THE INFLUENCE OF VARIOUS FACTORS ON THE INDUCTION OF BITTERNESS IN STORED CARROTS BY ETHYLENE AND ITS DETECTION BY FLUORESCENCE

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By

JAMES ERNEST ELLS

AN ABSTRACT

Submitted to the College of Agriculture, Michigan State University
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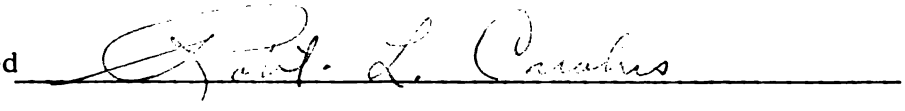
Approved 

The development of bitterness in stored carrots has become an economic problem of considerable importance in recent years, chiefly in carrots grown for processing. The presence of ethylene in the atmosphere caused non-bitter carrots to develop a bitter flavor after a few months in cold storage.

Three tests (organoleptic, fluorescent, and spectrophotometric) were used to determine the degree of bitterness. The organoleptic tests were made on raw carrots which were rated on a scale of 0 to 4, with 0 being non-bitter and 4 being highly bitter. Fluorescence determinations were made by examining the cross sectional cut surfaces of a carrot root, one-half inch below the crown with an ultra-violet lamp having a 2537 Å filter. The rating was on a 0 to 4 basis with no fluorescence having a rating of 0 and an intense yellow-green fluorescent speckling in the phloem denoting a 4 rating. The spectrophotometric rating was arrived at on the basis of the absorption of ultra-violet light at 240 mμ, 265 mμ, and 290 mμ, by a solvent used to extract the bitter principle (3-methyl-6-methoxy-8-hydroxy-3, 4-dihydro-isocoumarin) from the carrot roots. These tests have been successfully correlated with one another.

Storage experiments involving variations in the variety and color

of carrots, soil type and temperature, and the storage atmosphere and temperature, were conducted on carrots stored in sealed 55-gallon drums held at four different temperatures. These drums received a continuous supply of air, which amounted to one complete change of air per day. Variations in the atmosphere were produced by injecting ethylene gas, apple emanations, and automobile exhaust into the air line supplying the drums. Carrot samples were removed from these drums periodically for testing.

Ethylene, whether as a pure gas, or as a constituent in apple emanations or automobile exhaust gas, produced a bitter flavor, fluorescence, and a quantity of the bitter principle in carrots, which was significantly higher in every case than the control carrots.

The quantity of ethylene present in the storage atmosphere and the length of the storage period was significantly related to the degree of bitterness induced in carrots.

The flesh color of the carrot had an apparent effect upon the induction of bitterness by ethylene, with orange carrots becoming most bitter, yellow carrots intermediately bitter, and white carrots least bitter, indicating that if the precursor to carotene is involved in the bitter principle, some of this precursor is present in white carrots.

Physiological age of the carrot was shown to be a factor in the degree

of bitterness induced by ethylene as determined by organoleptic, fluorescent, and spectrophotometric tests, with mature carrots attaining a higher degree of bitterness than either immature or overmature carrots.

In conclusion, ethylene as a pure gas or as a constituent in other gases, was found to be effective in inducing bitterness, fluorescence, and the bitter principle (3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin) in stored carrots. Although all carrots responded to the ethylene treatment, the degree of bitterness induced was influenced by the quantity of ethylene, the time in storage, the flesh color, and the physiological age of the carrots.

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INTRODUCTION

Producers and processors of carrots have recently observed that the roots frequently become bitter during storage. In a few cases carrots scheduled for processing were found to be bitter and were used in soups, where the off-flavor was masked or in extreme cases the carrots were dumped. Since such practices inevitably result in economic loss, investigations were undertaken to determine the cause of this bitterness and to find means of preventing its occurrence.

Considerable preliminary work on this problem has already been done. The bitter principle has been identified as 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin (37). Methods for its detection and quantitative procedures for measuring it have been devised. Several possible causes for the trouble have been explored with varying degrees of success.

One of the more promising approaches has been from the standpoint of atmospheric conditions prevailing in carrot storages. Bessey (4) observed a relationship between the degree of bitterness and the presence of apples in storage. To verify these observations, a study was undertaken to determine the influence of a number of environmental factors and variety on the development of bitterness in carrots when subjected to various storage environments.

REVIEW OF LITERATURE

Bitterness in Crops

Bitterness is a quality which is desirable in certain beverages, such as coffee, cocoa and beer, but is quite undesirable in most horticultural crops. Truscott (39) has reported an instance of bitterness in celery, which was so severe that the crop was unmarketable. However, only one person in three could detect this bitterness which was described as hot and peppery, and an exaggeration of the normal celery flavor. Since it disappeared in storage, it was regarded as a temporary phenomenon. Enslin (11) observed that bitterness in cucumbers was traced to an enzyme, elaterase and occurred in thirty-three different species in the Cucurbitaceae family. Higby (19) has identified isolimonin in navel orange juice which develops bitterness upon exposure to air or pasteurization. He also refers to narigin, the bitter glycoside in grapefruit skin, and limonin, the bitter substance on orange seeds.

Off-flavors have been reported by Himreimer (20) and Gilpin (14) in root crops grown on soils which had been treated with either benezene hexachloride (B. H. C.) or lindane. Lean (23) reported off-flavor in carrots caused by aster yellows, and Yamaguchi et al. (44) reported another off-flavor in carrots associated with green "shoulders".

Bitterness in Carrots (Isocoumarin Type)

The carrot bitterness with which this study is concerned was reported by Truscott (39) in 1953. He described two types of bitterness. The first flavor was a peppery taste which imparted a painful burning sensation in the mouth and disappeared upon processing. The other flavor was a strong, spicy-hot, flavor which also left a burning sensation in the mouth, and this flavor was not destroyed by processing. In 1955, Yamaguchi et al. (44) reported a bitterness in carrots not due to aster yellows, green shoulders or weed control chemicals. This bitterness which developed in storage, was found in 10 to 90 per cent of the carrot roots. Atkins (2), Sondheimer et al. (36), and Dodson et al. (9) have experienced a taste sensation in stored carrots similar to that described by Truscott (39) and Yamaguchi et al. (44).

In a more recent publication Yamaguchi et al. (44) found a difference in the alpha-carotene content between lots of bitter and non-bitter carrots. Petroleum ether extracts of the chromatograms of magnesium oxide and "Hy-flo Super Cel"[1] indicated that the bitter fraction was strongly adsorbed on the top portion of the column. Phillips (28) extracted the bitter fraction from the top of the column with methanol (spectrograde) and determined the bitter substance quantitatively with a model DK-2 Beckman spectrophotometer.

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[1] A product marketed by John's Mansville Corporation.

Concurrently, Sondheimer (36) found that a petroleum ether extract of a sample of pureed carrots of known bitterness prior to canning, produced a bell-shaped absorption curve when measured with a Beckman spectrophotometer. The minimum absorption values were 240 mμ and 290 mμ, and the maximum absorption value was 265 mμ. The following formula was used to express these results:

$$\text{Opitcal density 265 m}\mu - \frac{\text{(optical density 290 m}\mu + \text{optical density 240 m}\mu)}{2}}{\text{Weight of sample (grams)}} \times 40 \text{ ml.}$$

A positive correlation was noted between the rating determined by this formula and that found by taste. He found that the phloem contained more of the bitter principle than the xylem, and that this analytical procedure worked equally well on fresh or processed carrots.

In a later publication, Sondheimer (37) reported the formula for the bitter principle as $C_{11}H_{12}O_4$, molecular weight 208, melting point 76°C, and named the compound 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin, which in this paper has been abbreviated to "isocoumarin". From this data he calculated the factor 2.2, which when multiplied by the reading at 265 mμ gave the weight of isocoumarin in milligrams per 100 grams of sample.

Steam distillation of the bitter carrots yielded a distillate of tiny oil droplets having a bitter taste. Isocoumarin could not be detected in the dis-

tillate by chemical analysis. However, isocoumarin was easily detected in the residue. Moreover, crystalling isocoumarin was only slightly volatile with steam (37). This supports Truscott's original contention that bitterness is derived from two compounds.

According to Sondheimer (37) isocoumarin placed directly on the tongue gave no bitter taste, but the taste panel found that a .01 per cent aqueous solution was bitter. Further tests showed that when .02 per cent isocoumarin was added to the steam distillate of non-bitter carrots, the tasting panel could not distinguish it from the steam distillate of bitter carrots.

Dodson et al. (9) extracted 100 grams of the principle from 14 bushels of bitter carrots with acetone. This crystalline compound produced an ultra violet absorption curve on the spectrophotometer similar to the one described by Sondheimer. The colorless platelets were soluble in chloroform, ethyl ether, water, and methanol.

Factors Influencing Bitterness in Carrots

Truscott (39) found that carrots lost their bitter flavor when held at room temperature, or when they were allowed to sprout in storage. Yamaguchi et al. (44) stored carrots at 32°, 50° and 77° F, and found no significant difference in the occurrence of bitterness. However, Bessey (4) concluded that carrots became more bitter at temperatures higher than 32° F.

Neither Atkins (2) nor Bessey (4) found any varietal difference pertaining to bitterness, although Yamaguchi et al. (44) found that the progeny of bitter carrots were more bitter than the progeny of non-bitter carrots. Atkins and Sayre (3) observed that minor elements lessened the degree of bitterness, and also that muck and sandy soils produced carrots more susceptible to bitterness. Neither Bessey (4) nor Yamaguchi et al. (44) could find any significant influence due to soil type; and Yamaguchi et al. (44) and Atkins (2) concluded from irrigation trials that soil moisture was not a factor in predisposing carrots to bitterness.

Bessey (4) in harvesting and handling studies was unable to relate rough handling to bitterness development, and indicated that early planted carrots developed lower levels of bitterness than the later planted carrots when placed in storage. Both Atkins (2) and Bessey (4) observed that immature carrots attained less bitterness in storage than did mature carrots.

In storage studies, Bessey (4) found no correlation between oxygen and carbon dioxide levels and bitterness; however, he obtained evidence that indicated that apple emanations caused bitterness.

Changes in Carrot Composition During Storage

During the first 30 days of storage, Brown (6) found an increase in carotene content of carrots on a dry weight basis. The carrots remained at

this higher level for the next 60 days. Platenius (29) observed that the moisture in stored carrots at 32°, 35°, 40° and 50° F increased slightly in all lots except those held at 32° F. He noted that sucrose was converted to reducing sugars and the latter substances were reconverted to sucrose, and that the conversions were accelerated by an increase in temperature.

Appleman (1) showed that the rate of respiration of fresh carrots rapidly declined with the age of the roots. Once in storage, the respiration rate was never as great as at the time of harvest, and there was no indication of a sharp rise of carbon dioxide in storage (climacteric). Wright et al. (43) showed that the respiration rate of carrots was three times as great as apples. Werner (41) found reducing sugars to increase in carrot varieties during storage, while Rygg (34) and Brown (6) observed an increase in carotenoid content. Newhall (26) reports an increase in water of some lots when the relative humidity was maintained above 94 per cent.

Influence of Apple Emanations and Ethylene on Stored Produce

Bessey (4) suggested apple emanations played a role in the development of the bitter principle in carrots. A review of the literature reveals that apples produce a vapor which delay abnormal sprouting in potatoes (13), and that apples, pears, peaches, tomatoes, and bananas yield a gas which stimulates ripening and produces a critical rise in respiratory activity of im-

mature fruit (13). Nelson (24) concluded that ethylene was the active ingredient in apple emanations and measured its evolution (substantiated by Hanson and Christensen (17) in 1939). Nelson (25) in another study showed that ethylene from the apple prior to its climacteric is consumed in the ripening process. Smock (35) noted the stimulating effect of one lot of apples upon another. Rood (33) observed that apple emanations as well as ethylene caused brown spot injury on lettuce and was most effective in producing injury at 44° F of firm heads.

Ethylene is an unsaturated hydrocarbon gas, non-poisonous, has a faint sweetish odor with a boiling point of -103. 9° C, and a specific gravity of 0. 975. It is soluble in water to the extent of 25. 6 cc per 100 grams of water at 0° C (30). Denny (8) showed that kerosene heaters produced ethylene which unduly ripened citrus while in transit to northern markets. Crocker (30) tested 28 gases and found that ethylene, propylene, acetylene, butylene, and carbon monoxide produced epinasty in tomato seedlings; but that ethylene was 500 to 500, 000 times more effective than the other four gases. Englis and Dykins (30) concluded that ethylene had no effect upon pure enzymes and that it acted directly on living protoplasm. Williamson (42) observed that ethylene caused epinasty, yellow coloration, and abscission of leaves, flowers, and fruits, and that tissue infected with certain pathogens showed a marked stimulation in ethylene production. He cited the black spot of rose and the

shot hole of cherry as examples. In 1951, Hall (16) stated that the only requirement for the production of ethylene is a fermentable substrate under aerobic conditions. He believed that sugar was the original ethylene producing substance; however, he suggests that a complex sequence of intermediates catalyzed by the necessary enzyme systems is required to produce ethylene. In fruit ripening, he suggested that ethylene functioned autocatalytically, thus accelerating its own production. Young et al. (45) proved that Penicillium digitalum evolved ethylene. In 1953, Curtis (7) showed that as little as 1 ppm of ethylene damaged dormant nursery stock of apple and pear. The damage occurred seven times as rapidly at 55° F as at 35° F. In one case the source of ethylene was an apple room adjacent to a well insulated wall.

Fenning (13) demonstrated that auto exhaust produced some unsaturated hydrocarbons, but did not identify them. Rahrbaugh (32) noted that automobile exhaust induced epinasty in pea seedlings, and that 25 ml. of motor exhaust produced the same effect as 0.05 ml. of ethylene. Further work by Crocker, Zimmerman and Hitchcock (30) found automobile exhaust to contain 9.3 percent carbon monoxide. Rahrbaugh (32) reasoned that exhaust gas contained ethylene since the epinastic response was too great to be caused by carbon monoxide alone.

The influence of ethylene and other storage conditions on the postharvest physiology of fruits and vegetables has received excellent treatment in three review articles; Biale (5) 1950, Pentzer and Heinze (27) 1954, and

Ulrich (40) 1958. However, the influence of ethylene on stored carrots is not mentioned.

Fluorescence

Fluorescence refers to the property of substances to emit visible light when excited with invisible radiation. The invisible radiation is generally produced by ultra-violet light, which was discovered by Ritter (22) in 1801. Fluorescence is generally defined as invisible light with a wave length between 40 and 4000 Å. Radley (31) cites examples where fluorescence had found practical application in the field of agriculture in distinguishing between barley seeds, between rye grass seedlings, in determining composition of animal feeds, in determining the amount of coumarin present in sweet clover, in detecting bruises on fruit, and to determine infection.

Bessey (4) noted that bitter carrots had a yellow-green fluorescence and the intensity was related to the degree of bitterness and to the isocoumarin content of the root. Radley (31) states that a number of three-carbon compounds show a greenish fluorescence in alkaline solution. Strain (38) observed a fluorescence above the beta carotene band in the adsorption column. The substance in this band has not yet been isolated into crystalline form. Zechmeister and Sandoval (47) reported that petroleum ether was a good solvent for extracting many fluorescent substances from plant tissue. The extracted substances could be measured quantitatively with a Beckman spectrophotometer. According to Goodwin and Kavanaugh (16) vitamin A has a yellow or green fluorescence.

GENERAL METHODS

Storage Apparatus

The carrots were placed in mesh bags, in 55-gallon air tight drums, fitted with removable heads, and placed in cold storage. The removable heads were equipped with inlet and outlet tubes, connected to a compressed air source, and regulated so that a rubber tube attached to the inlet tube of each drum conveyed 144 ml. of air per minute, or a complete change every 24 hours, through a metering bottle to the drum.

To produce apple emanations, a 16-liter, wide mouthed bottle filled with apples of various seasonal varieties and fitted with a gas tight lid having inlet and outlet hoses attached to the drum (Figure 1). Air was passed into the bottle and then through a sodium hydroxide wash bottle to remove carbon dioxide, and then into the drum stored in the cooler. The exhaust from the drum was passed through an outlet hose, and bubbled through a water seal.

The control and the ethylene drums received their air directly from the metering bottles, the only difference being that a removable section of hose with a volume of 42 ml. was filled with ethylene gas and inserted into the air flow-line every 48 hours. One such charge of ethylene provided a temporary maximum of 200 ppm of ethylene gas in the atmosphere of the drum. Since 55 gallons of air were passing through the drum each day, the ethylene was soon dissipated and had to be readministered.

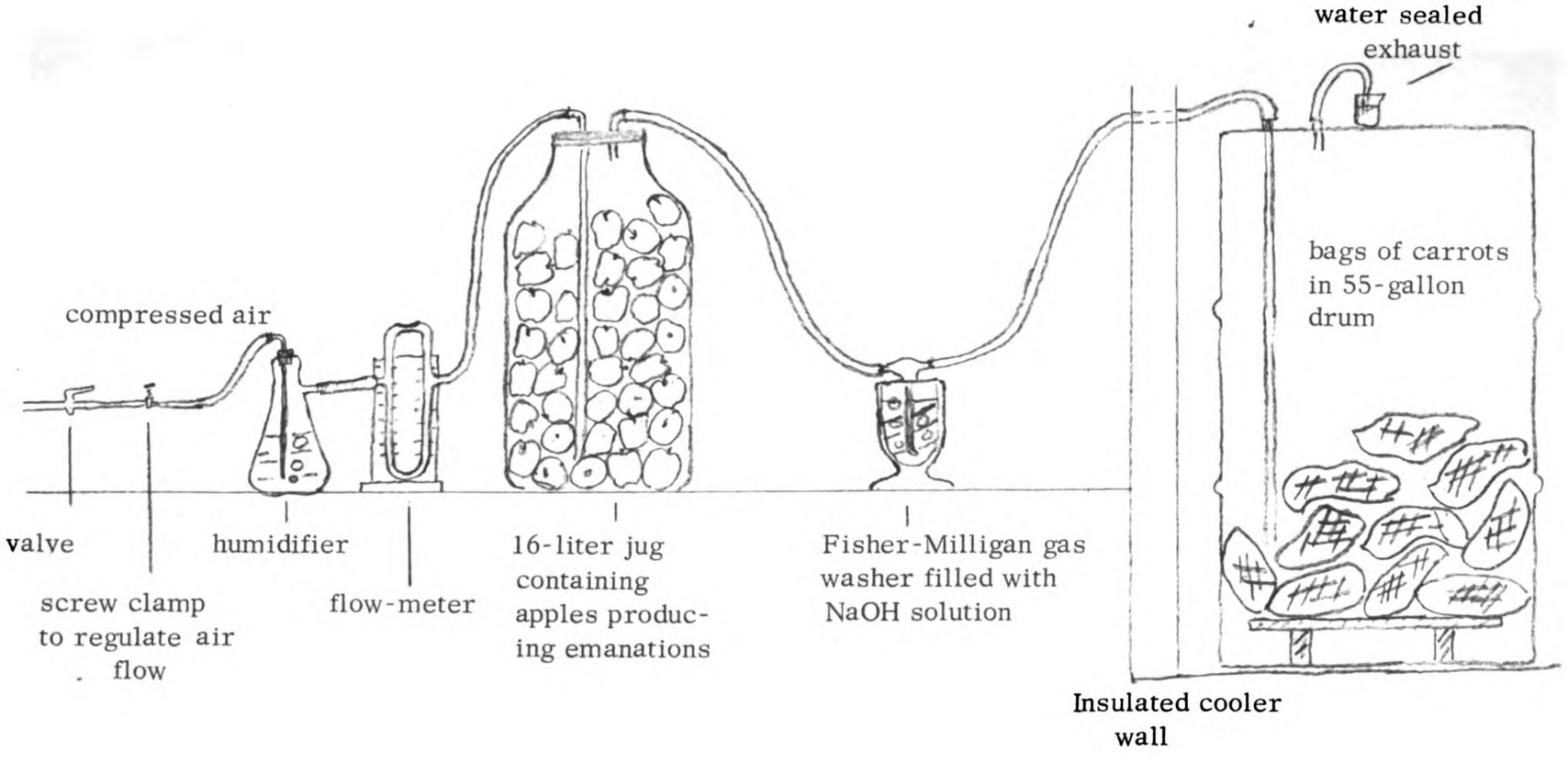

Figure 1. Diagram of apparatus used for administration of apple emanations to carrots in storage.

Analytical Procedure

At each sampling a representative bag of carrots was removed, and small segments were taken out of several carrots and tasted. An organoleptic rating for the sample was designated on the basis of a 0 to 4 scale with 0 being non-bitter and 4 being extremely bitter.

A fluorescent rating was made on the same carrots by slicing a half inch of the crown off of each carrot and examining it in a dark room under a ultra-violet lamp with a 2537 Å filter[1] for fluorescence. Each root was assigned a value on the basis of a 0 to 4 scale, with 0 being non-fluorescent, and 4 being highly fluorescent. The values for each root were averaged. The threshold for bitterness by fluorescence was approximately 1.5. The type of fluorescence associated with bitterness is shown in Figure 2. In this figure is shown the three enlarged photographs of a normal and bitter carrot in cross section. The lower photograph reveals the difference between the carrots under the 2537 Å lamp. The speckled fluorescent pattern in the phloem is typical of bitter carrots, with this particular carrot having a fluorescence intensity rating of 4.

The spectrophotometric determinations were made using a longitudinally cut quarter from each carrot of the sample. These quarters were pureed in a Waring Blendor with an amount of water equal to their weight. A five-gram sample was weighed into a 50 ml. ground glass stoppered, Erlen-

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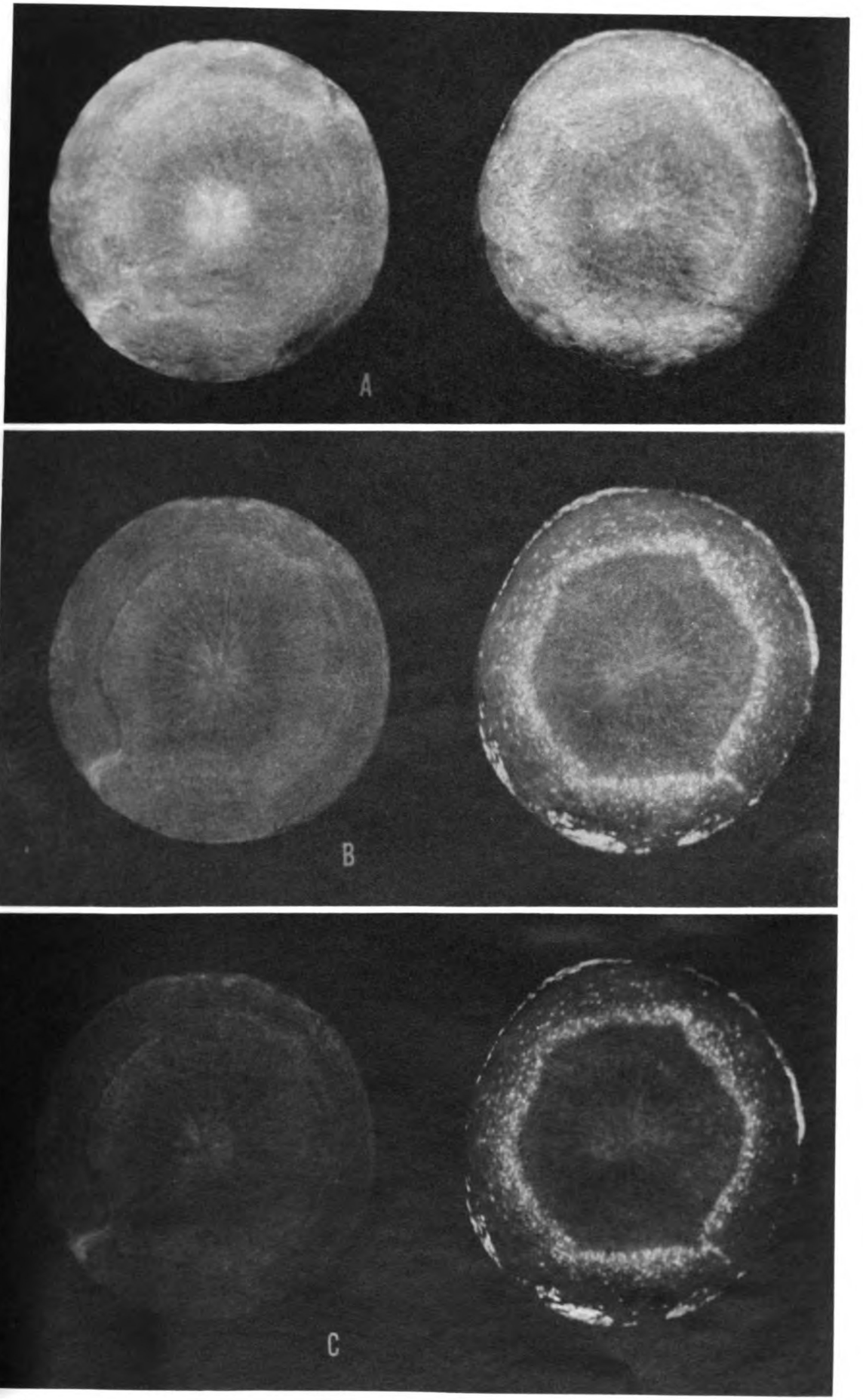
[1] "Mineral Light" model SL 2537 short wave. Ultra-Violet Products, Inc., South Pasadena, California.

Figure 2. Fluorescence of cross sections of normal and bitter carrot roots. (Normal on left, bitter on right).

A. Under photoflood reflector lamp.
B. Under ultra violet light 3650 Å wave length.
C. Under ultra violet light 2537 Å wave length.

(X1 filter used with all lights)


A
B
C

meyer flask and shaken with 40 ml. of "Skelly-solve B"[1] three times for 15 seconds each, to extract the bitter principle. The supernatant was decanted into a spectrophotometer cell and absorption determined with a DK-2 Beckman spectrophotometer. The ultra violet light was passed through the cell at wave-lengths of 240 mμ, 265 mμ, and 290 mμ. The absorption of light at these wave lengths was recorded on a graph, and the data was interpreted with the aid of Sondheimer's formula to estimate the quantity of isocoumarin. This procedure is a modification of Sondheimer's method (34). The spectrophotometric determination of isocoumarin ranged from 0.00 for non-bitter carrots to 17.00 for highly bitter carrots, with the taste threshold for bitterness evaluated at 0.75. These results are more exact than those obtained by either the organoleptic or fluorescent methods of analysis, which are subjective in nature.

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[1] Skelly-solve, a product marketed by the Skelly Oil Company.

STUDIES OF THE INFLUENCE OF CULTURAL AND STORAGE PRACTICES ON THE DEVELOPMENT OF BITTERNESS IN CARROTS

Many of the original suspected causes of bitterness, such as nutrition and spray chemicals often having been explored were eliminated as possible causes by several workers; however, there still remained some question as to the influence of planting date, time of harvest, and soil type.

Bessey (4) observed that the soil types were of little consequence in predisposing carrots to bitterness, while Atkins (2) believed that muck and sandy soils produced carrots more inclined to bitterness than upland soils. In regard to harvest dates, Atkins (2) showed that earlier harvested carrots became more bitter than later harvested carrots, which was confirmed by Bessey (4). In addition, Bessey found that earlier planted carrots became more bitter in storage than those that were planted later.

As work on bitterness progressed, it became apparent that post-harvest handling influenced the development of bitterness. Bessey (4) observed that the bitterness developed in carrots stored with apples, and thought it was due to ethylene emanations from the fruit.

The purpose of this experiment was to test the influence of apple emanations and ethylene on the development of bitterness in carrots, of different maturities, that had been grown on different soils.

Methods and Procedure

Through the courtesy of the Gerber Products Company, Long Type Chantenay carrots were furnished for the experiment. Samples were taken from two muck fields and from a mineral soil planting. One of the muck plots was seeded on April 10 and designated "early", the other muck plot was planted on May 1 and designated "late", and the mineral plot also planted on May 1, was designated "mineral". Carrots from these three plots were harvested at different times and subjected to various storage conditions.

The first harvest was made on August 8 from the two muck areas. At time of harvest, there was no organoleptic, fluorescent, of spectrophotometric evidence of bitterness. Five 20-root lots of carrots from each of two fields were placed in storage.

The carrots were sampled at weekly intervals during storage for bitterness by organoleptic, fluorescent, and spectrophotometric determinations. Estimations were made on six, 20-root samples at weekly intervals for five weeks.

A second harvest was made from the same plots on September 6, and in addition, carrots from the mineral loam were included. Seven lots from each of the three fields were placed in each drum. This time, however, a fourth drum was added, which was given only an initial charge of 42 ml. of ethylene. This treatment is referred to as "ethylene prime".

The third and final harvest was made from these plots on October 11. The carrots were not bitter at time of harvest. A bushel of carrots from each plot was also stored at this time in a pit at the horticultural farm as an additional control for the refrigerated carrots that were in the drums. A sample of carrots from the "late" muck was packed in ice at the time of harvest, and placed in the ethylene drum because it had been suggested that carrots might not become bitter when the respiratory activity was retarded from the time of harvest.

The carrots which were held in pit storage were dug on December 3, and found to be non-bitter, and non-fluorescent. They were then divided into two lots and placed in the control and ethylene drums for 10 days at which time they were again observed for fluorescence.

Results

Table I A, B and C show the influence of pre-harvest environment and storage under various conditions on the development of bitterness as indicated by organoleptic, fluorescent, and spectrophotometric tests. The figures in Table I A are averages of carrots from both muck fields and the mineral field.

Regardless of the date of harvest (A, B, or C), the averages of carrots subjected to apple emanations, ethylene gas, and "ethylene prime" were

TABLE I

The Influence of Age and Storage Treatment on Development of Bitterness in Chantenay Carrots Stored at 32° F

A. Early Harvest

Weeks in Storage	Control	Apple	Ethylene	Average
	Organoleptic Rating of Bitterness[a]			
1	0.0	1.0	2.0	0.7
2	0.0	2.0	2.0	1.3
3	1.0	3.0	3.0	2.3
4	0.0	1.0	2.0	2.0
5	0.0	1.5	1.0	0.8
Ave.	0.2	1.7	2.0	1.3
	Fluorescence Rating of Bitterness[b]			
1	0.0	0.0	0.5	0.2
2	0.5	1.0	2.0	1.2
3	1.0	2.0	2.0	1.7
4	0.5	3.0	3.5	2.3
5	0.5	2.0	2.0	1.5
Ave.	0.5	1.6	2.0	1.4
	Spectrophotometric Rating of Bitterness (Isocoumarin)[c]			
1	.045	.075	.135	.087
2	.250	.690	1.390	.780
3	.735	2.055	1.820	1.537
4	.380	2.575	5.330	2.762
5	.495	7.040	5.760	4.428
Ave.	.383	2.487	2.885	1.919

	Treatment		Storage		T X S	
L. S. D.	5%	1%	5%	1%	5%	1%
Organoleptic	.65	.98	.84	1.26	-	-
Fluorescence	.41	.60	.53	.77	.92	-
Spectrophotometric	1.02	1.48	1.31	1.90	2.29	3.31

[a]Rating on a 0-4 basis; taste threshold: 1

[b]Rating on a 0-4 basis; taste threshold: 1.5

[c]Ratings have ranged from 0.0-17.0; taste threshold: 0.75

TABLE I (Continued)

B. Intermediate Harvest

Weeks in Storage	Control	Apple	Ethylene	Ethylene Prime	Average
	Organoleptic Rating of Bitterness[a]				
1	0.0	0.6	1.0	0.0	0.4
2	0.0	1.0	1.7	1.3	1.0
3	0.3	2.0	2.7	1.3	1.6
4	0.0	2.3	2.7	1.3	1.6
5	0.0	1.6	1.3	0.3	0.8
6	0.3	2.6	2.7	2.0	1.9
7	0.0	2.6	2.0	2.0	1.7
8	0.0	2.3	1.7	2.0	1.5
Ave.	0.1	1.9	2.0	1.3	1.3
5-week ave.	0.1	1.5	1.8		
	Fluorescence Rating of Bitterness[b]				
1	0.17	1.17	1.17	1.83	1.09
2	0.00	2.00	2.33	2.33	1.67
3	0.00	2.50	3.00	2.17	1.92
4	0.00	3.67	3.67	2.00	2.34
5	0.00	2.00	2.67	1.67	1.59
6	0.00	2.67	3.33	2.67	2.17
7	0.10	2.43	2.10	2.37	1.75
8	0.07	2.80	2.73	1.90	1.88
Ave.	0.04	2.41	2.63	2.12	1.80
5-week ave.	1.03	2.27	2.57		
	Spectrophotometric Rating of Bitterness (Isocoumarin)[c]				
1	.073	.380	.613	.919	.496
2	.390	1.883	2.483	2.173	1.732
3	.510	6.707	6.583	2.510	4.077
4	.700	4.800	5.337	4.383	3.805
5	.087	5.457	10.410	2.857	4.703
6	.180	5.580	7.653	3.460	4.218
7	.120	7.143	7.177	2.757	4.299
8	.150	8.523	8.930	3.357	5.240
Ave.	.277	5.059	6.148	2.802	3.322
5-week ave.	.352	3.845	5.085		

L. S. D.	Treatment 5%	Treatment 1%	Storage 5%	Storage 1%	T X S 5%	T X S 1%
Organoleptic	.4	.6	.6	.8	-	-
Fluorescence	.25	..34	..36	.48	.72	.99
Spectrophotometric	1.19	1.60	1.68	2.27	3.35	4.53

a, b, c See footnotes Table I A.

TABLE I (Continued)

C. Late Harvest

Weeks in Storage	Control	Apple	Ethylene	Ethylene Iced[d]	Average
	Organoleptic Rating of Bitterness[a]				
1	0.0	0.7	0.3	1.0	0.3
2	0.0	1.3	1.3	1.0	0.9
3	0.0	2.7	1.3	0.0	1.3
4	0.7	3.0	2.7	2.0	1.9
Ave.	0.2	1.9	1.4	1.0	1.2
	Fluorescence Rating of Bitterness[b]				
1	0.00	1.51	0.98	1.07	0.83
2	0.00	2.53	2.07	1.64	1.53
3	0.00	2.34	2.15	0.85	1.50
4	0.44	2.78	2.58	1.80	1.93
5	0.14	2.88	2.73	1.94	1.92
6	0.27	2.95	2.67	1.93	1.96
Ave.	0.14	2.50	2.20	1.54	1.61
5-week ave.	0.11	2.41	2.10		
	Spectrophotometric Rating of Bitterness (Isocoumarin)[c]				
1	.440	.913	.517	.702	.623
2	.440	1.647	1.813	.976	1.301
3	.387	3.733	3.170	1.560	2.430
4	.217	4.383	4.143	2.370	2.581
5	.490	4.430	3.603	1.440	2.841
6	.367	5.560	3.867	2.200	3.265
Ave.	.390	3.444	2.852	1.541	2.229
5-week ave.	.394	3.021	2.649		

	Treatment		Storage		T X S	
L.S.D.	5%	1%	5%	1%	5%	1%
Organoleptic	.60	.85	.70	.97	-	-
Fluorescence	.21	.29	.30	.41	-	-
Spectrophotometric	.41	.56	.58	.78	1.01	1.37

[a, b, c] See footnote Table I A.

[d] Ethylene iced carrots are not included in statistics.

TABLE II

Analysis of Variance Summary for the Influence of Age and Storage Treatment on Development of Bitterness in Chantenay Carrots

Factor	D. F.	Organoleptic Variance	Fluorescence Variance	Spectrophotometric Variance
		A Harvest		
Source	1	1.10	.27	8.53
Treat.	2	6.75**	5.99**	178.15**
Store. T	4	1.60*	3.79**	176.09**
S x T	2	.35	.37	5.62
T x ST	8	.20	.67*	50.49*
S x ST	4	.13	.06	13.46
Error	8	.35	.16	9.78
		B Harvest		
Source	2	3.00**	.26	39.23
Treat.	2	17.67**	33.90**	2342.40**
Store. T	7	3.14**	1.75**	327.83**
S x T	4	.83	.26	61.22
T x ST	14	.52	.66**	18.86
S x ST	14	.43	.19	106.18*
Error	28	.52	.19	40.45

Factor	D. F.	Organoleptic Variance	D. F.	Fluorescence Variance	D. F.	Spectrophotometric Variance
				C Harvest		
Source	2	.58	2	33.03**	2	1.56
Treat.	2	9.75**	2	275.97**	2	472.29**
Store. T	3	5.07**	5	17.75**	5	97.17**
S x T	4	.84	4	10.76**	4	10.22*
T x ST	6	.83	10	2.14	10	29.17**
S x ST	6	.22	10	1.73	10	1.89
Error	12	.46	20	.92	20	3.47

*Significant to the 5% level.

**Significant to the 1% level.

all significantly more bitter than the controls under all three methods of analysis (organoleptic, fluorescence, and spectrophotometric).

Organoleptically, the average of the first sample from storage was significantly more bitter than the first sample in harvests B and C; while in harvest A there was no significant difference between the first sample and the last sample. Using fluorescent and spectrophotometric analysis in all harvests (A, B and C), the averages of the last samples were significantly more bitter than the averages of the first samples.

The spectrophotometric analysis under all harvests (A, B and C) show figures which are significantly more bitter for treatment x storage, while fluorescence analysis under harvests A and C show figures which are significantly more bitter than others. Organoleptic estimations show no significance for treatment x storage.

In terms of isocoumarin, on the five-week averages for treatments, carrots which were harvested in September (B) became considerably more bitter than those harvested in a more immature (A) or more mature (C) condition.

It is also observed that "ethylene prime" carrots did not become as bitter as those receiving ethylene and apple emanations continuously, but more bitter than the controls. In the third harvest, the iced carrots were observed to lag behind the non-iced in the development of isocoumarin.

As indicated in Table II, the organoleptic estimations for source were significant for harvest B, and the fluorescence estimations for source were significant in harvests A and C.

Pit-stored carrots were found non-bitter and non-fluorescent after a month in storage. After treatment in the drums for ten days it was revealed that these carrots which were placed in the ethylene drum became fluorescent, with the mineral carrots having as estimation of 1.6, the late planted muck carrots having an estimation of 0.9, and the early planted muck carrots having a rating of 0.5. The controls remained practically non-fluorescent with a rating of 0.1 for both the early and late and 0.0 for the mineral.

Discussion

A statistical analysis of the data indicated that neither soil type nor time of planting had a significant effect on the development of isocoumarin according to the spectrophotometric rating, which is the most exact quantitative test. The organoleptic and fluorescent estimations showed highly significant results for source in three cases, indicating that soil type and time of planting made a difference; however, it must be remembered that these are only subjective methods. The statistical analysis of the data also indicate that ethylene, whether in the form of apple emanations, or as a pure gas, promoted bitterness, fluorescence, and isocoumarin development in carrots. The pit-stored carrots provided further evidence of this phenomenon when they became bitter after subjection to ethylene.

The fact that "ethylene prime" carrots were significantly less bitter than the ethylene and apple emanation treated carrots, and more bitter than the control, suggests that the degree of bitterness may be related to the quantity of ethylene present.

The higher values of ethylene treated carrots under all three methods of analysis as compared with the apple emanation treated carrots from the early and intermediate harvests on the basis of the five-week average, and the reversal of this tendency in the late harvest, suggests that the more mature apples used in the later harvest produced a larger quantity of ethylene than was being administered to the ethylene drum.

The figures show that the iced carrots lagged behind non-iced carrots in production of isocoumarin suggesting that the icing slowed down the metabolism of the carrots which, in turn, slowed down the production of the bitter principle.

The fact that both the spectrophotometric and fluorescent ratings for the ethylene treatment on a five-week average are higher for B than for A or C suggests that there is a physiological age when carrots are most susceptible to the development of isocoumarin. The reason for using the five-week average was to put A, B and C on a comparable basis. The reason for considering the ethylene treatment and not the apple emanation treatment was because the ethylene was administered in like quantities to all harvests, whereas the amount of ethylene emanation for apples varied with the variety and maturity of the apples used. The fact that there was no statistical difference in spectrophotometric measurements between planting dates (source) was probably because plantings were too close together.

THE INFLUENCE OF VARIETY ON THE DEVELOPMENT OF BITTERNESS IN STORED CARROTS

Although neither Bessey (4) nor Atkins (2) found a difference in varietal susceptibility of bitterness, six varieties were subjected to storage studies involving ethylene.

Methods and Procedure

Arrangements were made with the Ferry Morse Company of Detroit to procure the following varieties, grown on muck: Touchon, Long Type Chantenay, Red Core Chantenay, Imperator, St. Valery, and Gold Pak. On September 25, the varieties were harvested and a sample of each was examined for fluorescence. None was found. The remainder of each variety was divided into four lots and 24 mesh bags were placed into two 55-gallon drums at 34° F. Both the "ethylene prime" and the control drum received 144 ml. of air per minute with the ethylene prime drum receiving a charge of 42 ml. of ethylene gas at the start of the treatment.

Results

After three weeks in storage, a sample was analyzed and although the taste was not affected by the ethylene treatment, the appearance of fluorescence was quite apparent. After six weeks when the second and final sample was taken, a bitter flavor had developed in these carrots. Table III shows

TABLE III

The Influence of Variety on the Development of Bitterness in Stored Carrots as Determined by Organoleptic, Fluorescent and Spectrophotometric Ratings at 34° F

Variety	Days to Maturity	Control			Ethylene		
		3 wk.	6 wk.	Ave.	3 wk.	6 wk.	Ave.
		Organoleptic Rating of Bitterness					
Touchon	68	0.0	0.0	0.0	0.0	2.0	1.0
L. T. Chantenay	70	0.0	1.0	0.5	0.0	1.0	0.5
R. C. Chantenay	77	0.0	0.0	0.0	0.0	2.0	1.0
Imperator	77	0.0	0.0	0.0	0.0	1.0	0.5
Gold.Pak	77	0.0	0.0	0.0	0.0	1.0	0.5
St. Valery	85	0.0	0.0	0.0	0.0	1.0	0.5
Ave.		0.0	0.1	0.1	0.0	1.3	0.7
		Fluorescent Rating of Bitterness					
Touchon	68	0.0	0.0	0.0	2.0	1.8	1.9
L. T. Chantenay	70	0.0	0.0	0.0	2.0	1.3	1.7
R. C. Chantenay	77	0.0	0.0	0.0	2.0	1.6	1.8
Imperator	77	0.0	0.0	0.0	2.0	0.9	1.5
Gold Pak	77	0.0	0.0	0.0	1.0	1.1	1.1
St. Valery	85	0.0	0.0	0.0	1.0	0.7	1.7
Ave.		0.0	0.0	0.0	1.7	1.1	1.4
		Spectrophotometric Rating of Bitterness					
Touchon	68	.100	.042	.071	.837	2.110	1.474
L. T. Chantenay	70	.075	.071	.073	.622	1.330	1.475
R. C. Chantenay	77	.204	.270	.237	.331	1.870	1.101
Imperator	77	.125	.090	.158	.678	1.370	1.024
Gold Pak	77	.149	.196	.173	.702	1.540	1.121
St. Valery	85	.055	.033	.044	.140	.270	.205
Ave.		.093	.084	.126	.552	1.415	1.067

L. S. D.	Treatment		Storage Time		T. x S. T.	
	5%	1%	5%	1%	5%	1%
Organoleptic	.511	.613	1.37	1.51	.786	-
Fluorescent	.357	.427	-	-	-	-
Spectrophotometric	.414	.649	1.45	-	-	-

the results of these findings, with the varieties listed in the order of maturity. Table IV indicates the variance summary for the influence of variety of bitterness development.

TABLE IV

Analysis of Variance Summary for the Influence of Variety on the Development of Bitterness in Carrots

Factor	D. F.	Organoleptic Variance	Fluorescent Variance	Spectrophotometric Variance
Variety	5	.08	.146	.204
Treatment	1	2.05*	12.586**	4.498**
Storage Treat.	1	3.38**	.286	1.115*
V x T	5	.14	.165	.159
V x S T	1	.07	.047	.066
T x S T	1	2.03*	.286	.621
Error	5	.14	.068	.157

*Significant to 5% level.

**Significant to 1% level.

Discussion

Carrots which gave a reading of less than 0.75 were not considered bitter; therefore, there is apparently good agreement between organoleptic and spectrophotometric ratings. The fluorescence rating of ethylene treated carrots in the first sample is shown to be higher than the rating of the second sample. This is probably due to the fact that the method of rating carrots for fluorescence was changed in the period between the two samplings. The rating scale remained the same; however, the carrots in the first sample were rated by estimating the amount of fluorescence of a particular lot and assigning a number to it. In the second lot, a value was assigned to each carrot in the lot, and their average value became the value for the lot. Had the individual root analysis been used on both the first and second lot, it is believed that the first lot would have been lower than the second.

Although the varieties all responded to the "ethylene prime" treatment, it is noted that the values for Touchon were higher than the values for St. Valery. While all varieties were planted on the same day, Touchon was more nearly mature than St. Valery on the basis of days to maturity. The difference in bitterness ratings may, therefore, be due to maturity rather than variety.

THE INFLUENCE OF SOIL TEMPERATURE ON THE DEVE LOPMENT OF BITTERNESS IN STORED CARROTS

Since carrots appear to vary in their degree of bitterness from year to year (4), it was thought that soil temperature during growth might influence the development of isocoumarin in stored carrots.

Methods and Procedure

On July 15, seeds of the variety Nantes, Red Core Chantenay, and Imperator were planted in a muck-loam soil mixture, in 48 2-gallon, glazed crocks. They remained outside until September 5, when they were moved to a greenhouse, placed in temperature tanks, and thinned to 12 carrots in each crock. There were 12 crocks for each of four tanks, with the water in these tanks regulated at 50°, 60°, 70° and 80°F. Three harvests were planned so that physiological age as well as variety and temperature could be included in the study of bitterness development. At each harvest, four carrots were taken from each crock, two of these carrots going into a control drum, and two into a drum which received only an initial charge of 42 ml. of ethylene gas (ethylene prime). The harvests were taken on October 8, November 23, and December 20, and the carrots were stored for 21, 24, and 38 days, respectively.

Results and Discussion

Although two analyses of each harvest were planned, there were not enough good carrots to make two tests from the last harvest; therefore, only the results of the first analysis for each harvest appears in Table V. The quantity of carrots in each sample was too small to obtain a spectrophotometric reading. However, fluorescence observations were made, and a representative sample was tasted and found to agree with the fluorescence evaluations. The data in Table V indicate the fluorescence ratings and the average weights for the roots. "Ethylene prime" carrots were more fluorescent than the controls, and temperature influenced the weight of the roots. Nantes variety grew best at 50° F, while Imperator and Red Core Chantenay grew best at 60° F. Temperature had no statistically significant effect upon the development of bitterness.

This experiment showed that ethylene was effective in producing fluorescence and bitterness in carrots. The more mature carrots in the last harvest were also more fluorescent than carrots in earlier harvests with the exception of Red Core Chantenay grown at 50° F.

TABLE V

The Influence of Soil Temperature on the Development of Root Weight and Bitterness in Stored Carrots

Temperature	Variety	Ave. Wt. (Gms)	Fluorescence Rating							
			Control				Ethylene Prime			
			Harvests				Harvests			
			1	2	3	Ave.	1	2	3	Ave.
50°	Nantes	30.4	0.0	0.0	0.0	0.0	1.3	0.8	2.8	1.6
	Chantenay	31.5	0.0	0.0	0.0	0.0	3.0	2.5	2.3	2.9
	Imperator	22.1	0.0	0.0	0.0	0.0	1.8	1.0	2.1	1.6
	Average	28.0	0.0	0.0	0.0	0.0	2.0	1.4	2.4	1.9
60°	Nantes	28.3	0.0	0.2	0.0	0.0	1.0	2.5	3.3	2.3
	Chantenay	41.7	0.0	0.0	0.0	0.0	1.3	2.2	3.3	2.3
	Imperator	30.0	0.0	0.0	0.0	0.0	0.0	2.5	2.3	1.6
	Average	33.3	0.0	0.1	0.0	0.0	0.8	2.4	3.0	2.1
70°	Nantes	24.6	0.2	0.0	0.1	0.1	2.3	0.7	2.6	1.9
	Chantenay	34.8	0.0	0.0	0.0	0.0	0.8	2.0	2.4	1.7
	Imperator	17.2	0.0	0.0	0.0	0.0	1.2	0.0	2.5	1.2
	Average	25.5	0.1	0.0	0.0	0.0	1.4	0.9	2.5	1.6
80°	Nantes	21.6	0.0	0.0	0.0	0.0	2.3	1.5	3.2	2.3
	Chantenay	22.3	0.0	0.0	0.0	0.0	0.8	2.5	1.7	1.7
	Imperator	13.8	0.0	0.0	0.0	0.0	2.5	2.2	2.7	2.4
	Average	19.2	0.0	0.0	0.0	0.0	1.9	2.1	2.5	2.2
Ave. for treatment						0.0				2.0

	Treatment	
L. S. D.	5%	1%
	.37	.50

THE INFLUENCE OF STORAGE TEMPERATURE, AREA OF PRODUCTION, AND SOURCE OF ETHYLENE ON BITTERNESS DEVELOPMENT

Ethylene treatment produced bitterness in different varieties of carrots grown at different soil temperatures, in carrots grown on different soils, and in roots of different physiological ages.

This experiment was to determine whether bitterness would occur at different storage temperatures. Stored carrots were also treated with automobile exhaust gas and compared with ethylene treated roots to determine whether the ethylene in the exhaust gas was sufficient to cause bitterness (33). In some storages in which bitter carrots were found, gasoline lift trucks had been used.

Methods and Procedure

On January 17, Imperator carrots from California, and Nantes and Long Type Chantenay carrots from Michigan were purchased. The Imperator carrots were harvested immaturely approximately three weeks prior to treatment. The Chantenay carrots were overmature, the Nantes carrots were mature at harvest, and both had been in storage for approximately three months prior to treatment. Samples of these carrots were tasted and examined for fluorescence prior to subjecting them to treatment, and no evidence of bitterness or fluorescence was found.

Three storages at temperatures of 32°, 42° and 52° F were available for the experiment. Eight drums, each containing seven bags of 16 carrots from each of the three sources were used. A control and ethylene drum were placed in each storage, and in addition, the 32° F storage contained carrots in drums receiving apple emanations and automobile exhaust gas.

A 16-liter jug of apples was placed in the 32° F storage and a gas analysis of the apple emanation indicated that ethylene was being evolved[1]. The drum receiving the exhaust treatment was taken to the delivery door entrance twice a week and connected to the exhaust pipe of a running truck engine for five minutes. The drum was returned to the storage and re-connected to the air line.

Duplicate, eight-root samples were taken from each drum every 10 days for spectrophotometric and fluorescence determinations.

Results

Since there was no significant difference between duplicate samples, the values shown in Table VI A and B are averages of the duplicates. The spectrophotometric rating for treatment and storage period at 32° F for the three varieties of carrots are given in Table VI A. In less than ten days after storage all treatments resulted in significant increases in the isocoumarin content of Imperator and Chantenay carrots. On the basis of the averages

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[1] Analysis made by Arleigh Dodson, Department of Agricultural Chemistry, Michigan State University.

TABLE VI

The Influence of Storage, Temperature, Area of Production, and Source of Ethylene on Bitterness Development of Carrots in Storage

A. Spectrophotometric Ratings (Temperature 32° F)

Days in Storage	Control	Apple	Ethylene	Exhaust	Average
		Imperator - Source: California			
10	.081	.741	.582	.772	.544
20	.056	.408	.563	.337	.341
30	.098	1.598	1.364	.950	1.003
40	.127	2.420	.894	1.020	1.115
50	.072	1.948	1.470	.710	1.050
90	.190	2.372	1.906	.819	1.322
Average*	.087	1.423	.975	.758	.811
		Long Type Chantenay - Source: Michigan			
10	.000	.267	.801	.692	.440
20	.179	.640	.530	.441	.304
30	.198	.842	.920	.810	.693
40	.172	1.646	1.284	.886	.997
50	.157	1.886	1.230	.606	.970
90	.313	1.312	1.502	.780	.977
Average*	.141	1.056	.953	.687	.681
		Nantes - Source: Michigan			
10	.291	2.495	1.955	2.145	1.722
20	.631	4.140	3.665	1.390	2.457
30	.701	3.046	5.600	2.186	2.883
40	.812	3.800	2.766	2.542	2.480
50	.646	3.676	3.950	3.052	2.831
90	1.157	6.740	3.900	2.860	3.664
Average*	.616	3.431	3.587	2.263	2.475
Ave. all var.	.281	1.970	1.838	1.236	1.322

	Treatment		Time in Storage		T x S	
L. S. D.	5%	1%	5%	1%	5%	1%
Imperator	.283	.397	.317	.444	.633	.887
Chantenay	.181	.254	.202	.283	.404	.567
Nantes	1.174	1.646	-	-	-	-

*Statistical evaluations and averages do not include the values for 90 days in storage.

TABLE VI (Continued)

B. Fluorescence Rating (Temperature 32° F)

Days in Storage	Control	Apple	Ethylene	Exhaust	Average
		Imperator - Source: California			
10	0.0	1.3	1.0	1.3	0.9
20	0.0	1.8	1.1	1.1	1.0
30	0.0	1.8	1.6	1.3	1.2
40	0.0	3.3	2.5	3.5	2.3
50	0.0	2.5	1.9	0.5	1.2
90	0.0	3.2	3.9	2.4	2.4
Average*	0.0	2.1	1.6	1.5	
Correlation with spectrophotometric rating: r .800					
		Long Type Chantenay - Source: Michigan			
10	0.0	0.3	0.7	0.5	0.4
20	0.0	0.0	0.5	0.1	0.2
30	0.1	0.6	0.7	0.9	0.6
40	0.1	0.9	0.6	1.1	0.7
50	0.0	1.2	0.8	0.0	0.5
90	0.8	0.6	1.0	1.5	1.0
Average*	0.0	0.6	0.7	0.5	
Correlation with spectrophotometric rating: r .827					
		Nantes - Source: Michigan			
10	0.1	1.7	1.9	0.9	1.2
20	0.6	1.3	1.5	0.3	0.9
30	0.7	2.1	1.7	1.1	1.4
40	1.1	3.1	2.7	3.1	2.5
50	0.8	3.0	2.9	2.1	2.2
90	2.3	3.9	3.6	1.7	2.9
Average*	1.7	2.2	2.1	1.5	
Ave. all var.	0.2	1.6	1.5	1.2	
Correlation with spectrophotometric rating: r .442					

	Treatment		Storage	
L. S. D.	5%	1%	5%	1%
Imperator	.25	-	-	-
Chantenay	.44	.62	-	-
Nantes	.57	.80	.64	.90

*Averages do not include the values for 90 days in storage.

of the five sampling periods, all treatments resulted in marked increases in the spectrophotometric readings for the Nantes variety. Figures 3 and 4 indicate that the isocoumarin or fluorescent content of Imperator and Chantenay carrots increased more slowly during storage than Nantes variety. After ten days in storage, the Nantes variety reached an average isocoumarin level which was higher than either of the other varieties.

The average for all varieties shows the spectrophotometric readings highest for apple emanations, lower for ethylene, and lowest for exhaust. These differences are probably due to the concentration of ethylene in the drums during the course of the experiment.

The fluorescent ratings of treatments at various storage periods for the same three varieties of carrots are given in Table VI B. All treatments increased fluorescence in all three varieties of carrots. Only Nantes showed a significant increase in fluorescence for continued storage after ten days. A correlation between spectrophotometric and fluorescent ratings were found for all varieties.

In Figures 5, 6, 7 and 8 are shown the comparative fluorescence effect of ultra-violet light of wavelengths of 3650 Å with the Imperator and Nantes carrots. Figures 5 and 6 show cross sections of Imperator and Nantes carrots which have been treated with ethylene, apple emanations and engine exhaust fumes, and photographed under three types of light. The

Figure 3. Spectrophotometric ratings of isocoumarin in three varieties of carrots under four storage treatments at 32° F.

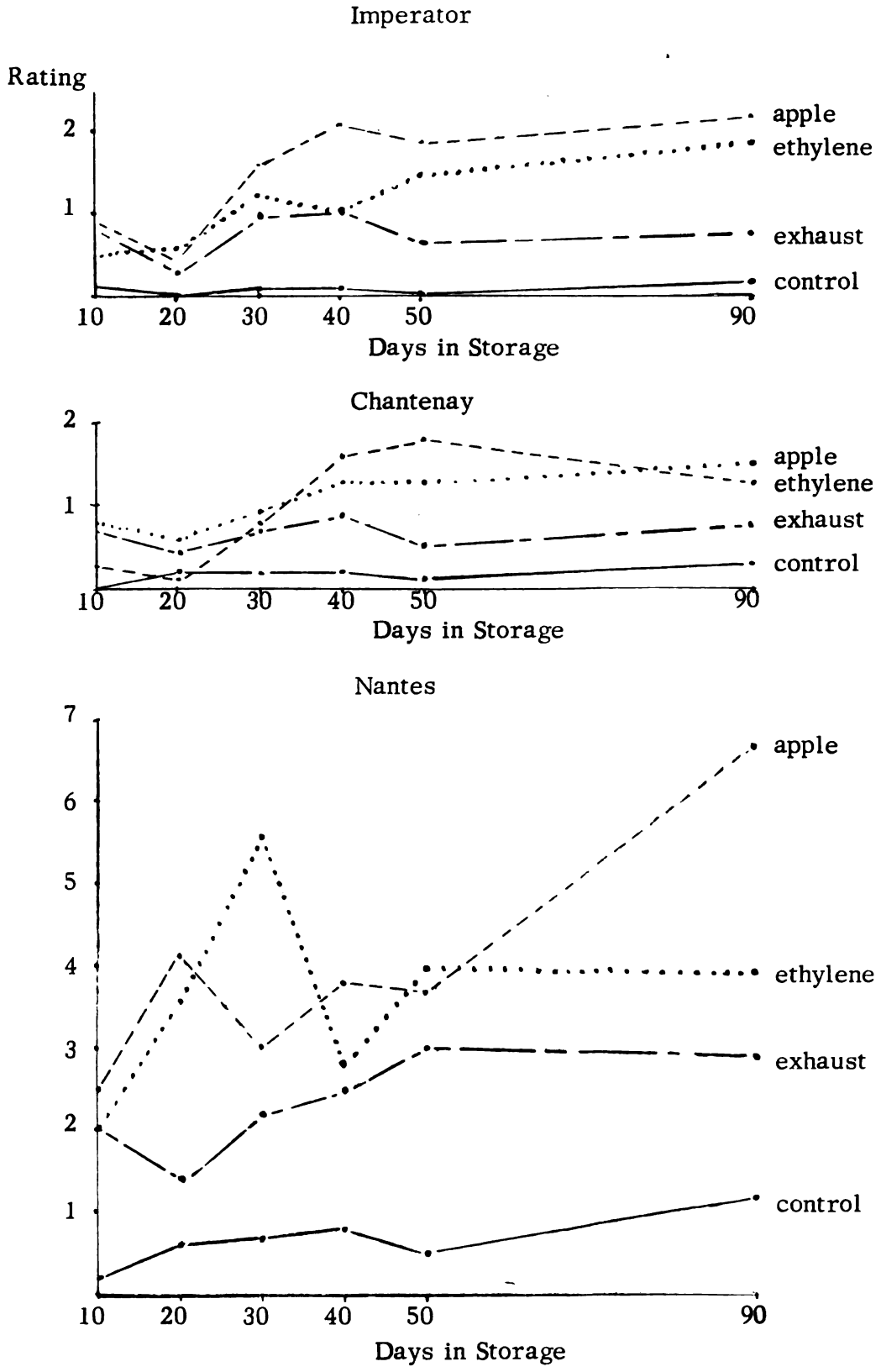
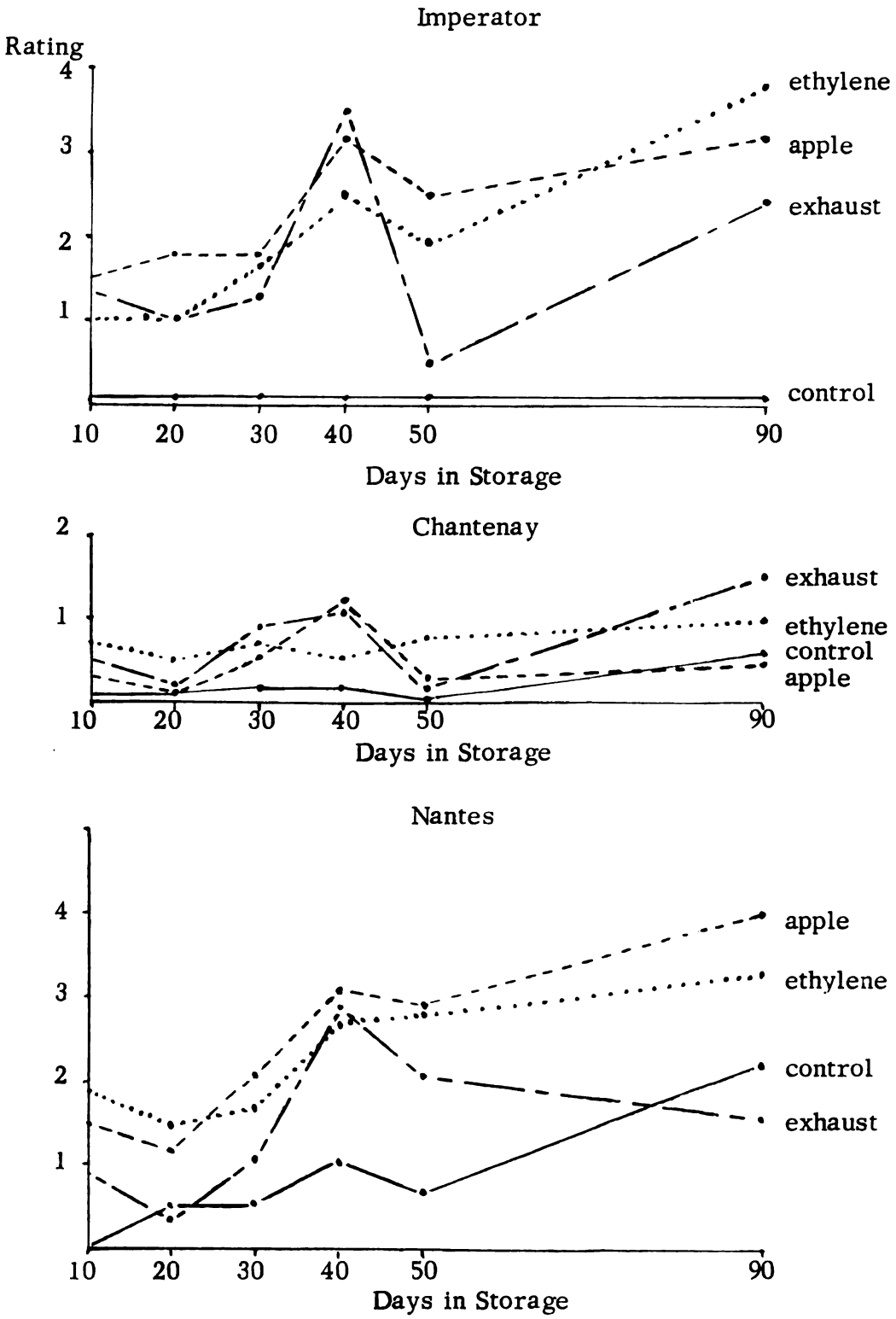


Figure 4. Fluorescent ratings of isocoumarin in three varieties of carrots under four storage treatments at 32° F.



lower photographs were taken under 2537 Å ultra-violet light, which was used exclusively for determining the fluorescence rating of carrots. The rating for each carrot increased as the intensity of the fluorescent speckling in its phloem. Figures 7 and 8 illustrate external fluorescence of Imperator and Nantes carrots also treated with ethylene, apple emanations and exhaust fumes, and photographed under three sources of light. It is noted that Imperator has only three fluorescent specks under 2537 Å ultra-violet light, while Nantes carrots are quite fluorescent under all treatments. Although this external fluorescence is not used as a criterion of bitterness, it is indicative of high intensity of speckled phloem fluorescence.

The influence of storage temperature upon the development of fluorescence and isocourmarin under control and ethylene treatment as determined by spectrophotometric and fluorescent ratings are given in Table VIII. Since Chantenay carrots deteriorated in 42° and 52° F storage, they were not included in the statistical analysis. The analysis of the data indicated that temperature was not a significant factor in the development of isocoumarin.

Discussion

On the basis of the results obtained, storage temperature in the range from 32° to 52° F did not differentially influence the development of bitterness. Carrots from each of the three sources all became bitter when treated with ethylene regardless of the temperature.

TABLE VII

Analysis of Variance Summary for the Influence of Treatment on the Development of Isocoumarin in Different Varieties of Stored Carrots at 32° F as Determined by Spectrophotometric and Fluorescent Methods

Factor	D. F.	Variance		
		Imperator	Chantenay	Nantes
		Spectrophotometric Analysis		
Replication	1	4, 601	257, 282	53, 144
Treatment	3	3, 096, 795**	1, 443, 344**	18, 839, 233**
Storage Period	4	956, 689**	767, 750**	1, 723, 432
T x SP	12	316, 129*	270, 803**	1, 239, 814
Error	19	101, 908	74, 448	1, 091, 586
		Fluorescence Analysis		
Treatment	3	425. 6**	40. 0*	268. 3**
Storage	4	132. 3*	16. 5	63. 1*
Error	12	32. 2	10. 3	17. 3

*Significant at 5% level.
**Significant at 1% level.

TABLE VIII

The Influence of Storage Temperature on the Development of Bitterness

Variety	Temperature						Average for Variety
	32° F		42° F		52° F		
	Control	Ethylene	Control	Ethylene	Control	Ethylene	
			Spectrophotometric Measurement				
Imperator	.087	.975	.173	1.814	.246	1.084	.730
Chantenay	.141	.953	.249	1.280	.146	.968	.622
Nantes	.616	3.587	.542	2.793	.319	2.447	1.717
Average	.281	1.833	.321	1.962	.237	1.500	
Ave. for Temp.	1.057		1.142		.869		
			Fluorescence Measurement				
Imperator	0.00	1.62	0.00	2.67	0.00	2.55	1.14
Chantenay	0.04	0.66	0.08	0.56	0.08	1.13	0.43
Nantes	0.65	2.14	0.50	2.26	0.55	2.48	1.43
Average	0.21	1.47	0.19	1.83	0.21	2.05	
Ave. for Temp.	0.84		1.01		1.13		

Imperator and Nantes are averaged from five sampling dates; Chantenay is averaged from five sample dates for 32° F, three sample dates for 42° F, and two sample dates for 52° F.

This experiment is in agreement with previous work and demonstrates that auto exhaust fumes which contain ethylene, can induce bitterness in carrots. It also indicated the possibility that the development of isocoumarin in carrots is a function of the quantity of ethylene and of time.

The fact that Nantes carrots responded more to treatment than either the Imperator or the Long Type Chantenay suggests that the effect is probably related to maturity. The Nantes carrots were grown to full maturity for fresh market, harvested and pit-stored until shipped to market in January. The Imperator carrots from California were harvested and packaged immature for the fresh market a week prior to being purchased on the market, while the Chantenay carrots were planted as early as weather permitted in the spring, and grown as long as the weather permitted in the fall, so that maximum yield was produced. The Chantenay carrots were probably overmature at time of harvest, while the Imperator carrots were probably too immature to respond to ethylene with as high a production of isocoumarin as produced by the Nantes carrots.

The treatment did not have identical effects on the three varieties, even though they were stored in the same drum. Chantenay had the highest bitterness rating under apple emanations, with both methods of analysis. Nantes was shown to be equally affected by ethylene and apple emanations.

Ethylene may produce metabolic changes that cause bitterness,

therefore the quantity of ethylene should be a factor in determining the amount of bitterness produced. It is possible that the quantity of ethylene administered exceeded the quantity of ethylene given off by the apples during the first few weeks of storage, and later the situation reversed itself. Linking this with the relative maturity[1] of the carrots, the Chantenay variety might have reached the end of their receptive period when the ethylene administrations were highest, and therefore became more bitter under ethylene treatment. The Nantes might have been nearing the end of their receptive period as ethylene in the apple emanations were surpassing the ethylene administrations. The Imperator carrots were quite immature, and retained their susceptibility to ethylene after the apple emanations of ethylene surpassed the administered ethylene and, therefore, they showed higher ratings with apple emanations.

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[1] A mature orange type carrot was considered to be one which has grown for more than 60 days, is orange to the tap root, and has stumped at the junction of the tap root and fleshy root.

An immature orange type carrot is one which has grown for less than 80 days, has no shoulders or color change to clearly define the junction of the root with the tap root.

An overmature orange type carrot is one which has grown for over 100 days.

Figure 5. Fluorescence of cross sections of Chantenay carrot roots. (Photographs of the same carrots taken under different lights).

A. Photoflood reflector lamp
B. Ultra-violet light 3650 Å wavelength
C. Ultra-violet light 2537 Å wavelength

Vertical rows from left to right:

Fluorescence intensity No. 4 rating
Fluorescence intensity No. 3 rating
Fluorescence intensity No. 2 rating
Fluorescence intensity No. 1 rating
Fluorescence intensity No. 0 rating (single carrot section)

Horizontal rows from top to bottom:

Ethylene treated carrots
Apple emanation treated carrots
Gasoline engine exhaust treated carrots

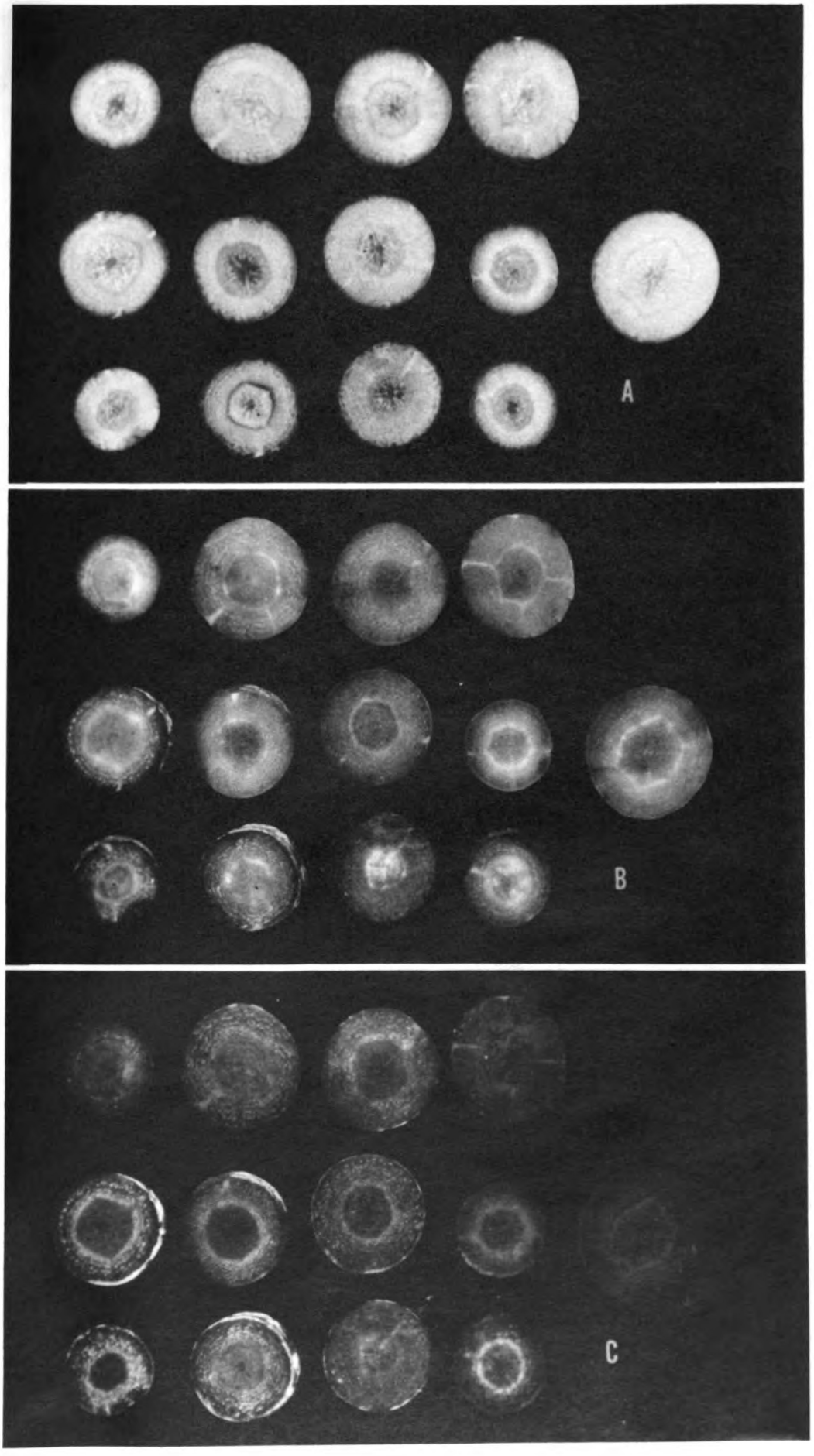

A
B
C

Figure 6. Fluorescence of cross section of Imperator carrot roots. (Photographs of the same carrots taken under different lights).

A. Photoflood reflector lamp
B. Ultra-violet light 3650 Å wavelength
C. Ultra-violet light 2537 Å wavelength

Vertical rows from left to right:

Fluorescence intensity of No. 4 rating
Fluorescence intensity of No. 3 rating
Fluorescence intensity of No. 2 rating
Fluorescence intensity of No. 1 rating
Fluorescence intensity of No. 0 rating (single carrot section)

Horizontal rows from top to bottom:

Ethylene treated carrots
Apple emanation treated carrots
Gasoline engine exhaust treated carrots

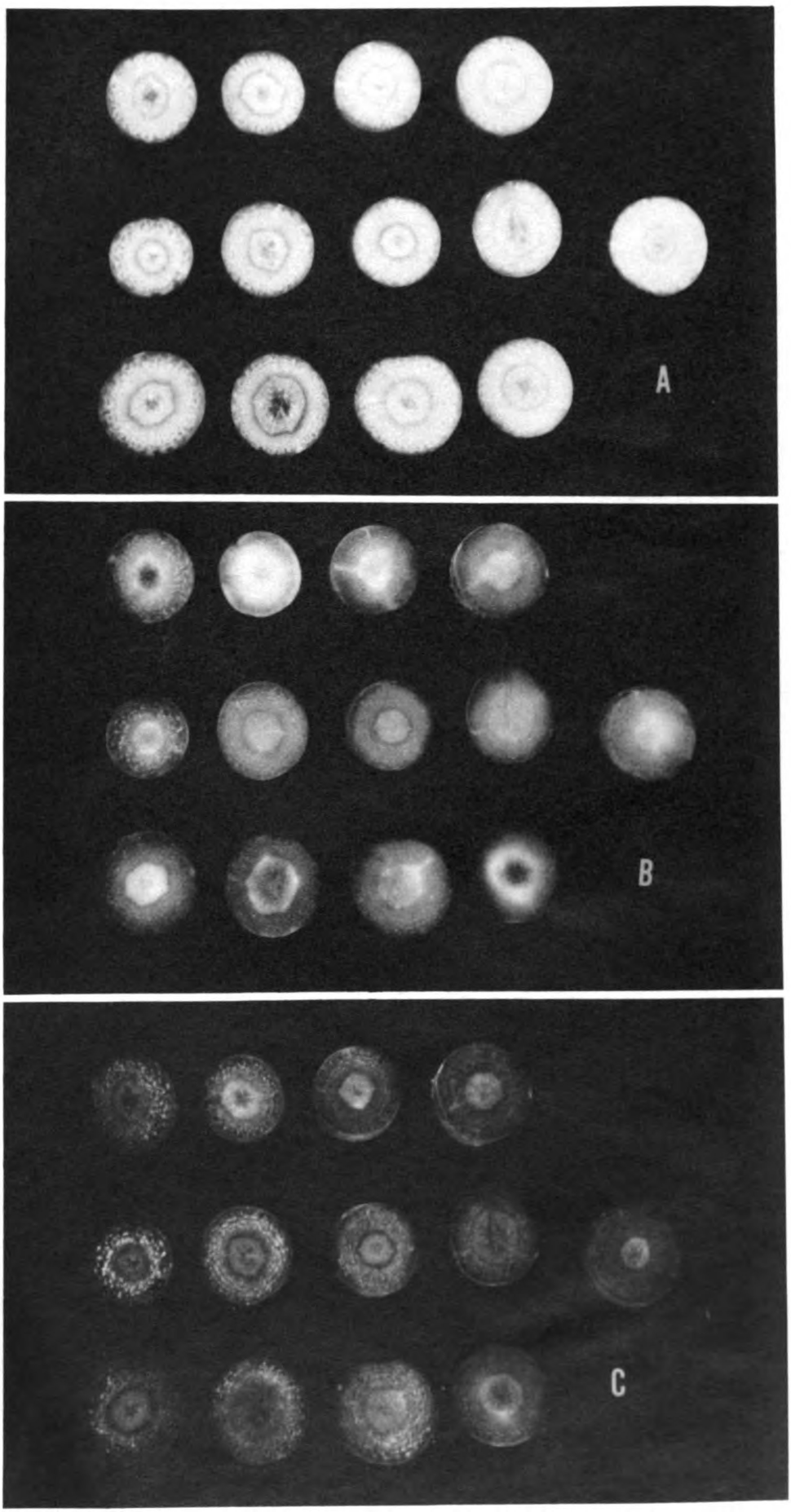

A
B
C

Figure 7. Fluorescence of Imperator and Chantenay carrots under ethylene treatment (photographs of two groups of carrots).

A and D - Under photoflood reflector lamp
B and E - Under ultra-violet light 3650 Å wavelength
C and F - Under ultra-violet light 2537 Å wavelength

The carrots on the left are Imperator (A, B and C), and the carrots on the right are Chantenay (D, E and F). The carrots in each photograph are arranged from left to right according to their cross sectional fluorescence rating, with No. 4 rating on the left to No. 1 rating on the right.

Note external fluorescence in Chantenay carrots (F) as compared to Imperator carrots (C), which show only three tiny specks as indicated by arrows.

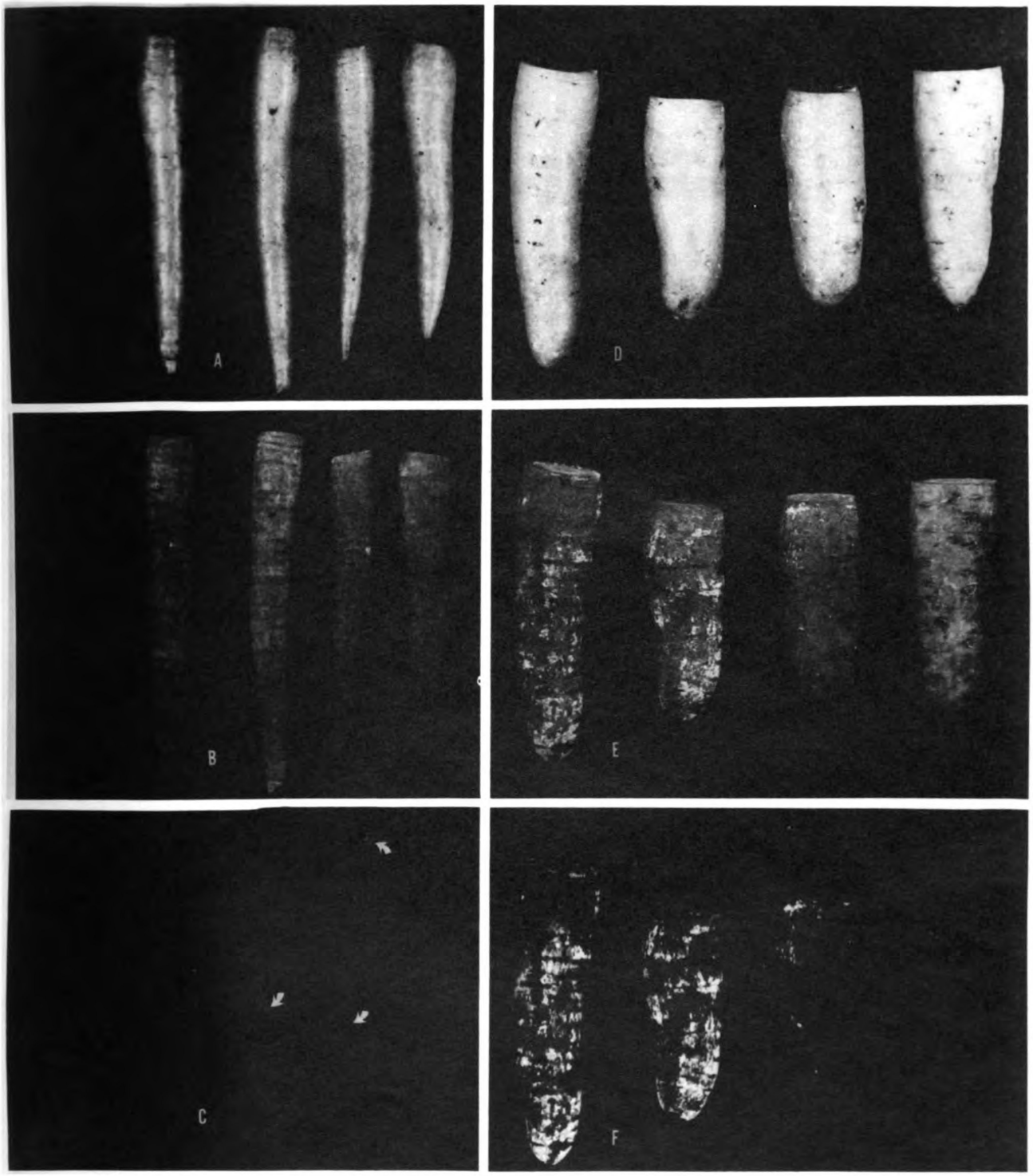
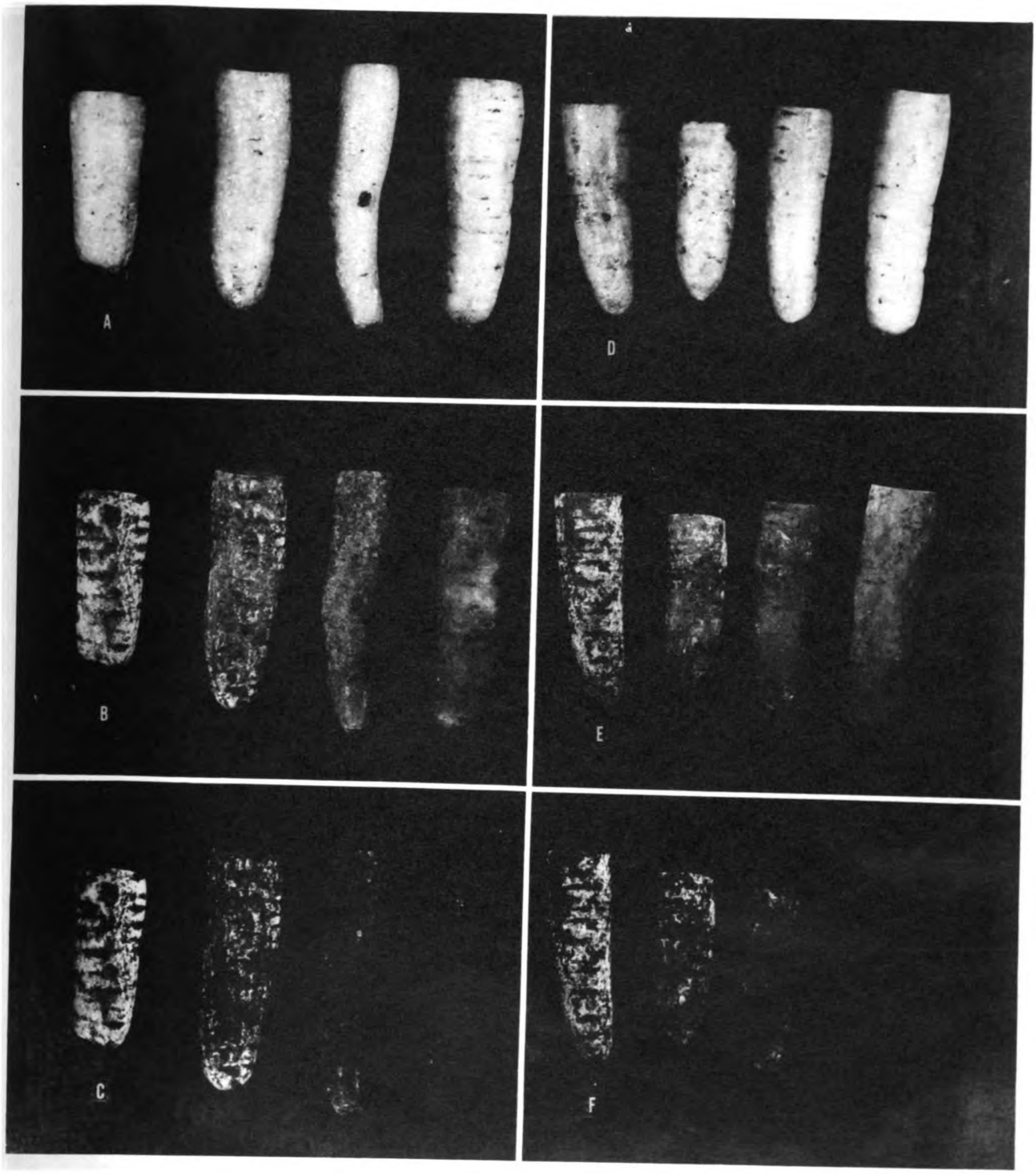

A
D
B
E
C
F

Figure 8. Fluorescence of Chantenay carrots under apple emanations and gasoline engine exhaust treatment (photographs of two groups of carrots).

A and D - Under photoflood reflector lamp
B and E - Under ultra-violet light 3650 Å wavelength
C and F - Under ultra-violet light 2537 Å wavelength

The carrots on the left were treated with apple emanations (A, B and C), while the carrots on the right were treated with gasoline engine exhaust (D, E and F). The carrots in each photograph are arranged from left to right according to their cross sectional fluorescence rating, with No. 4 on the left, and No. 1 on the right.


A
D
B
E
C
F

THE EFFECTS OF ETHYLENE ON WHITE, YELLOW AND ORANGE CARROTS FROM WISCONSIN

On November 9 a sample of white, yellow and orange fleshed carrots were obtained from Wisconsin. These carrots were all examined for fluorescence with the yellow and white carrots revealing no fluorescence, while some of the orange carrots revealed fluorescence of two types. The first type was the speckled isocoumarin type, located in the phloem, and the other type consisted of glowing areas located in the core.

After a month of ethylene treatment the yellow carrots, Yellow A (Yellow Belgium selection A-1630-5 D5CD) and Yellow B (Yellow Belgium University of Wisconsin), and the white carrots showed the following spectrophotometric values:

	Control	Ethylene
White	.098	2.13
Yellow A	.000	3.12
Yellow B	.123	4.15

The orange carrots exhibited a strong fluorescence, indicating a high isocoumarin content.

Discussion

Because carotene content increases in storage (5), it has been suggested that the bitter principle is formed from some precursor of carotene.

This experiment with yellow and white carrots indicates that this is not the case since white carrots developed an isocoumarin content of considerable magnitude. However, as the yellow carrots developed more isocoumarin than white roots, carotene may have a role in the development of high isocoumarin values.

The fact that the orange carrots which showed a core fluorescence were not bitter, indicates that isocoumarin was not causing the fluorescence. When they were treated with ethylene, they developed the isocoumarin-type fluorescence in addition to the core fluorescence.

THE EFFECTS OF ETHYLENE GAS UPON THE RESPIRATION OF CARROTS IN STORAGE

Since ethylene was presumed to influence some metabolic processes in carrots, it was suggested that it might effect the respiration rate. The atmosphere of the control and ethylene drums were sampled for carbon dioxide to determine if there was any increase in respiration due to the addition of ethylene.

Methods and Procedure

An Orsat gas analyzer was used to determine the carbon dioxide in the drums which contained the Chantenay carrots under storage treatment. The analyzer was inserted into the air line to the drum, and three samples were removed. The first two were expelled, and the third was analyzed for carbon dioxide.

Results

Figure 9 shows the results of periodic analysis of the carbon dioxide contents of the control and ethylene drums during a six-week period. The graph indicates a generally declining quantity of carbon dioxide until September 6, when fresh carrots were added. The carrots in both the control and ethylene drums responded by an increase in carbon dioxide accumulation; however, the carbon dioxide of the ethylene drum increased until September

16, when it reached a high of 3.4 per cent, compared to a high of 1.4 per cent for the control on that same date. In both cases the carbon dioxide concentration fell off until October 5, when more carrots were put into the drums for treatment, after which it started to rise again.

Discussion

This experiment was conducted to check the respiration of the carrots which were undergoing storage treatment. The results were so striking that carbon dioxide concentrations were measured in the apple emanation drum and the ethylene prime and control drums. These results further indicated that ethylene stimulated the evolution of carbon dioxide.

Since the Orsat analyzer is not designed to make respiration determinations, it was decided to forego these samplings and conduct an experiment on the effects of ethylene on carrot respiration at a later date with the proper equipment. The attempt which was made, using the proper respiration equipment was unsuccessful; therefore, the previous results have been presented to indicate the effects of ethylene upon the respiration of carrots.

The immediate rise of carbon dioxide concentration in the control drum is due to the high respiration of the warm roots. As the roots are cooled, their respiration and carbon dioxide evolution decreased. It further decreased as carrot samples were removed. When the next lot of carrots was added, only a few samples of the original lot remained.

GENERAL DISCUSSION

This study has demonstrated that ethylene in the storage atmosphere will cause rapid metabolic changes to take place in the roots of carrots that result in the production of bitterness of the isocoumarin type. This effect takes place in less than ten days, and may be brought about not only by ethylene gas, but also by emanations from apples and gasoline engine exhaust fumes. The incipient development of this type of bitterness in carrot roots is readily detected by the fluorescence of the bitter product of this metabolic activity in the phloem tissue adjacent to the cambium. At this stage the concentration of isocoumarin is too low to be readily detected organoleptically and the roots are still edible. The high degree of correlation between the isocoumarin content in the root and its fluorescent value introduces a rapid method of detection of bitterness in carrots. Fluorescent measurements may be utilized in detecting carrots that will become bitter before they are organoleptically bitter.

There is evidence that immature or overmature carrots do not develop isocoumarin as rapidly, or to as high a content as that found in mature carrots. In immature carrots perhaps the substrate required for the development of isocoumarin may be partially limiting; in overmature carrots the low respiratory rate and the possible loss or conversion of

some of the precursor of isocoumarin reduces the rate of development and total quantity of the bitter principle formed. Therefore, in fresh market carrots, which are harvested immature and consumed shortly after harvest, bitterness is not likely to be a problem. Carrots that are harvested at full maturity for subsequent processing should be stored in an atmosphere free of ethylene and processed at once if any fluorescence is detected.

The variety, root color, soil temperature or type, storage temperature, or area of production, seemed to have little bearing upon the qualitative development of bitterness in storage after ethylene was injected into the storage atmosphere.

Whether or not ethylene enters into the synthesis of the bitter principle or the carotene and the isocoumarin precursors are the same compound remains to be demonstrated.

The association of ethylene with these biochemical and physical phenomena has not been heretofore reported in the literature for any crop and may possibly find use in evaluation of the influence of ethylene on other products.

SUMMARY AND CONCLUSION

Ethylene has been demonstrated to be effective in producing bitterness, fluorescence, and isocoumarin as determined by organoleptic, ultraviolet, and spectrophotometric tests, in all carrots subjected to ethylene treatment. The carrots used in these tests included carrots of three different colors (orange, yellow, and white), of nine different varieties, of three physiological ages (immature, mature, and overmature), produced on two major soil types (loam and muck), in two states (Michigan and California), grown at four soil temperatures (50°, 60°, 70° and 80° F), planted and harvested during four different months, and stored at five different temperatures (32°, 34°, 42° and 52° F).

The ethylene was administered in two ways to the storage atmosphere, which was receiving one complete change of air each day. The first method was to apply ethylene to a concentration of 200 ppm every 48 hours, and the second method was to apply an initial atmosphere of 200 ppm and allow it to be dissipated without being replenished. Both treatments were effective in producing bitterness, fluorescence, and isocoumarin in carrots, although the second method was not as effective as the first, leading to the assumption that the degree of bitterness is related to the quantity of ethylene.

Two other treatments consisted of apple emanation and automobile exhaust fumes, both of which contain ethylene. These treatments were also effective in producing bitterness, fluorescence and isocoumarin in carrots to a lesser or greater extent than the ethylene treatments. The degree to which these phenomena were expressed is believed to be contingent on the amount of ethylene contained in these gases.

Of the variable factors among the carrot samples (color, variety, physiological age, soil temperature and type, planting and harvesting time, area of production, and storage temperature) only color and physiological age had any modifying effect on the development of bitterness, fluorescence, and isocoumarin. These factors were not statistically proven, however, possibly because the experiment was not designed to specifically test these factors.

The white carrots did not become as bitter as the yellow ones, nor the yellow carrots as bitter as the orange, indicating that, if the precursor to carotene is involved in the formation of the bitter principle, as has been suggested, this precursor is also present to a limited degree in white carrots.

Physiological age of the root seemed important since mature carrots became much more bitter and fluorescent and contained more isocoumarin after identical treatment than either the immature or overmature carrots.

This phenomenon was noted in three experiments.

Further experiments will be required to determine whether similar results can be obtained in successive years, and to statistically test the observations regarding physiological age and color.

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