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Enhancement of Citrus Regreening and Peel Lycopene by Trickle Irrigation[1]

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Abstract. Regreening of 'Valencia' oranges (*Citrus sinensis* L., Osbeck) is more intense with trickle irrigation than flood irrigation. Peels of fruit from trickle-irrigated trees contain more chlorophyll and less carotenoids than peels of fruit from flood-irrigated trees. Peels of 'Redblush' grapefruit (*C. paradisi* Macf.) from trickle irrigated trees have higher chlorophyll and lycopene contents, but do not differ in ß-carotene content.

Citrus grown in Superstition sand (90% sand) on the Yuma Mesa is commonly irrigated by border-flooding, which results in water application considerably in excess of a trees estimated consumptive use (16). Accordingly, alternative irrigation methods that might improve water use efficiency have been under investigation at the University of Arizona Experiment Station for some time. In addition, marketing considerations encourage delayed harvesting of 'Valencia' oranges and 'Redblush grapefruit.

Flowering of citrus in Arizona occurs during the early weeks of April, and 'Redblush' grapefruit is usually harvested from the first of December through May, while 'Valencia' orange is harvested about March of the following year. In response to cool winter temperatures citrus fruit lose chlorophyll (12, 19) and 'Valencia' orange develops its best color in February or March (3). 'Redblush' grapefruit also loses chlorophyll during the winter months, but also tends to lose the lycopene (10, 13, 14) responsible for the desired red color.

During the spring and early summer 'Valencia' oranges and 'Redblush' grapefruit begin to regreen, apparently in response to warmer air and soil temperatures (2, 9, 20). Regreening is heightened by nitrogen application and reduced by ground covers (3, 7). Results reported here compare levels of chlorophyll and carotenoids in peels of 'Valencia' oranges and 'Redblush' grapefruit harvested between February and July from trees watered by border flooding and trickle irrigation.

On the dates indicated, 10 'Valencia' oranges were randomly collected from 6 trees on 'Alemow' (*C. macrophylla* Wester) rootstocks in a single row irrigated by border flooding (173 cm water/year). A second set of 10 fruit were collected from 6 similar trees irrigated by trickle irrigation (74 cm water/year). Grapefruit were collected in groups of 10 from 3 trees on 'Rough lemon' (*C. jambhiri* Lush.) rootstocks irrigated by border flooding (218 cm water/year), 3 trees trickle irrigated with 152 cm water/year and 3 trees trickle irrigated 122 cm water/year. Trickle irrigated trees were internal trees within a randomly mixed block of 'Marsh' and 'Redblush' varieties. Flood irrigated trees were in the same mixed block, but within a single row. Trickle irrigations were timed to closely match 125% and 100% of the trees estimated daily consumptive use (5, 16), and flood irrigations were done when tensiometer readings reached 60 centibars.

Since chlorophyll and lycopene content of grapefruit peels from trees trickle irrigated at the two different rates did not differ significantly, the data were combined into a single set of trickle irrigation data.

Chlorophyll content of fruit was estimated *in vivo* by the difference in absorbance at 675 and 735 nm ($\Delta A_{675-735}$) using a Bausch and Lomb Model 340 spectophotometer fitted with an integrating sphere reflectometer attachment (6). One measurement was made at the stem end and another in the equatorial region of each fruit, and the 10 measurements for each location and irrigation treatment averaged. The reflectometer is sensitive to the low levels of chlorophyll that cause observable differences in the color of degreened fruit but are troublesome to estimate by extraction, requiring relatively large samples and much concentrating of extracts.

Total carotenoids were estimated in 'Valencia' orange flavedo by extracting 20 discs, 1 cm in diameter, cut from the equators of 10 fruit. The extraction procedure was a modification of that of Lime et al. (10). discs were first homogenized with methanol, then water, discarding the extracts. The residue was then extracted with a 50:50 mixture of acetone and hexane. These extracts were washed with water to remove acetone, dried with Na_2SO_4 and their spectra recorded from 400 to 600 nm. Total carotenoids in 'Valencia' orange extracts were calculated using 2500 as the average carotenoid extinction coefficient (8) and lycopene and ß-carotene in 'Redblush' grapefruit extracts were calculated from absorbances at 451 nm and 502 nm (10).

In February, 'Valencia' oranges on trees irrigated by border flooding had apparently lost all chlorophyll from the equatorial region although there still remained some cholorphyll near the stems (Table 1). In contrast, fruit from trickle irrigated trees retained some chlorophyll in the equatorial regions of the fruit. Carotenoid levels were the same in both sets of fruit. During spring and into summer, fruit from both sets of trees regreened somewhat, with fruit from trickle irrigated trees consistently having higher chlorophyll levels than fruit from flood irrigated trees. Contrary to observations reported elsewhere (3, 5), both sets of fruit continued to accumulate carotenoids while regreening, and flood irrigated trees consistently yielded fruit with significantly higher levels of carotenoids in the peel than trickle irrigated trees (Table 1). Thus, fruit from flood irrigated trees had superior ratios of carotenoids to chlorophyll throughout the harvest period.

Although carotenoids have been observed to decline during regreening, this decline is not directly related to chlorophyll accumulation, for untreated fruit at Riverside, California continued to accumulate carotenoids during the first 3-4 months of regreening (1). In Australia, El-Zeftawi (4) observed that carotenoids began to decline 3 months in advance of chlorophyll accumulation during one season, but during the following season carotenoids accumulated along with chlorophyll for 3 months. Thus, carotenoids must be affected in part by factors independent of those affecting chlorophyll accumulation, but why 'Valencia' oranges in this study continued to accumulate carotenoids during regreening is unknown.

Chlorophyll levels in the peel of 'Redblush' grapefruit tended to increase into May, and again the peel of fruit from

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Table 1. Chlorophyll and carotenoid levels in peels of 'Valencia' oranges from trees watered by border flooding and trickle irrigation, 1979.

	Chlorophyll ($\Delta A_{675-735}$)				Carotenoids ($\mu g/cm^2$)		Carotenoids/ chlorophyll	
	Stem End		Equator		Equator		Equator	
Date	Flood	Trickle	Flood	Trickle	Flood	Trickle	Flood	Trickle
Feb. 15	.06 ± .05[z]	.03 ± .02	.00 ± .002	.03 ± .01*	15 ± 1[y]	15 ± 1	∞	500
May 1	.20 ± .01	.26 ± .02*	.15 ± .01	.18 ± .01*	29 ± 2	22 ± 1*	193	122*
July 1	.33 ± .02	.41 ± .03*	.24 ± .01	.30 ± .02*	44 ± 3	30 ± 3*	183	100*

[z]Values for chlorophyll are means ± SE for 10 fruit from 6 trees.
[y]Values for lycopene are means ± SE of 4 determinations on a combined sample of 10 fruit from 6 trees.
*Value for trickle irrigation is significantly different from corresponding value for flood irrigaiton at 5% level.

trickle irrigated trees consistently had higher chlorophyll levels than the peel of fruit from flood irrigated trees (Table 2), especially near the stem end.

While 'Redbush' grapefruit tends to lose lycopene in the fall and winter (10, 12, 15) there was a marked accumulation of lycopene in the peel during April and May (Table 2) increasing from 0.1 $\mu g/cm^2$ in February and March to 1 or 2 $\mu g/cm^2$ by May 15. The peel of fruit from flood irrigated trees contained consistently less lycopene than did that of fruit from trickle irrigated trees. In contrast, levels of ß-carotene in the peel were not significantly affected by irrigation method (data not shown). The seasonal increase in lycopene is apparently a response to warmer temperatures (11, 15) similar to regreening.

Thus, while the peel of 'Valencia' oranges from trees irrigated by border flooding clearly has more desirable combinations of chlorophyll and carotenoids than the peel of similar fruit from trickle irrigated trees, 'Redblush' grapefruit from flood irrigated trees have more desirable chlorophyll levels, but less desirable lycopene levels.

How trickle irrigation increases chlorophyll in citrus peels is not readily apparent. One possibility is that trickle irrigation has the same effect as increasing nitrogen application, for some of us have observed (17, 18) that one effect of trickle irrigation is an increase in leaf-nitrogen levels, apparently through improved nitrogen up-take. If so, adjusting nitrogen applications should obliterate any effects of irrigation method on regreening. Improved nitrogen utilization does not, however, explain how the irrigation method effects lycopene levels in grapefruit peels.

Table 2. Chlorophyll and lycopene levels in peels of 'Redblush' grapefruit watered by border flooding and trickle irrigation, 1980.

	Chlorophyll ($\Delta A^{675-735}$)				Lycopene ($\mu g/cm^2$)	
	Stem end		Equator		Equator	
Date	Flood	Trickle	Flood	Trickle	Flood	Trickle
Feb. 15	0.04 ± 0.01[z]	0.10 ± 0.01*	0.02 ± 0.01	0.07 ± 0.01*	0.00 ± 0.01[y]	0.11 ± 0.02**
March 15	0.09 ± 0.01	0.17 ± 0.02*	0.06 ± 0.02	0.08 ± 0.01	0.00 ± 0.01	0.07 ± 0.02**
April 15	0.06 ± 0.02	0.16 ± 0.02*	0.03 ± 0.01	0.06 ± 0.01	0.21 ± 0.01	0.35 ± 0.02**
May 15	0.15 ± 0.01	0.21 ± 0.02*	0.11 ± 0.01	0.16 ± 0.02*	0.98 ± 0.07	2.12 ± 0.05**

[z]Chlorophyll values for flood and trickle respectively are means ± SE for 10 and 20 fruit from 3 and 6 trees.
[y]Lycopene values for flood and trickle respectively are means ± SE of 4 and 8 determinations for combined samples of 10 and 20 fruit from 3 and 6 trees.
*,**Value for trickle irrigation is significantly different than corresponding value for flood irrigation at 5% (*) and 1% (**) level.

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