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Investigation into "bud blast" in the Easter lily (*Lilium longiflorum* Thunb)

Mason, Michael Regis, M.S.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

INVESTIGATION INTO "BUD BLAST" IN THE EASTER LILY (*LILIUM LONGIFLORUM* THUNB).

by

Michael Regis Mason

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SIGNED: Michael Regis Nason

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Dr. William B. Miller
Assistant Professor of Plant Sciences

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Date

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LIST OF CHEMICALS

Common Name	Chemical Name
Banrot (trade name) ethazol	5-ethoxy-3-trichloro-methyl-1,2,4-thiadiazole
thicphanate-methyl	dimethyl [1,2-phenylenebis (iminocarbonothioyl)] bis[carbamate]
Benlate (trade name) benomyl	methyl[1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamate
Ethephon	2-chloroethylephosphonic acid
STS	silver thiosulfate
Subdue (trade name) metalaxyl	N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester
Terraclor (trade name) quintozene	pentachloronitrobenzene
Truban (trade name) ethazol	5-ethoxy-3-trichloro-methyl-1,2,4-thiadiazole

ABSTRACT

Ethylene and carbohydrate deprivation were investigated as possible causes of bud abortion in *Lilium longiflorum* Thunb. Silver thiosulfate (STS) was investigated as an inhibitor of ethylene-induced abortion.

Fourteen days of 92.5% irradiance reduction increased bud abortion when plants were exposed to 2.07 mM ethephon. Percent bud abortion was 39% and 60% for plants grown in full irradiance and reduced irradiance, respectively. Ethephon resulted in 54% abortion, regardless of irradiance at 4.15 mM. A 70% irradiance reduction for 14 days did not increase bud abortion when plants were treated with ethephon.

STS was applied to plants at visible bud + 2 weeks followed by ethephon application 2 days later. Bud abortion was reduced from 69 to 13% with 2 mM STS; the STS × ethephon interaction was significant. STS inhibited ethephon-induced bud abortion when applied at visible bud, 4 weeks prior to ethephon application; However, STS application at flower bud initiation did not prevent/reduce ethephon-induced bud abortion.

INTRODUCTION

Easter lily (*Lilium longiflorum* Thunb.) is an important commercial floriculture crop in the United States. In 1987, potted flowering Easter lily sales reached $28 million wholesale (USDA Natl. Agr. Stat. Serv., 1987). Approximately 90% of the Easter lilies forced in greenhouses in the United States and Canada originate from bulbs grown along the coast in northern California and southern Oregon (Green, 1986). The cultivar 'Nellie White' accounts for 65% of all bulbs grown with 'Ace' and 'Harbor' making up the majority of the remaining 35% (Boodley et al., 1984). In their native environment, Easter lilies flower in the summer but, are forced to flower for the Easter holiday by commercial growers. Bulbs are received in the fall and placed in cold storage (1.7 to 4.4C) for six weeks of vernalization. After vernalization, the bulbs are forced in the greenhouse at 15.5C night temperature and 21C day temperature. Plants typically reach anthesis in 110 to 120 days after planting.

Many commercial growers of Easter lilies have flower buds that experience a physiological disorder known as bud blast or bud abortion which is the death of a flower bud after it has formed. The death of these buds leads to a loss in profit since lilies are partially priced according to the number of flower buds per plant. Many environmental and cultural factors have been attributed as causal to bud blast but no single factor has been determined to be the major cause.

Previous studies with a range of plant species have suggested that carbohydrate depletion can result in flower bud abortion. The controlling factor in Easter lily bud blast appeared to be the supply of carbohydrates available to developing buds (Mastalerz, 1965). During winter, with prevailing low irradiance and short days, flower bud abortion is a major problem during forcing of *Iris* 'Wedgwood' (Mae and Vonk, 1974). Premature abscission of *Hibiscus* flowers often occurs under conditions that limit the supply of photosynthates to developing buds (Force et al., 1988). Weinbaum and Muraoka (1978) suggest that an increased sensitivity to ethylene (as measured by abscission of prune fruit) is brought about by a carbohydrate deprivation. Miller and Langhans (1989b) concluded reduced irradiance has a greater effect on plant carbohydrate status than on bud abortion *per se*. They speculated that greenhouse-grown lilies forced under extended periods of reduced irradiance may be more susceptible to ethylene injury due to reduced carbohydrate levels in the leaves and buds.

Results from the above experiments imply that carbohydrate deprivation increases sensitivity to ethylene. In Easter lilies, increased sensitivity to ethylene would be expressed by increases in ethylene-induced bud abortion. If an ethylene source is present in greenhouses, it could be a major factor responsible for Easter lily bud abortion in commercial greenhouses.

Therefore, experiments were designed to determine sensitivity of Easter lily buds to ethylene as measured by bud abortion and to examine the effect of carbohydrate depletion on plant response to ethylene. Also, the use of

silver-thiosulfate was investigated as an economically feasible treatment to prevent bud abortion in commercial operations.

LITERATURE REVIEW

INDUSTRY

Modern bulb production is both labor-intensive and time consuming. To produce a "commercial" bulb from a scale takes three years and the first two years the bulbs are dug, sized, and then replanted (Laurie et al., 1969). During the first year, scales from a bulb are planted outdoors in a field and allowed to grow. The following fall, the small, new bulbs, referred to as scalets, are dug from the ground. The scalets are replanted for another years growth. When dug the next fall, the bulbs are referred to as "yearlings" and are now two years old. After one more year of growth, a "commercial" is produced and ready for sale to a forcer who eventually sells the final product as a flowering potted plant (Miller, 1985). Other vegetative forms of propagation exist but, primarily for economic reasons are not commonly practiced.

Greenhouse forcing of Easter lilies begins with obtaining bulbs in the fall of the year. The bulbs must be exposed to vernalizing temperatures in order to flower and may be vernalized four different ways. The easiest vernalization method is case-cooling. Case-cooling requires the forcer to have enough cooler capacity for the size of crop intended. Also, good control of temperature is needed to obtain optimum vernalization temperatures for 1000 hours (6 weeks). Pre-cooled bulbs (commercial case cooling) can be obtained from the supplier. However, the forcer does not know exactly what treatment the bulbs have

received other than what the supplier tells him. Thus home-case cooling gives the grower better control.

The final two methods of cooling (below) are not as popular because of time and space restraints (controlled temperature forcing, CTF) or lack of control (natural cooling). CTF consists of potting non-cooled bulbs, irrigating, and giving the bulbs 3 weeks of warm (17C) temperatures in the greenhouse to promote root growth. The potted bulbs are then cooled for 1000 hours at the optimum vernalizing temperature (De Hertogh et al., 1969). The cultivars 'Ace' and 'Nellie White' have optimum temperatures of 1.7 to 4.4C and 4.4 to 7.2C, respectively. If Easter is early in a given year, the 3 weeks to promote root growth must be sacrificed to allow time for the required 1000 hours vernalization. Also, additional cooler space is needed to cool the potted bulbs which take up an enormous amount of space compared to case-cooled bulbs.

Natural cooling is probably the least used. Non-cooled bulbs are obtained from the supplier and potted upon arrival. The bulbs are watered and exposed to the prevailing temperatures of October to early December. The major problems with natural cooling are lack of temperature control resulting in the risk of possible freeze damage or too much or not enough vernalization.

Timing of an Easter lily crop is crucial. Easter lilies that flower too early or too late relative to Easter, are not saleable and the crop is lost. Therefore, plants must be timed to flower in a seven to ten day window just prior to Easter. One way of timing a lily crop is by the leaf counting method described by Wilkins

(1970). Since the plant is determinate, after the flower buds have been initiated (usually in late January), no more leaf primordia are formed. Thus, the number of leaves on a plant is fixed after flower initiation. The number of leaves that are unfolded are counted on several plants soon after the flower bud initiation stage (FBI). An unfolded leaf is one that has bent away from the apex by 45 degrees. This number is subtracted from the total number of leaves on the plant which is obtained by counting both unfolded and folded leaves in a destructive harvest. By subtraction, the number of folded leaves is determined. To use this information, the grower must first determine the desired number of days from VB to sale (usually 35 to 40). Once the VB date is established, the number of days between FBI and VB is calculated and the number of leaves yet to unfold are divided by the number of days to VB. This gives the number of leaves needed to unfold per day to reach the VB date. Leaf unfolding rates are manipulated by decreasing or increasing the greenhouse air temperature. Increasing the temperature increases the unfolding rate and decreasing the temperature has the opposite effect. Plants are monitored by marking the last unfolded leaf and counting how many leaves unfold after 5 to 6 days. Dividing the number of leaves by days gives the unfolding rate since the last count. Temperatures are adjusted accordingly.

A useful tool used later in the development of the crop is the "bud stick" (Miller, 1985; Healy and Wilkins, 1984). The pointed end of the bud stick is placed at the juncture of the bud and pedicel. At the tip of the bud, the grower

can read the number of days to flowering at several temperatures. This enables the grower to change temperatures accordingly.

The medium used to grow Easter lilies must be well drained and the pH should be in the range of 6.5 to 7.5. To assure optimum crop nutrition, fertility levels determined by Spurway test procedures (Cornell Floriculture Soil Test Lab) should be maintained as follows (Boodley et al., 1984):

Nitrates	30 to 35 ppm
Phosphorous	3 to 4 ppm
Potassium	25 to 30 ppm
Calcium	150 to 200 ppm

Total soluble salts should be less than 150 $dS \cdot m^{-1}$ in mineral soils (1 soil to 2 water), and less than 200 $dS \cdot m^{-1}$ in peat-lite mixes (1.5 and 2.0 $\mu mho \cdot m^{-1}$, respectively).

A fertilization program should be started when shoots emerge from the soil. Constant liquid fertilization at each watering with 300 $mg \cdot liter^{-1}$ N and K is commonly used.

Fungicide drenches are needed to prevent fungal diseases, with root rot being the major problem. The root rot complex is caused by one or more of the following pathogens, *Pythium, Rhizoctonia* and *Fusarium.* A fungicide drench is given after potting and then a rotation of fungicides are given every four weeks.

FLOWER BUD ABORTION

Terminology. Flower bud blasting and flower bud abortion are terms which have been used interchangeably by researchers and industry personnel. Flower bud blasting has been defined as flower bud death occurring after flower differentiation is completed. After flower bud blasting, visible remnants of the floral organs are evident. Blasted flower buds are greater than ≈5 mm in length. To industry personnel, an aborted bud is one that ceases development at an extremely early stage. Thus, at anthesis, the remnants of aborted buds are tiny bumps at the base of the inflorescence, usually subtended by narrow, non-developed axillary leaves of 5 to 20 mm in length. Technically, flower bud abortion is defined as the cessation of floral bud development at any stage (De Hertogh et al., 1971). Thus, in a technical sense, flower bud abortion encompasses flower bud blasting. However, in this thesis, all events of flower bud abortion would be termed "blasting" by industry personnel. The term flower bud abortion is used to follow the earlier published definition (De Hertogh et al., 1971).

Historical Perspective. There has been diverse research conducted in the area of bud abortion in Easter lilies as well as in other bulbous species. Hitchcock et al. (1932) found illuminating gas (containing 3% ethylene) caused abortion of young flower buds in Easter lilies. Post (1949) attributed the cause of bud abortion in Easter lilies to light, water, temperature, fertilizer, and "other conditions". Post

felt that inadequate light intensity and high temperature deplete carbohydrates in the buds, thereby causing abortion. Post (1949) suggested that root damage from excess fertilizer or decreased water supply to the plant may be additional causes of bud abortion. Thus, Post was suggesting that a carbohydrate depletion and/or water stress influences bud abortion. Seeley (1950), using the old 'Croft' cultivar reported calcium deficient sand cultures caused an increase in bud abortion while other mineral deficiencies had no significant effect. Mastalerz (1965) investigated bud abortion as affected by the environmental factors of air, soil, and stem temperature, water stress, or light reduction. In treatments where a constant air temperature was maintained at 4.4C or soil was maintained at 4.4C, abortion was increased significantly. When soil temperatures were 4.4C day and 27C night, abortion was also increased. Withholding water for two weeks (at the visible bud + one week stage) caused 18% bud abortion compared to 2% in plants without water deficit. Bud abortion of 83, 37, and 19% was observed for plants placed in complete darkness for 10 days at 27C to deplete carbohydrates for two tests with 'Croft' and one with 'Creole', respectively. When night temperatures were reduced from 27C to 10 or 0C, bud abortion was significantly decreased. A higher percentage of bud abortion was observed when plants were shaded for approximately one month before treatment of complete darkness. Complete bud abortion (100%) was observed in these plants after 8 days of darkness. After two and four days of darkness, 12 and 56% of the buds aborted respectively. Mastalerz studied the effect of removing 10, 33, 66, or 90% of the leaves when

buds were 0.64 to 1.3 cm in length. Leaf removal of 10, 33, 66 and 90% resulted in 0, 4, 21 and 33% bud abortion, respectively. Thus, it was necessary to remove 66% or more of the leaves to significantly increase bud abortion. From these experiments, Mastalerz (1965) concluded that the primary cause of flower bud abortion in several cultivars of *Lilium longiflorum* was low leaf and flower bud carbohydrate levels. Unfortunately, Mastalerz did not measure carbohydrate levels in his experiments. He assumed the treatments of complete darkness at high temperatures and leaf removal lowered plant carbohydrate levels. This assumption seems reasonable due to the extreme nature of these treatments, especially when compared to the environment in commercial greenhouses. Einert and Box (1967) conducted studies showing more than 50% irradiance reduction was required to induce significant amounts of bud abortion in *Lilium longiflorum* 'Georgia' while shading up to 50% had no effect on abortion. Flower bud abortion for plants grown in full sun and 50% shade was 17% and 26%, respectively. Miller and Langhans (1989a) reported 63% bud abortion in Easter lily when irradiance was reduced by 85%. However when the experiment was repeated the following year, only 12% abortion was observed. Irradiance reduction treatments began after floral initiation was complete and continued to anthesis. Heins et al., (1982) reported that 50% irradiance reduction had no effect on flower abortion in Easter lilies. Plants were exposed to the 50% irradiance reduction at several developmental stages for various durations. Weiler, (1978) treated Easter lilies with 70% irradiance reduction for the

duration of the crop cycle. In this experiment, there were no differences in the number of flower buds between the 70% irradiance reduction and the control. de Munk (1973) observed flower bud abortion in tulips exposed to 0.3 ppm (parts per million) ethylene when applied during storage of the bulbs. Rhoades et al. (1973) observed ethylene applied exogenously at concentrations of 1 ppm caused abortion in younger flower buds of Easter lilies. Plants were exposed to ethylene for two days in a static system. They noted that the stage of development of buds at the time of ethylene exposure affected the severity of injury. Small buds (i.e. less than 5 cm in length) aborted, while buds between 5 and 10 cm in length ceased elongation, prematurely whitened and either did not open or opened prematurely. Larger buds were reported to open with no adverse effects. Mae and Vonk (1974) attributed bud abortion in *Iris hollandica* 'Wedgwood' to insufficient light (95% reduction), high temperature and shortage of water. They also found that injection of cytokinins into flower buds during complete darkness helped overcome occurrence of bud abortion. Durieux (1975) stated that, although higher temperatures in the greenhouse promoted the occurrence of abscission, the primary cause of bud abortion in the Enchantment cultivar was a shortage of light during the winter months. The shortage of light is due to shorter day length and decreased photosynthetic photon flux.

de Munk and Gijzenberg (1977) concluded the primary cause of bud abortion in tulips was alterations in hormonal status of the plant. In their experiments, injection of GA_3, GA_{4+7}, BA, or kinetin into flower buds prevented

ethylene-induced flower bud abortion. They suggested that flower buds compete with daughter bulbs for substrates that are mainly derived from the mother bulb. Flower bud sink strength is reduced by ethylene and increased by cytokinins and gibberellins. Susceptibility of tulips to bud abortion increased when bulbs did not receive proper cooling (de Munk et al., 1980). Bulbs cooled for 2 weeks at 17C, rather than the typical 4 or 6 weeks had a higher rate of bud abortion.

van Meeteren and de Proft (1982) reported ethylene was produced in 3 cm long flower buds of *Lilium* × 'Enchantment' when the photoperiod was shortened to 4 hours or plants were placed in darkness. They suggested ethylene was instrumental in light controlled flower bud abscission. In one experiment, plants were placed in darkness for 7 days commencing when flower buds reached a length of 3 cm. Treatments consisted of buds receiving an injection of 0.2 mM silver thiosulfate (STS) prior to being placed in the darkness, STS and darkness, and a third treatment of no STS and no dark treatment. Approximately 47% of buds receiving dark treatment and no STS abscised, while only 8 to 9% of dark treated buds abscised if treated with STS. Buds remaining in light with no STS showed no abscission.

In Asiatic hybrid lilies, additional photosynthetic lighting was needed during winter to prevent flower bud abortion and abscission (van Tuyl et al., 1985). van Tuyl et al. (1985) concluded a short period of very low light intensity coupled with the critical (2 to 3 cm) stage for bud abscission (as determined by Durieux et al., 1983), will cause a disproportionate increase in percent flower bud

abortion.

Growth medium water saturation for one to several days lead to bud abortion in Easter lilies (Miller and Langhans, 1986). In this case, abortion could be caused by an ethylene mechanism. Bradford and Dilley (1978) saw increased rates of ethylene production in shoots of water saturated tomato plants. Bradford and Yang (1980a, 1980b) identified the cause of this surge of ethylene as increased ACC synthesis. They proposed that root anoxia stimulated an increase of ACC and increased its accumulation. After translocation to the aerobic shoot, the ACC was rapidly converted to ethylene, leading to characteristic epinasty.

Miller and Langhans (1986) studied the effects of ethephon (a.i. 2-chloroethylphosphonic acid), an ethylene releasing compound, on Easter lily bud abortion. They found that ethephon altered ^{14}C-assimilation products within the plant, and increased movement of assimilated ^{14}C toward the bulb. These data support previously mentioned work conducted by de Munk and Gijzenberg (1977) using tulips where assimilate partitioning was affected by the hormonal status of the plant. Ethylene weakened the sink of the flower bud and caused the daughter bulb to became a stronger sink for assimilates. Kinet and Hachimi (1988) suggested that ethylene is involved in control of tomato inflorescence abortion and the detrimental effects of a low light regime on reproductive development are, at least partly, mediated by ethylene.

In summary, previous studies examining flower bud abortion in bulbous species used extreme treatments to induce bud abortion. These extreme

conditions are not seen in commercial greenhouses where bud abortion occurs therefore it does not seem likely that these factors individually are the primary cause in bud abortion. It does seem possible that a combination of factors could result in bud abortion. Carbohydrate deprivation and a source of ethylene could be found in commercial greenhouses and could combine to cause bud abortion. While both factors have been studied individually as possible causes of bud abortion, no studies have examined the interactions.

ETHYLENE

The simple chemical structure of ethylene has made the task of identifying the biosynthetic pathway very difficult. In 1864, streets were lighted with illuminating gas. In certain German cities, when the gas pipes leaked, leaves fell off the shade trees. Ethylene is presumed responsible since it causes the senescence and abscission of leaves (Salisbury and Ross 1985). A Russian physiologist named Dimitry N. Neljubow (1876 to 1926) was the first person to establish that ethylene affects plant growth. He identified ethylene in illuminating gas and showed that it causes a triple response in pea seedlings which are: Inhibition of stem elongation, increase in stem thickening and horizontal growth habit (Salisbury and Ross 1985).

Abeles and Rubenstein (1964) reported successful production of ethylene when they combined a non-protein extract and an enzyme fraction isolated from pea seedlings. They also observed that Fe^{++} and flavin mononucleotide (FMN)

could cause non-enzymatic evolution of ethylene from their non-protein fraction at a rate exceeding that of the enzyme catalyzed reaction. Yang et al. (1966), confirmed the FMN-mediated ethylene production reported by Abeles and Rubenstein (1964). They identified the active substrate as methionine. The ethylene carbon skeleton was derived from carbons 3 and 4 of methionine and identified by paper chromatography and ^{14}C-methionine.

Lieberman and Mapson (1964) described a non-enzymatic system that produced both ethylene and ethane. The system required copper, rather than iron, as a catalyst and ascorbic acid as the reducing agent for the metal. Linolenic acid was incubated with the ascorbic acid and copper in acetate buffer at a pH of 4.5. Large amounts of ethylene and much smaller amounts of ethane were produced. In the absence of the reducing agent, ethylene was produced, but in smaller amounts. This led the authors to believe the ethylene carbons must derive from linolenic acid. After completion of these experiments they found methionine could also react in the copper-ascorbate system to form significant amounts of ethylene. The methionine system produced no ethane, (unlike the linolenate system), but required aerobic conditions for ethylene evolution. This system was very similar to the natural ethylene system in apples and other fruit. Additionally, methionine stimulated ethylene production in apple slices. Lieberman and Kunishi (1965) described a reaction that forms ethylene from a Cu^{+}-catalyzed breakdown of methionine in phosphate buffer in an aerobic environment. Hydrogen peroxide was an intermediate and supported production

of ethylene under anaerobic conditions. The model system consisted of L-methionine, Cu^{+2}, ascorbate and phosphate buffer, pH 6.8. These components were sealed in 25 ml flasks containing clamped capillary tubes and were incubated at 30C. Methional was the most effective producer of ethylene and appeared to be an intermediate. Tracer studies suggested ethylene was derived from the number 3 and 4 carbons of methionine. The methionine system produced 3 to 5 times as much ethylene per mole of substrate as did the linolenate system described above. The use of copper binding chelating agents inhibited ethylene production in these tissues. These experiments suggested that methionine is a precursor of ethylene biosynthesis in plant tissues and the enzymic system associated with the biosynthesis of ethylene involves a copper enzyme, perhaps a peroxidase.

Baur et al. (1971) working with avocados, suggested that ethylene biosynthesis is not controlled by the level of methionine, but by a step after synthesis of methionine. During three different stages of fruit ripening, preclimacteric, climacteric rise, and climacteric peak, different levels of methionine were observed in the tissues as well as different ethylene evolution rates. During the preclimacteric stage, high levels of methionine were present while no ethylene evolution was observed. Methionine was efficiently converted to ethylene at both the climacteric rise and climacteric peak stages and when methionine levels were low in the tissue. Baur et al. (1971) demonstrated the oxygen dependence of ethylene formation in apple tissues. Under a nitrogen

atmosphere, apple tissue fed ^{14}C-methionine released no ethylene. Upon return to air an hour later, rapid conversion of methionine to ethylene was observed. When ^{14}C-methionine was administered to the apple tissue and left in air, ^{14}C-ethylene evolution was observed after a 40 minute lag. These data suggested that the processes occurring during the lag phase do not require oxygen. Apparently, methionine is converted to an intermediate followed by an oxygen-dependent reaction to convert the unidentified intermediate to ethylene.

Burg (1973) concluded that of methionine activation most likely occurs at the sulfer atom, probably forming S-adenosylmethionine (SAM). In order for the apple to continue to produce ethylene over time, Baur and Yang (1972) believed the sulfer of methionine must be recycled so as to provide an adequate supply of methionine for continued ethylene production. Murr and Yang (1975) proposed if SAM is the direct precursor of ethylene, then 5'-methylthioadenosine formed during ethylene synthesis must donate its methylthio group to an acceptor compound leading to the formation of methionine. Murr and Yang (1975) demonstrated that when ^{14}C-5'-methylthioadenosine-methyl was administered to apple tissue, the radioactivity was incorporated into methionine.

Adams and Yang (1979) identified 1-aminocyclopropane-1-carboxylic acid (ACC) as an intermediate in the conversion of methionine to ethylene. They fed uniformly labeled L-methionine to apple tissue incubated in nitrogen where it was converted into labeled ACC. Labeled ACC was efficiently converted by to ethylene when aerobic conditions were imposed. In the presence of unlabeled

ACC, the conversion of labeled methionine to ethylene was greatly reduced while conversion of labeled ACC to ethylene was not affected by the presence of unlabeled methionine. The application of a strong inhibitor of pyridoxal phosphate-mediated enzyme reactions, 2-amino-4-(2'-aminoethoxy)*trans*-3-butenoic acid, greatly inhibited the conversion of methionine to ethylene but had no effect on the conversion of ACC to ethylene. With these results, Adams and Yang (1979) proposed the following sequence for the pathway of ethylene biosynthesis in apple tissue:

methionine ----> S-adenosylmethionine ----> 1-aminocyclopropane-1-carboxylic acid ----> ethylene. A more detailed pathway for ethylene biosynthesis is given in Figure 1.

ETHYLENE RELEASING COMPOUNDS

Ethephon (2-chloroethylphosphonic acid) is one of several chemicals that release ethylene when applied to plants. There are several commercially available formulations, including Florel (21.7% a.i.) and Ethrel (3.9% a.i.). Ethylene evolution from ethephon is caused by chemical breakdown in plant tissues (Cooke and Randall, 1968; Warner and Leopold, 1969). This degradation of ethephon results in responses characteristic of ethylene treatments in plants (Cooke and Randall, 1968; Russo et al., 1968; Warner and Leopold, 1967). Ethephon is stable in aqueous solutions below pH 4, but rapidly disintegrates into ethylene in alkaline solutions (de Wilde, 1971). The mechanism of ethylene

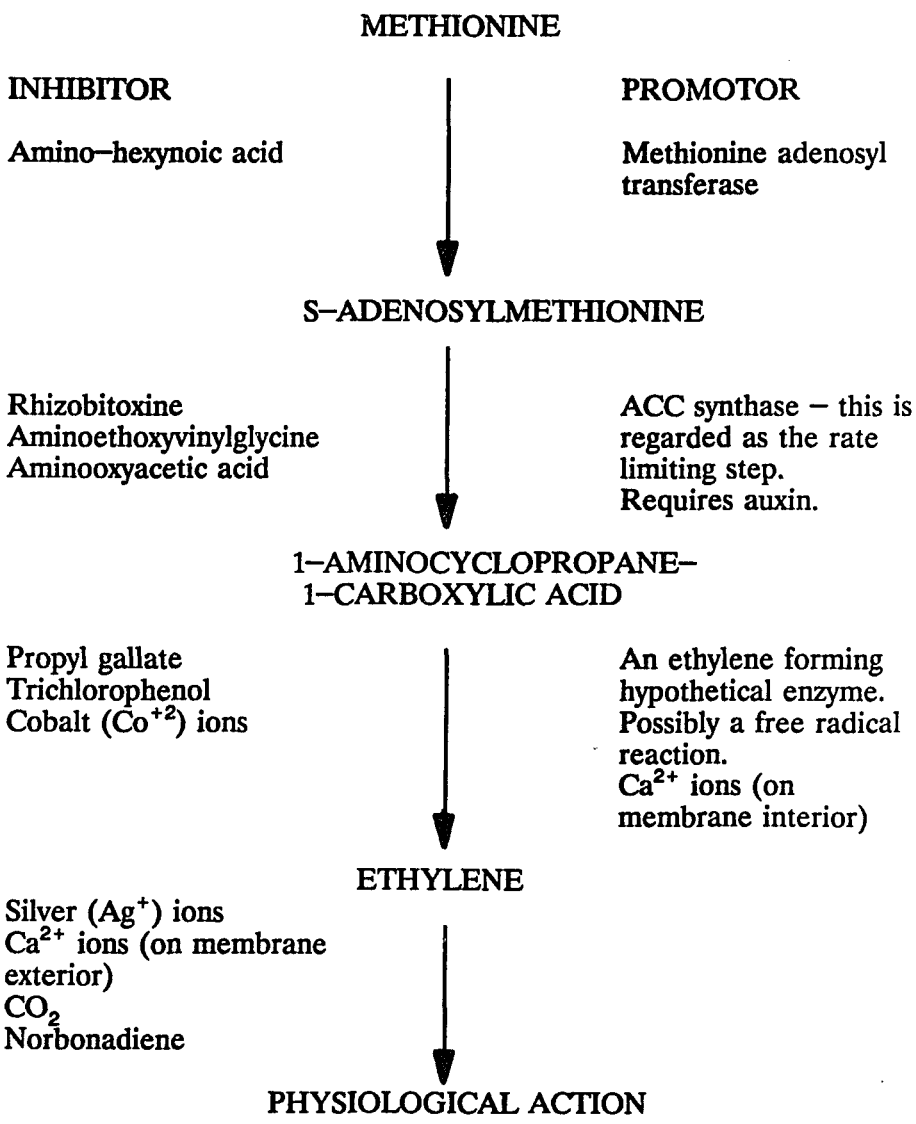

Figure 1. Pathway for ethylene biosynthesis in plant tissue. Adapted from Lesham et al. (1986) and Beyer et al. (1985).

release from ethephon involves the nucleophilic attack on the phosphonate dianion by a water molecule and the concerted elimination of chlorine, leading to direct formation of ethylene, phosphate, and chlorine (Maynard and Swan, 1963). Ethephon is often substituted for ethylene in agricultural experimentation because of cost and ease of application under field or greenhouse conditions.

According to Abeles (1973), ethephon is nearly entirely converted into ethylene within the plant. Weaver et al. (1972) found that when grape berries were injected with ^{14}C-labeled Ethrel, only 9% of the label was recovered after 7 days and when applied to the base of the first leaf above the cluster, only 0.7% of label was recovered. When the labeled Ethrel was applied to the surface of the berries, 26% was recovered. Martin et al. (1972) reported that 2% or less of labeled ethephon was recovered from walnuts 5 days after application. Thus, these studies indicate that most of the ethephon is rapidly broken down to ethylene in plant tissues.

CARBOHYDRATE STATUS

Carbohydrate levels in plant tissues will vary according to the environment the plant is exposed to. Photosynthesis is affected by CO_2 concentration surrounding the photosynthetic cells, light intensity and its quality, and water availability (Salisbury and Ross, 1985). For most plant species, increases in temperature up to 30 and 35C, causes increases in respiration rate. High temperatures typically cause increased respiration rates, presumably resulting in

lowered carbohydrate levels in the plant (Post, 1949). Lower irradiance would result in lowered photosynthetic rates also resulting in reduced plant carbohydrate levels (Miller and Langhans, 1989b). Miller and Langhans (1989a) reported that flower bud and open flower dry weights were progressively reduced as applied irradiance was reduced immediately following flower bud initiation. In the same experiment, 17.5 cm bulbs had 63% bud abortion and in a different year 20.0 cm bulbs only showed 12% abortion. Larger bulbs have more carbohydrate reserves available to the developing buds and flowers and may help reduce bud abortion in some circumstances.

Mayak and Dilley (1976) found floral longevity of cut carnation (*Dianthus caryophyllus* L.) was enhanced by solutions containing kinetin and sucrose compared to solutions containing kinetin alone. Flowers developing under low light intensity did not respond to kinetin treatments in the post harvest stage. Therefore, the extension of longevity by kinetin seemed to depend on prevailing light energy during floral development. Mayak and Dilley believed the lack of response to kinetin treatments was related to the carbohydrate reserves in the flowers.

When Mayak and Dilley (1976) exposed flowering stems of cut carnations to 2 μl/liter ethylene for 8 hours, sucrose-pretreated stems were insensitive to ethylene and did not begin to wilt and in-roll until 60 hours after exposure controls (no added sucrose) showed signs of in-rolling after just 10 hours. Force et al. (1988) grew *Hibiscus* plants under two different irradiance regimes, full and

50% reduction, and then placed them into dark storage for 5 days. After 5 days, plants were returned to full irradiance and the extent of the resulting bud abscission was determined. Plants grown under 50% irradiance reduction showed 78% abscission while the controls had 55% abscission. They suggested dark-storage-induced flower bud abscission in *Hibiscus* is regulated partially by the availability and partitioning of photosynthates to developing flower buds.

ETHYLENE AND ASSIMILATE PARTITIONING

Application of CGA-15281, an ethylene releasing compound, was found to affect carbon transport into young peach fruits (Krewer and Daniell, 1983). Fruits on the respective limbs were treated with CGA-15281. After 0.25, 24, 48, 96, or 144 hours, terminal leaf clusters were exposed to $^{14}CO_2$. Tissues were separated and analyzed via autoradiography. A substantial inhibition of photosynthate transport was observed after 24 hours and continued thereafter. These data were illustrated by the absence of autoradiograph exposure of the fruit beginning at 24 hours on CGA-15281-treated fruit. Robinson (1983) found that Ethrel treatments significantly decreased the percentage of translocated ^{14}C accumulated by the inflorescence and increased the percentage of ^{14}C accumulated by the stem, leaves, and new corm in *Gladiolus grandiflorus*. Nichols and Ho (1975) found that the sink strength of carnation nectar, stem and ovary increased during ethylene-accelerated senescence of the flower and that the petals were one of the sources of translocated substrate.

ETHYLENE IN GREENHOUSES

In order for ethylene to be a practical production limitation in greenhouses, ethylene must be in the greenhouse atmosphere. Hannan (1973) stated that tests in the Denver, Colorado region showed that ethylene may reach significant levels (up to 0.75 ppm) in unit-heater flue gases. According to Davison and Wharmby (1980) many flueless gas burners are used for heating greenhouses in England. They also stated that these burners generate a complex mixture of gases that includes ethylene. Therefore, venting exhaust into a greenhouse inevitably leads to a risk of plants being affected; of bud and leaf drop, poor growth, and necrosis. Hand (1982) stated that ethylene levels of 20 to 1,000 ppb (parts per billion) have been measured in moderately well-sealed glasshouses and film-plastic structures equipped with direct-fired CO_2 generators and heaters. He also measured ethylene levels ranging from 5 to 50 and 5 to 500 ppb in rural and industrial areas, respectively. Abeles (1973) suggests that vehicular emissions in urban areas of California are responsible for damage to bedding plants amounting to tens of thousands of dollars annually. Ethylene pollution in greenhouses has been often observed due to incomplete combustion of fuels for CO_2 generation (Mortensen, 1987). Ashenden et al. (1977) measured ethylene levels of 3.8 ppm in a commercial glasshouse receiving CO_2 enrichment. Abeles (1973) stated that concentrations above .01 ppm have a discernable effect on plant growth.

PREVENTION OF ETHYLENE ACTION

Beyer (1972, 1975a, 1975b, and 1976) showed that Ag^+ applied as a nitrate salt effectively prevented ethylene action and the effect persisted for many days following a single foliar treatment of intact tomato, cucumber, and bean plants. Beyer (1976) also stated that the ability of silver to block the action of ethylene to the extent reported is unprecedented in plant biology. Beyer also stated that Ag^+ is specific, persistent, and not phytotoxic at effective antiethylene concentrations. Reid et al. (1980) found that the vase life of carnation flowers was doubled by immersing stems in solutions containing a silver thiosulfate (STS) complex prepared by combining silver nitrate with sodium thiosulfate (molar ratio 1:4). This effect could be achieved by treating stems with as little as 1.0 mM Ag with a pulse as short as 10 minutes. Dimalla and van Staden (1980) found that after immersing cut stems of carnations in silver thiosulfate, the silver accumulated in the gynaecia of the flowers. This treatment extended the floral longevity by almost 100%. Mor et al. (1981) found that treatment of standard ('Scania') and spray ('Elegance') carnations with STS and Physan, a biocide, markedly extended shelf life in deionized water after shipment from California to Maryland. The extension of vase life over controls was 11.5 and 5.2 days for 'Scania' and 'Elegance', respectively. van Meeteren and de Proft (1982) found that when flower buds of *Lilium* × 'Enchantment' were injected with 0.2 mM silver thiosulfate or bulbs were soaked for 24 hours in 2.0 mM silver thiosulfate,

flower bud abscission, induced by darkness, could be prevented. Prince et al. (1987) found that whole plant STS sprays (0.5 to 2.0 mM Ag) prior to harvest reduced storage-induced Easter lily bud abortion and increased floral longevity. They also found that STS application (1.0 mM Ag) prior to storage reduced ethephon-induced disorders. In the Netherlands, van Meeteren and Slootweg (1986) found that flower bud abscission of *Lilium* × 'Enchantment' was reduced to ≈20% of the control by foliar sprays with silver thiosulfate in greenhouse experiments during winter. Moe and Smith-Eriksen (1986) found that begonias treated with 0.03 mM STS were more resistant to ethephon-induced flower bud abscission. At 1.05 mM STS, flower bud abscission and malformation was almost eliminated while at 6.25 mM STS became phytotoxic.

Sisler et al. (1983) proposed a possible scheme by which ethylene could cause its dramatic effects on plant growth and development. They believe an ethylene molecule possibly binds to a specific site which in turn releases a "second message", a molecule which itself or through mediation of subsequent messenger molecules, results in the transcription of new mRNA. The mRNA codes for proteins which are the enzymes responsible for ethylene effects. Changes in protein and mRNA populations during the senescence of carnation petals were investigated by Woodson (1987). Woodson observed both increases and decreases in certain polypeptides during senescence. An increase of mRNA's was also observed during the climacteric rise of ethylene associated with the onset of senescence in carnation. These results suggested that carnation petal

senescence is associated with changes in gene expression. Later experiments conducted by Woodson and Lawton (1988) found that at least 6 mRNA's accumulate in carnation petals following ethylene exposure of 7.5 μl/l in a flow through system. Significant increases in most mRNA's were observed 3 hours following exposure. Another group of mRNA's decreased at the same time. These results indicate that ethylene-induced changes in petal physiology may be the result of rapid changes in gene expression.

Heavy metals such as Ag^+ are known to interfere with a number of enzymatic processes in plants and are non-competitive enzyme inhibitors (Veen, 1983). Veen (1983) stated that silver ion is very efficient in its inhibiting action on ethylene. Reid et al. (1989) showed that the effects of exogenous ethylene on cut rose flowers could be overcome by pretreatment with STS. They also stated that the Ag^+/ethylene interaction was competitive, suggesting that Ag^+ binds reversibly to the ethylene forming enzyme and denies ethylene access to the active site.

MATERIALS AND METHODS

Bulbs of *Lilium longiflorum* 'Nellie White' were received from Dahlstrom and Watt Bulb Farms, Inc. and placed into 4C dark storage on 27 Oct. 1987. On 8 Dec. 1987, bulbs were removed from the cooler, planted in 1.8 liter plastic containers, and placed into a greenhouse using 26C venting and 15C night temperatures. The growth medium consisted of a 1 soil: 2 sphagnum peat: 2 perlite (v/v/v) mixture amended with 4.75 kg ground dolomitic limestone, 890 g treble superphosphate, 593 g KNO_3, 593 g $MgSO_4$, and 74 g Frit Industries Trace Element No. 555 (Peters Fertilizer Products, W.R. Grace and Co., Fogelsville, Pa.) per cubic meter. The plants received constant liquid fertilization at each watering with 300 mg·liter $^{-1}$ N and K, each supplied from 776 and 550 mg KNO_3 and NH_4NO_3 per liter, respectively. Fertilizer solution was maintained at 6.0 pH by injecting 75% (w/w) technical grade phosphoric acid into the system, supplying 37 mg P/liter at every watering. A fungicide solution containing 14.8 ml Subdue 25 EC (a.i. metalaxyl) + 227 g Terraclor 75 WP (a.i. quintozene) per 379 l was applied as a drench of 237 ml per pot two days after planting. Four weeks after planting, 227 g Banrot 40 WP (a.i. ethazol + thiophanate-methyl) per 379 l was applied in the same manner. Eight weeks after planting, 227 g Truban 30 WP (a.i. etridiazole) + 76 g Benlate 50 WP (a.i. benomyl) per 379 l was applied in the same manner. Twelve weeks after planting, the Banrot drench was repeated and after 16 weeks, the Truban + Benlate drench was repeated.

During the 1989 growing season, bulbs were received on 25 Oct. 1988, and placed in 4C dark storage for 6 weeks. On 6 Dec. 1988, bulbs were potted as described above. Cultural practices for all 1989 experiments were identical to 1988 practices unless otherwise stated.

SOLUBLE CARBOHYDRATE EXTRACTION

The procedures for carbohydrate extraction were as described by Boersig and Negm (1985) with modifications described by Miller and Langhans (1989b).

Bud and leaf tissues were harvested in late afternoon and placed into aluminum foil packets. All leaves from one plant were combined into the same packet while buds were packaged individually. The tissue was frozen in liquid nitrogen and placed in a freeze drier for a minimum of 72 hours. After freeze-drying, the tissue was ground in a Wiley mill through a 20 mesh screen or powdered in a mortar and pestle. All ground samples were stored in a desiccator at room temperature.

Fifty milligrams of freeze dried tissue were loaded into pasteur pipets fitted with glass wool plugs and with tubing fitted onto the tips of the pipets. Tissue was extracted with 4 × 1.5 ml volumes of methanol:chloroform:water (MCW, 12:5:3, v/v/v), for 1 hour each. An internal standard of mannitol (1.0 mg) was added during the first extraction. After the last extraction, samples were rinsed with 2 ml MCW. The extracts were collected into 15 ml centrifuge tubes. Five ml of glass distilled water were added to each extract and mixed using a

stirring rod. The solution was placed in a table top centrifuge for 10 minutes at approximately 1000 g's. The upper, aqueous phase was removed and applied to Bio-Rad poly-prep columns (Bio-Rad Laboratories, Richmond, Calif.) containing 1 ml Dowex 50–W (100 to 200 mesh) cation exchange resin layered over 1 ml Amberlite IRA–45 (16 to 50 mesh) anion exchange resin. Each resin was extensively washed in distilled water and equilibrated with methanol:water (MW, 1:1) prior to use. The aqueous phase was applied to these columns and soluble carbohydrates eluted with 4 ml of MW. Eluate was evaporated to dryness under a vacuum at 40C. The residue was dissolved in 2 ml HPLC water and passed through a 0.45 μm membrane filter (Gelman, Ann Arbor, Mich.).

SOLUBLE CARBOHYDRATE ANALYSIS

Soluble carbohydrate analysis was conducted by injecting samples into a HPLC system (LKB 2150 pump, Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) using a Rheodyne (Cotati, Calif.) 7010 injector equipped with a 20-μl sample loop. The system was operated at 0.6 ml $\cdot$ min^{-1} using degassed HPLC water as the mobile phase. HPLC water was prepared from glass distilled water, using a Waters (Bedford, Mass.) Norganic ion exchange cartridge followed by vacuum filtration through a 0.45-μm membrane. Soluble sugars were separated using a Bio-Rad HPX–87C stainless steel column (300 × 7.8 mm i.d.) maintained at 85C with a Bio-Rad column heater. A guard column was used at room temperature between the injector and analytical column. Refractive index

(Knauer 198 Rainin Instrument Co. Inc., Woburn, Mass.) was used for detection, and carbohydrate retention times were determined using a Hewlett Packard (Avondale, Pa.) 3390A integrator. Quantitation was based on peak area from the integrator. Equal mass carbohydrate standards had identical peak areas.

STARCH DETERMINATION

Starch was estimated from dried tissue remaining in the pasteur pipets following soluble carbohydrate extraction. Each pipet, was broken off, and the remaining pipet portion with tissue residue placed into 13 × 100 mm test tubes. Each sample received 4.0 ml of Na-acetate buffer (0.1 M, pH 4.2) then placed in a boiling water bath for 20 minutes to gelatinize the starch (Haissig and Dickson, 1979). After cooling to room temperature, 1.0 ml of amyloglucosidase solution (Sigma Chemical Co. St. Louis, Mo., No. A–7255) (50 units $\cdot$ ml^{-1} in 0.1 M pH 4.2 Na-acetate buffer) was added to each sample. Samples were incubated 48 hours at 50 to 55C, with periodic agitation. A glucose oxidase method (Miller and Langhans, 1989b) was used for glucose determination on an aliquant or dilution of this mixture. A 5.0 ml volume of ice-cold enzyme solution containing 25 units glucose oxidase (No. G-6125), 5 units peroxidase (No. P-8125), and 0.2 mg o-dianisidine (No. D-3252) in 0.1 M pH 6.0 Na-phosphate buffer was added to a 100 μl fraction and incubated for 30 minutes in a 35C water bath. After incubation, 1.0 ml of 2.2 N HCl was added to stop the reaction and stabilize the colored product. Absorbance was measured at 450 nm using a Beckman DU 64

spectrophotometer (Beckman Instruments Inc., Fullerton, Calif.). Glucose standards prepared in distilled water were used for the calibration line. Tissue glucose content was multiplied by 0.9 to obtain the starch content.

ETHEPHON SOLUTIONS

Ethephon solutions were made using Florel (a.i. 2–chloroethylphosphonic acid, Union Carbide, Research Triangle Park, N.C.) which contains 39.5 g a.i./l Solutions used in 1988 experiments contained 1 drop of dishwashing detergent per 100 ml. Solutions used in 1989 experiments contained 0.1% Tween–20 as a surfactant.

SILVER THIOSULFATE (STS) SOLUTIONS

Stock solutions were made as described by Reid et al. (1980). Stock solutions consisted of $AgNO_3$ (0.1 M) and $Na_2S_2O_3$ (0.1 M) and were stored in dark bottles at room temperature. Dilute STS was prepared on the day of application by adding appropriate amounts of each stock to distilled water and then adding the $AgNO_3$ solution to the $Na_2S_2O_3$ solution to prevent Ag^+ precipitation. A molar ratio between silver and thiosulfate of 1 to 4 was used in all experiments. Solutions contained 1 drop of detergent per 100 ml in 1988 and 0.1% Tween–20 in 1989 as surfactants.

EXPERIMENTS

Plants for all experiments were selected for uniformity, using developmental stage and height as selective criteria.

EXPT. 1 (1988).

To determine if carbohydrate deprivation increases sensitivity to ethylene as determined by bud abortion, plants were given the following treatments: 0 or 92.5% irradiance reduction and 0, 2.07, or 4.15 mM ethephon in a factorial design. Ten plants were used per treatment. Irradiance reduction was achieved by suspending 3 layers of shade cloth over and around the bench on which the plants were placed. Irradiance reduction treatments started at visible bud + 2 weeks, and lasted 14 days. The day the plants were removed from the irradiance reduction, plants were given the appropriate ethephon treatment by spraying 10 ml of ethephon solution on the flower buds and upper 1/4 of the plant canopy. Control plants received water + surfactant. Plants were observed daily for bud abortion after application of ethephon treatments until anthesis. Total number of flower buds and number of aborted flower buds were recorded, and percent bud abortion was calculated for each treatment.

A destructive harvest was performed to determine soluble carbohydrate and starch concentrations in leaves and buds at the end of the irradiance reduction treatment.

All data were analyzed as a randomized complete block design with one

replication of irradiance treatment using Costat (CoHort Software, Berkeley, Calif.).

EXPT. 2 (1988).

To determine the effect of STS on ethephon efficacy, plants received the following treatments: 0, 2.07, or 4.15 mM ethephon, and 0, 1, or 2 mM STS in a factorial design with 10 plants per treatment. STS was applied at visible bud + 2 weeks. Two days after STS treatment, ethephon was applied as described previously. Plants were observed daily for bud abortion for each treatment until anthesis. Total number of buds and number of aborted buds per plant were recorded. Statistics were conducted on arcsine transformed data of percent bud abortion per plant. Data were analyzed in a complete randomized design using Costat (CoHort Software, Berkeley, Calif.).

EXPT. 3 (1989).

Irradiance was reduced by using 1 layer of 70% shadecloth. Photosynthetic photon flux was monitored throughout the experiment for each irradiance treatment using LICOR LI-190SA Quantum Sensors connected to a LICOR LI-1000 Data Logger. Irradiance levels were 16.8 and 5.01 $M \cdot day^{-1}$ for full irradiance and irradiance reduction, respectively. Irradiance levels were recorded using The experimental design was a randomized complete block split plot design with irradiance being the main plot and ethephon the sub plot. On 29 Feb., ten

plants in each of three blocks received the following treatments: 0 or 70% irradiance reduction and 0, 1.38, or 2.76 mM ethephon in a factorial design. The irradiance treatment period lasted 14 days and began at visible bud + 2 weeks (29 Feb.). Irradiance treatments were terminated on 14 Mar. and ethephon treatments were applied. After ethephon application, plants were observed for bud abortion daily until anthesis. Five plants from each irradiance treatment were destructively harvested on 14 Mar. and tissue analyzed for carbohydrate concentration. Data were analyzed using SAS (SAS Institute Inc., Cary, N.C.).

EXPT. 4 (1989).

Ten plants each received the following treatments: 0, 1, or 2 mM STS and 0, 1.38, or 2.76 mM ethephon in a factorial design. STS was applied at visible bud + 3 weeks on 9 Mar. On 11 Mar., ethephon sprays were applied and plants were observed for bud abortion daily in each treatment until anthesis. Total number of buds per plant and number of aborted buds were recorded. Statistics were run on arcsine transformed data of percent bud abortion per plant. Data were analyzed in a complete randomized design using Costat.

EXPT. 5 (1989).

To determine the length of effectiveness of STS sprays on Easter lily, plants were treated with STS at three growth stages: 1) flower bud initiation (FBI), 2) visible bud (VB), or 3) visible bud + 3 weeks (VB + 3 weeks) using 0,

1, or 2 mM STS. FBI, VB, and VB + 3 weeks occurred 25 Jan., 21 Feb., and 5 Mar., respectively. At visible bud + 4 weeks (12 Mar.), plants were treated with 0, 1.38, or 2.76 mM ethephon. Fifteen plants were used per treatment. Plants were observed daily for bud abortion. Total number of buds per plant and number of aborted buds per plant were recorded. Statistics were conducted on arcsine transformed data of percent bud abortion using Costat.

PRELIMINARY ETHYLENE EXPT. (1989).

Preliminary experiments were conducted with ethylene gas in a static system. Low ethylene concentrations were established in sealed 60 × 60 × 60 cm (210 liter) plexiglass chambers by injecting pure ethylene. Typically ethylene concentration decreased 10-fold, from 5 ppm to 0.5 ppm over a twelve hour period. Plants were exposed to 0, 0.25, 0.5, or 1 ppm ethylene gas for a twelve hour period. These concentrations represented initial (maximum) values. Based on the ethylene loss rates described above, actual ethylene levels in the box were somewhat less. Ethylene decay rates and concentrations were determined by injecting samples into a gas chromatograph (Tracor 550, Tracor Westronics, Fort Worth, Texas) equipped with a flame ionization detector. A stainless steel column (1.8 m × 0.22 cm i.d.) was used for separation and filled with porapak N, 80/100 mesh (Alltech Assoc. Inc., Deerfield, Il.). The column temperature was 100C and N_2 was used as the carrier gas.

RESULTS

EXPT. 1 (1988).

Both 92.5% irradiance reduction and ethephon treatments increased bud abortion in *Lilium longiflorum* (Table 1). Due to a malfunction of teh freezedrier, carbohydrate analyses for this tissue could not be reliably reported. As ethephon levels increased from 0 mM to 4.15 mM, bud abortion increased in full irradiance. In the irradiance reduction treatment, percent bud abortion increased, then leveled off as ethephon increased from 0 to 4.15 mM. At 2.07 mM ethephon, there was more bud abortion on plants receiving reduced irradiance than on plants in full irradiance (Table 1). The buds that did not abort were longer than tose that did abort. In all experiments, larger buds usually opened prematurely in response to ethephon. Elongation of flowers, stamens and pedicels was inhibited by ethephon.

EXPT. 2 (1988).

Ethephon increased the incidence of bud abortion as levels increased from 0 mM to 4.15 mM and STS decreased the incidence of bud abortion as levels increased from 0 mM to 2 mM (Table 2.). The ethephon × STS interaction was highly significant (Table 2). It is likely that the highly significant nature of the interaction is due to the great response of STS when ethephon is present, *vesus* when ethephon is absent. With no STS and 4.15 mM ethephon, 100% abortion

Table 1. Percent bud abortion in *Lilium longiflorum* Thunb. 'Nellie White' as influenced by 14 days of 92.5% irradiance reduction and subsequent ethephon application (1988). Irradiance reduction began at visible bud + 2 weeks. Ethephon was applied at the termination of irradiance reduction.

Ethephon	Percent bud abortion	
	Natural irradiance	Irradiance reduction (92.5%)
0 mM	0[z]	3.3
2.07 mM	38.6	60.1
4.15 mM	53.7	53.8
Ethephon	***[y]	
Irradiance	*	
Eth × Irr	NS (.0739)	

[z]Means based on 10 plants per treatment.
[y]Treatment effect nonsignificant (NS), or significant at $0.05 \geq \alpha > 0.01$ (*), or $\alpha \leq 0.001$ (***), respectively.

Table 2. Ethephon-induced flower bud abortion inhibited by silver thiosulfate (STS) in *Lilium longiflorum* Thunb. 'Nellie White'. STS was appied at visible bud + 2 weeks. Ethephon application was two days later.

STS concentration	Ethephon concentration		
	0 mM	2.07 mM	4.15 mM
	Percent bud abortion		
0 mM	0[z]	73.6	100
1 mM	0	20.8	67.6
2 mM	0	3.3	54.0
Ethephon	***[y]		
STS	***		
Eth × STS	***		

[z]Means based on 10 plants per treatment.
[y]Treatment effect significant at $\alpha \leq 0.001$ (***).

was observed (Table 2.), and 2 mM STS reduced abortion by almost 50%.

EXPT. 3 (1989).

Irradiance reduction of 70% for 14 days did not affect bud abortion but ethephon increased bud abortion significantly (Table 3). Both irradiance treatments showed about 60% bud abortion at the highest ethephon concentration of 2.76 mM (Table 3). Total carbohydrate concentrations in both leaves and buds were reduced by irradiance reduction (Table 4).

EXPT. 4 (1989).

In this repeat of the 1988 experiment, the interactive effect of ethephon and STS on flower bud abortion was highly significant (Table 5). As in the earlier experiment it is felt that the interaction is probably due to the lack of an STS effect in the abscence of ethephon. This experiment was conducted at visible bud + 3 weeks while experiment 2 was conducted one week earlier in the developmental stage (visible bud + 2 weeks). This is reflected in the fact that , in Experiment 2, the younger plants treated with 2.07 mM ethephon had more bud abortion than older plants treated with 2.76 mM in 1989. Plants treated with 2.76 mM ethephon showed 69% bud abortion which was reduced to 12.7% in the presence of 2 mM STS. Plants treated with 1.38 mM ethephon showed 49% abortion which was reduced to 2.9% by 2 mM STS (Table 5).

Table 3. Bud abortion in *Lilium longiflorum* Thunb. 'Nellie White' as influenced by 14 days of 70% irradiance reduction and subsequent ethephon application (1989). Irradiance reduction began at visible bud + 2 weeks. Ethephon was applied at termination of the irradiance reduction regime.

Ethephon		Natural irradiance	Irradiance reduction (70%)
0 mM		2.1[z]	3.8
1.38 mM		49.3	50.8
2.76 mM		56.7	61.4
Ethephon	***[y]		
Irradiance	NS		
Eth × Irr	NS		

[z]Means based on 30 plants per treatment.
[y]Treatment effect nonsignificant (NS) or significant at $\alpha \leq 0.001$ (***).

Table 4. Total carbohydrate concentration ($mg \cdot g^{-1}$ dry weight) of leaves and buds of *Lilium longiflorum* Thunb. 'Nellie White' after 14 days of 70% irradiance reduction[z].

	Total carbohydrate conc. ($mg \cdot g^{-1}$ dry wt.)	
Treatment	Leaves	Buds
Full irradiance	305[y]	264
Irradiance reduction	264	214
Significance	**[x]	**

[z]Total carbohydrate is the sum of sucrose, glucose, fructose and starch.
[y]Means based on 5 plants per treatment.
[x]Treatment effect (in columns) significant at $0.01 \geq \alpha > 0.001$ (**).

Table 5. Ethephon-induced flower bud abortion inhibited by silver thiosulfate (STS) in *Lilium longiflorum* Thunb. 'Nellie White'. STS was applied at visible bud + 3 weeks. Ethephon application was made two days later.

	Ethephon (mM)		
STS	0	1.38	2.76
	Percent flower bud abortion		
0 mM	0[z]	49.0	69.0
1 mM	0	1.3	26.8
2 mM	0	2.9	12.7
Ethephon	***[y]		
STS	***		
Eth × STS	***		

[z]Means based on 10 plants per treatment.
[y]*** Treatments significant at $\alpha \leq 0.001$.

EXPT. 5 (1989).

All treatments and interactions affected flower bud abortion. The three way interaction between time, STS and ethephon in Table 6 can be partially explained as previously mentioned. In the absence of ethephon, STS had no effect while in the presence, ethephon efficacy was reduced. This most likely resulted in the significance of the interaction between STS and ethephon. However, at the FBI stage, STS did not affect ethephon efficacy. This is most likely the basis of the interaction between time and the other two factors. Since STS did not reduce ethephon efficacy at the FBI stage but did at the later two developmental stages, the interaction was found to be significant.

PRELIMINARY ETHYLENE EXPT. 1989.

Plants exposed to a maximum of 1 ppm ethylene gas for twelve hours showed 48% flower bud abortion while plants exposed to 0.5 ppm showed 17% abortion. No flower bud abortion was observed in the presence of 0.25 ppm ethylene or the controls.

Table 6. Duration of STS effectiveness against ethephon-induced bud abortion in *Lilium longiflorum* Thunb. 'Nellie White'. STS was applied at three growth stages: 1) flower bud initiation (FBI), 2) visible bud (VB), or 3) visible bud + 3 weeks (VB + 3). Ethephon was applied at VB + 4 weeks.

	Developmental stage when STS was applied								
	FBI			VB			VB + 3		
	Ethephon concentration (mM)								
STS	0	1.38	2.76	0	1.38	2.76	0	1.38	2.76
	Percent flower bud abortion								
0 mM	0.8[z]	39.7	55.6	0.8	38.9	60.9	0	32.8	61.3
1 mM	1.1	39.8	55.9	0	13.9	39.4	0.9	3.1	6.3
2 mM	0	31.9	59.4	0	5.5	23.4	0.8	0.8	0.8

Time	***[y]
Ethephon	***
STS	***
Time × STS	***
Time × Eth	***
STS × Eth	***
Time × Eth × STS	***

[z]Means based on 15 plants per treatment.
[y]Treatment effect significant at $\alpha \leq 0.001$ (***).

DISCUSSION

The literature reviewed indicates that several sources of ethylene exist in commercial greenhouses. Exhaust from gas-fired heaters can produce damaging levels of ethylene if the heaters are not properly adjusted and installed. Urban and industrial areas can contain substantial amounts of ethylene from vehicular and industrial emissions. This ethylene can enter greenhouses through venting and cause damage. The plants themselves are sources of ethylene from wounds (Abeles, 1973) or waterlogging (Bradford and Yang, 1980a, 1980b) of the root system.

Southern Arizona is an ideal area to conduct these experiments. Due to the high natural light, various irradiance levels can be achieved through shading. The opposite situation exist in other areas: photosynthetic irradiance must be added (usually by the use of high intensity discharge lighting). This is expensive both in capital investment and in operating cost. Another advantage is the high winter day temperatures that allow for greenhouse venting. The ability to vent the greenhouse in the winter reduces the possibility of ethylene accumulations. In other parts of the country, winter ventilation is severely restricted due to cold outdoor temperatures. Thus, experiments conducted in Arizona eliminate possible ethylene pollution from sources other than ethylene treatments. A third advantage of Arizona again concerns winter ventilation, but from the standpoint of carbohydrate production. With the relatively high rates of winter ventilation,

plants in our greenhouses are much more likely to take advantage of our high irradiance due to the constant addition of CO_2.

Data from the experiments conducted in this thesis support the hypothesis that ethylene could be major cause of Easter lily bud abortion in greenhouses. Ethephon increased bud abortion as shown in Tables 1–3, 5, and 6; damage from ethephon was noted within 7 days after exposure. Irradiance reduction (92.5%) for 14 days increased bud abortion when plants were treated with 2.07 mM ethephon (Table 1). With irradiance reductions of 70%, (i.e. 4.96 ± 0.68 mols per day for 14 days), there were no significant differences in percent bud abortion (Table 3) regardless of ethephon treatment. Apparently, 70% irradiance reduction for 14 days does not result in carbohydrate depletion to the point of enhancing susceptibility to ethephon.

Arizona has high levels of greenhouse irradiance. Measurements taken during the 1989 growing season averaged 14.3 ± 4.4 mol photons per day in January and 16.6 ± 2.5 mol photons per day in March. An irradiance reduction of 70% for 14 days does not compare with the low irradiance levels endured by Easter lilies during the entire growing season in other areas of the country such as New York, the upper midwest and the pacific northwest (Figure 2). A 70% irradiance reduction in Arizona is equivalent to full irradiance in these other areas. Carbohydrate levels in plant tissue of Easter lilies grown in these regions are much lower than those found in Arizona. Miller and Langhans (1989b) reported flower bud total carbohydrate levels of 107.9 mg per g dry weight in

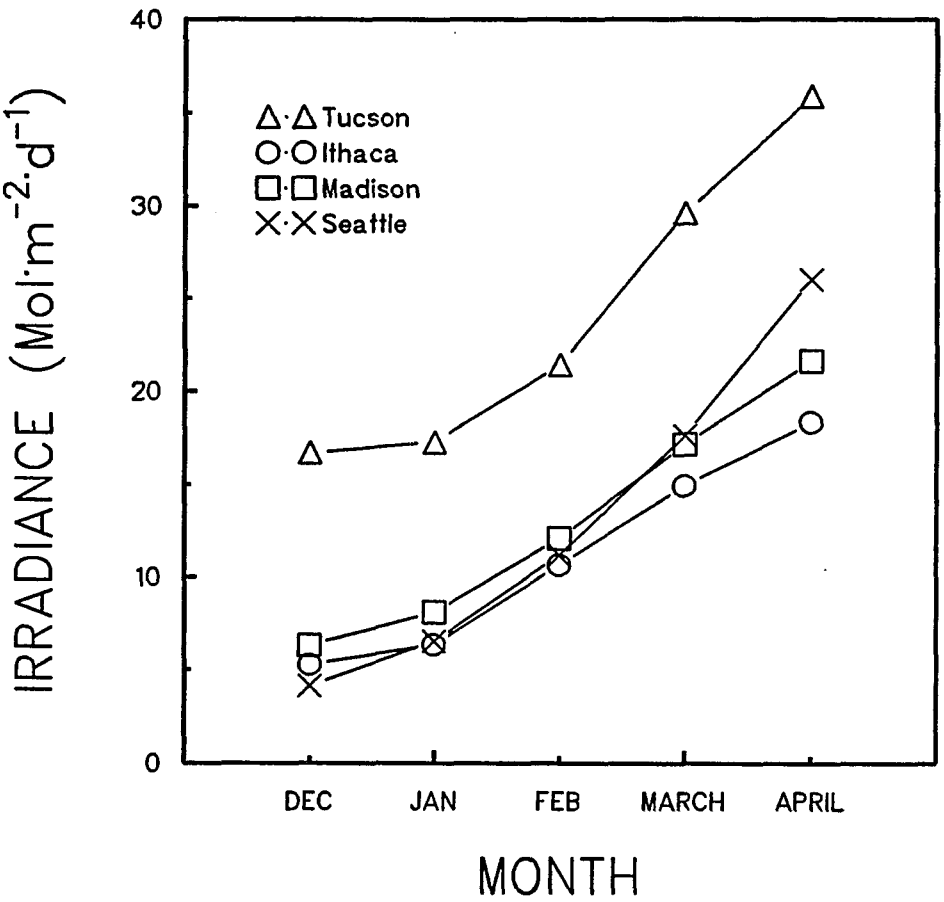

Figure 2. Greenhouse irradiance (mol · m^{-2} · d^{-1} PPF) in Tucson, Ariz., Ithaca, N.Y., Madison, Wis., and Seattle, Wash. Solar radiation was obtained from United States Dept. of Commerce, Environ. Data Serv. (1968) and was converted from langleys to mol · m^{-2} · d^{-1} PPF using conversions from Thimijan and Heins (1983). Greenhouse irradiance was calculated using information from Langhans (1980).

Easter lily plants grown in full irradiance in Ithaca, New York. Values obtained from plants grown under 70% irradiance reduction in Arizona were almost double (214.5 mg per g dry weight, Table 4). A significant difference in percent bud abortion may be observed if the irradiance levels of these other areas were simulated here in Arizona for the duration of the growing season. Carbohydrate levels may then be of more importance in bud abortion.

Silver thiosulfate was effective against ethephon-induced bud abortion, at both concentrations tested (1 and 2 mM), (Tables 2, 5, and 6). With higher levels of STS (4 mM), phytotoxicity was observed in the form of brown spots on the buds and leaves (data not shown). Silver thiosulfate applied early in crop development was less effective in inhibiting ethephon-induced bud abortion compared with later STS applications (Table 6). With early applications of the STS, it is likely that less STS was absorbed by the plant since the leaf surface area was much smaller at this stage. This would lead to increased STS run-off into the soil. Another possibility is that the silver ion was somehow made immobile in leaf tissue during the long period from STS application to ethephon treatment. The use of STS to prevent ethylene injury in the form of bud abortion is both effective and cost efficient on a material basis. Using the price of silver nitrate ($478.95 per 500 g) from Sigma Chemical Co. (St. Louis, Mo. 1989) it was calculated that the silver cost per pot was 0.164 and 0.325 cents for 1 and 2 mM STS treatments respectively.

Prevention of bud abortion, however, should include an understanding of

what causes ethylene and eliminating ethylene sources. Also to be considered is the fact that Ag^+ is a heavy metal and long term use in greenhouse operations could cause potential problems with accumulations from runoff. In southern California, runoff from several large greenhouse operations is already being monitored (Whitesides, 1989). Therefore, eliminating the sources of ethylene should be the main priority in prevention of bud abortion rather than use of Ag^+.

It is interesting to note that ethylene sensitivity of individual Easter lily buds decreased as buds elongated. Adverse effects, such as epinasty, inhibition of stamen elongation and premature anthesis are still observed in later stages of bud development, but bud abortion did not occur. These observations suggest tissue age plays a critical role in determining ethylene sensitivity of buds. Trewavas (1981, 1982) stated that sensitivity to plant growth regulators, such as ethylene, generally refers to the tissues ability to respond to these regulators. Trewavas believes tissue sensitivity to hormones is the limiting factor in the plants physiological or biochemical response rather than the concentration of the hormone. This hypothesis is supported by the fact that, in general, flowers are more sensitive to ethylene at later stages of development than are buds. The molecular basis of these fluctuations in sensitivity is not understood. He feels that it is most likely dependent upon the presence or absence of specific receptor proteins in the various tissues. It is likely that the silver ion interferes with the binding sites for ethylene. Sisler (1982) demonstrated that Ag^+ lowers ethylene binding in extracts of mung bean sprouts. Trewavas (1982) stated that the

specific protein receptors are membrane-located and alterations in factors which change membrane structures will likewise have an effect on the interaction of the growth substance with its receptor. He named several factors which would have this capability such as ion levels and fluxes, electric potentials, pressure, lipids, membrane proteins and hydrophilic molecules. If the presence or absence of carbohydrates have the capability of altering membrane structure, they could also have a possible effect on ethylene action.

Woodson (1987) and Woodson and Lawton (1988) believe that coordination and regulation of petal senescence is related to ethylene-induced gene expression. Woodson (1987) showed that there were changes in protein and mRNA populations after carnation petals were exposed to ethylene. He found that three classes of mRNAs were associated with carnation petal senescence and another class decreased with the climacteric rise in ethylene production that accompanied the beginning of senescence. Several mRNAs appeared to decrease in abundance during petal senescence. Extrapolation of results from these experiments would suggest Easter lily bud abortion is, at least partially, caused by ethylene-induced gene expression.

FUTURE EXPERIMENTS

If future experiments were conducted, plants should be exposed to various ethylene concentrations in a flow through system. A time course study of ethylene exposure would also be conducted. Using time course studies and

several concentrations of ethylene, critical exposure periods and critical concentrations could be determined. These results would be more valid than those obtained with ethephon induced bud abortion as well as being more definitive.

Further research should also be conducted to determine the reason Easter lily buds become less sensitive to ethylene later in their development. After a length of approximately 7.5 cm, ethylene exposure tends to cause earlier and abnormal flowering. The larger the bud is during exposure, the lower the damage is to the bud after the critical developmental stage of around 7.5 cm. One approach to investigate this is to determine whether there is any difference in assimilate partitioning between the different developmental stages of the bud. Carbohydrate levels could be measured on a gram dry weight basis for increasing lengths of buds. This could be followed by another experiment where various size buds would be treated with ethylene rates and exposure times as determined by the previous experiments mentioned. Then, carbohydrate levels could be measured to see if assimilates are diverted from the bud when exposed to ethylene and if that changes as the bud size increases.

Another experiment to determine why buds become less sensitive to ethylene at the later stage is to see if this process occurs after meiosis in the stamens. Durieux et al. (1983) discovered that the critical period for bud abscission in *Lilium* 'Enchantment' coincided with a peak in endogenous ethylene production at the end of the meiotic phase of the stamens.

A long term approach might be to investigate changes in proteins and mRNAs induced by ethylene at various stages prior to anthesis. If mRNA groups are present during early (sensitive) bud development that are not found in later (less sensitive) bud development, it would be concluded that the mRNA groups were associated with ethylene sensitivity.

Although experiments conducted in this thesis do not fully support the hypothesis that a carbohydrate deprivation increases sensitivity to ethylene, I believe it deserves further research. Plants grown for 14 days in 92.5% irradiance reduction showed a significant stimulation of ethephon-induced bud abortion due to the irradiance treatment while plants grown in 70% irradiance reduction for 14 days did not show a significant difference. To better test the hypothesis, Easter lilies would be grown under reduced irradiance for the duration of the crop cycle rather than for only 14 days. The irradiance reduction would be similar to natural irradiance of Oregon, northern California, New York, and Minnesota where many Easter lilies are forced. At visible bud stage, the buds and leaves would be analyzed for soluble carbohydrates and starch to determine the level of carbohydrate deprivation. With a long term irradiance similar to the northern climates, carbohydrate concentrations in lilies should be much lower than ones forced in Arizona under natural irradiance. When these plants are exposed to ethylene in the flow through system, the plants grown in reduced irradiance should exhibit higher percentages of bud abortion when exposed to ethylene.

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