

Ethephon Increases Carotene Content and Intensifies Root Color of Carrots

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Abstract. Poor root color is a recurring problem in carrot (*Daucus carota* L.) production. Consumers prefer dark orange carrots that are high in carotene. However, unfavorable environmental conditions and certain production practices can lead to light orange roots with low carotene content. Growers sometimes refer to this as “white root.” No one has clearly established the causes or cures for this disorder. Several environmental factors are known to affect the color of carrots, but to date there is no practical treatment. High-density planting often reduces carotene content. Field studies were conducted in the 1995–96 and 1996–97 winter growing seasons to determine if foliar applications of ethephon would improve carrot color, carotene content, and yield. Carotene content and root color increased as the number of applications or the amount of ethephon applied with each application increased. Root weight was not significantly affected.

Increased consumer demand has led to a dramatic rise in fresh carrot production in the last 10 years. Production in the United States has increased from 6,243 ha in 1986 to 37,608 ha harvested in 1996 (U.S. Dept. of Agriculture, 1997). Beta and alpha carotene are the pigments principally responsible for the orange color of carrot roots (Gross, 1991). Many consumers purchase carrots and other carotene-rich fresh vegetables because of their perceived health benefits as sources of antioxidants (Packer et al., 1981). Consumers recognize carrots as the most important vegetable source of carotene (Senti and Rizek, 1975).

Environmental factors, including soil fertility, temperature, and water content, all affect root color (McGiffen et al., 1997). High or low temperatures during the critical 3- to 6-week period before harvest can limit color development (Bradley and Smittle, 1965). Large acreages of fresh carrots have been rendered unmarketable when roots failed to develop the desired orange color. Growers sometimes refer to widespread light-colored roots as “white root” (McGiffen et al., 1997).

Increased consumption of fresh carrots has coincided with the introduction of cut-and-peel or “baby” carrots (McGiffen et al., 1997). Cultural and handling practices associated with

cut-and-peel carrots can decrease the intensity of orange color in the roots. Cut-and-peel carrots are planted at high densities to ensure long, thin roots (Lazcano et al., 1998), but increasing the planting density can reduce root color (Fluery et al., 1993). Carotene content is highest in the exterior cells of the root (Werner, 1941), and the processing of cut-and-peel carrots removes these cells. These factors have made root color an even more critical quality factor.

Growth regulators and pesticides can either increase or decrease the carotene content of carrots. Decreased carotene content followed foliar application of chlormequat (2-chloroethyltrimethyl ammonium chloride) (Linser and Zeid, 1975) and various herbicides (Rouchad et al., 1982). Increased carotene content and sometimes greater root weight have been observed after the application of gibberellic acid (GA) (Linser and Zeid, 1975), indolebutyric acid (IBA) (Maurya, 1986), or ethephon (Bewick et al., 1987; Furutani and Zandstra, 1982). Furutani and Zandstra (1982) found that foliar applications of ethephon increased carrot root length, but decreased leaf growth. Bewick et al. (1987) reported that ethephon increased the carotene content of processing carrots, especially when applied early in the growing season.

Although color is critical to consumer perception of quality, no study has directly determined whether growth regulators can enhance carrot root color, particularly for the increasingly important fresh market. In this study, we tested the efficacy of ethephon as an enhancer of fresh carrot root color, carotene content, and yield.

Materials and Methods

Field experiments were conducted in grower fields in Imperial County, Calif. The planting dates were 18 Sept. 1995 in Westmoreland and 25 Oct. 1996 in Calexico. Each year, carrots were planted on raised beds

1.6 m apart. Each bed had eight rows of carrots (‘Apache’) arranged as two sets of four rows, with the outer row of each four-row set 10 cm from the edge of the bed. The four rows within a set were 4 cm apart. Plots were three adjacent beds 10 m long. All fields were sprinkle irrigated until the seedlings were established, then furrow irrigated from seedling establishment until 1 month prior to harvest. The fields were not irrigated during the last month of the season in preparation for harvest. Ethephon was applied as a foliar spray at rates of 0, 100, 200, 300, and 400 g·ha⁻¹ a.i. Each application rate was applied 1, 2, 3, or 4 times (1×, 2×, 3×, or 4×). The experimental design was a randomized complete block of four replications arranged factorially in all possible combinations of the five application rates and the four numbers of applications. Applications began 1 month after planting and continued every 2 weeks. The ethephon solution was applied at 180 L·ha⁻¹ using a CO₂ backpack sprayer at 275 kPa pressure with two 8003 nozzles spaced 50 cm apart.

Harvest dates were 5 Mar. 1996 at Westmoreland, and 13 Mar. 1997 at Calexico. Two meters of row was dug from each plot, and the carrots were weighed. Harvest data included the fresh weight of all roots, marketable roots, and carrot tops.

Color analysis by spectrophotometer. A modified standard assay of plant tissue (Association of Official Agricultural Chemists, 1965) was used to determine the total amount of carotenoids present in root tissue, using subsamples of 20 roots from each replicate. The central 10 cm was excised from each root and the tissues combined, ground, and lyophilized. A subsample (2 g) of the lyophilized tissue was weighed into a 125-mL Erlenmeyer flask, 70 mL of acetone was added, and the mixture was refluxed for 1 h to extract carotenoids. After cooling, the mixture was vacuum filtered into a volumetric flask and diluted to 100 mL with acetone. A 5-mL aliquot was diluted to 50 mL and the absorbance at 450 nm determined with a spectrophotometer. Beta-carotene standards were used to provide regression data for a standard curve. Linear regression produced an equation that converted the values into milligrams carotene per gram of carrot root dry weight.

The accuracy of results was evaluated by analyzing three, independent, fresh carrot root samples using our methods vs. those from previous total carotene content studies (Bewick et al., 1987; Umiel and Gabelman, 1971). Each sample was analyzed four times using each analytical method. All methods agreed on the amount of carotene present in each sample ($P < 0.01$).

Visual rating of color. A randomly selected subsample of 20 roots was taken from each replicate. Color categories were selected to correspond with those used by production-line graders who accept or reject carrots based upon color. Each root was given a numeric score based upon one of three color categories: 1 = light orange, 2 = orange, and 3 = deep orange. Carrots in Category 1 are considered too light to be acceptable.

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Statistical analysis. Pearson correlation coefficients (r , SAS 1993) were calculated for all possible combinations of the carrot harvest parameters. Multiple linear regression determined the relationship between carrot yield parameters and the number and rate of ethephon application. Error sum of squares were used to develop F tests for the significance of a model in each year, and to determine whether the same model fit both years (Draper and Smith, 1981).

Results and Discussion

The winter growing season in the low desert is typified by maximum daily temperatures $>35^{\circ}\text{C}$ in early September. Temperatures decline through January to below 10°C , then rise in late February and March (Table 1). The 1995–96 growing season had greater temperature differences than did the following year; average maximum temperatures were greater in September–November and minimum temperatures were lower in December–January than in the 1996–97 season.

The most striking difference in weather between the 1995–96 and 1996–97 growing seasons was precipitation. Rainfall in the desert is a rare event. Most rains occur in the winter months. Rainfall was recorded during each month of the 1995–96 growing season, for a seasonal total of 45 mm. No rain fell during the 1996–97 season until January, and the seasonal total was only 22% of the 1995–96 season. The 17 mm of precipitation in Feb. 1996 was more than the total rainfall from Sept. 1996 through Feb. 1997.

Carrot yield data reflect the differences in weather between the two growing seasons. While the 1995–96 growing season was 1 month longer, carrots were not as heavy or dark as in the 1996–97 season (Table 2). Root, top, and marketable weights in 1996 were generally half as great as in 1997. In 1996, 43% of the controls were graded as light orange, vs. only 18% of the controls in 1997.

However, many of the relationships between the carrot yield parameters were similar in both years (Table 3). As carrot tops became heavier, so did the roots ($r = 0.73$ in 1996 and $r = 0.69$ in 1997). Heavier roots increased marketable yield in both years ($r = 0.89$ in 1996 and $r = 0.83$ in 1997), but the increase was correlated with a decline in carotene content ($r = -0.51$) and an increase in orange roots ($r = 0.63$) only in 1996. Increasing root weights were associated with a decline in the number of dark orange roots in 1996 ($r = -0.60$). Root weight was not correlated with the percentage of light orange roots in either year ($P > 0.05$). Top weight had no effect on the percentage of light roots but was negatively related to both carotene content ($r = -0.61$ in 1996 and $r = -0.58$ in 1997) and the percentage of dark orange roots ($r = -0.65$ in 1996 and -0.74 in 1997). Increases in top weight was positively correlated with increases in the percentage of orange roots in 1996 ($r = 0.77$) and 1997 ($r = 0.64$) and marketable weight in 1997 ($r = 0.49$).

Marketable weight, carotene content, and

Table 1. Monthly rainfall and average temperature data for Westmoreland (1995–96) and Calexico (1996–97), Calif.

Month	Total precipitation (mm)		Avg min. temp ($^{\circ}\text{C}$)		Avg max. temp ($^{\circ}\text{C}$)	
	1995–96	1996–97	1995–96	1996–97	1995–96	1996–97
September	15	0	22	21	41	37
October	6	0	12	14	35	31
November	4	0	11	9	29	26
December	1	0	5	6	22	22
January	2	8	3	7	22	21
February	17	2	10	6	23	23

the root color classes were inconsistently related over the two field seasons. In 1996, the correlation between the increasing percentage of marketable roots classified as orange ($r = 0.51$) was the inverse of the correlation between the percentage of dark orange and marketable roots ($r = -0.51$). Marketable weight was not correlated with the percentage of light, orange, or dark orange roots in 1997 ($P > 0.05$). Marketable weight was unrelated to carotene content in either 1996 or 1997 ($P > 0.05$). Carotene content increased as both root ($r = -0.51$ in 1996, ns in 1997) and top mass ($r = -0.61$ in 1996 and $r = -0.58$ in 1997) declined, but carotene content had no effect on marketable yield. Carotene content was negatively correlated with the percentage of roots graded as light in 1996 ($r = -0.58$), and was not correlated with light roots in 1997 ($P > 0.05$). The untreated controls had a much higher percentage of light roots in 1996 (43%, Table 2) than in 1997 (17%). Differences in water stress were probably not responsible for these differences on the irrigated, sandy loam soils. It is more likely that the cloudier days of the 1995–96 season provided less sunlight for carbohydrate production, ultimately resulting in lower yield and carotene production than during the 1996–97 growing season (Tables 2 and 3).

Ethephon applications did not affect total root weight or weight of marketable roots in either growing season ($P > 0.05$, Table 4). This contradicts previous findings of an increase in carrot root yield following ethephon application, apparently due to a shift in the partitioning of carbohydrates from top to root mass (Furutani and Zandstra, 1982). We did find that a decline in top growth was related to both the rate and number of ethephon applications in both years (Table 4, $R^2 = 0.59$). The decline in top weight was a linear function of the frequency and rate of ethephon application, as the interaction term, i.e., the total amount of ethephon applied, was nonsignificant.

In the regression for carotene content, the coefficients for the number of applications and the interaction with application rate were significant, but the application rate coefficient was not (Table 4). The significant interaction coefficient shows that the increase in carotene content with increasing rate of ethephon was not constant for each number of applications. The response was greater with increased number of applications (Table 2). Carotene content generally increased when ethephon was applied, but not always as a linear function of application rate (Table 2). Despite lower root, top, and marketable weights in 1996, carotene

content was generally higher than in 1997. This is consistent with the negative correlation between carotene content and root or top weight (Table 3).

The percentage of roots classified as light orange was negatively related to both the number and rate of ethephon application, i.e., increasing the number or rate of ethephon application generally decreased the percentage of light roots. The interaction coefficient was small but significant (0.013, Table 4), suggesting that as the total amount of ethephon applied increased the percentage of light orange root increased. Whenever ethephon was applied to carrots, the percentage of light orange roots was half or less than the percentage of light roots in the untreated control. Further, the percentage of light roots was zero in both 1996 or 1997 when $400\text{ g}\cdot\text{ha}^{-1}$ of ethephon was applied four times, and in 1997, there were no light roots when $400\text{ g}\cdot\text{ha}^{-1}$ was applied one or three times.

Top weight also declined as either number of applications or rate of ethephon increased (Table 4). Some minimum mass of tops is required for root growth and color development. Apparently the decline in top weight from high doses of ethephon applications resulted in too little carbohydrate production to support adequate root color.

The number of ethephon applications negatively affected the percentage of orange roots. However, the percentage of orange roots was insensitive to the amount applied per application (rate of application) or the total amount applied during the season (Table 4). In contrast, the percentage of dark orange roots increased with more applications, higher rates, and greater total amounts of ethephon. Increases in dark orange roots were directly correlated with declines in light (Table 3, $r = -0.71$ in 1996; $r = -0.66$ in 1997) and orange roots ($r = -0.75$ in 1996; $r = -0.95$ in 1997) and increased carotene content ($r = 0.82$ in 1996; $r = 0.77$ in 1997). The net effect of ethephon applications on carrot root color was to increase carotene content and, in turn, shift the root color categories toward dark orange.

Our results agree with previous reports of increased carotene content following the application of ethephon to processing carrots in Wisconsin (Bewick et al., 1987). However, carotene content was significantly higher for any treatment in our study than that found in carrots treated with either 260 or $520\text{ g}\cdot\text{ha}^{-1}$ of ethephon (Bewick et al., 1987). The increase in carotene with increasing dosage of ethephon was also more consistent for our data than that reported by Bewick et al. (1987). Our data also

Table 2. Treatment means for carrot response to ethephon application.

Year	No. appl. ^c	Rate (g·ha ⁻¹)	Wt/root (g)		Wt of tops (g/plant)	Carotene content (mg·kg ⁻¹)	Light orange roots (%)	Orange roots (%)	Dark orange roots (%)
			All	Marketable					
1996	0	0	69	76	9	1041	43	28	28
	1	100	76	84	9	1115	8	53	40
	1	200	70	75	9	1103	15	30	55
	1	300	69	74	10	1120	3	38	60
	1	400	78	83	10	1222	13	28	58
	2	100	75	76	13	1200	8	50	43
	2	200	71	75	9	1233	5	30	65
	2	300	67	71	8	1228	8	23	70
	2	400	65	68	7	1364	0	18	83
	3	100	69	72	8	1267	15	18	68
	3	200	67	74	7	1324	10	35	55
	3	300	71	80	9	1358	3	35	63
	3	400	63	70	6	1533	3	18	80
	4	100	76	86	8	1364	10	23	68
	4	200	63	68	7	1381	8	18	75
	4	300	68	76	8	1601	3	18	80
4	400	65	70	7	1539	0	18	83	
1997	0	0	143	132	33	990	18	53	30
	1	100	146	158	33	1041	10	45	45
	1	200	137	147	29	956	0	45	55
	1	300	138	141	27	1013	3	38	60
	1	400	146	150	29	1047	0	38	63
	2	100	139	148	29	1013	0	38	63
	2	200	134	133	27	1154	3	28	70
	2	300	112	125	22	1064	0	38	68
	2	400	129	141	24	1109	5	15	80
	3	100	137	148	28	1047	0	28	73
	3	200	124	127	27	1086	8	18	75
	3	300	120	128	24	1369	5	15	78
	3	400	144	147	25	1245	0	8	93
	4	100	132	141	23	1200	0	23	78
	4	200	143	143	26	1256	0	20	80
	4	300	122	128	21	1262	0	18	83
4	400	145	151	27	1352	0	5	95	

^cTwo weeks between applications.

Table 3. Pearson correlation coefficients (*r*) for carrot yield parameters. When *r* = 0, no correlation exists. *r* = 1.0 is an exact correlation, e.g., the correlation of the parameter with itself.

	Root wt (g/plant)		Tops wt (g/plant)		Marketable wt (g/plant)		Carotene content (mg·kg ⁻¹)		Light orange roots (%)		Orange roots (%)		Dark orange roots (%)	
	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
	Root weight (g/plant)	1.0	1.0	0.74	0.69	0.89	0.83	-0.51	NS	NS	NS	0.63	NS	-0.60
Tops weight (g/plant)			1.0	1.0	NS	0.49	-0.61	-0.58	NS	0.58	0.77	0.64	-0.65	-0.74
Marketable weight (g/plant)					1.0	1.0	NS	NS	NS	NS	0.51	NS	-0.51	NS
Carotene content (mg·kg ⁻¹)							1.0	1.0	-0.58	NS	-0.60	-0.84	0.82	0.77
Light orange roots (%)									1.0	1.0	NS	NS	-0.71	-0.66
Orange roots (%)											1.0	1.0	-0.75	-0.95
Dark orange roots (%)													1.0	1.0

^{NS}Nonsignificant, *P* > 0.05.

show that ethephon applications decrease the percentage of roots graded as light and increase the percentage of dark orange roots. In both 1996 and 1997, carrots with higher top mass had lower carotene content and a lower percentage of dark orange roots (Table 3). Similar correlations with carotene content and the percentage of dark orange roots were found for root weight in 1996 but not in 1997.

Maintaining high color may have become more difficult over the last decade because of two changes in carrot production. Acreage has expanded into areas where environmental conditions may not be ideal for carrot production; e.g., studies have shown that carotene synthe-

sis is particularly sensitive to temperature (Barnes, 1936). Carotene synthesis is also sensitive to plant density (Fleury et al., 1993). The increased densities used for cut and peeled carrots may make that production method particularly vulnerable to poor root color (McGiffen et al., 1997).

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Table 4. Parameter estimates (and standard errors) for regressions of the effect of ethephon applications on carrot yield, carotene content, and color category. Regression equations were nonsignificant ($P > 0.05$) for root weight or marketable yield and are not presented.

	Intercept	No. of applications	Application rate (g-ha ⁻¹)	Total ethephon applied (g-ha ⁻¹) ^z	R ²
Top weight (g/plant) ^y	32.6 (1.4)	-1.50 (0.46)	-0.011 (0.004)	NS	0.59
Carotene content (mg·kg ⁻¹) ^w	988.1 (38.1)	46.0 (20.7)	NS	0.20 (0.06)	0.64
Light orange roots (%)	23.3 (3.7)	-5.11 (1.57)	-0.056 (0.016)	0.013 (0.006)	0.48
Orange roots (%)	43.7 (3.4)	-6.81 (1.29)	NS	NS	0.47
Dark orange roots (%)	30.9 (3.0)	8.49 (0.95)	0.066 (0.009)	NS	0.84

^zTotal ethephon applied, i.e., the interaction term, is the product of the number of applications multiplied by the application rate.

^yThe regression equation for 1996 was nonsignificant. The regression parameters for 1997 data are presented.

^wThere were no significant year × treatment interactions for carotene content or any of the color classifications. Data were pooled for both years for regression parameter estimation.

^{ns}Nonsignificant, $P > 0.05$.

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