THESIS

EFFECT OF ETHYLENE ON CARNATION KEEPING LIFE

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY <u>LAURA ELIZABETH BARDEN</u> ENTITLED <u>EFFECT OF ETHYLENE ON CARNATION KEEPING</u> <u>LIFE</u> BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE,

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ABSTRACT

EFFECT OF ETHYLENE ON CARNATION KEEPING LIFE

Carnations are susceptible to flower damage when exposed to relatively low ethylene dosages. Experiments conducted at Colorado State University attempted to determine the effect of the following factors on the susceptibility of cut carnations to ethylene injury: 1) ethylene concentration, 2) length of exposure, 3) exposure temperature, and 4) age of flower. Keeping studies were used to evaluate ethylene-induced injury.

Ethylene concentration and length of exposure were evaluated simultaneously by use of a dosage term, ppb-hours. Keeping life correlated closely with dosages expressed in this manner. Beyond threshhold dosage values, keeping life declined as dosage increased. Increasing the exposure temperature decreased both threshhold dosage values and dosages at which keeping life, in relation to control flowers, was reduced to zero. Buds were less susceptible to ethylene injury than open flowers at all exposure temperatures. Sleepiness and subsequent flower collapse characterized severe ethylene injury to open flowers. Outer petal sleepiness and burn characterized severe ethylene injury to buds.

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Results in this study were comparable with results reported previously in the literature. Bud-cutting and refrigeration of cut flowers were recommended to minimize ethylene injury to carnations.

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INTRODUCTION

Ethylene was first recognized in the early 1900's as a physiologically active gas having many growth initiating and modifying effects. It was not until the 1930's, however, that proof of the endogenous production of ethylene led to recognition of its role as a plant growth hormone (6). Today, ethylene is unique in being both a natural plant growth regulator, effective at very low concentrations, and a serious phytotoxic air pollutant (39).

Significance and Objectives

The problem of ethylene-induced plant damage is of importance to carnation growers, wholesalers, and retailers, particularly in urban areas, because carnations are susceptible to flower injury when exposed to relatively low ethylene dosages. Carnations may be damaged by ethylene at any step from the growers' level to the retail sales outlet.

The carnation grower located in or near a metropolitan area faces a two-fold ethylene problem. First, his plants are exposed continuously to ambient ethylene levels higher than those found in rural areas (39). Chronic exposure to these levels may subtly diminish the yield and quality of his crop. Secondly, when large amounts of ethylene are produced in or near the greenhouse, or during severe air pollution episodes, peak ethylene levels may exceed threshholds for acute plant damage (36), resulting in visible injury to carnations and other greenhouse crops.

Urban cut flower wholesalers and retailers also handle carnations in ethylene-polluted air. By the time a cut carnation reaches the consumer level, it may be visibly damaged, or may lose substantial vase life because of "hidden" ethylene damage. Either type of injury represents an economic loss to the carnation industry.

To evaluate the extent of occurrence of ethylene injury to carnations, and take preventive or corrective measures, basic facts concerning the sensitivity of carnations to ethylene must be known. Conditions of temperature, time of exposure, and ethylene concentration at which damage will occur must be defined. Sensitivity to ethylene damage at different developmental stages as well as symptoms characteristic of ethylene injury should be investigated. With these basic facts, the carnation industry can reliably assess the problem. If ethylene-polluted air is causing injury, air quality standards for ambient ethylene can be legislated. Such standards have already been established in California on the basis of ethylene damage to greenhouse crops (36). Ways may be found to minimize or prevent damage to carnations in storage and shipment by maintaining conditions least favorable for the occurrence of ethylene injury.

Previous studies in New York, California, and England provided valuable information on the sensitivity of carnations to ethylene. These studies, however, were based on approximated ethylene dosages (7), small flower samples (28, 34, 35), or on injury evaluation that did not take total keeping life into account (40, 41).

The objective of this study was to provide information on factors affecting the susceptibility of Colorado carnations to ethylene-induced damage. Factors investigated were:

1. The effect of ethylene dosage (exposure to an ethylene concentration over a given period of time) on carnation keeping life.

2. The effect of temperature at which the ethylene dosage is applied on subsequent keeping life.

3. The sensitivity of two carnation flower stages, buds and open flowers, to ethylene-induced injury.

LITERATURE REVIEW

This review surveys the effects of ethylene on carnations and other plants, sources of ethylene, and means of preventing ethylene damage to cut flowers. A section on bud-cut carnations is also included.

Effects of Ethylene

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Crocker and Knight (7), in 1908, were the first to investigate the effects of ethylene on carnations. Initially they investigated the effects of illuminating gas (4% ethylene) because plant damage sometimes occurred when this gas leaked into greenhouses. Ethylene was found to be the only constituent of illuminating gas present in sufficient quantity to be toxic.

Since the 4% level of ethylene in illuminating gas was believed to exceed the amount of ethylene needed to cause flower damage, Crocker and Knight sealed uncut flowers and buds in bell jars containing smaller concentrations of ethylene in air. They found that 1 ppm ethylene for three days could prevent the opening of older buds and kill the youngest. Medium-sized buds were least damaged. Exposure to 500 ppb for 12 hours closed open flowers. Vegetation was undamaged even by the highest ethylene levels. Unable to analytically detect ethylene concentrations below 4.0 ppm (22), early workers relied on serial dilutions to reach reported concentrations.

More recently, Smith and Parker (34) and Smith, et al. (35) reported a significant decrease in vase life of cut carnations exposed to 50 ppb for 48 hours at 18.3° C or 50 ppb for three to four weeks at -0.4°C. Two samples of five flowers each were used for each treatment. Ethylene levels were measured by gas chromatography.

Nichols (28) found that 100 to 200 ppb ethylene for six hours had no visible effect on open carnation blooms, but treatment for 24 hours at these levels caused inrolling of outer petals (sleepiness), from which the flowers slowly recovered. There appeared to be a seasonal variation in sensitivity to ethylene, but 200 ppb for 48 hours consistently resulted in accelerated senescence of cut carnations. All treatments were made at 18.3° C in CO₂-free flowing air. Nichols used three to five flowers per treatment.

Uota (40), in California, reported the threshhold concentration of ethylene causing sleepiness in fresh cut carnations to be 125 ppb for 24 hours at 20[°]C. When blooms were held two days at 5[°]C prior to exposure, this threshhold dropped to 30 to 60 ppb.

In another report, Uota (41) studied the effects of time, temperature, and ethylene level on damage thresholds. He found that 60 ppb ethylene was a damage threshold for carnations exposed one day at 20° C, two days at 10° C, or four days at 5° C. Three days at 10° C

and eight days at 0[°]C both had threshhold values of 30 ppb. Blooms responded to lower levels of ethylene when exposure time was increased. Higher temperatures caused greater damage at ethylene concentrations above the threshhold values of 30 and 60 ppb.

Uota exposed carnations to ethylene in flowing air and, like Nichols (28), used gas chromatography to monitor ethylene levels. Treatments were replicated twice, using 12 to 15 flowers for each replication.

There is marked variation in response among plant species exposed to ethylene. Doubt (13), in 1917, exposed 42 species of plants to illuminating gas and ethylene. Dosages ranged from 100 ppb to 400 ppm ethylene for two or more days. Plants sensitive to ethylene were tomato, castor bean, scarlet sage, Jimson weed, and mimosa. All five showed epinasty, or downward bending of the leaves, at dosages similar to or lower than those causing damage to carnations. Because of their characteristic response to low ethylene levels, these plants were recommended as "indicator plants" to warn of the presence of illuminating gas in greenhouses. Some species of bluegrass and maple showed no response at the highest dosages of ethylene.

Working with several varieties of roses in 1931, Zimmerman, et al. (45) found epinastic responses in young leaves at a minimum dosage of 300 ppb ethylene for 24 hours at room temperature. Mature leaves showed no epinasty, but began abscission after 48 hours of

40 ppm. Young and "middle-aged" leaves were last to abscise. Cut rose buds exhibited accelerated opening and early petal fall after exposure to a minimum of 3 ppm for 24 hours. Bud and leaf abscission were minimal for dosages applied below 10°C. Other symptoms of ethylene damage such as leaf discoloration, stimulation of shoot production, and interference with shoot elongation occurred with minimum dosages much above those causing injury to carnation blooms.

Crocker, et al. (8) exposed 202 species and varieties of plants to varying dosages of ethylene and found epinastic responses in 89. These responses continued only in the presence of ethylene. The most sensitive plants were buckwheat and pigweed, showing epinasty after 12 hours at 50 ppb ethylene, and African marigold, showing epinasty after 24 hours at 50 ppb. Of 38 gases tested, five caused epinastic responses in tomato. These were ethylene, acetylene, propylene, carbon monoxide, and butylene. The latter four required concentrations 500 to 500,000 times that of ethylene to induce similar epinastic responses.

In 1935, Crocker, et al. (6) exposed plants to various levels of ethylene for 48 hours at 25[°]C. Epinastic responses were reported for sweet pea seedlings at 25 ppb ethylene, African marigold at 16 ppb, lupins at 12.5 ppb, and tomatoes at 100 ppb.

Davidson (9) reported in 1948 that Cattleya buds beginning to open were sensitive to dry sepal damage when exposed to 2 ppb ethylene for

24 hours. Severe petal injury and abnormal blossom opening resulted from a dosage of 100 ppb for eight hours. Davidson, like all earlier workers, used serial dilutions to reach these ethylene levels, but could not analytically confirm the concentrations.

Heck and Pires (20) fumigated 114 plants with 2, 5, or 10 ppm ethylene for ten days, then divided the plants into six groups according to recovery rate after exposure. Herbaceous plants, particularly their inflorescences, were most sensitive to ethylene and slowest to recover after exposure. Woody plants were intermediate in response, grasses most resistant. Although these ethylene levels resulted in severe stunting of most of the plants, Heck and Pires stated that ethylene was not a true toxicant, but a physiologically active gas.

A recent survey summarized the effects of ethylene on three broad plant groups (3). In general, broad leaf plants exhibited ethylene injury in the form of leaf epinasty or abscission, stimulation of lateral development, bud abscission, and/or failure of floral structures to open. More resistant broad leaf plants showed only growth retardation and occasional loss of apical dominance. Grasses exhibited growth retardation and increased suckering, although damage was minimal even at high concentrations. Among conifers, needle abscission, retardation of new needle elongation, and poor development or abscission of cones characterized ethylene injury.

Sources of Ethylene

In the early 1900's, illuminating gas was the major source of ethylene causing plant damage (7, 42). Today, motor vehicles are probably the major source of ambient ethylene, particularly in urban regions (36, 39). Auto exhaust is known to contain up to several hundred ppm ethylene (26). Although rural ethylene values average less than 3 ppb (39), peak values of 0.5 to 1.0 ppm ethylene can be expected in some metropolitan areas (36). Most damage from ethylene air pollution occurs in winter during periods of light winds and stable air masses (36).

Other sources of ethylene include burning or decaying organic matter, growth regulators, and improperly vented or adjusted greenhouse heaters (19).

Ethylene is also produced by plants themselves. Denny and Miller (11) and Denny (10) demonstrated that fruit, flower, and vegetative tissue produced volatiles causing epinastic responses in indicator leaves identical to ethylene-induced responses. Differences between tissue types in the ability to cause epinasty were shown to be quantitative, not qualitative.

In 1949, Fischer (16) reported that snapdragons and calceolarias produced a "toxic" gas that caused shelling (flower drop) of those plants. When stored with snapdragons and calceolarias, carnations became sleepy, and sweet pea seedlings showed etiolation, a typical

ethylene response. In a later study, Fischer (17) reported ethylene production by cut orchids, carnations, mums, marigolds, tulips, lilacs, gladiolus, and gardenias. On a short term basis, these flowers produced insufficient ethylene to damage themselves. Better Times roses, larkspur, and dendrobium, however, were visibly affected by self-produced gas.

Nichols (27) measured daily ethylene production by carnations from day of cut to post-senescence. At 18.3°C, Nichols reported a 10 to 30 fold surge in ethylene production at senescence, rising to 200 to 600 ul/flower/hr. At temperatures above 7.2°C, the ethylene surge was accompanied by petal collapse and rapid water loss, after which ethylene production declined. Below 7.2°C, ethylene production remained negligible as flowers slowly lost weight and petals became flaccid.

Diseased or injured tissues are a source of ethylene. Shredded rose and cherry leaves and leaves infested with red spider mite produced more ethylene than normal leaves (44). Virus infected plants were also an ethylene source (31). Carnations became irreversibly sleepy and snapdragons and calceolarias began shelling when confined two days at room temperature with chrysanthemums infected with ray blight fungus (12).

Snapdragon and chrysanthemum rust, chrysanthemum Septoria leaf spot, carnation Alternaria leaf spot, and Botrytis cinerea on mums

and carnations produced moderate amounts of ethylene (43). Chrysanthemums infected with <u>Botrytis</u> produced 0.01 ul/capitulum/hr ethylene, equivalent to an accumulation of 500 ppb in 24 hours (27). Carnations innoculated with <u>Botrytis</u> exhibited a surge of ethylene production much earlier than the normal surge (15, 33).

Prevention of Ethylene Damage

Ethylene damage to carnations and other cut flowers may be prevented by separating flowers from ethylene sources or by altering the immediate environment of the flowers to minimize damage from ethylene already present.

Cut flowers should not be stored with fruit, because fruit produce ethylene at a rate of 0.02 to 200 ul/kg/hr (4). This high ethylene production may damage flowers held with fruit in a confined area. Different flower types should not be stored or shipped in the same box, as sensitive flowers may be damaged by flowers producing large amounts of ethylene (16, 17). Diseased or injured tissues should be removed from the vicinity of cut flowers (12, 15, 19, 27, 33, 43).

If ethylene is ubiquitous in the air, as in a metropolitan area, air may be passed through a brominated charcoal filter to remove ethylene (16). It has been suggested that shipping and storing containers be lined with paper impregnated with mercuric perchlorate $(Hg(CIO_4)_2)$, an effective ethylene absorbant (29). The toxicity and caustic nature of this chemical, however, has precluded its widespread use. Ethylene oxide, a gas described as a metabolic antagonist to flower senescence, reduces ethylene damage if present in the air when flowers are exposed to ethylene (2, 24, 28). Ethylene oxide can also be toxic to flowers, depending on the concentration used.

Increased CO₂ or decreased O₂ levels have proved effective in reducing susceptibility of vegetation and flowers to ethylene damage. Denny (10) noted in 1935 that CO₂ concentrations of 5% could reduce epinastic responses to ethylene. Flower drop of calceolarias and snapdragons in the presence of ethylene was reduced when CO₂ was added to the container (16). Smith, et al. (35) reported that vase life reduction resulting from exposing carnations to 50 ppb ethylene at -0.4 °C for three to four weeks could be eliminated by inclusion of 5% CO₂ in the treatment air. Flowers exposed to 50 ppb ethylene for two to four days at 18.3 °C were undamaged if simultaneously exposed to 2.2% CO₂ (34).

Nichols (28) reported that CO_2 at 2 to 3% could prevent damage when carnations were exposed to 200 ppb ethylene for 48 hours at 18.3°C. The surge of endogenous ethylene occurring at senescence of carnation flowers could be suppressed by CO_2 accumulation or O_2 depletion, the threshold concentration for each being about 4%. Accumulation of respiratory CO_2 delayed but did not prevent ethyleneinduced sleepiness. Uota (40) found that during a 24 hour exposure at 20° C, 10% CO₂ could prevent damage by 250 ppb ethylene, 20% CO₂ could counteract 500 ppb ethylene, and 40% CO₂ could prevent damage by 1000 ppb ethylene. These CO₂ concentrations were damaging, however, causing blueing in petals and bleaching of leaves. Uota found, as did Nichols (28), that higher than normal CO₂ concentrations inhibited endogenous ethylene production by carnations.

Although it is an effective treatment, use of CO_2 to reduce ethylene injury to cut carnations has not been applied commercially. Cut flower packing and storing is usually done in large, open areas where maintenance of high CO_2 levels would be unfeasible. Compact and economical CO_2 generators have not yet been developed for use in shipping boxes or small display cases.

Probably the simplest and most economical means of reducing ethylene damage to cut flowers is to maintain low temperatures during storage and shipment. Carnations and other flowers produced little ethylene and were less susceptible to ethylene damage at temperatures below 7.2°C (17, 19, 27, 41). The suggestion made by Lumsden, et al. (25) in 1940, that carnations would keep best at 0.5 to 2.2°C, is of practical value today.

Bud-cut Carnations

In initial work with bud-cut carnations, Kohl and Smith (23) stated that flowers opened off the plant had quality and vase life similar to those opened on the plant. Buds showing 3/4 to 1 inch of color opened at the same rate as those on the plant, but required a preservative solution to open. Holley (21) reported that after storage for 18 days, the mean life of bud-cut carnations was the same as opencut flowers. Buds most advanced at the time of cutting kept longest; buds showing 1/2 inch or more of color proved most satisfactory.

Cheng (5) found Cornell solution best for opening buds, and Everbloom, a commercial cut flower preservative, best for keeping the flowers. Optimal opening room temperature was 21.1 to 23.9°C. Useful life, flower size, and color of bud-cut carnations equaled that of open-cut flowers. Buds stored dry up to three weeks at 0.5°C kept as well or better than open flowers stored for the same period. Buds withstood the stresses of high temperature or long periods in shipment better than open flowers.

Other advantages of bud-cut carnations included the possibility of bi-weekly harvest, smaller space requirement in storage and shipment, faster cutting and bunching, and the possibility of storing buds and opening them as needed for weekend or holiday demand (1, 21). Differences in flower variety or season did not seem to affect the performance of bud-cut flowers (1). Buds did require careful grading and bunching to open on the same day, as advanced buds opened in one to two days, while tighter buds required three to four days (18).

Since buds withstand stresses better than open flowers (5), buds may be more resistant to ethylene injury. No work has been done in this area, however.

Summary

Carnations belong to a small group of herbaceous plants that are highly sensitive to ethylene injury. Carnation flowers can be damaged by exposure to ethylene produced by motor vehicle exhaust, fruits, flowers, and diseased tissues. Injury can be reduced by removing carnations from ethylene sources, using filters or absorbants, treating with CO_2 , or by reducing storing and shipping temperatures. Although bud-cut carnations require careful grading and use of preservative solutions, they do have advantages in cutting, shipping, and storing. General performance of bud-cut flowers equals or exceeds that of open-cut flowers.

MATERIALS AND METHODS

General

Observations made in exploratory studies helped in the selection of materials and methods used in this study (Appendix I).

Carnations (<u>Dianthus caryophyllus</u> L., cv 'White Sim') grown at Lake Street Research Greenhouses were used for all experiments. Uniform open flowers were cut from first year plants. Buds showing 1/2 in color were cut from second year plants. Flowers and buds, graded fancy and standard, were cut to a 40 cm stem length before treatment with ethylene.

All experiments were conducted between January 16 and March 1, 1972. Because this was a period of low flower production, open flowers from two successive cuts were sometimes combined to provide enough uniform flowers for an experiment. When accumulated, flowers were held in dry storage at 0.5° C for no more than three days. On the day an experiment was to begin, freshly cut and stored flowers were completely randomized. Fresh cut buds were used for all bud experiments.

Definitions

For the purposes of this study, the following terms are defined: Experiment: exposure of 120 buds or flowers to four ethylene levels at a given temperature for a specified amount of time.

<u>Ethylene level</u>: one of four ethylene concentrations used per experiment, thirty flowers being exposed to each level. In order of increasing concentration, Level A was the control, Level B a "low" concentration, Level C a "medium" concentration, and Level D a "high" concentration.

<u>Group</u>: forty flowers taken from a cross section of the ethylene levels, e.g. 10 flowers from each of Levels A, B, C, and D. Each experiment had three groups. Dividing into groups of forty was justified from two standpoints: 1) owing to space limitations, one group may have been exposed to ethylene several days subsequent to the other two groups, and 2) one group may have been exposed to uniformly higher or lower concentrations for reasons to be explained later.

The 14 experiments in the study are listed in Table 1.

Method of Exposure

Open flowers and buds were sealed in airtight chambers for exposure to ethylene in flowing air. The chambers, plastic cylinders 10 cm in diameter and 60 cm long, each held a maximum of 20 flowers or 30 buds. Air was occasionally passed through two chambers joined in series. As a result of leakage, however, ethylene concentrations were usually slightly lower in the second chamber. When two chambers

Experiment	t Flower Temperature Exposure			Ave ppb	per Level		
Number	Stage	°C	Period (days)	A	В	С	D
1	open	1.7	3	5.0	67.2	174.9	227.3
2	open	1.7	5	5.1	63.0	138.2	214.2
3	open	1.7	10	7.0	64.3	170.5	214.5
4	bud	1.7	5	5.1	63.0	138.2	214.2
5	bud	1.7	10	7.0	64.3	170.5	214.5
6	open	10.0	1	8.1	52.7	106.6	142.9
7	open	10.0	2	5.9	58.2	97.0	149.3
8	open	10.0	3	2.9	51.5	121.0	174.1
9	bud	10.0	3	9.0	60.6	112.9	154.1
10	bud	10.0	6	8.2	67.8	110.7	157.3
11	open	21.1	$\frac{1}{2}$	8.4	46.0	96.3	103.0
12	open	21.1	1	6.3	55.8	125.6	165.8
13	bud	21.1	1	3.7	62.5	138.5	188.2
14	bud	21.1	2	7.9	54.6	165.4	175.6

Table 1. Experiments conducted on White Sim carnations between January 16 and March 1, 1972.

were joined in series at each of Levels A, B, C, and D, the result was that group(s) in the first set of chambers were exposed to slightly higher ethylene levels than group(s) in the second set of chambers.

A compressor with an outdoor intake provided air. Flowboards, with appropriate manometers, barostats, and valves, maintained a flow rate of approximately 200 ml/min through each chamber. Prior to the addition of ethylene, air for all four levels was passed through Purafil¹ to reduce the background level of ethylene below an average of 10 ppb. Air was then bubbled through water to increase humidity. One line passed this air directly to Level A (control) chambers. A tank of compressed ethylene-in-air (with an ethylene concentration known to within \pm 1%), equipped with a three-stage regulator and a manifold with three outlet capillaries, metered the required amounts of ethylene into separate air lines for Levels B, C, and D.

Analytical Method

During each treatment, 50 ml air samples were drawn periodically at each chamber outlet to monitor ethylene levels. Intervals between samples ranged from 6 to 24 hours, depending on the length of the experiment. Gas samples were analyzed in a Hewlett-Packard, model 5750b, gas chromatograph with flame ionization detectors.

¹ activated alumina impregnated with potassium permanganate; a product of Borg-Warner Corp., U. S. A.

The chromatograph was equipped for light hydrocarbon analysis following procedures outlined by Stephens and Burleson (37, 38). Air samples were first concentrated by injection into a 1/8-in OD stainless steel sample loop packed with 10% dimethyl sulfolane on 42/60 mesh C-22 firebrick and submerged in liquid oxygen. The air sample was simultaneously volatized at 0° C and injected into a column (1.52 m long, inside diameter 2.38 mm) packed with 100/120 mesh poropak N. Nitrogen flowing at 80 ml/min was used as a carrier gas in this column. Column temperature was maintained isothermally at 60° C. Detector temperature was 210° C.

The amount of ethylene in each sample was determined by measuring peak height. To calibrate the analyzer, samples from a pressure cylinder containing 1.18 ppm (\pm 1%) ethylene were diluted in 100 ml hydrocarbon-free air (less than 0.1 ppm total hydrocarbons), then injected into the chromatograph using the regular analytical procedure. By plotting known ppb against scale deflection for each sample, calibration charts were drawn up. A variation of \pm 5% could be expected with identical air samples run in succession.

Sensitivity of the chromatograph seemed to vary over the experimental period. Because the ethylene metering system remained unchanged for all 14 experiments (capillary lengths, pressures, and flow rates remained the same), one set of calibration charts, obtained at the end of February, was applied to all air sample data.

Calculation of Dosage

Between and within experiments, the ppb ethylene for all four levels fluctuated because of changes in factors such as barometric pressure and outside temperature. At the highest concentration, peak values at times exceeded the average value by 30 ppb. To correct for fluctuations, and allow direct comparison of the different levels, a dosage term, ppb-hours, was derived by plotting ppb ethylene against hours of exposure for each of Levels A, B, C, and D within groups. Integrating the area under each curve with a planimeter yielded ethylene dosage in ppb-hours. Dividing the total dosage by hours of exposure gave average ppb values for each group. Experiment dosages are indicated in Table 2.

Keeping Life Determinations

After exposure to ethylene, open flowers and buds were moved immediately to a keeping room where stems were recut. Each replication of ten flowers was placed in a glass jar containing one liter of Cornell solution (4% sugar, 50 ppm $AgNO_3$, 200 ppm HQC in distilled water) (32). Keeping solutions were not replenished during a trial. The keeping room was maintained at 21.1°C, $\pm 2°C$, at a relative humidity of 35 to 45%. Air in the room was recirculated through a Purafil filter to maintain ambient ethylene levels at 10 ppb or less.

Flowers were checked every 24 hours for symptoms of petal burn or wilt, and were discarded at the first sign of senescence.

Experiment	Group No.		Dosage	(ppb-hours)	
No.	-	Level A	Level B	Level C	Level D
1	1,2	392.0	4,728.0	14,192.0	17,000.0
	3	304.0	5,048.0	9,392.0	15,096.0
2,4	1	672.0	7,896.0	17,112.0	25,880.0
	2	672.0	7,184.0	16,176.0	25,168.0
	3	504.0	7,600.0	16,488.0	26,056.0
3,5	1	1,536.0	15,360.0	42,864.0	50,304.0
	2	1,680.0	14,736.0	41,976.0	49,032.0
	3	1,848.0	16,224.0	37,920.0	55,08 0.0
6	1,2	238.4	1,249.6	2,192.0	3,041.6
	3	102.4	1,291.2	3,294.4	4,204.8
7	1,2	348.0	2,776.0	4,460.0	6,828.0
	3	160.0	2,824.0	5,040.0	7,848.0
8	1,2	160.0	3,616.0	9,096.0	12,832.0
	3	304.0	3,888.0	7,944.0	11,936.0
9	1, 2, 3	648.0	4,360.0	8,128.0	11,096.0
10	1, 2, 3	1,184.0	9,760.0	15,936.0	22,656.0
11	1,2	100.8	552.0	1,156.0	1,236.8
12	1,2	152.0	1,355.2	3,017.6	4,035.2
	3	150.4	1,308.8	3,011.2	3,868.8

Table 2. Dosages for Experiments 1 to 14.

Table 2 (continued)

Experiment	Group No.		Dosage (ppb-hours)	
No.	10.02	Level A	Level B	Level C	Level D
13	1, 2	73.6	1,523.2	3,332.8	4,529.6
	3	115.2	1,451.2	3,305.6	4,494.4
14	1,2	356.0	2,652.0	8,208.0	8,628.0
	3	424.0	2,560.0	7,396.0	8,032.0

Vase life for open flowers was the total number of days from placement in solution to one day before being discarded. For buds, vase life was the total number of days from opening (circle of outer petals perpendicular to stem) to one day before being discarded.

To express keeping life on a relative rather than absolute basis, the vase life of the ten flowers in each of Levels A, B, C, and D within a group was first averaged. The averages of Levels B, C, and D within a group were then expressed as a percent of Level A, the control.

All regression data were analyzed on a Hewlett-Packard calculator, model 9810, in conjunction with a Hewlett-Packard plotter, model 9862a. The calculator was programmed for analysis and plotting of polynomial regressions according to procedures outlined by Draper and Smith (14).

RESULTS AND DISCUSSION

Data from separate experiments were combined to give a comprehensive view of the response of carnations to ethylene over wide dosage ranges. Experiments were pooled according to stage of flower development and exposure temperature (Table 3). By pooling data, keeping life could be simultaneously evaluated for short period, high concentration exposures, or long period, low concentration exposures having similar dosage values. Dosages and keeping lives for all experiments are listed in Appendix II.

Experiments Pooled (cf Table 1, p.18)	Flower Stage	Exposure Temp. (^o C)		
1, 2, 3	open	1.7		
4,5	bud	1.7		
6,7,8	open	10.0		
9,10	bud	10.0		
11, 12	open	21.1		
13, 14	bud	21.1		

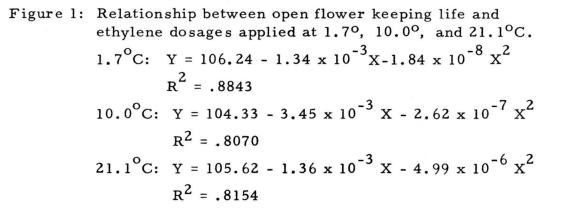
Table 3. Pooling of experimental data according to stage of flower development and exposure temperature.

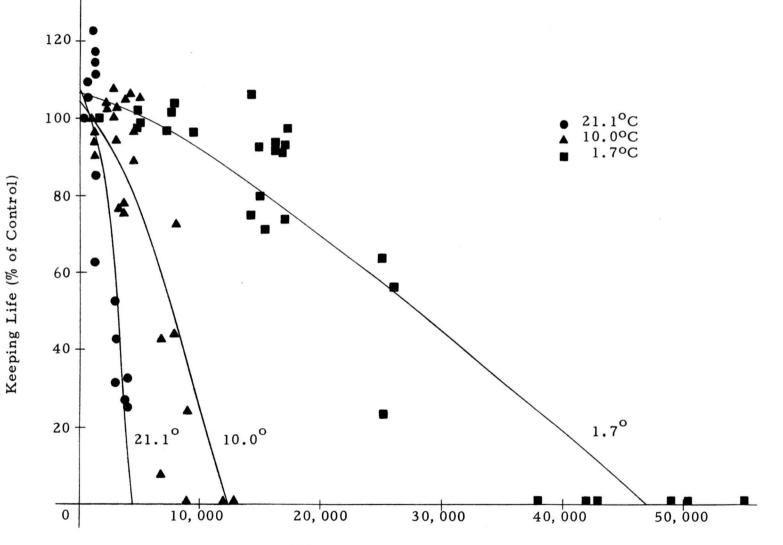
When keeping life was plotted against dosage for pooled experiments (Fig. 1, 2, 4, 5, 6), control (Level A) data points fell near the Y intercept, since control keeping life was always expressed as 100% and control dosages were very low. Control keeping life actually varied from 4.6 to 7.9 days for open flowers, and from 4.4 to 10.5 days for buds, largely as a result of temperature and length of exposure during ethylene treatment. Expressing keeping life as a percent of the control within groups removed much of this variability. Control dosages varied according to background ethylene levels, but always fell well below damaging levels.

Effect of Temperature

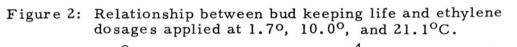
Exposure temperature caused substantial differences in the ethylene dosage-keeping life relationship of open flowers (Fig. 1). To reduce keeping life to 0.0% of the control (X intercept of regression) at an exposure temperature of 21.1°C, a dosage of approximately 4,400 ppb-hours was required. At 10.0°C, however, 12,000 ppb-hours were necessary to cause the same loss of keeping life. To reduce open flower keeping life to 0.0% at 1.7°C, 47,000 ppb-hours had to be applied. The dosage causing zero vase life at 1.7°C was therefore over ten times higher than the dosage causing zero vase life at 21.1°C.

A similar temperature-regulated relationship occurred between bud keeping life and ethylene dosage (Fig. 2). There was nearly a three-fold increase in zero-life dosage between buds exposed at 21.1° C and those exposed at 10.0° C (8,400 vs 25,000 ppb-hours). At 1.7° C, no keeping life decrease was recorded for buds, even at the highest dosage applied (55,080 ppb-hours). These results indicate



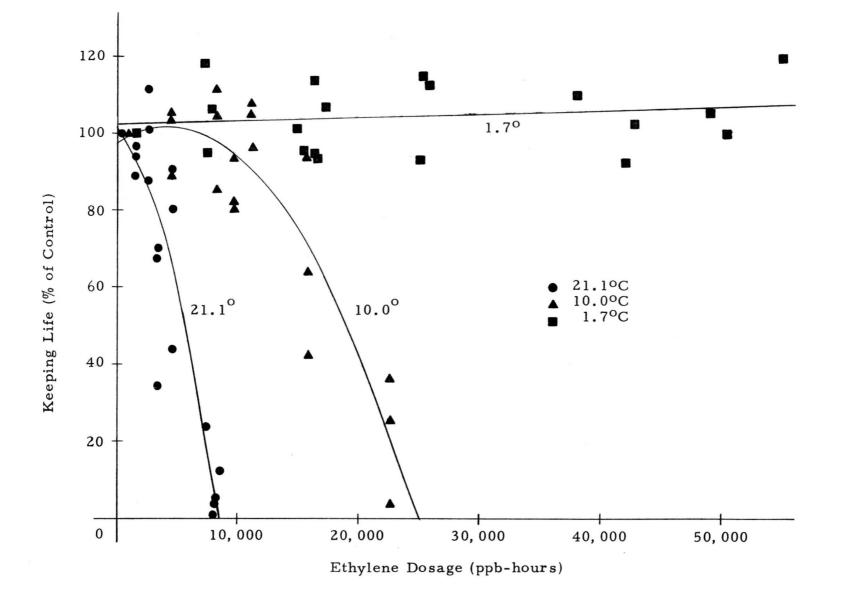


Ethylene Dosage (ppb-hours)



1.7°C:
$$Y = 101.07 + 1.12 \times 10^{-4} X$$

 $R^{2} = .0572$
10.0°C: $Y = 97.68 + 1.99 \times 10^{-3} X - 2.36 \times 10^{-7} X^{2}$
 $R^{2} = .8200$
21.1°C: $Y = 101.44 - 3.24 \times 10^{-3} X - 9.99 \times 10^{-7} X^{2}$
 $R^{2} = .9055$



that buds are comparatively insensitive to ethylene applied at temperatures just above $0^{\circ}C$.

Each zero-life dosage reported above could result from different combinations of ethylene concentration and exposure period. For example, 8,400 ppb-hours, causing 0.0% keeping life for buds exposed at 21.1°C, might represent 200 ppb for 42 hours or 150 ppb for 56 hours. Keeping life was found to be a function of dosage, however, irrespective of the time and concentration factors comprising that dosage.

The regression lines best representing keeping life response of open flowers and buds to ethylene were curvilinear in all but one case. (For regression analysis of variance, see Appendices III through VIII). Thus, keeping life of flowers exposed at 1.7° , 10.0° , and 21.1° , and buds exposed at 10.0° and 21.1° C, did not decrease at the same rate for each additional dosage increment. Dosage ranges were too large and data points too few, however, to detect specific threshold values where keeping life began a substantial decline from 100%, or specific points where keeping life decreased most rapidly. The regression of keeping life on dosage was not significant for buds exposed at 1.7° C (Appendix VIII).

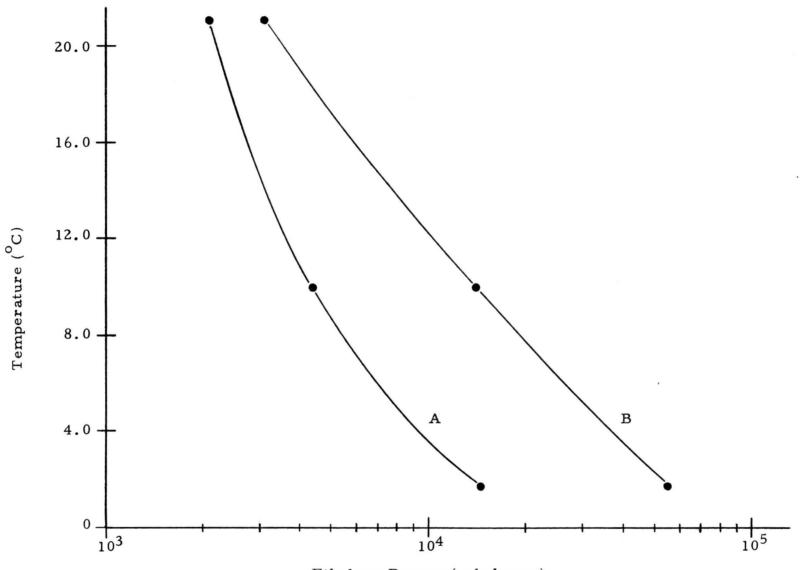
Because threshhold values could not be quantitatively defined, a 20% decrease in keeping life was arbitrarily chosen as a "significant" change from control keeping life, and the corresponding ethylene

dosage became the threshhold value. Dosages corresponding to 20% loss of keeping life were plotted against temperature (Fig. 3) to provide two types of information: 1) For a given dosage, exposure temperature needed to cause 20% keeping life decline for buds or open flowers. The temperature needed to damage buds was higher than the temperature for open flower damage at the same threshhold dosage. As dosages were increased, exposure temperature required for a 20% loss decreased. 2) For a given temperature, dosages at which buds and open flowers would lose 20% keeping life. Buds had to be exposed to higher threshhold dosages at all temperatures to cause the same keeping life decline.

Effect of Flower Age

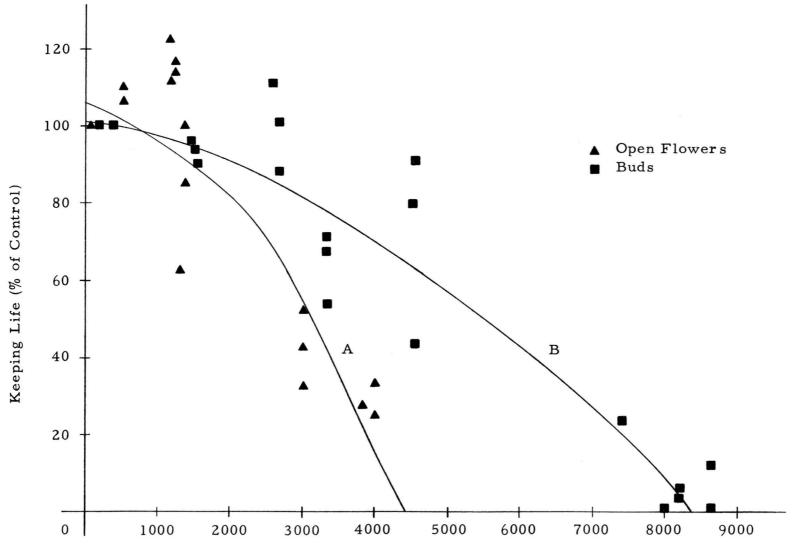
The dosages required to reduce keeping life to 0.0% of the control were higher for buds than for flowers. At exposure temperatures of 21.1° and 10.0° C, dosages corresponding to 0.0% bud keeping life were nearly twice as high as dosages required for 0.0% vase life in open flowers at the same temperature (Fig. 4, 5). Buds exposed to 55, 080 ppb-hours at 1.7° C had an average keeping life equivalent to control buds. Open flowers showed 0.0% vase life after exposure to 47, 000 ppb-hours at the same temperature (Fig. 6). In general, carnation buds withstood higher ethylene concentrations, longer exposures, and higher temperatures better than open flowers under similar conditions.

Figure 3: Relationship between temperature and ethylene dosage at which 20% loss of carnation keeping life occurred. Line A represents open flowers; Line B represents buds.



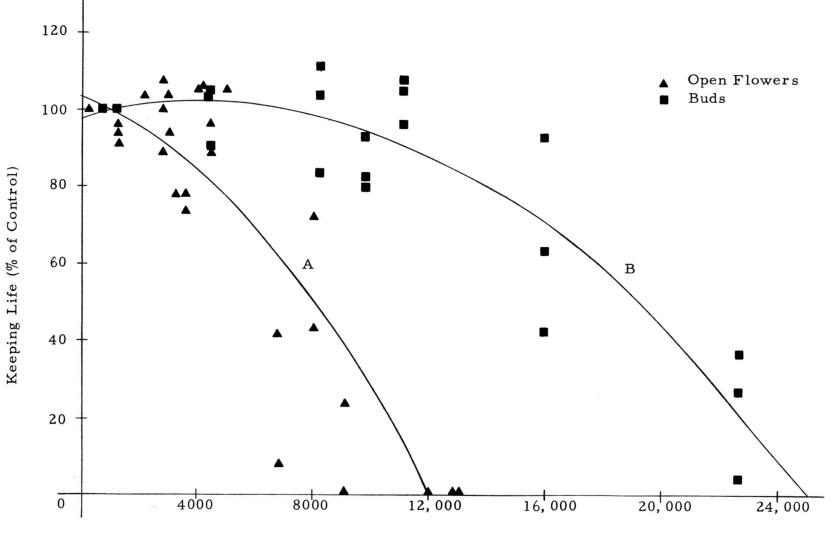
Ethylene Dosage (ppb-hours)

Figure 4: Relationship between carnation keeping life and ethylene dosage applied at 21.1°C. Line A represents open flowers; Line B represents buds.



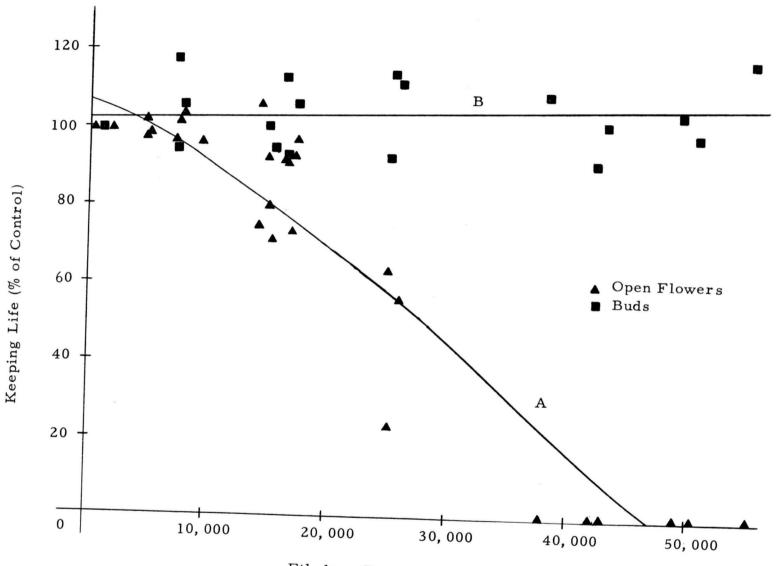
Ethylene Dosage (ppb-hours)

Figure 5: Relationship between carnation keeping life and ethylene dosage applied at 10.0°C. Line A represents open flowers; Line B represents buds.



Ethylene Dosage (ppb-hours)

Figure 6: Relationship between carnation keeping life and ethylene dosage applied at 1.7°C. Line A represents open flowers; Line B represents buds.



Ethylene Dosage (ppb-hours)

High ethylene dosages caused different damage symptoms in buds and open flowers at all exposure temperatures. Flowers having a keeping life of 0.0% were visibly sleepy when removed from exposure chambers after an experiment. All petals, both outer and inner, were curled and slightly yellow. After 24 hours in the keeping room, entire inflorescences collapsed and turned brown. The striking difference between control and ethylene-damaged flowers can be seen in Figure 7.

When buds were discarded on or before the day after opening, as indicated by a keeping life of 0.0%, the usual symptom was severe outer petal burn (Fig. 8). When removed from the chambers after exposure, these petals were sleepy. Burning usually appeared before the bud opened. Since inner petals were unaffected, the flowers appeared uninjured if outer petals were removed. Damaged buds opened normally and at the same rate as other buds.

Open flowers or buds not damaged by ethylene showed symptoms of petal burn or wilt within 13 days. These symptoms showed up earlier in flowers and buds partially affected by ethylene. Buds usually took one to three days to open. Bud controls in general had vase lives averaging over two days longer than open flower controls (8.6 vs 6.2 days). Leaves and stems were undamaged in all experiments.

Effect of Altitude on Dosage

Ethylene concentrations used in this study were established and evaluated at an altitude of 5,000 ft. (.836 atm) under variable



Figure 7. Open flowers exposed to ethylene for 10 days at 1.7°C (Group 3, Experiment 3). From left to right, average ethylene concentrations were 7.7 ppb, 67.6 ppb, 158.0 ppb, and 229.5 ppb. Photograph taken two days after exposure.



Figure 8. Buds exposed to an average of 179.8 ppb ethylene for 48 hours at 21.1°C (Experiment 14). Photograph taken three days after exposure.

temperatures. These data should be corrected to standard temperature and pressure $(1.0 \text{ atm}, 25^{\circ}\text{C})$ or conditions matching those of other studies if valid comparisons of results are to be made.

Data reported in relative units of ppb_v (parts per billion by volume) at this altitude can be corrected to sea level by using the formula:

$$P_1V_1 = P_2V_2$$

where $P_1 = 1.0$ atm, $V_1 = ppb$ at sea level, $P_2 = .836$ atm, and $V_2 = ppb$ at this altitude. A concentration of 150 ppb ethylene in Fort Collins is therefore equal to 125 ppb at sea level. A difference of 25 ppb could be important in establishing threshold values.

Concentrations reported in ppb can be converted to absolute concentration (ug/m^3) by using the perfect gas law:

$$pv = (w/m)RT$$

where p = pressure (atm), v = volume (liters), w = weight of gas (g), m = molecular weight of gas (g/M), R = 8.21 x 10^{-2} l-atm mole⁻¹ ($^{\circ}$ K)⁻¹, and T = temperature ($^{\circ}$ K). Expressing concentrations in ug/m³ automatically corrects for pressure and temperature differences. Examples of changes in absolute concentration under different conditions are given in Table 4.

Expressing concentrations of ethylene and other air pollutants in absolute units is now preferred by the National Air Pollution Control Administration (39). For the purposes of this study, however, expressing concentrations in ppb provided sufficient accuracy and information.

	absolut	e concentration	1 (ug/m ³)	
ethylene (ppb)	21.1°C ^{1 a}	<u>tm</u> 1.7 [°] C	21.1° ^{.836} C	atm 1.7 [°] C
100	116	124	97	104
300	349	373	291	312

Table 4. Effect of temperature and pressure on ethylene absolute concentration.

Comparison with Previous Studies

Previous studies of the effect of ethylene on open carnations were conducted at altitudes close to sea level. When ethylene treatments causing flower injury in previous work are converted to dosages and corrected for altitude, a comparison can be made with the results in this study (Table 5). Of five dosages reported to cause flower injury or decreased keeping life at sea level, three would cause a loss of more than 20% keeping life at .836 atm. Dosages that would cause less than 20% keeping life decline at .836 atm were reported to be threshold dosages at sea level.

Commercial Application

Ethylene damage to carnations can be minimized two ways: 1) by reduction of temperatures around cut flowers, and 2) by cutting flowers in the bud stage.

Reference	Exposure Temp(^o C)	Dosage ¹ (ppb-hr) 1 atm	Effect l atm	Dosage ² (ppb-hr) .836 atm	Effect ³ .836 atm
(40)	10.0	2,880	sleepiness threshhold	3,445	13% loss of keeping life
(33)	18.3	2,400	significant loss of vase life	2,871	41% loss of keeping life
(28)	18.3	9,600	consistant senescence acceleration	11,483	100% loss of keeping life
(39)	20.0	3,000	sleepiness threshhold	3,589	68% loss of keeping life
(40)	20.0	1,440	sleepiness threshhold	1,722	13% loss of keeping life

Table 5. Summary of ethylene dosages causing carnation flower injury or decrease in keeping life.

 1 derived from ethylene concentrations and exposure times reported in the literature

² derived from dosage at 1 atm by using formula $P_1V_1 = P_2V_2$ (page 44)

³ found by entering .836 atm dosage into curve for regression of keeping life on ethylene dosage at nearest exposure temperature (Fig. 1).

Carnation growers cannot realistically use lower greenhouse temperatures to avoid ethylene damage. Carnations can be cut as buds or shortly after opening, however, to avoid prolonged exposure to ambient ethylene levels. Flowers should be refrigerated soon after being cut, and cut flower wholesalers and retailers should store and ship flowers at temperatures as close to 0°C as possible.

Precautions should still be taken to keep cut carnations away from ethylene sources during handling. Use of CO₂, ethylene oxide, air filters, or absorbants will be largely unnecessary, however, if temperatures are kept at low levels and flowers are cut tight.

Suggested Research

The following research is suggested:

1. Obtain more data in the threshhold regions to define dosages at which a significant decline in keeping life begins.

2. Study the response of other carnation cultivars to ethylene.

3. Run ethylene studies at different times of the year to

determine if ethylene sensitivity varies with season.

4. Expose carnations to ethylene and other air pollutants simultaneously to check for synergistic effects.

5. Expose entire plants to ethylene continuously from time of planting to time of cut. Compare flower yield, quality, and keeping life with carnations grown in ethylene-free air.

SUMMARY AND CONCLUSIONS

To evaluate the effect of ethylene on carnation keeping life, four factors were taken into account. These were ethylene concentration, length of exposure, temperature of exposure, and stage of flower development when exposed to ethylene.

Ethylene concentration and time of exposure were expressed simultaneously by use of a dosage term, ppb-hours. Stating dosages in ppb-hours was important because it accounted for fluctuations in ethylene concentration during the exposure period and facilitated comparisons between different ethylene treatments.

Carnation keeping life decreased as ethylene dosage increased after a threshhold dosage value was exceeded. Although each dosage applied may have been the product of different ethylene concentration and exposure period combinations, keeping life correlated closely with dosage at each exposure temperature.

Lowering the temperature at which carnations were exposed to ethylene increased damage threshhold dosages as well as dosages needed to cause zero vase life (in relation to control flowers). Open flowers were over twice as sensitive to injury when exposed to ethylene at 21.1°C as they were at an exposure temperature of 10.0°C. Flowers exposed at 10.0°C were over three times as susceptible to injury as those exposed at 1.7°C. Extent of bud damage was also temperature dependent. Buds exposed at 21.1°C were nearly three times as susceptible to injury as those exposed at 10.0°C. Buds exposed to 55,080 ppb-hours ethylene, the highest dosage in this study, showed no loss of keeping life.

Buds were less susceptible to ethylene injury than flowers at all three exposure temperatures. At 21.1° and 10.0° C, flowers showed zero keeping life at dosages half those causing zero keeping life for buds. At 1.7° C, bud keeping life was not adversely affected by a dosage causing complete loss of open flower keeping life. Budcut carnations kept at 1.7° C were least susceptible to ethylene injury.

Symptoms of severe ethylene damage differed for open flowers and buds. Entire open flowers became sleepy during exposure to high dosages, and subsequently desiccated. Only the outer petals of damaged buds were affected, becoming sleepy during treatment and later showing severe burn. Ethylene did not affect carnation leaves and stems.

When ethylene treatments applied at sea level were corrected for the altitude of Fort Collins (5,000 ft), comparisons could be made between results of this study and previously published studies. Three of five dosages reported to cause flower injury or loss of keeping life in other studies would have caused keeping life declines of greater than 20% when fitted into regressions of keeping life on dosage in this study.

To minimize ethylene-induced damage on the commercial level, cutting carnations in the bud stage and refrigerating cut flowers from time of cut to time of retail sales was recommended.

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APPENDICES

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Appendix I. Exploratory Studies

Studies of the effect of ethylene on carnation and rose keeping life were conducted in summer and fall, 1971. The following observations were made:

1. Carnations cut from plants of different age or grown under different conditions varied in keeping life, Roses obtained commercially were visibly of different age and quality. This variability may have masked small differences in keeping life resulting from ethylene treatment.

2. Commercially obtained roses frequently developed neck droop from water loss, preventing a meaningful evaluation of ethylene's effect on keeping life. Increasing the humidity, recutting stems, and providing water before and after treatment did not decrease occurrence of neck droop. Work with roses was therefore discontinued.

3. A Mariotte bottle system (30) was unsatisfactory for metering ethylene in the low ppb range for flower treatment, since ethylene levels fluctuated widely as a result of small temperature and pressure changes.

4. Occasional bacterial contamination occurred in keeping solutions composed of 4% sugar and 200 ppm HQC per liter distilled water. Incorporating 50 ppm AgNO₃ into this solution solved the problem.

		Leve	1 A	Lev	el B	Leve	el C	Level	D
Exper.	Group	Dosage (ppb-hr)	Keeping Life (%)						
1	1	392.0	100.00	4,728.0	98.04	14,192.0	107.84	17,000.0	74.51
	2	392.0	100.00	4,728.0	101.75	14,192.0	75.44	17,000.0	92.98
	3	304.0	100.00	5,048.0	98.23	9,392.0	96.86	15,096.0	80.49
2	1	672.0	100.00	7,896.0	103.23	17,112.0	98.39	25,880.0	56.45
	2	672.0	100.00	7,184.0	96.83	16,176.0	92.06	25,168.0	23.81
	3	504.0	100.00	7,600.0	101.61	16,448.0	91.94	25,056.0	62.90
3	1	1,536.0	100.00	15,360.0	71.43	42,864.0	0.00	50,304.0	0.00
	2	1,680.0	100.00	14,736.0	92.59	41,976.0	0.00	49,032.0	0.00
	3	1,848.0	100.00	16,224.0	93.10	37,920.0	0.00	55,080.0	0.00
4	1	672.0	100.00	7,896.0	106.32	17,112.0	107.37	25,880.0	112.63
	2	672.0	100.00	7,184.0	118.54	16,176.0	114.61	25,168.0	115.73
	3	504.0	100.00	7,600.0	95.15	16,448.0	93.20	25,056.0	93.20
5	1	1,536.0	100.00	15,360.0	95.74	42,864.0	102.13	50,304.0	100.00
	2	1,680.0	100.00	14,736.0	101.08	41,976.0	92.47	49,032.0	106.45
	3	1,848.0	100.00	16,224.0	94.62	37,920.0	110.75	55,080.0	119.35
6	1	238.4	100.00	1,249.6	96.20	2,192.0	102.53	3,041.6	102.53
	2	238.4	100.00	1,249.6	93.67	2,192.0	103.80	3,041.6	
	3	102.4	100.00	1,291.2	90.77	3,294.4		4,204.8	

Appendix II. Ethylene dosages in ppb-hours and corresponding keeping lives expressed as a percent of control (Level A) keeping life within each group.

		Leve	1 A	Leve	1 B	Leve	1 C	Leve	1 D
Exper.	Group	Dosage (ppb - hr)	Keeping Life (%)	Dosage (ppb-hr)	Keeping Life (%)	Dosage (ppb-hr)	Keeping Life (%)	Dosage (ppb-hr)	Keeping Life (%)
7	1	348.0	100.00	2,776.0	100.00	4,460.0	88.89	6,828.0	7.94
	2	348.0	100.00	2,776.0	89.06	4,460.0	96.88	6,828.0	42.19
	3	160.0	100.00	2,824.0	107.02	5,040.0	105.26	7,848.0	43.86
8	1	160.0	100.00	3,616.0	74.14	9,096.0	0.00	12,832.0	0.00
	2	160.0	100.00	3,616.0	77.94	9,096.0	23.53	12,832.0	0.00
	3	304.0	100.00	3,888.0	105.66	7,944.0	71.70	11,936.0	0.00
9	1	648.0	100.00	4,360.0	89.00	8,128.0	84.21	11,096.0	95.69
	2	648.0	100.00	4,360.0	103.23	8,128.0	104.30	11,096.0	108.60
	3	648.0	100.00	4,360.0	105.38	8,128.0	110.75	11,096.0	105.38
10	1	1,184.0	100.00	9,760.0	80.41	15,936.0	63.92	22,656.0	4.12
	2	1,184.0	100.00	9,760.0	82.00	15,936.0	42.00	22,656.0	37.00
	3	1,184.0	100.00	9,760.0	93.02	15,936.0	93.02	22,656.0	26.74
11	1	100.8	100.00	552.0	105.71	1,156.0	111.71	1,236.8	114.29
	2	100.8	100.00	552.0	109.52	1,156.0	122.22	1,236.8	117.46
12	1	152.0	100.00	1,355.2	100.00	3,017.6	31.37	4,035.2	25.49
	2	152.0	100.00	1,355.2	84.78	3,017.6	52.17	4,035.2	32.61
	3	150.4	100.00	1,308.8	61.67	3,011.2	41.67	3,868.8	26.67
13	1	73.6	100.00	1,523.2	93.10	3,332.8	54.02	4,529.6	90.80
	2	73.6	100.00	1,523.2	89.47	3,332.8	70.53	4,529.6	44.21
	3	115.2	100.00	1,451.2	96.20	3,305.6	67.09	4,494.4	79.75

Appendix II (continued)

Appendix II (continued)

		Lev	el A	Leve	lВ	Level	ιc	Level	D
Exper.	Group	0	Keeping Life (%)	Dosage (ppb-hr)	Keeping Life (%)	Dosage (ppb-hr)	Keeping Life (%)	Dosage (ppb-hr)	Keeping Life (%)
14	1 2 3	356.0 356.0 424.0	100.00 100.00 100.00	2,652.0 2,652.0 2,560.0	88.24 101.10 111.36	8,208.0 8,208.0 7,396.0	5.88 3.68 22.73	8,628.0 8,628.0 8,032.0	11.76 0.00 0.00

Source of Variance	df	MS	F
Regression	2	22,078.42	126.08**
Residual	33	175.11	
Total	35		

Appendix III. Analysis of variance for regression of keeping life on ethylene dosage for open flowers exposed at 1.7°C.

Appendix IV. Analysis of variance for regression of keeping life on ethylene dosage for open flowers exposed at 10.0°C.

Source of Variance	df	MS	F
Regression	2	18,621.82	69.01**
Residual	33	269.83	
Total	35		

Appendix V. Analysis of variance for regression of keeping life on ethylene dosage for open flowers exposed at 21.1°C.

F	MS	df	Source of Variance
2 37.55**	9,075.02	2	Regression
7	241.67	17	Residual
		19	Total
		19	Total

Appendix VI.	Analysis of variance for regression of keeping life
	on ethylene dosage for buds exposed at $1.7^{\circ}C$.

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Source of Variance	df	MS	F
Regression	1	88.78	1.33 n.s.
Residual	22	66.56	
Total	23		

Appendix VII. Analysis of variance for regression of keeping life on ethylene dosage for buds exposed at $10.0^{\circ}C$.

Source of Variance	df	MS	F
Regression	2	7,758.78	47.84**
Residual	21	162.17	
Total	23		

Appendix VIII. Analysis of variance for regression of keeping life on ethylene dosage for buds exposed at 21.1°C.

Source of Variance	df	MS	F
Regression	2	15,981.92	100.66**
Residual	21	158.78	
Total	23		