T H E S I S

EFFECTS ON CARNATION CUT FLOWERS
OF STORAGE
WITH GROCERY PRODUCE

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ROBERT P. WINTZ

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Chapter I

INTRODUCTION

The value of the floriculture industry of this country over the 1948-1949 period has been estimated by Fossum (15) to be approximately 250 million dollars. This figure represents an impressive increase of over twelve and one half times the value for the year 1900 of 19 million dollars. Colorado is rapidly growing in floral crop production, especially in carnation production. In the 1950 preliminary census for Colorado the number of flowers sold of the three leading cut flower crops were carnations - 29,332,584; roses - 6,619,765; pompon chrysanthemums (bunches) - 107,105; and standard chrysanthemums - 272,142, for a total dollar value of $3,932,620. These figures clearly tend to show the marked increase in the floral industry over the United States over this half century.

Such an increase in production naturally would give rise to a need for more sales outlets. With the development and increase in the number of supermarkets, the consumer has virtually been able to supply the entire family needs at one store. Thus, it is highly conceivable that soon cut flowers will find their place on the market shelves. A supermarket selling fresh flowers, economically priced, could greatly aid in filling this need for more outlets for flowers.

One of the problems that would confront such an operation would be to transport fresh flowers and keep them fresh until they were purchased by the consumer. It is generally known that certain fruits cause cut carnations to "go to sleep" or close up. This would present a serious problem in storage and keeping since the flowers would very likely come in close proximity to various fruits and vegetables.

It is the purpose of this study to determine what fruits and vegetables can safely be stored with cut carnations.

The problem

With the advent of flowers in stores that sell grocery produce, it is necessary to know the effects of various fruits and vegetables on the keeping quality of cut carnations.

Problem analysis.--An analysis of the problem raises these questions:

1. How do various fruits and vegetables affect ethylene-sensitive plants?

2. How do various varieties of cut carnations affect ethylene-sensitive plants?

3. What are the effects on cut carnations when stored with various fruits and vegetables?

4. What effects do cultures of common molds and soft-rot organisms isolated from fruits and vegetables have on the keeping quality of cut carnations?

Delimitations.--This study has been limited to the fruits and vegetables as they would normally be found in supermarkets.

The cut carnations used were Crowley's Pink Sim or William Sim, cut fresh each day from the Colorado A and M experimental greenhouse. The variety in adequate supply at the time was used.

Definition of terms.--Sleepiness - is a term used to denote an undesirable condition of cut carnations characterized by an incurving of the edges of the petals, the petals become somewhat discolored, and they lose their turgor.

Ethylene-like - any gas or vapor that would give a similar effect upon indicator plants to that of ethylene.

Triple response - as defined by Knight and Crocker (30), is a change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of growth in length.

Epinasty - this condition is typified by rolling and curling of leaflets, and downward bending of entire leaves from their points of attachment.

Ethylene-sensitive plants - etiolated pea seedlings, potato and tomato seedlings, and African marigold seedlings. These plants are extremely sensitive to minute amounts of ethylene gas, and are therefore used extensively to determine the presence of ethylene.

Etiolated pea seedlings - are pea seedlings that have been sprouted and grown in an absence of light.

Survival - refers to the number of days cut carnations will remain in a desirable condition.

Desirable condition - as long as the flowers have the typical appearance of carnations.

Climacteric - is the period during ripening that is accompanied by a sharp rise in respiratory activity.

Chapter II

REVIEW OF LITERATURE

Injurious effects of illuminating gas and ethylene on vegetation

The fact that certain gaseous emanations have caused injurious effects on various plants was known as early as 1864 when Girardin (21) called attention to the phenomenon of illuminating gas injury to trees. Experimental proof of this type of injury was made by Kny (31) and Harvey and Rose (26).

The first reported work done with carnations was by Crocker and Knight (3). They emphasized the fact that the so called "sleep" or closing of carnations was a serious problem for flower growers and retailers, for once the flowers closed they never again opened. In several homes lighted with gas, carnations could be kept only a few hours before they would go to sleep. Through various experiments these workers found that there was much evidence to support the fact that the injury caused by illuminating gas depended on the amount of ethylene it contained. To verify this they ran parallel experiments with illuminating gas and known amounts of pure ethylene gas. The illuminating gas used in these experiments was a manufactured gas.

Doubt (13) tested several plant tissues for the effects due to illuminating gas and ethylene. She noted several different types of responses to the gases, and degrees to which the plants responded. The responses noted were: dropping of leaves, production of epinasty of petioles, development of proliferation tissue in lenticels, leaf scars, etc., and production of root tubercles.

Zimmerman, Hitchcock, and Crocker (50) tested the effects of illuminating gas and ethylene upon roses. They noted the following responses: slight epinasty of young leaves, abscission, color change in the leaves, rapid opening of the flower bud followed by early petal fall, slight retardation of growth in the actively growing regions, and a forcing effect of ethylene which caused an abnormally large number of latent rose buds to produce shoots.

Hitchcock, Crocker, and Zimmerman (27) reported that lily, narcissus, tulip, and hyacinth were retarded in growth with treatments of illuminating gas ranging from one part ethylene in 75 parts air to one part ethylene in 40,000 parts air, without causing death or abscission of leaves. In a later work, the above named authors (4) tested some 202 different species and varieties of plants. Of these, they found that 72 showed marked epinasty of leaves, 17 showed slight epinasty, and 113 showed no epinastic response.

Lumsden and others (32) reported on the effects of ethylene on carnations, roses, snapdragons, stocks, and narcissus in cold storage rooms. They found that ethylene caused identical injury in cut flowers as did storage of cut flowers in rooms with apples or even in rooms close by. They also found that flowers stored separately at 70°, 50°, and 36° F remained in good condition longer than corresponding lots stored in the presence of fruit or ethylene at the same temperatures. They noted that the damage to carnations in full bloom was indicated by an incurving of the edges of the petals, and they became discolored and lost their turgor. Similar results were earlier reported by Crocker and Knight (3). The injury to cut roses and snapdragons consisted of discoloration and early dropping of the petals and flowers. Narcissus and stocks reacted by deterioration of color and shriveling of the flowers. These writers felt that since most fruits were not generally stored at 50° F, cut flowers stored at this temperature would not be so apt to be injured by gases from apples or other fruits.

According to Davidson (5) orchid flowers are very sensitive to traces of ethylene gas. Exposure of orchid flowers to an atmosphere containing 0.002 ppm ethylene gas for 24 hours produced injurious effects. The injury to orchid flowers is characterized by a progressive drying and bleaching of the sepals, beginning at the tips and extending toward the bases.

Ethylene-sensitive plants used as indicator plants

Neljubow (35) noted that the epicotyl of the pea and some other legumes developed in a peculiar way in laboratory air. He found that this modified growth habit was due to traces of artificial illuminating gas in the air. He mentioned ethylene and acetylene, both constituents of illuminating gas, as being especially effective in producing an effect on the pea seedlings. One part of ethylene in three million of air induced a slight effect in a portion of the epicotyl, whereas two to four times that concentration gave an effect in all portions.

Knight and Crocker (30), with reference to the effects produced on pea seedlings, coined the term "triple response" meaning a change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of growth in length.

Harvey (25) noted that the castor bean plant showed nastic drooping of leaves after the plant had been brought into the laboratory, and felt that this plant could be used as a delicate test for the presence of ethylene gas. He found that one part ethylene per 10 million of air caused a definite response, and felt that since the response was so definite, that even a lower concentration might be quite effective. From this experiment he recommended that the castor bean plant could be effectively used as an indicator plant in the laboratory or greenhouse to warn of gas leakage.

Zimmerman, Crocker, and Hitchcock (49) reported on the effects of various concentrations of illuminating gas on plant tissue. They found the most sensitive plant tested to be tomato, which was found to be sensitive to one part illuminating gas to 100,000 parts of air (equivalent to one part ethylene in three million parts of air).

Crocker, Zimmerman, and Hitchcock (4) tested several other plants for epinastic effects, and found that tomato, sunflower, buckwheat, and African marigold were sensitive to traces of ethylene gas, and could be used as indicator plants. They felt the tomato plant was the best to use as a test plant because of the ease of growing and speed of response combined with sufficient sensitiveness for practical purposes. They cautioned, however, that in using the epinastic response of tomato leaves, as well as other plants, young vigorously growing plants should be used because they are more sensitive and respond more quickly. In reviewing the literature these authors noted that Singer (45) had mentioned that potato sprouts were sensitive to about one part of illuminating gas to 100,000 parts of air (one part ethylene to three million parts of air).

Crocker, Hitchcock, and Zimmerman (2) tested leaves of the potato to determine the minimum concentration of ethylene necessary to produce epinasty. They also made tests to redetermine the minimum concentration of ethylene necessary to produce epinasty in the tomato and African marigold. They found the tomato to be affected by a minimum of one part of ethylene per 10 million of air; potato one part ethylene per 40 million of air; and African marigold one part per 60 million of air. Although the tomato was only one fourth as sensitive as the potato, it had certain advantages above the potato. In addition to being easy to grow, having a quick response, it could be grown at any time of the year, even during the shortest days of winter; it could stand darkness, and all the leaves responded, including the oldest mature leaves. Potatoes were more difficult to grow and suffered somewhat even with two days' exposure to darkness.

Denny and Miller (12) used young potato plants as test plants since they found them easy to handle and much more sensitive than tomato plants. Denny (7), in further studies, instead of using the entire potato plant with roots, cut the stem of the plant off at the surface of the soil, and this cutting was then placed in a vial of water. This cutting was found quite capable of producing the typical epinastic response.

In a recent book on the growth of plants, Crocker (1) summarized the work done on ethylene-sensitive plants. Following are listed the more popular test plants used today and their degree of sensitivity:

Plant	Minimum concentration of ethylene necessary to produce epinasty
Carnation flower	0.5 ppm
Sweet pea seedling	0.2 ppm
Tomato	0.1 ppm to 0.04 ppm
Potato	0.04 ppm
African marigold	0.001 ppm

<u>Gases and vapors causing epinastic responses</u>

Knight and Crocker (30), testing the toxicity of smoke, found that of the gases that are found in smoke, carbon monoxide, acetylene, ethylene, propylene, and perhaps methane produce an effect similar to that produced by smoke. However, considering the magnitude at which carbon monoxide, acetylene, propylene, and methane must be used to show a response, it seemed they could not play a part in the toxicity of smoke. The great toxicity of ethylene made it highly probable that it was the gas that determined the toxic limit of smoke. Moreover, the toxicity of smoke was greatly reduced when washed with bromine, a system well known to absorb heavy hydrocarbons.

Crocker, Zimmerman, and Hitchcock (4) listed the following gases as producing epinastic responses in tomato petioles:

Comparative Effectiveness of Gases in Producing Epinasty in Tomato Petioles and Declination in Sweet Pea Seedlings

Gas	Minimum parts per million needed to produce: Declination in sweet pea seedlings, according to Knight and Crocker*	Minimum parts per million needed to produce: Epinasty in tomato petiole**
Ethylene	0.2	0.1
Acetylene	250.0	50.0
Propylene	1,000.0	50.0
Carbon Monoxide	5,000.0	500.0
Butylene	---	50,000.0

* 3 days' exposure   ** 2 days' exposure

From this table it is evident that if the minimum concentration of ethylene necessary to produce the response is considered as 1, the minimum concentrations of the other gases are; acetylene and propylene 500, carbon monoxide 5,000, and butylene 500,000. Acetylene and propylene then are 1/500 as effective as ethylene in producing epinasty in the petioles of the tomato; carbon monoxide 1/5,000 as effective; and butylene 1/500,000 as effective. It will be noted that these workers added butylene to the list of gases that produce epinasty, but they found that it was effective only at extremely high concentrations. Of some 38 other gases and vapors tested by Crocker, Zimmerman, and Hitchcock, none were found to produce an epinastic response.

Of 77 volatile chemicals tested for ability to induce epinasty of potato leaves, Denny (8) found only three that gave positive responses; ethyl bromide

(C_2H_5Br), ethyl iodide (C_2H_5I), and propyl chloride (C_3H_7Cl). However, he stated that the epinasty-inducing volatile product from various organs of plants could not be any of these three, since mixtures of these with air, when passed through a tube immersed in a freezing mixture of carbon-dioxide-snow and alcohol, gave negative tests for epinasty. Moreover, the volatile products from plants were not condensed by the freezing mixture, and the uncondensed gas which issued from the tube retained its effectiveness. Denny also tested the other volatile chemicals which have been shown to cause epinasty, (ethylene, acetylene, propylene, carbon monoxide, and butylene). By the use of the mercuric nitrate reagent used by Hansen and Hartman (24) in their work with pears, and in a modified form by Denny himself, he showed that the only gas which behaves like the effective volatile constituent from plant tissue in being absorbed by the reagent and released again without loss of epinasty-inducing power was ethylene.

Denny (7) also tested essential oils from 41 different species of plants for their effect with respect to epinasty. All results proved negative, which seemed to indicate that it was unlikely that epinasty was caused by volatile oils from the tissues.

Through further experiments, Denny (9) found that various species of the Cruciferae seedlings, especially those of radish, produced, in addition to the epinasty-inducing volatile product, another gas which killed potato test-plants. This appeared to be a mustard oil (allylisothiocyanate, C_3H_5NCS), set free from the mustard oil glucoside during the germination of the seed and growth of the seedlings. However, when the mustard oil was present in a concentration sufficient to injure the leaf, the leaf could not respond to ethylene by epinastic bending because the tocicity prevented the growth necessary to produce epinasty.

From this section of the review of literature, and especially from the results obtained by Denny, there is a definite indication that ethylene is the only effective constituent from plant tissues known today possessing epinasty-inducing-power.

<u>Production of ethylene by plant tissue</u>

Denny and Miller (12) proposed the view that ethylene is produced in the normal life processes of plants and is given off from the tissue into the surrounding atmosphere. Their beliefs concerning this phase of plant physiology were based upon the observations made by O. H. Elmer.

Elmer (14) declared that a volatile substance normally produced by apple fruits caused striking morphological abnormalities in the potato.

Huelin and Barker (28), working on the effect of ethylene gas on the sprouting of potatoes, noted a direct analogy between these effects and those produced by a volatile product of ripening apples.

Gane (16) first showed by chemical means that the active volatile substance produced by apples was actually ethylene. He found that the amount of ethylene produced was very small, in the nature of about 1 cc during the whole life of the fruit.

Denny and Miller (12) substantiated the work done by Gane, and found that two grams of apple tissue in a seven liter space would produce within one day a quantity of ethylene sufficient to cause epinasty. On this basis the amount of ethylene within the seven liter container should be about one part in 20 million. These results compare satisfactorily with those obtained by Gane. Moreover, they found by using the epinastic response of leaves as an indication of the presence of ethylene in the emanations from plant tissue, that apple, pear, tomato, banana, cantaloupe, squash, eggplant, avocado, loquat, asparagus, and several other plant tissues produced epinastic responses. However, they did not prove definitely that the emanation from these tissues was ethylene. They did feel that if some other chemical was the factor, then some proof must be included that ethylene was not present as an impurity in amounts sufficient to give a concentration of at least one part in 20 million of air. Hansen and Hartman (24), working with pears came to the same conclusions as did Denny and Miller.

Niedrl and others (38) found conclusive qualitative evidence of the evolution of ethylene by bananas during ripening.

Pratt and others (40) identified ethylene as a volatile product of ripening avocado fruit.

Skok (46) pointed out that on the basis of the evidence collected to date, it is probable that gaseous emanations from ripe fruits present on the tomato vines are a major contributing factor resulting in defoliation of tomato plants as grown under commercial field culture.

Through other experiments Denny (7) listed several plant organs as producing an epinastic response similar to that of ethylene.

From these and other results obtained by the Boyce Thompson Institute and other groups, Crocker (1) concluded that all respiring plant tissue produces ethylene, although the amount produced varies greatly with the species of plants, the plant organ, and the age of the organ.

Ethylene as a metabolic product of diseased or injured tissue

Crocker (1), citing Gane (17), pointed out that aerobically growing yeast, but not anaerobically growing yeast, produced a substance that prevented the growth of a pea seedling.

Denny (7) obtained negative results from the fermentation of sugar with baker's yeast, and from the actively growing mycelium of Rhizopus nigricans in test tubes with potato dextrose agar (two per cent) as substrate. The mature sporophores of two species of mushroom, Russula emetica and Lepiota spp., caused no epinasty of potato leaves.

Miller and others (34) found that oranges and grapefruit inoculated with Penicillium digitatum produced epinasty in test plants sooner than did normal fruit under the same conditions. Positive results were also obtained when pure cultures of the organism were tested for evolution of ethylene gas. From previous work done with ethylene gas, they were prone to believe that the maximum evolution of ethylene by apple fruits occurred during the "climacteric". Since Penicillium is a fast growing fungus, and produces spores very readily, they felt it was quite possible that during the formation of fruiting bodies, a fungus, like an apple, may attain a climacteric. It is well to note that a small quanity of the fungus produced the same effect as 24 oranges. This could well mean that decaying fruit in stored lots could cause very harmful effects. In addition to Penicillium digitatum these workers also tested pure cultures of several other organisms, but only *Penicillium digitatum* proved harmful.

According to Williamson (47), large quantities of ethylene were produced by rose leaves infected with *Diplocarpon* rosae, cherry leaves infected with *Coccomyces hiemalis*, and chrysanthemum flowers infected with *Ascochyta chrysanthemi*. Several other organisms produced smaller quantities of ethylene. Several isolates were made of these organisms and tested for the production of an active emanation, but only *Penicillium digitatum* produced such an emanation. Williamson also found that even healthy leaves in general produced some small quantities of ethylene. Shredded healthy rose leaves and shredded healthy cherry leaves produced considerably more ethylene than did uninjured leaves. Ethylene production was most abundant while the leaf was green, decreased as the leaf yellowed, and ceased when the leaf died and became brown.

Ross and Williamson (44) inoculated several plant species with various viruses and the result was a production of a physiologically active emanation in amounts much greater than that produced by comparably healthy leaves. Ethylene production seemed to be the result of necrosis and not the cause. It was greatest in leaves inoculated with viruses that caused numerous large necrotic lesions, The relative amounts of ethylene produced by different types of tissues were estimated from the extent of the triple response.

Coloring and ripening of fruit with ethylene

Denny (10) reviewed the "forced coloring" method for ripening lemons where the fruit was placed in rooms or tents heated with kerosene stoves, with the result that the lemons turned yellow in one or two weeks. Through further experiments he found that ethylene with a concentration of one part ethylene to 200,000 parts air colored lemons in five to eight days.

Rosa (43) reported that ethylene gas in concentrations of one part to 4,300 parts air greatly accelerates the development of the red pigment (lycopene, $C_{40}H_{56}$) of the tomato.

Rosa (42) reported that fruits of Honey Dew and Casaba melons picked slightly unripe and exposed to varying concentrations of ethylene showed a marked acceleration in the rate of softening, in change of color from green to yellow, and in conversion of reducing sugars to sucrose.

Acceleration of the coloring and ripening of fruits with ethylene gas also was reported with bananas, Wolfe (48), celery, Mack (33), and Japanese Persimmon, Davis and Church (6).

Relation of ethylene to temperature and rate of respiration

Gore (22) in studies on fruit respiration, summarized that in general fruits which grow and mature quickly and soon become overripe respire rapidly. This is true of the small fruits. On the other hand, fruits that have a long growing season and mature slowly, such as the citrus fruits, are very inactive physiologically. Fruits such as plums, peaches, apples, pears, and grapes are intermediate in this respect. Measurements were given for the rate of respiration at different temperatures for several of these fruits, and although the respiration intensity of the fruits varied greatly, there was a correlation between temperature and rate of respiration. The rate of respiration increased from 1.89 to 3.01 times, with an average of 2.376 times for each 10 degrees rise in temperature. This was in line with earlier reports made upon plant tissue as reviewed by Gore.

Gane (18), citing Kidd and West (29), pointed out that with the onset of ripening, there is an accompanying sharp rise in respiratory activity. Kidd and West coined the word "climacteric" for this phenomenon. This stage in the life process of the apple is coincident with the maximum rate of production of CO_2 and corresponds to the period when the apple is fully ripe. Furthermore, Kidd and West have shown that the volatiles from postclimacteric apples stimulate the climacteric in unripe fruit and also that the same is true in the case of bananas. Gane confirmed their observations with regard to bananas. He also pointed out that there is a strong probability that bananas behave like apples and begin to produce ethylene with the onset of the climacteric.

Conversely, Wolfe (48), using concentrations of ethylene from one part to 100 parts air to one part to 10,000 parts air, found that respiring activity of bananas treated with ethylene differed little or not at all from untreated ones when 12 hour periods were considered.

Regeimbal, Vacha, and Harvey (41) noted a decided increase in respiration of bananas when ethylene used at a concentration of one part to 1,000 parts air was administered, followed in a few minutes by a rapid fall in the rate, and then eventually the normal rate was regained.

Denny (11) reported that ethylene used at concentration of one part to 1,000 parts air to one part to 1,000,000 parts air increased respiration of lemons from 150 to 250 per cent.

Huelin and Barker (28) reported that ethylene in varying concentrations caused an increase in respiration of potatoes to a maximum during the first two days after exposure, followed by a fall in the respiration to an adjusted state above the control value.

Increases in the respiration rates of fruits and vegetables were also noted in Japanese Persimmon by Davis and Church (6), in celery by Mack (33), and in avocado by Pratt and Biale (39).

Methods of ethylene determination

Delicate tests for ethylene gas determination have been made by Gane (19), Gerhardt and Ezell (20), Hansen and Hartman (24), Denny (11), Nelson (36 and 37), and Hall (23). However, according to Crocker (1), to obtain reliable and delicate tests for traces of ethylene gas the use of test plants, such as pea seedlings or young plants of tomato, potato, or African marigold should be used.

Chapter III

METHODS AND MATERIALS

A series of experiments was designed to determine what fruits and vegetables produce harmful substances in sufficient quantities to injure carnations or reduce their keeping quality. Preliminary tests showed that all of the varieties available at the Colorado A and M experimental greenhouse displayed sleepiness when stored in close confinement with apple tissue. Of these varieties Crowley's Pink Sim and William Sim proved to be the most reliable indicators of sleepiness. Consequently, these two varieties were used throughout the experiments.

Glazed four gallon water crocks, using window glass sealed with stopcock grease as a cover, were found suitable as air tight containers. These were large enough to hold cut carnations and fruits and vegetables to be evaluated, and yet small enough to be easily moved about. These containers had an adequate opening through which observations could be made.

Twenty-seven fruits and vegetables were selected at the local supermarket to be evaluated for their ability to induce sleepiness of carnations. They were apple, avocado, banana, celery, onion, summer and winter squash, potato, lettuce, green onion, yam, grape, tangerine, orange, grapefruit, pineapple, bell pepper, lime, tomato, cucumber, pear, broccoli, cabbage, cauliflower, radish, and carrot. Three crocks were used for each fruit or vegetable to be evaluated. Each respective produce item was weighed and placed in a crock along with a small flask holding three freshly cut carnations and a beaker containing 40 ml of 2 N KOH. The KOH absorbed the CO_2 given off during respiration.

As soon as the flowers in each crock showed sleepiness or signs of old age, the crocks were removed from the room, opened, and the contents taken out. The crocks were allowed to air overnight. This procedure was followed to insure against contaminating the air in the crocks to be used for subsequent trials. When two of the three flowers in each crock had gone to sleep or withered with age, the test was terminated. When the flowers in two of the three crocks had gone to sleep or withered with age, the test for that particular produce item was terminated. Daily observations were made and the results recorded.

The above mentioned procedure was followed in Experiments I, II, III, and IV. The apparatus used in these four experiments is shown in Figure 1.

Experiment I

A basement room in the Horticulture building was selected for this experiment since the temperature was rather constant at 60° F. The produce, cut carnations, and KOH were placed in the crocks and the tops sealed.

Experiment II

The crocks were placed in an upstairs room in the Horticulture building where an even temperature of 75° F could be maintained. Each crock and glass were thoroughly sterilized with a 200 ppm solution of BK, a chlorinated disinfectant. (Active ingredients--Calcium hypochloride 50%, inactive ingredients, 50%. Penn Salt Mfg. Co., Philadelphia 7, Pa.) This material was used hereafter for sterilization. For surface sterilization the plant produce was placed in 200 ppm of this solution and allowed to stand for 10 to 15 minutes. The produce was removed, placed in the crocks, and allowed to air dry before the lids were sealed. To reduce humidity 30 grams of anhydrous $CaCl_2$ were placed in the lid of a petri dish. Anhydrous $CaCl_2$ maintained the humidity at a low value (0-25%), approximating the humidity of a supermarket.

Experiment III

The sterilized crocks were placed in a storage refrigerator at an even temperature of 38° F. Thirty

Figure 1.--The apparatus used in evaluating fruits and vegetables for their ability to induce sleepiness of carnations.

grams of $ZnSO_4 \cdot 7\ H_2O$ were used to maintain the humidity at a high value (75-95%). This closely approximated the humidity of the storage refrigerator. Apple, pear, and banana were the produce items evaluated in this test.

Experiment IV

Sterilized crocks were placed in storage at 38° F for 48 hours. The produce items evaluated in this test were apple, pear, avocado, and tomato. Humidity was controlled with $ZnSO_4 \cdot 7\ H_2O$. The flowers were removed from the crocks and placed at room temperature. The length of survival was noted.

Experiment V

Young African marigold plants were used as indicator plants to test for gaseous emanations from various varieties of cut carnations. The varieties were William Sim, Aparahoe, Aura, Crowley's Pink Sim, Scarlet #18, Pelargonium, Colorado Gold, Durango, Frosted Pink Patrician, Miller's Yellow, White Sim, and Fanfare. In order to determine the degree of epinasty produced, it was necessary to get a full view of the indicator plants, Gallon milk jars, using window glass sealed with stopcock grease as covers, proved adequate for making these observations. Three jars were used for each variety of carnation tested.

Into each gallon jar was placed a small flask containing three freshly cut carnations and a potted African marigold plant four to five inches tall. The experiment was set up in a room where the temperature varies from 50° to 80° F. No attempt was made to control humidity. This was purposely done to allow for fluctuating humidities as well as fluctuating temperatures. Daily observations were made of the marigold plants to note any epinasty produced.

Experiment VI

Cultures of molds and soft-rot organisms found on fruits and vegetables used in Experiment I were isolated and grown on potato dextrose agar. These cultures found on tangerine, orange, grape, pineapple, squash, bell pepper, cucumber, radish, and cauliflower were evaluated for their ability to induce sleepiness of carnations. Three bell jars of five liter capacity were used for each culture, using square pieces of window glass as a base. All of the containers and equipment were sterilized with a one per cent solution of $HgCl_2$.

Three fresh carnations were put in a small flask and placed on the glass base along with the petri dish containing the desired culture. Forty ml of a 2 N KOH solution were used to absorb the CO_2 given off during respiration. The inside of each bell jar was wiped clean

Figure 2.--The apparatus used in evaluating molds and soft-rot organisms isolated from fruits and vegetables for their ability to induce sleepiness of carnations.

with the $HgCl_2$ solution and quickly lowered over the carnations, the KOH, and the culture. The lid on the culture was removed just before the jar was set in place. To keep the jar firmly in place stop-cock grease was applied. Daily observations were made to note any evidence of sleepiness. The apparatus used in this experiment is shown in Figure 2.

Chapter IV

RESULTS

Experiments I and II

These tests revealed that most of the fruits and vegetables evaluated in these trials are harmful when stored with cut carnations. Tables 1 and 2 show the number of days cut carnations survived with these fruits and vegetables at temperatures of 60° and 75° F respectively. These fruits and vegetables were arbitrarily placed into four groups based on the length of survival of the test flowers. Group (1) apple, banana, avocado, pear, tomato, pineapple, celery, squash (winter), broccoli, radish, and green onion were the most harmful of the kinds of produce evaluated. To be placed in this group the test flowers survived either 0-4 days at 60° F or 0-3 days at 75° F. Group (2) bell pepper, lime, squash (summer), and lemon were the second most harmful produce items. Test flowers survived either 5-8 days at 60° F or 4-5 days at 75° F. Group (3) orange and dry onion were slightly harmful. Test flowers survived either 9 days at 60° F or 6 days at 75° F. Group (4) grape, grapefruit, lettuce, cucumber, cabbage, tangerine, carrot, potato, yam, and cauliflower showed no noticeable effects. Test flowers survived either 10 or more days at 60° F or 7 or more days at 75° F. Sleepiness of carnations produced by ethylene gas from apple tissue is shown in Figure 3.

Table 1.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. AVERAGE TEMPERATURE, 60° F.

Fruit or vegetable	Weight in grams	Number of days flowers survive	Average number of days flowers survive
(harmful)			
Apple	169.8	1	1
	163.2	1	
	172.7	1	
Avocado	185.2	1	1
	169.9	1	
	187.8	1	
Pear	205.5	1	1
	202.9	1	
	211.6	1	
Tomato	268.2	1	1
	273.9	1	
	285.0	1	
Squash (sliced, winter)	421.9	2	2
	433.2	2	
	434.7	2	
Brocooli	867.5	4	4
	870.5	4	
	787.9	4	
Bell pepper	258.8	4	4
	277.8	4	
	284.2	4	
Pineapple	906.8	4	4
	949.1	4	
	866.6	4	
Banana	201.3	5	5
	206.8	5	
	205.8	5	

Table 1.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. AVERAGE TEMPERATURE, 60° F. (Continued)

Fruit or vegetable	Weight in grams	Number of days flowers survive	Average number of days flowers survive
(harmful)			
Radish	208.6	6	5+
(with tops)	195.5	5	
	224.9	5	
Lime	212.8	8	7+
	214.7	7	
	228.8	7	
Squash	216.5	8	8-
(whole, summer)	216.0	5*	
	202.1	7+	
Celery	262.3	8+	8+
	327.6	9	
	269.5	8	
Onion	177.1	8	9-
(green)	184.9	9	
	193.3	9	
Check			10 or more
(not harmful)			
Cabbage	1033.1	12	11+
	1034.1	10	
	1063.8	12	
Grapefruit	475.7	12	12
	476.4	12	
	465.7	12	
Orange	195.0	12	12
	195.8	12	
	199.0	12	
Carrots	476.3	13	13
(with tops)	441.1	13	
	455.9	13	
Tangerine	202.9	12	13-
	188.4	13	
	184.5	13	

Table 1.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. AVERAGE TEMPREATURE, 60° F. (Continued)

Fruit or vegetable	Weight in grams	Number of days flowers survive	Average number of days flowers survive
(not harmful)			
Cucumber	245.5	4*	14
	257.9	11*	
	267.5	14	
Grape	386.5	14	14
	403.3	14	
	362.4	14	
Onion (dry)	332.7	15	15
	320.4	15	
	302.4	15	
Potato	457.5	16	16
	447.3	16	
	437.0	16	
Lettuce	447.0	17	17
	487.2	17	
	449.1	17	
Yam	389.6	17	17
	329.1	12*	
	297.8	17	
- - - -			
Cauliflower	409.6	7*	6-
	402.8	6*	
	387.3	6*	

* Mold or soft-rot infection

+ or - = <0.5

Table 2.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. AVERAGE TEMPERATURE, 75° F.

Fruit or vegetable	Weight in grams	Number of days flowers survive	Average number of days flowers survive
(harmful)			
Apple	167.9	½	½
	170.7	½	
	167.6	½	
Pear	172.2	½+	½+
	164.5	½+	
	162.1	½+	
Avocado	183.6	1	1
	202.9	1	
	214.5	1	
Onion (green)	180.1	1	1
	191.2	1	
	218.7		
Tomato	139.7	1	1
	132.9	1	
	138.0	1	
Banana	167.0	1½	1½
	164.0	1½	
	155.4	1½	
Pineapple	1019.4	2	2
	991.1	2	
	1104.5	2	
Radish (with tops)	231.4	2	2
	243.1	2	
	301.6		
Celery	357.4	2	2+
	352.9	2	
	283.3	3	
Broccoli	615.4	3	3
	559.7	3	
	583.7	3	
Bell pepper	120.9	4	4-
	132.0	3	
	138.3	4	

Table 2.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. AVERAGE TEMPERATURE, 75° F. (Continued)

Fruit or vegetable	Weight in grams	Number of days flowers survive	Average number of days flowers survive
(harmful)			
Lime	129.6	4	4
	135.3	4	
	138.4	4	
Squash (whole, summer)	212.8	2	4
	216.1	5	
	230.1	5	
Lemon	253.3	5	5
	260.5	5	
	257.7	5	
Onion (dry)	255.7	6	6-
	266.0	6	
	270.6	5	
Orange	212.7	6	6
	216.6	6	
	216.8	6	
Check			7 or more
(not harmful)			
Cabbage	648.3	8	7
	592.5	8	
	631.7	5	
Cucumber	269.8	7	7
	280.4	7	
	271.3	7	
Yam	273.6	8	8-
	278.3	7	
	284.2	8	
Carrot (with tops)	565.4	8	8
	607.2	8	
	636.8	8	

Table 2.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. AVERAGE TEMPERATURE, 75° F. (Continued)

Fruit or vegetable	Weight in grams	Number of days flowers survive	Average number of days flowers survive
(not harmful)			
Lettuce	488.6	8	8
	531.0	8	
	506.6	8	
Potato	438.6	8	8
	368.3	8	
	369.5	8	
Grape	446.0	9	9
	472.7	9	
	459.2	9	
Grapefruit	506.4	9	9
	513.5	9	
	481.1	9	
Cauliflower	468.2	10	10
	422.7	10	
	402.3	10	

+ or - = 0.5

Experiment III

Carnations, with some of the more harmful produce items (apple, pear, and banana), showed no effects of sleepiness after three weeks in the refrigerator at a temperature of 38° F. (Table 3)

Table 3.--THE EFFECTS ON CUT CARNATIONS OF STORAGE WITH FRUITS AND VEGETABLES. TEMPERATURE, 38° F.

Fruit or vegetable	Weight in grams	Weeks of survival* First	Second	Third
Apple	156.5	--	--	--
	154.9	--	--	--
	166.2	--	--	--
Pear	162.5	--	--	--
	162.9	--	--	--
	157.9	--	--	--
Banana	172.3	--	--	--
	155.5	--	--	--
	158.2	--	--	--

* All flowers survived the three week period.

Experiment IV

Table 4 shows the results of storing carnations for 48 hours at a temperature of 38° F with some of the more harmful produce items. Of the four items tested with carnations in this experiment (apple, pear, avocado, and tomato), only apples produced sleepiness in carnations. This effect began to occur at room temperature two days after the flowers were removed from the crocks. The other flowers, when removed from the crocks of their respective treatments, showed no sleepiness and kept equally as well as the check flowers.

Table 4.--THE EFFECTS ON CUT CARNATIONS OF 48-HOUR STORAGE WITH FRUITS AND VEGETABLES. TEMPERATURE, 38° F.

Fruit or vegetable	Weight in grams	Days flowers survive at room temperatures													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Apple	157.2	-	1	-	-	-	-	1	-	3	-				
	167.3	2	1	-	-	-	-	-	-	1	-				
	150.5	3	-	-	-	1	-	1	-	-	-				
Pear	151.5	-	-	-	-	-	-	-	-	5	-				
	161.5	-	-	-	-	-	-	-	-	3	2				
	151.9	-	-	-	-	-	-	-	-	5	-				
Check		-	-	-	-	-	-	-	-	3	1				
Tomato	152.5	-	-	-	-	-	-	-	-	-	-	2	1	2	-
	129.5	-	-	-	-	-	-	-	-	-	4	-	1	-	-
	127.2	-	-	-	-	-	-	-	-	1	2	1	1	-	-
Avocado	192.3	-	-	-	-	-	-	-	-	-	2	1	1	-	1
	198.7	-	-	-	-	-	-	-	-	2	-	1	1	1	-
	193.7	-	-	-	-	-	-	-	-	1	2	1	1	-	-
Check		-	-	-	-	-	-	-	-	2	-	-	1	1	1

Experiment V

Table 5 shows that, of the 12 varieties of carnations tested for their ability to produce harmful gaseous emanations, there were none that produced a noticeable epinastic response on the African marigold indicator plants. It was noted, however, that the varieties Arapahoe, Durango, and Scarlet #18, when tested under these conditions, showed a decided bleaching of the petals and in some instances a brown discoloration and curling of the petals. The test was concluded after six days due to the drying of the soil of the potted marigolds. Epinasty of African marigold plants produced by ethylene gas from apple tissue is shown in Figure 4.

Table 5.--THE EFFECTS ON AFRICAN MARIGOLD PLANTS OF CUT CARNATION VARIETIES.*

Carnation variety	Days of survival					
	1	2	3	4	5	6
William Sim	--	--	--	--	--	--
Arapahoe	--	--	--	--	--	--
Aura	--	--	--	--	--	--
Crowley's Pink Sim	--	--	--	--	--	--
Scarlet #18	--	--	--	--	--	--
Pelargonium	--	--	--	--	--	--
Colorado Gold	--	--	--	--	--	--
Durango	--	--	--	--	--	--
Frosted Pink Patrician	--	--	--	--	--	--
Miller's Yellow	--	--	--	--	--	--
White Sim	--	--	--	--	--	--
Fanfare	--	--	--	--	--	--

* No carnation varieties produced epinasty in African marigold plants.

Experiment VI

Table 6 shows the average number of days cut carnations survived with cultures of molds and soft-rot organisms isolated from the fruits and vegetables used in Experiment I. Cultures from tangerine and orange caused

Figure 3.--Sleepiness of carnations produced by the evolution of ethylene gas from apple tissue.

Figure 4.--Epinasty of an African marigold plant produced by the evolution of ethylene gas from apple tissue.

quick and decided sleepiness of carnations. Cultures from cucumber, cauliflower, and radish produced slight sleepiness. The other cultures tested produced no sleepiness and the test flowers kept equally well as the check flowers.

Table 6.--THE EFFECTS ON CARNATIONS OF MOLDS AND SOFT-ROT ORGANISMS ISOLATED FROM FRUITS AND VEGETABLES.

Source	Sleepiness	Average number of days of survival
Tangerine	+	2
Orange	+	1
Grape	–	12
Pineapple (1)	–	13
Pineapple (2)	–	11
Squash	–	12
Bell pepper	–	13
Cucumber	+	8
Radish	+	7
Cauliflower	+	7
Check		12

Chapter V

DISCUSSION

The problem of keeping cut flowers fresh and in good condition is an ever pressing one for wholesalers and retailers alike. The customer wants freshness and keeping quality priced within his means. Supermarkets are capable of meeting these demands. From the information already known and from that found in this study on the harmful effects of fruits and vegetables on cut carnations, it is evident that segregation of flowers and produce is essential.

Twenty-seven fruits and vegetables that could be found in the supermarkets the year around were stored with cut carnations and their effects noted. No attempt was made to provide experimental proof that the gas produced from these fruits and vegetables was actually ethylene. However, from the facts and findings gathered to date, Denny (8) has shown that ethylene is the only gas from fruits and vegetables that could produce these harmful effects. To adequately determine the intensity of these harmful effects several factors must be taken into consideration: rate of respiration, temperature, humidity, stage of ripening, and the kind and condition of the produce.

Kidd and West, Gane, and others (29,18,22,41,11, 28) have shown that there is a decided rise in the rate of respiration of fruits and vegetables at the onset of ripening. This stage, termed the climacteric, is coincident with the maximum rate of production of CO_2 and occurs when the fruit or vegetable is fully ripe. These workers also found considerable evidence that the maximum rate of ethylene production occurred at this same stage in the case of apples and bananas. In recent studies Crocker (1) has concluded that all ripening plant tissue produces some ethylene gas. It would seem highly probable that all fruits and vegetables attain a maximum rate of ethylene gas production at this stage. This would mean that the same fruits and vegetables could be more or less harmful depending on their stage of ripening. This could account for the observations made in this study where the flowers in one crock survived longer than those in another.

Temperature is also a very important factor in this relationship of respiration and gas evolution. For with every 10 degrees rise in temperature respiration doubles. This would consequently mean an increase in the output of ethylene gas. In these experiments, with an increase in temperature from 60° F to 75° F, the keeping quality of the cut carnations was greatly reduced and the harmful effects of the fruits and vegetables were more pronounced. At a temperature of 38° F keeping quality was greatly increased and the effects produced by the fruits and vegetables were slight.

A high humidity (85-99%) is quite undesirable if the temperature is above 50° F. With such an environment, conditions are adequate for the growth and development of molds and soft-rot organisms.

Fruits or vegetables that were contaminated with molds or soft-rot produced considerably more harmful effects on carnations than sterilized produce items. This was especially true with tangerine and orange, which is in accord with the findings of Miller (34). He stated that a small quantity (petri dish culture) of the fungus <u>Penicillium digitatum</u> found on citrus fruits, produced the same harmful effect as did 24 oranges in the same volume of air. This fact was very evident in this study, for survival of test flowers was much lower in crocks where the produce was diseased. Few isolations from these diseased fruits and vegetables produced harmful effects on culture however. Only the cultured isolates of the organisms found on tangerine and orange have been reported from previous studies. Harmful organisms were found on cucumber, radish, and cauliflower in this study. These have not been described to date by other authors.

For the most part citrus fruits were found to be just slightly harmful to cut carnations. Limes produced a moderately harmful effect at 60° F, and lemons at 75° F.

Oranges produced a slight effect at the higher temperature. Miller (34) reported that oranges and grapefruit produced decided epinasty to indicator plants. However, in reviewing this report, it was found that Miller used several times more produce within the same volume, which would account for these differences.

Twelve carnation varieties were tested for ethylene gas production through their ability to produce epinastic response on young African marigold plants. These plants were used for they had been found to display epinastic response to minute amounts of ethylene gas. None of the carnation varieties tested produced epinasty on the marigold test plants.

If cut flowers are to be stored or displayed with fruits and vegetables, then rate of respiration, temperature, humidity, stage of ripening, and the kind and condition of the fruits and vegetables are the major points that must be considered. Should these factors be present under adverse conditions, the problem of keeping flowers fresh would be a difficult one. With these points in mind, it would be best to store and display cut flowers in supermarkets segregated from fruits and vegetables.

Suggestions for further study

1. Tests with other cut flower varieties should be made to determine if any harmful effects are

produced on these by grocery produce or minute amounts of ethylene gas.

2. Tests with other cut flower varieties should be made to determine if any of these produce ethylene gas in damaging amounts.

3. A study should be made of packaging different cut flowers together and separately, noting any differences in keeping quality.

4. A survey of supermarkets should be made to learn of their shipping methods and storage facilities. In conducting such a survey figures should be obtained on:

(a) the different storage temperatures maintained.

(b) the number of storage refrigerators per supermarket.

(c) the number of managers that would consider it possible to use a separate refrigerator for cut flowers.

(d) the number of managers that would consider selling cut flowers.

5. A study should be made to determine practical methods for the surface sterilization of grocery produce.

Chapter VI

SUMMARY

An effort was made through these studies to determine the effects on cut carnations when stored with common fruits and vegetables. When cut flowers are handled through supermarkets, information of this nature would be extremely beneficial.

1. Most fruits and vegetables produce gaseous emanations, presumably ethylene, in sufficient quantities to be harmful to cut carnations. It is generally believed and accepted that all plant tissue produces some ethylene in its life processes.

2. The major factors contributing to the production of harmful gaseous emanations from fruits and vegetables are rate of respiration, temperature, humidity, stage of ripening, and the kind and condition of the fruits and vegetables. As these factors are altered, so too is the evolution of ethylene gas.

3. Fruits and vegetables infected with molds or soft-rot organisms exhibit considerably more harmful effects to cut carnations than do fruits and vegetables that have been throughly cleaned and sterilized.

4. Carnation flowers do not produce gaseous emanations harmful enough to show epinastic response to young African marigold plants, which are known to be sensitive to one part ethylene in one billion parts of air.

A P P E N D I X

APPENDIX

The following experiment was made early in the course of this study, and the results obtained indicated that etiolated pea seedlings would be most unsatisfactory for use as indicator plants.

High quality Alaska peas were placed in a one per cent solution of $HgCl_2$ for 10 to 15 minutes. This provided surface sterilization. The peas were then counted and sown in small individual tarpaper containers. Vermiculte was used as the growing medium. These containers were placed in a darkened chamber free from any source of light. A pan of water also was placed in the chamber to provide an even humidity. The peas were left in this chamber until they had sprouted and were one to two inches tall. The seedlings were then removed from this chamber and placed under bell jars with Crowley's Pink Sim carnations. A small beaker of 2 N KOH also was placed under each bell jar to absorb the CO_2. Two of the small containers of seedlings were placed under each of the four bell jars used. Three cut carnations were used for each of three jars. A fourth bell jar served as a check. All handling of the seedlings was done in almost total darkness. The only source of light was a small amber globe. Daily observations were made.

The results of this experiment were most inconclusive. None of the seedlings displayed any signs of the triple response, and the growth behavior of the seedlings under treatment was the same as that of the check plants. Moreover, the growth behavior of the seedlings was not at all consistent.

Etiolated pea seedlings should be grown and handled in total darkness or the response will not be accurate. In any tests where daily observations are necessary, this would be impossible. Although the degree of sensitivity of etiolated pea seedlings is quite adequate to measure minute amounts of ethylene gas, in tests of this nature these seedlings should not be used as indicator plants.

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