low temperature than at high temperatures.

Continuous darkness speeds senescence in excised tobacco leaves (Vickery, et al., 1937). Seedlings exposed to darkness lose chlorophyll, protein, and RNA

more rapidly than lighted plants (Frank and Kenny, 1955; Goldthwaite and Leatch, 1961).

Drought stress hastens senescence. This also happens when plants are watered excessively and then allowed to dry out. Plants under low water situations when transferred to high water conditions showed less leaf death (Morton and Watson, 1948). Whole plant senescence is sometimes delayed rather than hastened by drought (Petri, et al., 1943; Gates, 1955).

Ethylene is another factor that may contribute to plant senescence during transit. Ethylene was related to leaf yellowing (Krone, 1937; Heck and Pires, 1962). The yellowing effect caused by ethylene could be related to its role in leaf abscission. Flowers have been shown to be delayed in opening (Abeles, 1967; Tjia, et al., 1969) or damaged by ethylene (Heck and Pires, 1962).

Ethylene concentration in transit could be affected by poor ventilation of containers and trucks, air pollution (Halevy and Kofranek, 1967), mechanical damage to plants (Jackson and Osborne, 1970), and natural plant production.

The natural production rate of ethylene has not been established for seed geraniums. Also, the sensitivity of the plants to various ethylene concentrations and durations is now known. Symptoms of ethylene

damage to the plant are unknown other than the obvious parameters of leaf yellowing and abscission.

This study was conducted to further define the effect of temperature, removal of plants from their flats for shipment, type of shipping container, darkness, and ethylene on geranium plants in simulated shipping. The synthesis of ethylene was investigated with respect to various water regimes and high temperature treatments. From this information it may be possible to set guidelines for manipulating the environment of the shipment to reduce plant senescence in transit.

Materials and Methods

Experiment 1 - Eighty day old seedling geraniums (cv. Sprinter Scarlet) were grown in Sun City, Florida. Expanded polystyrene trays consisting of seventy-two cubes per tray were used as a growing container. Each cube was 5 cm. deep, 3.7 cm. square at the top tappering to 0.94 cm. at the base, and 1.5 cm. apart. Plants were removed from the trays and placed in a waxed cardboard shipping container with four side vents (regular) or an expanded polystyrene (EPS) container with no ventilation. All yellow leaves were removed from the plants before the following treatments were applied:

1. Stored at 25°C - 35°C (no cooling).

2. Precooled to 10°C before the containers were closed and stored at 10°C.
3. Stored at 10°C.
4. Precooled to 2°C before the containers were closed and stored at 2°C.
5. Stored at 2°C.

Four containers of 72 plants of each container type were used for each treatment. At 0, 24, 36 and 72 hours after treatment one container from each treatment combination was flown to Lansing, Michigan. Ethylene samples (1 ml) were taken by syringe as the containers were opened. Thirty plants were removed randomly from each container and assigned to three blocks with ten observations per treatment combination. Plants were potted in 10 cm. pots containing equal parts of soil, peat, and perlite by volume. The number of yellow leaves per plant was recorded, but the leaves were not removed from the plants. Plants were grown in March at 21°C days and 17°C nights on a constant liquid feed rotation of 200 µl/liter of 20-20-20 and 25-0-25. After two weeks the number of dead leaves was recorded.

Experiment 2 - The effects of ethylene and handling procedures on leaf yellowing, leaf death, leaf abscission, and ability of seedling geraniums (cv. Sprinter Scarlet) to recover were investigated. Plants

received from Sun City, Florida, were grown for two weeks in East Lansing, Michigan, in polystyrene trays with seventy-two plants per tray as described in experiment 1. Twenty-four, ninety day old plants were selected and placed in four blocks with one observation for each treatment combination. Half of the plants were randomly left in their containers and half were removed to expose the root mass. This treatment was to observe what effect the damage from pulling the plants from the tray would have on the yellowing of plants in darkness. Four pulled and four non-pulled plants were placed in ten liter buckets with lids fitted with two ports. The inlet port was connected to an air stream containing 10 µl/liter of ethylene as described by Saltveit (1978), or to a stream of ethylene-free air from a Purafil scrubber. The air flow was held constant at 110 ml/min with a capillary flow meter. The outlet port was used for gas sampling to ascertain the ethylene level.

The experiment was performed in growth chamber at a temperature of 20^{o}C. At 0, 24, 48, 72, and 96 hours, plants were removed from each treatment combination and transplanted into 10 cm. pots in an equal mix by volume of soil, peat, and perlite by volume. The number of yellow leaves was recorded. Plants were grown at 21^{o}C days and 17^{o}C nights on a constant liquid feed

of 200 µl/liter alternated between 20-20-20 and 25-0-25. The number of dead or abscissed leaves was recorded after one week.

Experiment 3 - To further define the effect of ethylene, ninety-nine day plants were exposed to four concentrations of ethylene (0, 0.1, 1.0 and 10 µl/liter) for 12, 24, 48 and 96 hours in a 20°C growth chamber. Concentrations were established as in the previous experiment. All plants were removed from their trays and placed in 10 ml. buckets for treatment application. Four replications and three observations per treatment combination was used. Upon removal from the buckets, plants were treated as in experiment 1. After six weeks of growth, plants were visually rated on a scale of 1 to 5 from poor to high quality, respectively.

Experiment 4 - To determine if ethylene action is promoting senescence of leaves, and if so could it be inhibited by CO_2. An experiment like experiment 2 was conducted except 5 percent CO_2 was used or was not used with ethylene at 0, 0.1, 1 and 10 µl/liter in air. Plants were 105 days old when treated. Four replications and three observations per treatment combination were used. Number of yellow leaves was recorded after treatment and number of dead leaves was recorded one week after treatment. Ethylene analysis was by gas

chromatography employing a flame ionization detector and a column of activated alumina using N_2 as the carrier gas.

Experiment 5 - Seventy day old plants received from Sun City, Florida, were grown in Lansing, Michigan, for eleven days before treatments were applied. Trays described in experiment 1 were cut into sections containing nine plants. Plants in twelve sections were exposed to 20^{o} or 35^{o}C for twelve hours. At each temperature, plants were watered: (1) on a normal watering schedule, (2) one in which the soil mass was saturated for 48 hours before data measurement, or (3) watered 48 hours before data measurement and not watered until transplanting 96 hours later, after the data were taken.

After the 12 hours at various temperatures, the trays were placed in 10 liter desiccators which were connected to continuous flow system as described in experiment 2 at a rate of 100 ml/min. The air was scrubbed free of ethylene with a Purafil filter before entering the containers. The experiment was replicated four times. One ml. samples were taken by syringe at 0, 24, 48, and 96 hours of darkness and analyzed for ethylene as in experiment 5. At 96 hours plants were transplanted into 10 cm. pots containing equal parts of soil, peat, and perlite by volume. The number of yellow leaves and plant height was recorded. Plants were grown at the

same temperature and on the same fertilization program as in experiment 1. After two weeks the number of dead leaves was recorded.

Results

Experiment 1 - The temperature, container type, and duration of treatment all affected the number of yellow and dead leaves per plant (Figures 1, 2, 3, and 4). At 0 and 24 hours there was very little difference in the number of yellow leaves between any of the treatments while at 48 hours the plants with no cooling showed more damage (Figures 1 and 2). Plants at the 72 hour period showed less damage when precooled or shipped at 2°C and precooled to 10°C.

The number of dead leaves in all treatments increased slightly over time. Apparently, some leaves which were yellow at planting time recovered since in some cases there were fewer dead leaves after 2 weeks than there were yellow leaves. The greatest damage was seen with plants precooled to 10°C at 72 hours of treatment (Figures 3 and 4). An increase in temperature did not result in an increase in leaf mortality.

Ethylene accumulation in EPS containers was greater when plants were not cooled or precooled to 10°C and stored for 10°C. In general, EPS containers accumulated more ethylene at warmer temperatures (Table 1).

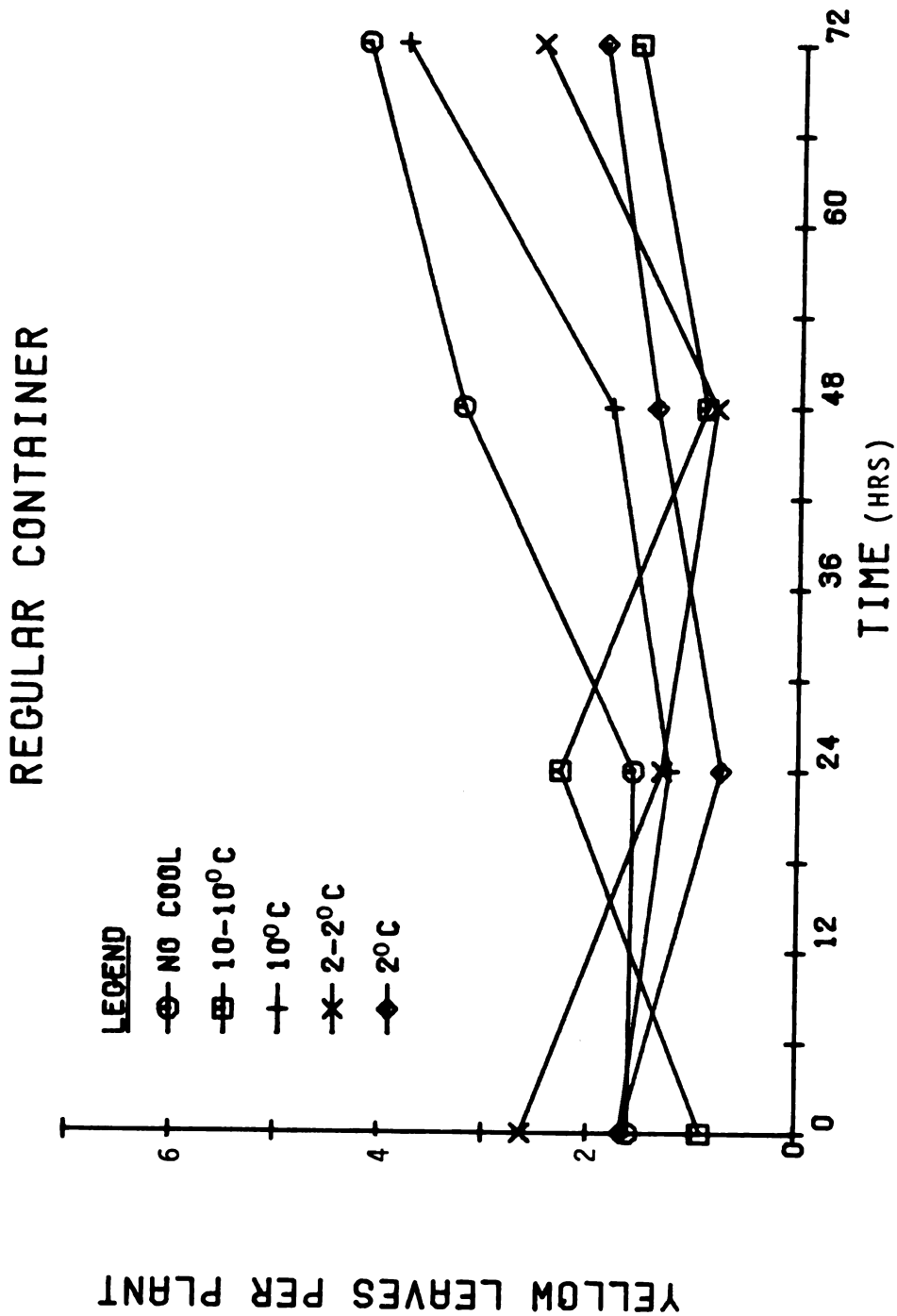

Figure 1.--Effect of temperature and duration of treatment, in regular containers on leaf yellowing of seedling geraniums two weeks after transplanting.

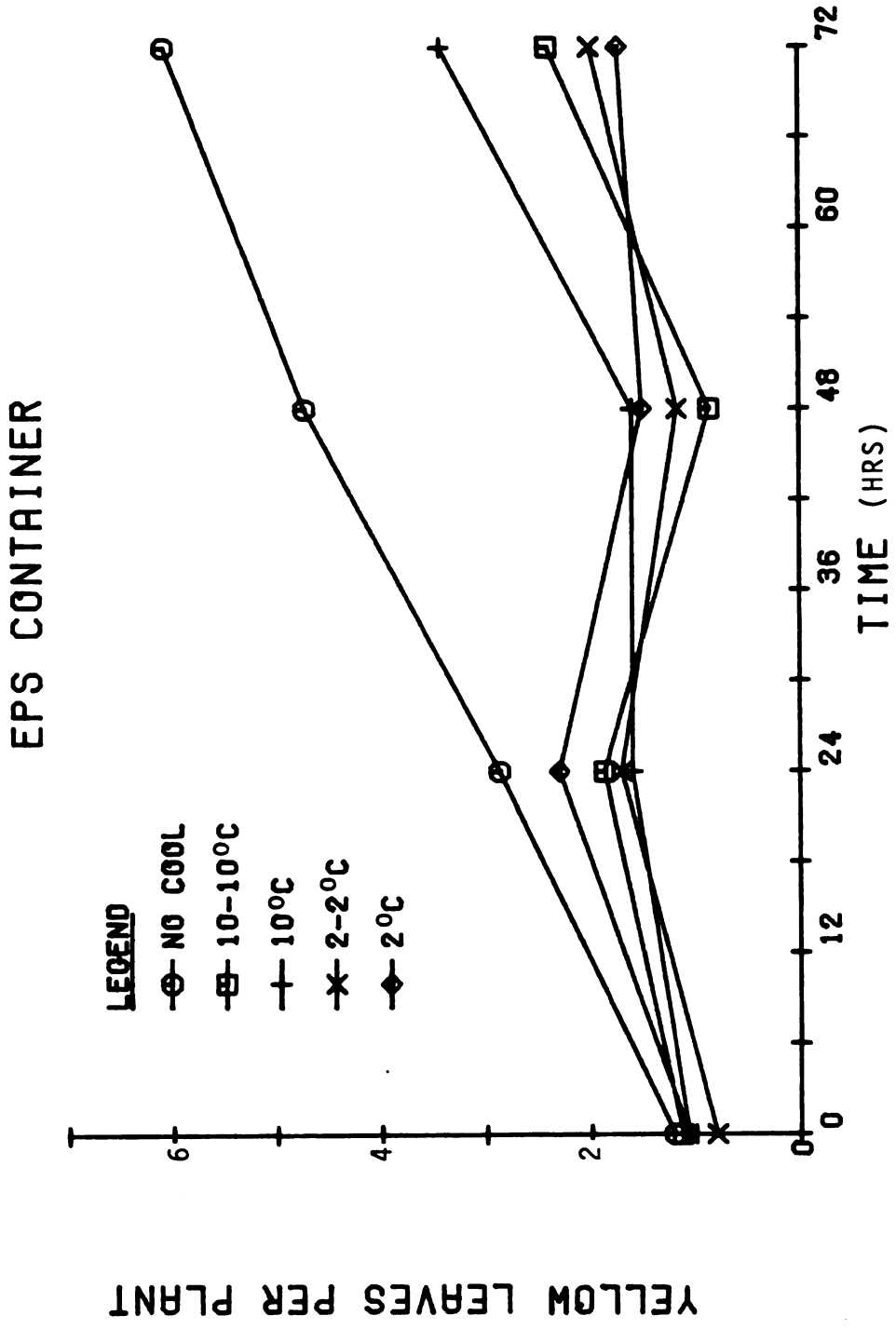

Figure 2.--Effect of temperature and duration of treatment, in EPS containers on leaf yellowing of seedling geraniums two weeks after transplanting.

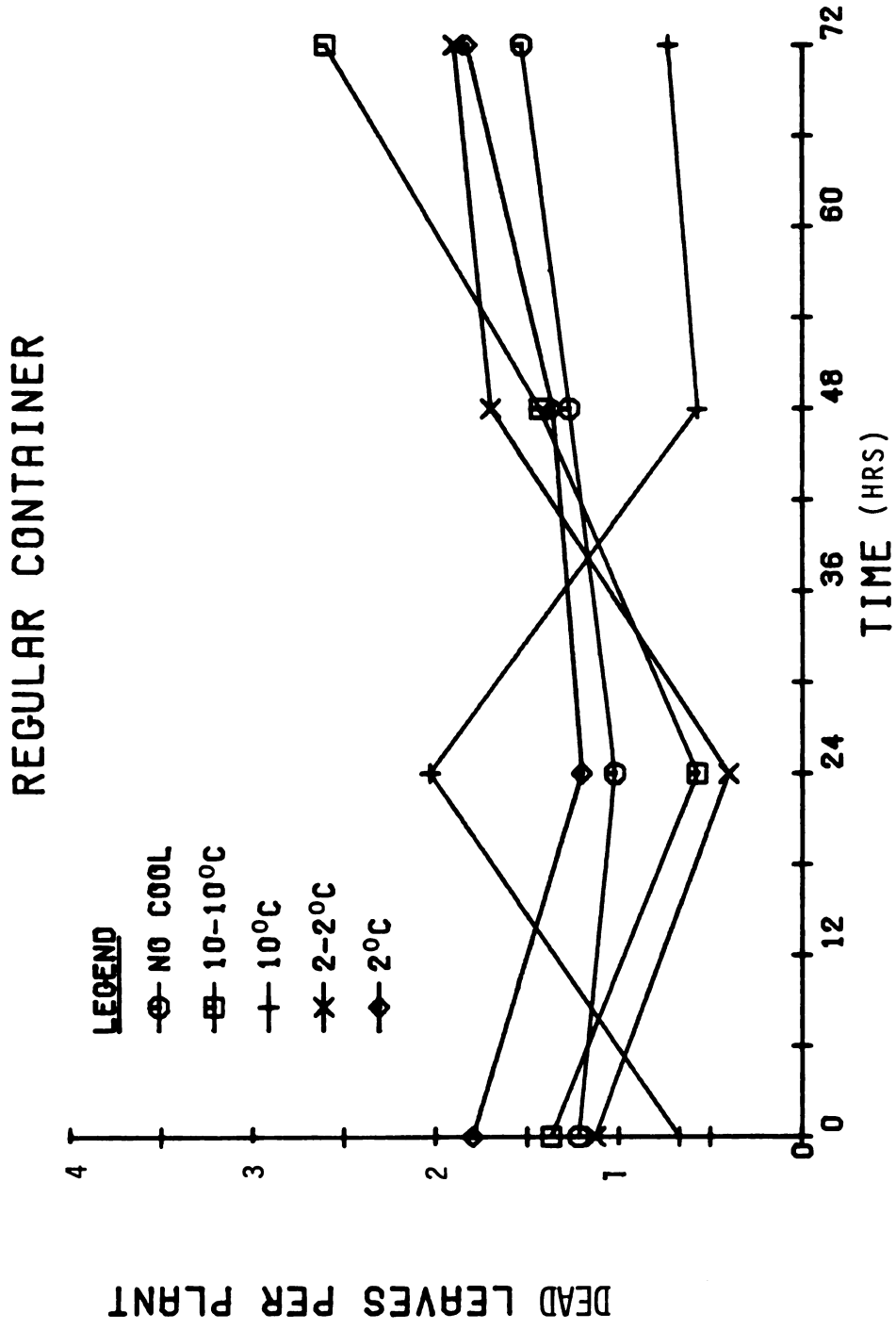

Figure 3.--Effect of temperature and duration of treatment, in regular containers on leaf death of seedling geraniums two weeks after transplanting.

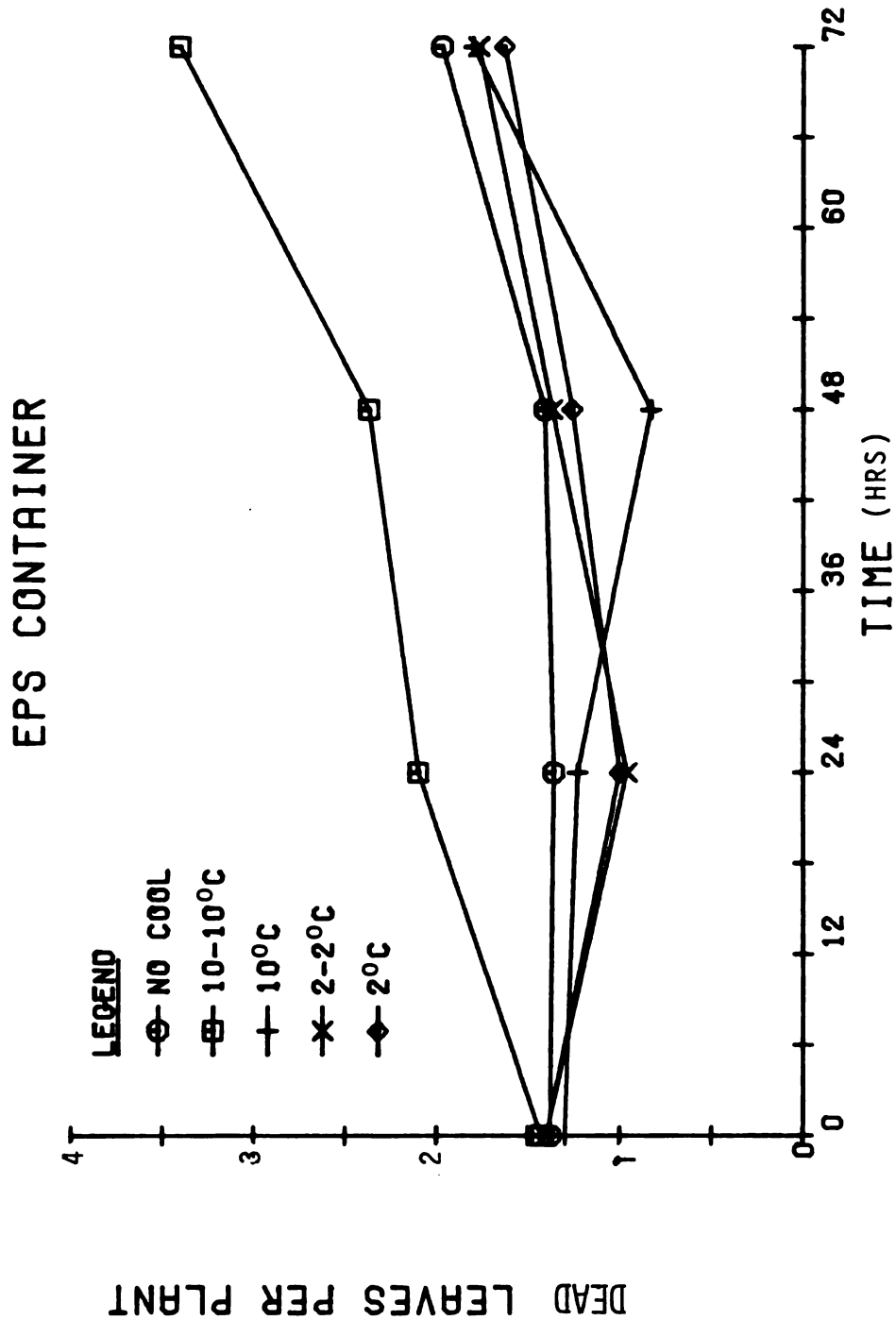

Figure 4.--Effect of temperature and duration of treatment, in EPS containers on leaf death in seedling geraniums two weeks after transplanting.

TABLE 1.--Effect of temperature and container type on ethylene concentrations in shipping containers upon arrival.

Temperature	Ethylene (nl/liter)	
	Regular container	EPS container
No cooling	7	100
Precooled 10°C and stored 10°C	10	142
Stored 10°C	6	68
Precooled 2°C and stored 2°C	7	40
Stored 2°C	39	83

$hsd_{.05}$ for two container means at the same level of cooling is 65.76.

$hsd_{.05}$ for two cooling means at the same or different levels of containers is 105.4.

Experiment 2 - The manner in which a plant was handled was not significant for any parameter except percent of abscised leaves where a two-way interaction involving duration of treatment and ethylene concentration was noted (Table 2). However, abscission occurred only with 10 µl/liter of ethylene when exposed for more than 48 hours. There was not significance found with respect to percent of yellow leaves which died (Table 2). It appears that pulling the plants may make them more sensitive to ethylene, but this must be examined further before a conclusion can be drawn.

TABLE 2.--Effect of ethylene concentration, duration of treatment, and handling of plants on the percent of dead or abscised leaves.

Ethylene concentration (µl/liter)	Time in hours	Percent[z] abscised		Percent yellow[y] dead	
		Pulled	Non-Pulled	Pulled	Non-Pulled
0	0	0	0	0	38
0	24	0	0	38	0
0	48	0	0	13	38
0	72	0	0	65	71
0	96	0	0	88	66
10	0	0	0	50	13
10	24	0	0	50	88
10	48	0	0	127	80
10	72	15	4	228	91
10	96	6	21	98	89

[z]Means were found to be involved in a 2-way interaction by the F-test at the 5% level.

[y]No significant difference was found by the F-test at the 5% level.

Rate of recovery on planting was significantly reduced by extending darkness. The longer the plant was exposed to darkness at 20°C the lower the quality of the plants when grown to salable product (Table 3).

The effect of ethylene on the percent of yellow leaves varied with time (Table 4). The data indicate that the longer the plants are in the dark beyond 72 hours the more leaf yellowing occurs and the presence of 10 µl/liter ethylene enhances this effect.

Experiment 3 - As the ethylene concentration increased, the percent of yellow leaves increased and generally more so the longer the duration of treatment (Table 5). As the treatment duration increased, only 48 and 96 hour treatments showed a significant increase in yellow leaves. However, at every level of duration, as ethylene increased percent yellow leaves increased significantly. As the duration of ethylene treatment increased the days to flower increased. This was also true for the percent of dead leaves (Table 6). When the effect of ethylene treatment is compared over all durations it is clear that flowering is delayed by 7 to 10 days at concentrations from 0.1 to 10 µl/liter (Table 7).

Recovery on planting was influenced by duration of treatment (Table 8). Plants exposed 96 hours showed

TABLE 3.--The effect of duration of treatment on recovery of seedling geraniums.

Duration of treatments	Recovery rating[z]
24 hours	4.5[y]
48 hours	4.0
72 hours	3.8
96 hours	3.2
$hsd_{.05}$	0.97

[z]Six weeks after treatment, plants growing in the greenhouse were rated from 1 to 5, lowest to highest quality, respectively.

[y]Means over handling and ethylene treatments.

TABLE 4.--The effect of duration of ethylene treatment in the dark on yellowing of leaves.

Duration in dark	Percent yellow leaves Ethylene (µl/liter)	
	0	10
0 hours	11	10
24 hours	10	28
48 hours	20	54
72 hours	45	62
96 hours	60	97

hsd at 5% level for two duration means at the same level of concentrations is 34.2.

hsd at 5% level for two concentrations means at the same level of durations is 15.83.

TABLE 5.--The effect of ethylene concentration and duration of exposure while in the dark on the percent of leaves which turn yellow.

Duration of treatment	Ethylene concentration (µl/liter)			
	0	0.1	1	10
12 hours	3	19	41	72
24 hours	23	33	50	92
48 hours	18	27	54	94
96 hours	25	32	62	100

hsd .05 of two concentration means at the same or different levels of duration is 17.45.

hsd .05 of two duration means at the same or different levels of concentration is 17.23.

TABLE 6.--The effect of duration of treatment on the percent of dead leaves and days to flower.

Duration of treatment	Percent dead leaves[z]	Days to flower[z]
12 hours	12	99
24 hours	18	101
48 hours	22	112
96 hours	42	127
$hsd_{.05}$	19.93	10.48

[z]Means over ethylene concentrations.

TABLE 7.--The effect of ethylene concentration on the number of days to flower a seedling geranium.

Concentration of ethylene (µl/liter)	Days to flower[z]
0.0	103
0.1	110
1.0	114
10.0	113
$hsd_{.05}$	5.79

[z]Means over duration of treatments.

TABLE 8.--The effect of duration of treatment on the recovery rating of seedling geraniums.

Duration of treatment	Recovery rate[z]
12 hours	3.58
24 hours	3.67
48 hours	4.02
96 hours	3.23
$hsd_{.05}$	0.34

[z]Six weeks after treatment, plants growing in the greenhouses were rated from 1 to 5, lowest to highest quality respectively. Ratings are means over ethylene treatments.

poorest recovery while 48 hour exposure had little effect.

Experiment 4 - As the ethylene dose increases, the percent of yellow leaves increase. Significant damage begins at 1.0 µl/liter or at 48 hours of treatment (Tables 9 and 10) which increases significantly at 96 hours. Leaf abscission starts after 96 hours and abscission increases as ethylene concentration increases. However, there were not significant differences between 1.0 µl/liter and 10 µl/liter (Table 11).

Five percent V/V CO_2 in air had no effect on any ethylene treatments (Table 12). However, 1 µl/liter with CO_2 at 48 hours shows more yellowing, while yellowing is more noticeable at 96 hours without CO_2. There is little difference seen at 12 or 24 hours when exposed to the air mixture.

Experiment 5 - Ethylene synthesis was significantly decreased and maintained between 3 and 7 nl/plant/hr after 24 hours of darkness in drought stressed plants. In normal or heavily watered plants, the decrease was significant after 48 hours and was maintained at a rate of 2 to 12 nl/plant/hr. When soil masses remained saturated ethylene production rose at 24 hours (Table 13).

TABLE 9.--The effect of ethylene concentration on percent leaf yellowing during darkness, of seedling geraniums.

Concentration (µl/liter)	Percent yellow leaves[z]
0	41
0.1	43
1.0	55
10.0	56
$hsd_{.05}$	7.78

[z]Means over durations of treatment.

TABLE 10.--The effect of duration of dark on the percent of yellow leaves on seedling geraniums.

Time	Percent of yellow leaves[z]
12 hours	29
24 hours	32
48 hours	54
96 hours	81
$hsd_{.05}$	6.85

[z]Means over concentrations of ethylene.

TABLE 11.--The effect of ethylene concentrations and duration of treatments on percent of leaf abscission.

Concentration of ethylene (µl/liter)	Duration of treatment			
	12	24	48	96
0	0	0	0	2
0.1	0	0	0	3
1.0	0	0	0	18
10	0	0	0	7
$hsd_{.05}$				11.92

TABLE 12.--Effect of air and CO_2, ethylene concentrations and duration of treatment on percent yellow and abscised leaves.

Ethylene concentration µl/liter	Time in hours	Percent yellow leaves		$\bar{x}$	Percent abscised leaves	
		Air	CO_2		Air	CO_2
0	12	23	32	28	0	0
	24	26	29	28	0	0
	48	45	37	41	0	0
	96	68	64	66	7	0
0.1	12	30	26	28	0	0
	24	26	34	30	0	0
	48	37	43	40	0	0
	96	83	68	76	29	6
1	12	25	32	28	0	0
	24	35	33	34	0	0
	48	61	72	66	0	0
	96	100	83	92	3	4
10	12	42	22	32	0	0
	24	37	31	34	0	0
	48	61	77	69	0	0
	96	100	80	90	3	0

TABLE 13.--Effect of various water regimes and duration of darkness of ethylene production.

Watering regimes	Ethylene (nl/plant/hr.)			
	0 hr.	24 hrs.	48 hrs.	96 hrs.
Normal	22	16	12	5
Soil saturated	21	31	9	2
Drought stressed	23	8	3	7

hsd at the 5% level for the differences between two times means at the same water regime is 8.3.

hsd at the 5% level for the difference between means of two water regimes at the same or different levels of time is 8.7.

Pretreating the plants for 12 hours at 20°C or 35°C did not alter ethylene production with plants from both temperatures producing 13 nl/plant/hr. However, due to the lack of replication of temperature this area should be investigated further.

Drought stressed plants had slightly fewer yellow leaves than the other two treatments (Table 14) but this was not significant. It is possible that the loss of leaves may have contributed to the lower rates of ethylene production noted with these plants (Table 13). Leaf mortality was greater in drought stressed plants as may be expected. Plant height, a measure of etiolation, was lower for drought stressed plants possibly because growth was limited by the lack of water directly as opposed to growth regulator effects (Table 14).

TABLE 14.--Effect of various water regimes on leaf yellowing, mortality, and plant height.

Water regimes	Percent yellow leaves[z,y]	Percent dead leaves[z]	Plant height[z]
Normal	92	28	4.8
Soil saturated	92	31	4.6
Drought stressed	82	38	3.9
$hsd_{.05}$	--	8.79	0.38

[z]Means over duration of darkness.

[y]Means were not found significantly different by the F test at the 5 percent level.

Discussion

The results of this study indicate leaf yellowing and leaf mortality of seedling geraniums shipped at cool temperatures (2°C to 10°C) are influenced by the duration of the shipment and the type of shipping container (Figures 1, 2, 3 and 4). Snapdragons, alyssum, petunia, salvia, and marigold have successfully been stored at 5°C for 4 to 6 weeks when supplied with 18 hours of light per day (Kumpf, et al., 1966). Also, tomato transplants have been successfully stored for 6 days at 5 to 15°C (Kretchman and Short, 1972). Kaltaler (1962) noted a slight amount of leaf yellowing occurred while storing geranium cuttings for 5 days at 5°C and for 1 to 3 days at 20-30°C. In this investigation, storage at 2°C or precooling at 10°C and storing at 10°C appeared to cause no damage for 48 hours in the dark (Figures 1 and 2). Some of the leaves that were counted as yellow at the end of the thermal-storage duration treatments regreened rather than died or abscissed (Figures 3 and 4).

The reduced occurrence of yellowing at the low temperatures in the dark may be caused by a general lowering of metabolism at the low temperature. Darkness normally is considered to speed senescence by decreasing protein and chlorophyll syntheses (Frank and Kenny,

1955; Vickery, et al., 1937, 1956; Goldthwaite and Laetsch, 1967).

In experiment 3 increasing the duration of darkness increased leaf mortality. However, this was not observed in experiment 1 (Table 2). This can not be explained directly, but the nine day age difference may play a role. Younger leaves are less prone to abscise than older leaves (Chaterjee and Leopold, 1964).

Darkness affected other parameters directly. The ability of plants to overcome the stress imposed by increasing darkness was impaired as the duration of darkness increased from 24 to 96 hours (Table 3). It appears to take 2 to 3 days of darkness to affect a significant decrease in recoverability.

As the duration of darkness in simulated transit increased the number of days required to flower increased (Table 6). A delay in flowering was observed after a 48 hour dark treatment.

Ethylene has been reported to cause leaf yellowing (Krone, 1937; Heck and Pires, 1962). The present work indicates leaf yellowing of seedling geraniums is affected by ethylene concentration as well as the duration of exposure to ethylene while the plants are in darkness (Tables 4 and 5). At 0.1 µl/liter ethylene,

yellowing was significantly enhanced in about 12 hours and at 10 µl/liter 72 percent of the leaves were yellow (Table 5). Increasing the duration of exposure to ethylene significantly enhanced leaf yellowing at all ethylene levels (Table 5). The longer plants were in the dark the more leaf yellowing occurs even without supplemental ethylene.

Removing seedlings from their growing containers could cause mechanical damage resulting in ethylene production. Several forms of stress cause plants to produce ethylene (Williamson, 1950; Jackson and Osborne, 1970). The only parameter significant in this treatment was abscission, but no trend was observed for pulled plants vs. non-pulled plants. However, it appears that pulling the plants free of their containers may make them more sensitive to ethylene with regards to leaf mortality (Table 2). Differences are not significant, and further examination must be made before a conclusion can be drawn.

Carbon dioxide (CO_2) treatment at a level of 5% v/v did not effectively negate ethylene action in reducing ethylene enhanced leaf yellowing (Table 12). The reason for this is not clear. Kays, et al. (1976) found similar results with pepper transplants and leaf abscission. The action of endogenous ethylene as the factor responsible for leaf yellowing during 96 hours in the dark was

also not affected by the presence of 5 percent CO_2. There is no clear indication of CO_2 inhibition of leaf abscission although less leaf abscission was observed in the presence of CO_2 at 0 and 0.1 µl/liter of ethylene. More experimentation is necessary to validate this point.

The injury or death to flower parts (Heck and Pires, 1962) following ethylene treatment is well established (Abeles, 1967; Tjia, et al., 1969). Flowering date was particularly affected by treating plants with exogenous ethylene (Table 7). Flowering date was inhibited by seven days at 0.1 µl/liter and did not vary significantly from this at 1 or 10 µl/liter ethylene. This may indicate that the threshold for ethylene action on this parameter is quite low and in keeping with other actions of ethylene shows no further activity beyond the saturating level. A possible action of ethylene would be bud abortion, but the flowers once formed seemed normal in outward appearance.

If ethylene is a contributing factor in leaf senescence during transit, a source of ethylene production needs to be present to produce concentrations high enough to cause damage. Healthy plants produce ethylene at low rates and diseased or injured plants produce much more (Williamson, 1949; Jackson and Osborne, 1970). Kays, et al. (1976) found pepper transplants produced ethylene in darkness while enclosed accelerating leaf abscission.

However, in lime plants whose leaves abscised in enclosed containers shipped from Florida contained no detectable ethylene. In the present work, ethylene production in plants shipped from Florida (Table 1) depended on the type of container used and the temperature of the shipment. Reflecting a slower metabolism rate, ethylene generally decreases as the temperature is dropped. This is significant only in EPS containers when precooled and stored at 10°C. EPS containers accumulated more ethylene at the two highest temperatures than regular containers. The EPS containers' lack of ventilation could be the reason for the accumulation.

In experiment 5 ethylene production generally decreased with exposure to darkness. The effect of darkness was also influenced by the way the plants were watered. Ethylene production by drought stressed plants decreased the fastest (Table 13). Morton and Watson (1948) found in sugar beets under a low water situation when transferred to high water condition had less leaf mortality than any other treatment. This supports the present study and the ethylene data because the seedlings were transferred to desiccators where humidity was high which would represent watering. However, dead leaves are shown to increase in dry regimes only (Table 14).

McAfee and Morgan (1971) found that the leaf petiole of cotton produced more ethylene when drought

stressed. It could be possible that the area around the abscission zone is producing more ethylene while the remainder of the plant is decreasing in synthesis causing a saturation.

Plant height is also the reverse of what might be predicted (Table 14). Ethylene is known to inhibit cell elongation (Harvey, 1915; Roberts, 1951). The failure of drought stressed plants to grow as high as plants watered adequately may merely reflect a water requirement as opposed to any action of ethylene. Plants will elongate in darkness if provided water.

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SECTION TWO

THE EFFECT OF BENZYLADENINE ON SEEDLINGS OF *PELARGONIUM* X *HORTORUM* BAILEY.

THE EFFECT OF BENZYLADENINE ON SEEDLINGS OF PELARGONIUM X HORTORUM BAILEY.

Abstract. BA (10^{-4}M) applied as a foliar spray when applied to geraniums decreased yellowing of plants held in the dark and reduced plant height, but 48 percent of the leaves on these plants were damaged by BA.

The market for seed geraniums to commercial growers is relatively new. A problem of this new market is transit induced senescence typified by leaf yellowing and abscission in transit or after transplanting (Carlson, 1977). This study was conducted to determine if a foliar application of the cytokinin, benzyladenine, would alternate senescence development of seed geraniums during simulated transit conditions.

Kinetin markedly delays senescence of excised leaves. This was first demonstrated by Richmond and Lang (1957). Cytokinins, N^6 benzylamino purine (BA) and kinetin, also delay senescence in intact wheat seedlings induced to senesce by darkness (Wittenbach, 1977), and in bean plants induced to senesce by natural fruiting (Fletcher, 1969).

Pre- or post-harvest applications of cytokinins delay senescence of green vegetables such as broccoli (Dedolph, et al., 1962). Also, leaf abscission and senescence in pot roses has been delayed by cytokinins (Halevy and Kofranek, 1976).

The present study investigated the effect of BA applied to seedlings geraniums as a treatment prior to simulated shipment in the dark for several days in an attempt to prevent or attenuate leaf senescence. Treatment of seed geraniums with BA could then serve as a simple treatment, by growers and shippers to maintain the quality of the plants during shipment.

Materials and Methods

Experiment 1 - Seventy day old seed geraniums (cv. Sprinter Scarlet) from Sun City, Florida, were grown in East Lansing, Michigan, for 14 days. Leaves were removed from the second and third node and surface sterilized with 10 percent ethanol. Leaf disc with a 1/2 cm. radius were cut from intervenial areas and transferred to petri dishes containing a 5 ml aqueous solution of BA at 0, 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7} M and a filter paper. Leaves were placed on 4 blocks according to size with 5 leaves per block. One disc per leaf was cut for each treatment. All procedures were carried out in a sterile transfer hood using sterile

materials autoclaved for 20 minutes at 115°C, and instruments were flamed between transfers. Petri dishes were then wrapped in aluminum foil to exclude light and placed in a 20°C chamber for six days. Chlorophyll was extracted in 80% ethanol, 5 ml/5 sections, using a 75-80°C water bath until the leaf discs were bleached (30-45 minutes). The optical density was determined at 665 nm using a spectorphotometer and the data are expressed as percent of the control (0 [BA]).

Experiment 2 - Seventy day old plants from Sun City, Florida were grown for 21 days in East Lansing, Michigan. Expanded polystyrene trays consisting of seventy two cubes per tray were used as a growing container. Each cube was 5 cm. deep, 3.7 cm. square at the top, tappering to 0.94 cm. at the base, and 1.5 cm. apart. Trays were cut into sections containing nine plants.

Plants were treated by spraying leaves and stems in benzyladinene solutions at concentration of 0, 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M, containing 0.1 percent tween 20. Twelve hours after treatment plants were placed in 10 liter desicators in a growth chamber at 20°C. Ethylene concentration in the treatment chambers was monitored by gas chromatography employing a

flame ionization detector and a column of activated alumina using N_2 as the carrier gas.

Results and Discussion

There was no significant difference in chlorophyll degradation found between water and any BA concentration. However, the control is less than any BA treatments (Table 1). BA reduced chlorophyll degradation slightly in leaf disc exposed to six days of darkness. This has been well supported in several species (Richmond and Lang, 1957; Suguira, et al., 1962; Leopold and Kawase, 1964).

TABLE 1.--Effect of BA on chlorophyll degradation of geranium leaf discs exposed to 6 days of dark.

Benzyladenine [M]	Chlorophyll (% of control) [z]
0 (control)	100
1×10^{-7}	32.5
1×10^{-6}	49.7
1×10^{-5}	40.0
1×10^{-4}	42.7

[z]No significant difference was found by the F-test at the 5 percent level.

Intact bean plants have been shown to be higher in RNA, protein and chlorophyll if BA (30 mg/l) was

painted on leaves before (Fletcher, 1969). In the case of potted roses in transit, an application of BA was found to reduce leaf senescence and abscission (Halevy and Kofranek, 1976). In the present work, leaf yellowing was reduced by 10^{-4} M BA (Table 2). The percentage of dead leaves was not affected by the treatment (Table 2). Plant height (Table 2) was also reduced which is an advantage, because control of plant height is a problem in seedling geranium production. The one problem encountered was a damage of 48 percent of the plants at 10^{-4} M. Other cytokinins (natural) may not have this toxic effect. Therefore, further investigation into this area is necessary before any conclusions can be made.

TABLE 2.--Effect of BA on leaf yellowing, death and damage, and plant height.

Benzyladine [M]	Percent Yellow[z]	Percent Dead[z,y]	Plant Height[z]	Percent Damaged[z]
0	32	6	4.7	0
10^{-6}	22	2	4.5	0
10^{-5}	21	5	4.5	0
10^{-4}	17	8	4.2	48
$hsd_{.05}$	11.59	8		

[z]Means over duration of darkness.

[y]Means were not significantly different by the F test at the 5 percent level.

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SUMMARY AND CONCLUSIONS

From the simulated shipping treatments, transit at cooler (2^{o} - 10^{o}C) temperature slows senescence and ethylene synthesis. Shipping containers with ventilation reduce ethylene synthesis.

Transit should be as short as possible, because darkness has been shown to enhance damage while delaying flowering by as much as 1 week.

Ethylene at concentrations as low as 0.1 µl/liter causes a later flowering date delaying the crop. Ethylene also caused leaf yellowing at 0.1 µl/liter for 48 hours, and at 1-10 µl/liter for 12 to 48 hours. Production of ethylene is not shown in darkness. However, this may be due to the drop in ratio ethylene products of the whole plant versus ethylene production of the abscission zone.

BA (10^{-4}M) can delay senescence, while reducing plant height. However, 48% of the leaves were damaged by this high of a concentration. Investigations of other cytokinins may not cause damage and should be further investigated.