Ethephon Increases Carotene Content and Intensifies Root Color of Carrots

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Additional index words. Daucus carota, growth regulator, cut-and-peel carrots, baby carrots

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 caro- tene-rich fresh vegetables because of their perceived health benefits as sources of anti- oxidants (Packer et al., 1981). Consumers recognize carrots as the most important vegetable source of carotene (Sentí 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-pearl or “baby” carrots (McGiffen et al., 1997). Cultural and handling practices associated with cut-and-pearl carrots can decrease the intensity of orange color in the roots. Cut-and-pearl 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-pearl car- rots 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 chloromequat (2-chloroethyltrimethyl ammonium chloride) (Linser and Zeid, 1975) and various herbi- cides (Rouchad et al., 1982). Increased caro- tene 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 in- creased 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 per- ception of quality, no study has directly deter- mined whether growth regulators can enhance carrot root color, particularly for the increas- ingly 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 irrig- gated until the seedlings were established, then furrow irrigated from seedling establish- ment 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 ran- domized complete block of four replications, arranged factorially in all possible combina- tions 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, market- able roots, and carrot tops.

Color analysis by spectrophotometer. A modified standard assay of plant tissue (Associa- tion 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 lyo- philized. 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 caro- tenoids. 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 re- gression data for a standard curve. Linear regression produced an equation that con- verted 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 > 0.1).

Visual rating of color. A randomly se- lected 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.
Results and Discussion

The winter growing season in the low desert is typified by maximum daily temperatures $>35^\circ$C in early September. Temperatures decline through January to below $10^\circ$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 were 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 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 photosynthesis and root growth, resulting in too little carbohydrate production to support adequate root color.

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 g ha$^{-1}$ of ethephon was applied four times, and in 1997, there were no light roots when 400 g 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 g 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

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

| Month   | Total precipitation (mm) | Avg min. temp ($^\circ$C) | Avg max. temp ($^\circ$C) |
|---------|--------------------------|---------------------------|---------------------------|
|         | 1995–96 | 1996–97 | 1995–96 | 1996–97 | 1995–96 | 1996–97 |
| September | 15      | 15      | 22      | 22      | 41      | 37      |
| October  | 6       | 6       | 12      | 14      | 35      | 31      |
| November | 4       | 4       | 11      | 9       | 29      | 26      |
| December | 1       | 1       | 5       | 6       | 22      | 22      |
| January  | 2       | 2       | 3       | 7       | 22      | 21      |
| February | 17      | 17      | 10      | 6       | 23      | 23      |
Table 2. Treatment means for carrot response to ethephon application.

| Year | No. appl. | 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 (%) |
|------|-----------|----------------|-------------|---------------------|--------------------------|-----------------------|-----------------|---------------------|
| 1996 | 0         | 0              | 69          | 76                  | 9                        | 1041                  | 43              | 28                  |
|      | 1         | 100            | 76          | 84                  | 9                        | 1115                  | 8               | 53                  |
|      | 1         | 200            | 70          | 75                  | 9                        | 1103                  | 15              | 30                  |
|      | 1         | 300            | 69          | 74                  | 10                       | 1120                  | 3               | 38                  |
|      | 1         | 400            | 78          | 83                  | 10                       | 1222                  | 13              | 28                  |
|      | 2         | 100            | 75          | 76                  | 13                       | 1200                  | 8               | 50                  |
|      | 2         | 200            | 71          | 75                  | 9                        | 1233                  | 5               | 30                  |
|      | 2         | 300            | 67          | 71                  | 8                        | 1228                  | 8               | 23                  |
|      | 2         | 400            | 65          | 68                  | 7                        | 1364                  | 0               | 18                  |
|      | 3         | 100            | 69          | 72                  | 8                        | 1267                  | 3               | 38                  |
|      | 3         | 200            | 67          | 74                  | 7                        | 1324                  | 10              | 35                  |
|      | 3         | 300            | 71          | 80                  | 9                        | 1358                  | 3               | 35                  |
|      | 3         | 400            | 63          | 70                  | 6                        | 1533                  | 3               | 18                  |
|      | 4         | 100            | 76          | 86                  | 8                        | 1364                  | 10              | 23                  |
|      | 4         | 200            | 71          | 75                  | 7                        | 1381                  | 8               | 18                  |
|      | 4         | 300            | 67          | 76                  | 7                        | 1661                  | 3               | 18                  |
|      | 4         | 400            | 65          | 70                  | 7                        | 1539                  | 0               | 18                  |

1997

| Year | No. appl. | 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 (%) |
|------|-----------|----------------|-------------|---------------------|--------------------------|-----------------------|-----------------|---------------------|
|      | 0         | 0              | 143         | 132                 | 33                       | 990                   | 18              | 53                  |
|      | 1         | 100            | 146         | 158                 | 33                       | 1041                  | 10              | 45                  |
|      | 1         | 200            | 137         | 147                 | 29                       | 956                   | 0               | 45                  |
|      | 1         | 300            | 138         | 141                 | 27                       | 1013                  | 3               | 38                  |
|      | 1         | 400            | 146         | 150                 | 29                       | 1047                  | 0               | 38                  |
|      | 2         | 100            | 139         | 148                 | 29                       | 1013                  | 0               | 38                  |
|      | 2         | 200            | 134         | 133                 | 27                       | 1154                  | 3               | 28                  |
|      | 2         | 300            | 112         | 125                 | 22                       | 1064                  | 0               | 38                  |
|      | 2         | 400            | 129         | 141                 | 24                       | 1109                  | 5               | 15                  |
|      | 3         | 100            | 137         | 148                 | 28                       | 1047                  | 0               | 28                  |
|      | 3         | 200            | 124         | 127                 | 27                       | 1086                  | 8               | 18                  |
|      | 3         | 300            | 120         | 128                 | 24                       | 1369                  | 5               | 15                  |
|      | 3         | 400            | 144         | 147                 | 25                       | 1245                  | 0               | 8                   |
|      | 4         | 100            | 132         | 141                 | 23                       | 1200                  | 0               | 23                  |
|      | 4         | 200            | 143         | 143                 | 26                       | 1256                  | 0               | 20                  |
|      | 4         | 300            | 122         | 128                 | 21                       | 1262                  | 0               | 18                  |
|      | 4         | 400            | 145         | 151                 | 27                       | 1352                  | 0               | 5                   |

*Two 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 1.0         | 0.74             | 0.89                    | 0.83                       | -0.51                 | NS              | NS                  |
| 1997 1.0         | 0.69             | 0.83                    | NS                         | NS                    | NS              | 0.60                |
| 1996 1.0         | NS               | 0.49                    | -0.61                      | -0.58                 | NS              | NS                  |
| 1997 1.0         | 0.77             | NS                      | 0.64                       | -0.65                 | NS              | -0.74               |
| 1996 1.0         | NS               | NS                      | 0.51                       | NS                    | NS              | NS                  |
| 1997 1.0         | 0.77             | NS                      | 0.82                       | 0.77                  | NS              | NS                  |
| 1996 1.0         | 1.0              | 1.0                     | -0.58                      | -0.60                 | -0.84           | 0.77                |
| 1997 1.0         | NS               | NS                      | -0.51                      | NS                    | NS              | NS                  |
| 1996 1.0         | 1.0              | NS                      | 0.71                       | -0.66                 | 0.66            | NS                  |
| 1997 1.0         | 1.0              | NS                      | 1.0                        | 1.0                   | -0.75           | -0.95               |

*Two weeks between applications.

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 synthesis 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.

| No. of applications | Application rate (g·ha$^{-1}$) | Total ethephon applied (g·ha$^{-1}$) | Intercept | Application rate (g·ha$^{-1}$) | $R^2$ |
|---------------------|-------------------------------|-------------------------------------|-----------|---------------------------------|-------|
| Top weight (g/plant)$^a$ | 32.6 (1.4) | -1.50 (0.46) | -0.011 (0.004) | NS | 0.59 |
| Carotene content (mg·kg$^{-1}$)$^a$ | 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 |

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

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

$^c$There 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.

$^d$Nonsignificant, $P > 0.05$.

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