hexacosanol, and octacosanol. Tetracosanol was the principle alcohol present in the wax esters. Coastal Bermuda grass contains a much broader spectrum of alcohols in its ester fraction than does ryegrass esters which contain 79% hexacosanol (Allebone and Hamilton, 1972).

Coastal Bermuda grass was found to contain octacosanol as its principle identified free alcohol. However, the major alcohol (Table II) has not been identified. The mass spectra of the unknown alcohol indicated a molecular weight of 426. It formed a monotrisilyl ether derivative indicating the presence of a single hydroxyl group. Its mass spectrum was similar in appearance to that of the triterpenol lanosterol. The degree of similarity suggests that it is a triterpene with a similar elemental composition as lanosterol. In ryegrass hexacosanol accounts for 93% of the free alcohols (Allebone and Hamilton, 1972).

The fatty acids were identified as their methyl esters. The major acids freed from the esters were eicosanoic and docosanoic acids with smaller amounts of tetracosanoic, octadecanoic, and hexadecanoic acids (Table II). The free fatty acids isolated contained the same broad spectrum of acids as found associated with the esters. However, the major free acid was hexadecanoic acid with lesser amounts of docosanoic, octadecanoic, and 9-hexadecenoic acids (Table II). Ryegrass wax esters have been reported to contain octadecanoic acid as its major acid with lesser amounts of eicosanoic and hexadecanoic acids (Allebone and Hamilton, 1972). The aldehyde fraction was reduced and the aldehydes identified as their corresponding alcohols. Hexadecanal was the principle aldehyde isolated along with lesser amounts of octadecanal, tetradecanal, and eicosanal (Table II).

The hydrocarbon components of Coastal Bermuda grass wax are unusual, since tritriacontane is rarely a major component of plant waxes (Tulloch, 1974). The major hydrocarbons of most plant waxes are heptacosane, nonacosane, or hentriacontane (Herbin and Robins, 1967; Kolattukudy, 1970). Plant waxes with tritriacontane as the major hydrocarbon have been isolated from members of the family Crassulaceae and Gymnosperms (Herbin and Robins, 1967). Tulloch (1974) has also identified tritriacontane as the major hydrocarbon in the common annual weed (*Portulaca oleracea* (L.)).

ACKNOWLEDGMENT

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Registry No. Hentriacontane, 630-04-6; tritriacontane, 630-05-7; pentatriacontane, 630-07-9; nonacosane, 630-03-5; octacosanol, 557-61-9; docosanoic acid, 112-85-6; lanosterol, 79-63-0; 9-hexadecenoic acid, 2091-29-4; hexadecanal, 629-80-1; octadecanal, 638-66-4; tetradecanal, 124-25-4; eicosanal, 2400-66-0; octadecanoic acid, 57-11-4.

LITERATURE CITED

Allebone, J. E.; Hamilton, R. J. *J. Sci. Food Agric.* **1972**, *23*, 777.
Allebone, J. E.; Hamilton, R. J.; Knights, B. A.; Middleditch, B. S.; Power, D. M. *Chem. Phys. Lipids* **1970**, *4*, 37.
Bianchi, B.; Avato, P.; Bertorelli, P.; Manini, G. *Phytochemistry* **1978**, *17*, 999.
Bianchi, B.; Avato, P.; Salamini, F. *Maydica* **1975**, *20*, 1965.
Eglinton, G.; Hamilton, R. J. *Science (Washington, D.C.)* **1967**, *156*, 1322.
Emery, A. E.; Gear, J. R. *Can. J. Biochem.* **1969**, *47*, 1195.
Hamilton, R. J.; Power, D. M. *Phytochemistry* **1969**, *8*, 1771.
Herbin, G. A.; Robins, P. A. *Phytochemistry* **1968**, *7*, 257.
Kolattukudy, P. E. *Lipids* **1970**, *5*, 259.
Pollard, A.; Chibnall, A. C.; Piper, S. H. *Biochem. J.* **1931**, *25*, 2111.
Tulloch, A. P. *Lipids* **1974**, *9*, 664.
Tulloch, A. P. *Phytochemistry* **1982**, *21*, 2251.
Tulloch, A. P.; Hoffman, L. L. *Phytochemistry* **1971**, *10*, 871.
Tulloch, A. P.; Hoffman, L. L. *Phytochemistry* **1973**, *12*, 2217.
Tulloch, A. P.; Weenink, R. O. *Can. J. Chem.* **1969**, *47*, 3119.
Wilkinson, R. E.; Cummins, D. G. *Crop Sci.* **1981**, *21*, 397.

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Fruit Development and Growth Regulator Effects on Normal Alkanes of "Washington" Navel Orange Fruit Epicuticular Wax

Mohamed El-Otmani[1] and Charles W. Coggins, Jr.*

The *n*-alkanes as percent of total wax were highest in the very young "Washington" navel orange (*Citrus sinensis* (L.) Osbeck) fruit, but *n*-alkanes per unit area were highest in maturing and senescing fruits. Odd-numbered alkanes dominated with a minimum of 81.5% in Sept. C_{25} dominated in the immature fruit. At maturity and during senescence, C_{31} was highest, followed by C_{29} and C_{27}. During fruit growth and maturation, a shift of *n*-alkanes from lower to higher molecular weight was observed, but the reverse of that occurred during the postmaturation phase. Gibberellic acid (GA_3 = 10 ppm) alone or in combination with (2,4-dichlorophenoxy)acetic acid (2,4-D = 16 ppm) reduced *n*-alkane accumulation, but 2,4-D alone did not produce any significant effect until the senescence stage, where it decreased the rate of *n*-alkane accumulation. GA_3, either alone or in combination with 2,4-D, reduced total percent of odd-numbered hydrocarbons in favor of the even-numbered fractions. Treatment effect on individual *n*-alkanes and possible relationships between wax *n*-alkanes and wax fine structure are discussed.

Plant surfaces are characteristically covered by several layers of hydrophobic material, the outermost being the epicuticular wax. Functional and physiological roles of epicuticular wax include: reduction in transpiration and maintenance of water balance; regulation of gas exchange; protection against pathogens and mechanical damage; protection against UV radiation; protection against atmospheric pollutants; and resistance to freezing. The appearance and quantity of epicuticular waxes vary greatly

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between and among species. These waxes consist of a mixture of long-chain hydrocarbons, alcohols, aldehydes, ketones, fatty acids, and related compounds.

In *Citrus*, leaf epicuticular wax paraffins are frequently second in quantity to primary alcohols (Freeman et al., 1979). However, paraffins varied from 2 to 67% of total wax depending on the cultivar and stage of leaf development. In fruit, paraffins ranged from 30 to 44% and 6 to 12% of total wax in immature and mature fruit, respectively (Freeman et al., 1979). Paraffins have been reported to account for 11.4% of the epicuticular wax of mature "Shamouti" orange fruit (Schulman and Monselise, 1970) and for 40% of the epicuticular wax of the mature "Valencia" orange leaf (Leece, 1976).

In *Citrus*, alkane analyses have been reported for hydrocarbons of the surface waxes of juice sacs (Nagy and Nordby, 1971, 1972, 1973; Nagy et al., 1975; Nordby and Nagy, 1974, 1975, 1977a), leaves (Baker et al., 1975; Nagy et al., 1975; Nordby et al., 1975, 1979a; Scora et al., 1982), peel (Baker et al., 1975; Nagy et al., 1975; Nordby and Nagy, 1977b), and seeds (Nagy et al., 1975). Alkanes have potential usefulness in differentiating nucellar from zygotic seedling trees (Nordby et al., 1975) and have been considered as useful chemotaxonomic markers (Nordby et al., 1979a; Nordby and Nagy, 1974). However, in an extensive study using 71 *Citrus* taxa and related biotypes, no biosystematically consistent groupings were found (Scora et al., 1982).

The alkane content of the surface waxes of leaves were 60, 42, 46 and 66%, and those of fruits were 23, 40, 42, and 36%, respectively, for "Adamopoulou" lemon, "Frost Valencia" orange, clementine mandarin, and "Willowleaf" mandarin (Baker et al., 1975). The dominant alkanes were *n*-hentriacontane (C_{31}, 43–53%) and *n*-tritriacontane (C_{33}, 31–39%) in leaf wax, and *n*-heptacosane (C_{27}, 9–32%), *n*-nonacosane (C_{29}, 21–33%), and *n*-hentriacontane (11–33%) in fruit wax.

In "Duncan" grapefruit, the linear chain alkanes represented 44 and 53% of total alkanes for juice sacs and seeds, respectively, while they represented at least 98% for stem, peel, and leaf (Nagy et al., 1975). C_{25} dominated in juice sacs and seeds, while C_{31} dominated in stem, peel, and leaf waxes.

In mandarin juice sacs, linear chains accounted for at least 47% of the alkanes, and at least 75% of the alkenes (Nordby and Nagy, 1975). In five mandarin x tangelo hybrids, linear chains accounted for approximately 47% of the alkanes and at least 70% of the alkenes (Nordby and Nagy, 1976).

Stage of maturity affects the profile and distribution; C_{20} to C_{26} alkanes and alkenes dominated in the peel epicuticular wax of immature fruit, whereas C_{27}–C_{33} chain length predominated in mature fruits (Nordby and Nagy, 1977b). Such changes from lower to higher carbon numbered alkanes upon maturation were also observed in citrus leaf waxes (Nordby et al., 1979a). However, the level of hydrocarbons in sweet orange juice sac wax remained relatively constant over the maturation period (Nordby and Nagy, 1977a), and the approximate ratio of alkanes to alkenes in the external wax of juice sacs was 15:1 (Nagy and Nordby, 1973; Nordby and Nagy, 1977a). In juice sac external wax of immature fruit, C_{23}–C_{27} alkanes comprised ca. 92–94% of the total alkanes (Nordby and Nagy, 1977a). From 40 to 45% of these were linear.

Rootstock type did not have a major effect on the alkane pattern of the *Citrus* leaf epicuticular wax nor did it influence the alkane profile of the juice vesicular wax (Nordby et al., 1979b).

In rice, it has been shown that brown spot disease incidence is negatively correlated with the proportion of alkanes of leaves (Gangopadhyay and Chattopadhyay, 1973) and cuticular resistance to transpiration paralleled total alkanes (O'Toole et al., 1979). Furthermore, in white pine, needle tolerance to ozone was reported to correlate positively with alkane content of the epicuticular wax (Trimble et al., 1982).

The relative abundance and composition of epicuticular wax alkanes have not been studied during fruit senescence. Senescence in citrus rind tissue can be delayed by treatment with growth regulators such as gibberellic acid (GA_3) (Coggins and Lewis, 1965), and better results have been reported with a combination of GA_3 with (2,4-dichlorophenoxy)acetic acid (2,4-D) (El-Zeftawi, 1980), 2,4-D being an abscission-delaying agent. GA_3 has also been shown to reduce rind staining (Coggins et al., 1963), improve rind protection against water spot (Riehl et al., 1966), reduce the rate of peel softening (Coggins and Lewis, 1965), reduce the incidence of puffy rinds (Kuraoka et al., 1977), delay the development of "sticky" rinds under preharvest as well as postharvest conditions, and reduce rind susceptibility to decay microorganisms such as *Penicillium* (Coggins, 1969).

The general objective of this study was to increase our understanding of citrus fruit development, maturation, and senescence. In particular, the objectives were to determine the quantitative and qualitative changes in alkane components of the "Washington" navel fruit epicuticular wax during fruit growth, maturation, and senescence, and to determine the effect of 2,4-D and GA_3 on these parameters during maturation and senescence.

MATERIALS AND METHODS

Fruit Material, Treatment Application, and Experimental Design. All fruit material was collected from a grove of 14-year-old "Washington" navel (*C. sinensis* (L.) Osbeck) trees on "Troyer" citrange (*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* Raf.) rootstock near Riverside, CA.

A randomized complete block design with six blocks and 16 trees per block was used for fruit sampling from May 25, 1983 through Oct 6, 1983, the date at which fruit color break occurred and the blocks were subdivided into four-tree plots receiving one of the following treatments at random: no treatment, 16 ppm of acid equivalent of the isopropyl ester of 2,4-D, 10 ppm of GA_3, or a combination of 2,4-D (16 ppm) plus GA_3 (10 ppm). A nonionic wetting agent (Western Farm Service Spreader), containing alkylaryl polyoxyethylene glycol (60%) as active ingredient, was used at a rate of 12 mL/100 L of the spray solution. The mixture was sprayed onto trees to the point of runoff.

Fruit Sampling and Wax Extraction. From May 25, 1983 through Oct 6, 1983, fruit samples were harvested from throughout each of the six replications by collecting fruits from all sides of each tree within a given block. Initially, each sample consisted of 1000 fruits. As fruit growth occurred, the number of fruits per sample was gradually decreased to 64 fruits per sample on Oct 6, at which date treatments were applied. Subsequent samples consisted of 24 fruits per replication per treatment. Fruits were collected at 4-week intervals throughout the experiment.

Epicuticular wax was extracted by immersing the fruit in warm chloroform for 1 min. The chloroform–wax solution was filtered, the solvent evaporated, and total epicuticular wax per replicate determined gravimetrically. Extracted wax was made up to 1% (wt/v) solution in chloroform and stored in a freezer until used.

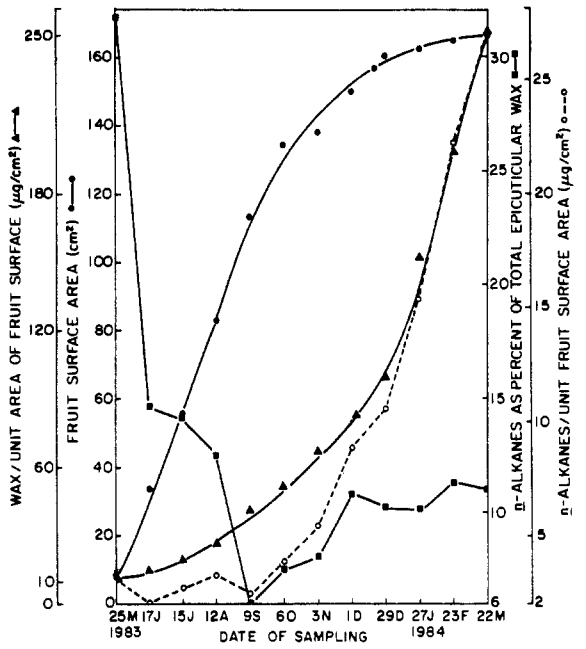

Figure 1. Seasonal variation of fruit surface area (cm^2), total epicuticular wax per unit of fruit surface area ($\mu g/cm^2$), the percentage of epicuticular wax *n*-alkanes, and the quantity of total wax *n*-alkanes ($\mu g/cm^2$) of the "Washington" navel orange fruit. Color break started during the first week of Oct, fruit were legally mature in early Dec, and some aspects of rind senescence were obvious in late Dec.

Fruit surface area was calculated from fruit length and diameter by using Turrell's tables (Turrell, 1946).

The *n*-Alkane Isolation and Quantitation. Glass columns, 0.9 cm in diameter and 30 cm in length, were packed with pentane-washed 28–200 mesh silica gel (Sigma Chemical Co., St. Louis, MO). A 2-mL sample of the 1% chloroform–wax solution (i.e., 20 mg epicuticular wax) was percolated into the prepared column and the *n*-alkane fraction eluted with 200 mL of pentane as shown by gas chromatography. The fraction was concentrated to a small volume, transferred to a small preweighed beaker, air dried, and weighed for total normal alkanes. The isolated *n*-alkanes were made up to 0.2% (wt/v) solution in isooctane and stored in a freezer until used.

Alkane Analysis. Four-microliter samples of the isooctane–alkane solutions were injected into a Hewlett Packard gas chromatograph Model 5830A with flame ionization detector equipped with an 18850A Hewlett Packard GC integrator. *n*-Alkanes were determined on a glass column (2 m × 2 mm i.d.) packed with 3% OV 210 (Hewlett Packard, Avondale, PA) on 80–100 mesh Chromosorb W. Injector heater temperature was 250 °C, and the detector was set for 300 °C. Oven temperature program consisted of an initial temperature of 60 °C for 10 min, after which the temperature was increased at a rate of 3 °C/min until a final temperature of 270 °C, which was held constant for 45 min. Nitrogen at a flow rate of 25 mL/min was used as the carrier gas. One analysis was done per replicate and standards of known composition were used to determine retention times of specific alkanes.

RESULTS AND DISCUSSION

Untreated Surfaces. The amount of wax per cm^2 of fruit surface increased with time (Figure 1), but the rate of increase was slower during the logarithmic phase of growth (May–Oct) and increased significantly during fruit maturation and senescence (Nov–March). Futhermore, the quantity of *n*-alkanes per unit surface area increased dramatically during maturation and senescence (Figure 1). Such increase in *n*-alkanes was reported for the *n*-alkane fraction of peach leaf adaxial and abaxial surface (Baker et al., 1979). In addition, the *n*-alkane fraction was relatively high in the very young fruit where it made up 32% of total wax (Figure 1). This percentage decreased rapidly to about 6% 4 weeks before fruit color break. This rapid decline coincides with rapid fruit growth (Figure 1). During maturation and senescence, fruit growth was slow and the *n*-alkane fraction of the epicuticular wax increased slowly to about 11%. The fact that the *n*-alkane fraction then remained relatively constant at 10–11%, is consistent with the parallel curves for quantity of *n*-alkane and quantity of wax per unit surface area during the latter half of the study and with previous reports on juice sac hydrocarbons (Nordby and Nagy, 1977a).

The alkane fraction consisted of a mixture of chain lengths varying from C_{23} to C_{33} (Figure 2 parts A and B). Odd-numbered chains were dominant throughout the season. They made up about 91.5% of the total hydrocarbon fraction in the very young fruit, declined to a minimum of 81.5% 4 weeks before fruit color break, and steadily increased during maturation and senescence to reach a maximum of about 93% (Figure 2 part A). In contrast, the even-numbered chains comprised between 8.5 and 18.5% of the total (Figure 2 part B). The maximum contribution from even-numbered chains was reached 4 weeks before color break. The dominance of odd-numbered chains in plants has been reported in numerous publications.

Within the odd-numbered *n*-alkane population, C_{25} made up about 54% of total hydrocarbon fraction of the most immature fruit followed by C_{27} with 26% (Figure 2 part A). Both of these compounds declined to reach a minimum of 4 and 10%, respectively, 4 weeks after color break. As fruit matured and senesced, they increased to reach 14 and 21%, respectively, in late March. C_{31}, which made up less than 2% of the total in May, i.e., in the small fruit, increased rapidly to about 46% at 4 weeks after fruit color break and declined thereafter to make up 24% of the total *n*-alkanes in late March. C_{29} exhibited its highest increase between May and July to reach about 20% and remained very constant thereafter. Very negligible amounts of C_{33} were detected in May, but a significant increase to 9% in Nov was recorded. Within the even-numbered chains, C_{26} dominated in early season but declined in importance after early Sept, the date at which the other even-numbered *n*-alkanes (except for C_{24}, which did not exhibit much change throughout the season) reached their maximum values with C_{30} predominating with about 6% (Figure 2 part B). Such changes in the distribution pattern of *n*-alkanes from lower to higher molecular weight chains upon maturation were also recorded in fruit peel epicuticular waxes of other citrus cultivars (Nordby and Nagy, 1977b), in juice vesicular wax of citrus fruit (Nordby and Nagy, 1977a), in leaf wax of citrus (Nordby et al., 1979a), in peach leaf wax (Baker et al., 1979), and in sorghum leaf wax (Atkin and Hamilton, 1982).

It is important to note that previous epicuticular wax studies of citrus have stopped at early stages of maturity. Our study continued for approximately 20 weeks after maturation and well into fruit senescence. Thus, we have confirmed developmental patterns previously reported, and we have learned that substantial quantitative and qualitative changes occur subsequent to maturation.

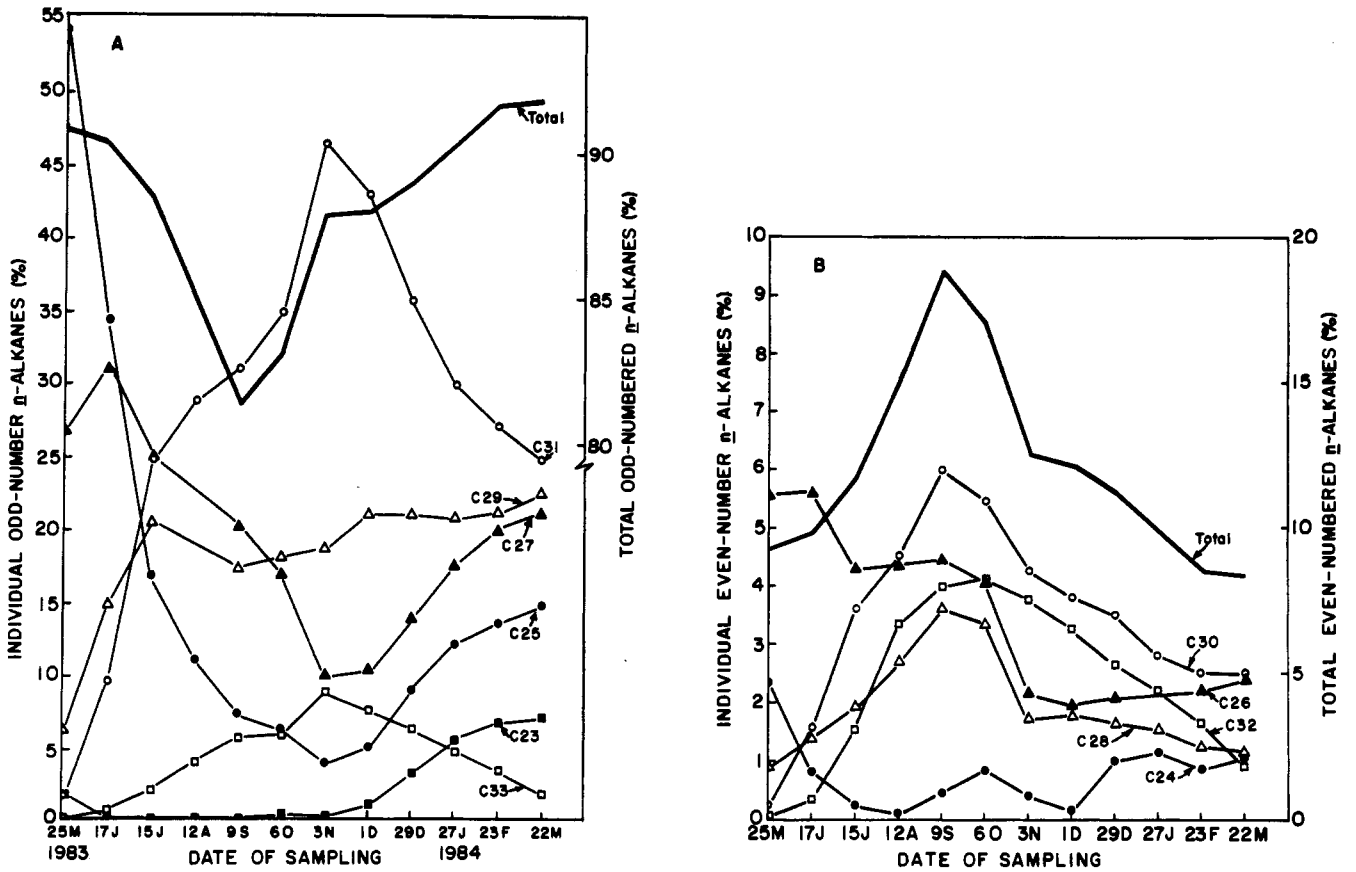

Figure 2. Seasonal variation of the distribution pattern (in percent of total *n*-alkanes) of odd-number *n*-alkanes (A) and of even-number *n*-alkanes (B) of "Washington" navel orange fruit epicuticular wax.

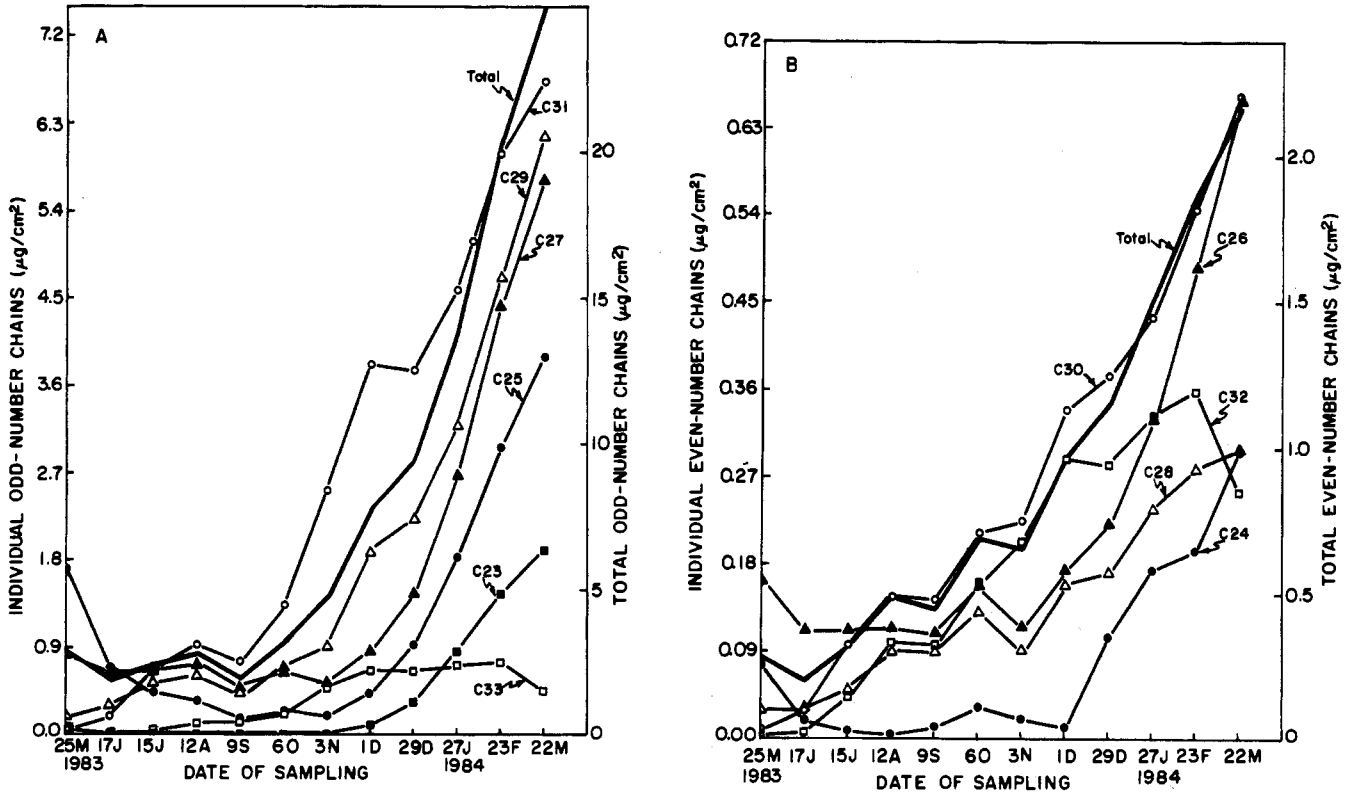

Figure 3. Seasonal variation of the quantity ($\mu g/cm^2$) of odd-number *n*-alkanes (A) and of even-number *n*-alkanes (B) of "Washington" navel orange fruit epicuticular wax.

The total *n*-alkane quantity per unit fruit surface area increased slowly during fruit growth and early maturation but rapidly during the postmaturation phase. However, the percent *n*-alkanes in the fruit epicuticular wax was relatively constant during the postmaturation stage (Figure 1). For individual *n*-alkanes, a slow increase in quantity ($\mu g/cm^2$) was observed during fruit growth and maturation, but a dramatic increase occurred during postmaturation

Table I. Effect of GA_3 (10 ppm), 2,4-D (16 ppm), and Fruit Age on the *n*-Alkane Fraction (%, w/w) of the Epicuticular Wax of the "Washington" Navel Orange Fruit[a]

	date of sampling						
	1983				1984		
treatment	Oct 6[b]	Nov 3	Dec 1	Dec 29	Jan 27	Feb 23	Mar 22
control	7.5	8.1 a[c]	11.0 b	10.6 c	10.2 a	11.3 b	11.0 b
GA_3		7.2 a	9.4 a	9.2 b	9.7 a	10.2 ab	10.5 b
GA_3 + 2,4-D		7.8 a	9.2 a	7.6 a	10.2 a	9.8 a	7.5 a
2,4-D		10.2 b	11.4 b	10.8 c	10.2 a	9.1 a	7.6 a
CV, %[d]		10	7	10	7	11	8

[a] All data are means of six replications. [b] Date of treatment application. [c] Mean separation within dates by Duncan's multiple range test at 5% level of significance. [d] Coefficient of variation.

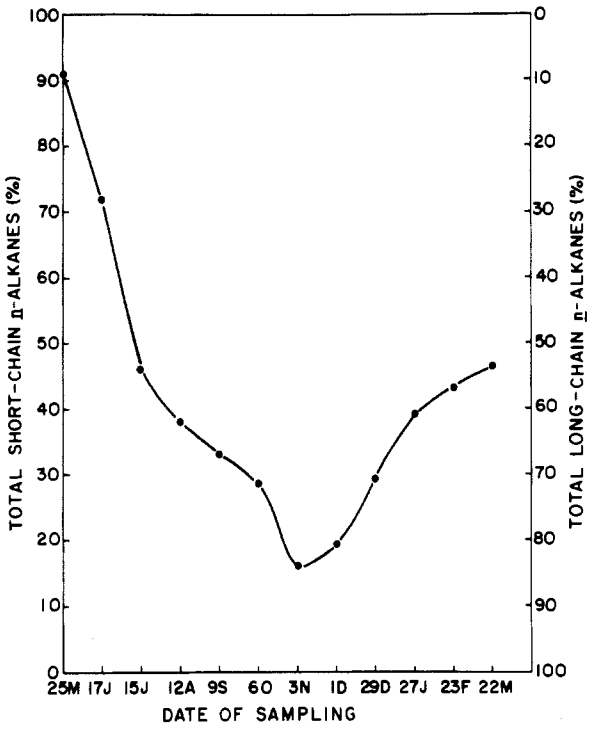

Figure 4. Seasonal variation of short-chain [$\sum$ (C_{23}–C_{27})] and long-chain [$\sum$ (C_{28}–C_{33})] *n*-alkanes (as percent of total) in the "Washington" navel orange fruit epicuticular wax.

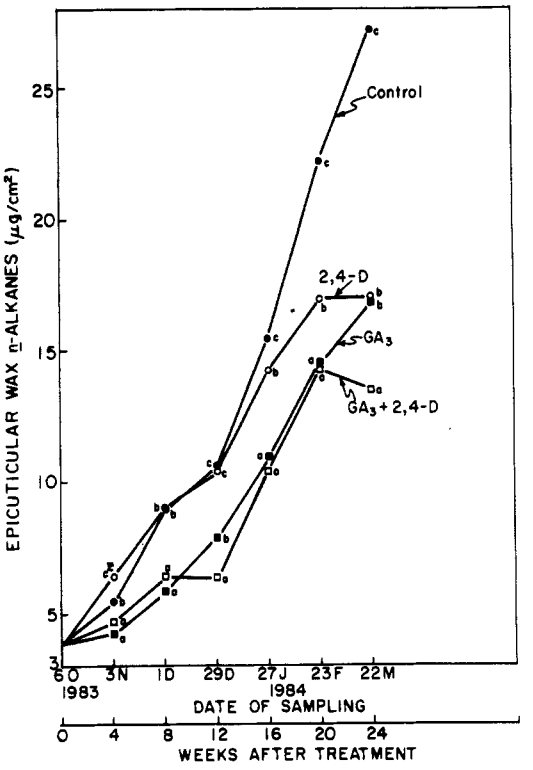

Figure 5. Effect of GA_3 (10 ppm), 2,4-D (16 ppm), and GA_3 + 2,4-D on the quantity of *n*-alkanes per unit fruit surface area ($\mu g/cm^2$) in "Washington" navel orange. (*Z* mean separation within dates by Duncan's multiple range test, 5% level.)

and senescence (Figure 3 parts A and B). The increase in the quantity of these individual alkanes per unit fruit surface area paralleled that of the total *n*-alkanes ($\mu g/cm^2$) but with different rates of accumulation; for odd-numbered chains, $C_{31} > C_{29} > C_{27} > C_{25} > C_{23}$ (Figure 3 part A). C_{33} did not change much with time. For the even-numbered chains, similar trends were found with $C_{30} > C_{32} > C_{26} > C_{28} > C_{24}$ for most dates (Figure 3 part B). While alkane quantity per unit surface area increased throughout growth, maturation, and senescence, there was a gradual shift in percentage of individual *n*-alkanes from shorter to longer chains observed during the stages of fruit expansion and maturation, but the reverse of that occurred during the postmaturity and senescence period with increasing proportions of shorter chains and decreasing proportions of longer chains (Figure 4).

In a morphology study (El-Otmani and Coggins, 1985), we showed that the surface of the epicuticular wax is amorphous during early stages of fruit development and during senescence. In between these two stages, the surface has a crystalline structure. Thus, we have concluded that amorphous wax contains relatively higher ratios of shorter *n*-alkanes and higher proportions of total odd-numbered *n*-alkanes than does a more crystalline wax. However, other wax components may also be involved in determining the wax morphology and fine structure, and this fact was discussed by Baker (1982) in his review on plant epicuticular waxes.

Treated Surfaces. The composition of the epicuticular wax with respect to percent of *n*-alkanes was not appreciably influenced by 2,4-D or GA_3 (Table I). However, growth regulators did significantly affect the quantity of *n*-alkanes per unit surface area (Figure 5). Fruit from GA_3-treated trees contained significantly lower quantities of *n*-alkanes than fruit from control and 2,4-D-treated trees for all except the last sampling date. At that point, fruit from each of the growth regulator treatments had lower quantities of *n*-alkanes than the controls. As seen in Figure 1 for control fruit, the quantity of wax and the quantity of *n*-alkanes per unit surface area were closely correlated during the postmaturation period. This is also true for growth regulator treatments as indicated by the fact that growth regulators caused a reduction in the quantity of wax (El-Otmani and Coggins, 1985) but caused no significant change in the percentage of total *n*-alkanes contained in the wax.

During Nov through Jan, fruit from GA_3-treated trees had epicuticular wax which was lower in odd-numbered chains and relatively richer in even-numbered chains than

Table II. Effect of GA_3 (10 ppm), 2,4-D (16 ppm), and Fruit Age on *n*-Alkane Components (Expressed as % of Total *n*-Alkanes) in "Washington" Navel Orange Epicuticular Wax[a]

hydrocarbon chain length	treatment	date of sampling: 1983 Oct 6[b]	1983 Nov 3	1983 Dec 1	1983 Dec 29	1984 Jan 27	1984 Feb 23	1984 Mar 22
C_{23}	control	0.4	0.3 a	1.2 a	3.3 a	5.5 b	6.7 b	7.1 a
	GA_3		0.3 a	1.4 b	3.1 a	4.7 a	5.2 a	6.3 a
	GA_3 + 2,4-D		0.4 a	1.4 b	3.2 a	4.7 a	6.6 b	6.9 a
	2,4-D		0.3 a	1.3 ab	3.2 a	5.5 b	6.8 b	6.9 a
C_{25}	control	6.3	3.8 a	4.9 a	8.8 a	12.0 b	13.4 b	14.6 b
	GA_3		4.9 b	6.3 d	8.6 a	10.2 a	11.8 a	12.2 a
	GA_3 + 2,4-D		5.0 b	6.0 c	8.7 a	10.9 a	12.7 b	13.1 a
	2,4-D		4.2 a	5.3 b	8.8 a	12.3 b	13.4 b	14.5 b
C_{27}	control	16.8	9.8 a	10.3 a	13.8 a	17.4 a	19.9 a	21.0 b
	GA_3		11.8 c	12.6 d	14.3 a	17.2 a	19.2 a	19.7 a
	GA_3 + 2,4-D		12.0 c	12.1 c	14.2 a	17.4 a	20.0 a	20.9 ab
	2,4-D		10.4 b	11.0 b	14.2 a	18.0 a	19.6 a	21.5 b
C_{29}	control	18.0	18.6 b	21.1 b	21.0 b	20.7 a	21.2 a	22.6 a
	GA_3		16.9 a	18.9 a	20.8 b	23.5 c	25.8 c	25.9 b
	GA_3 + 2,4-D		16.7 a	18.8 a	20.1 a	22.3 b	22.5 b	24.4 b
	2,4-D		18.3 b	21.3 b	21.0 b	20.7 a	20.6 a	22.2 a
C_{31}	control	34.7	46.4 b	42.9 c	35.7 a	29.8 a	27.0 c	24.8 a
	GA_3		43.3 a	39.4 a	34.3 a	28.9 a	26.1 b	24.1 a
	GA_3 + 2,4-D		43.2 a	39.8 a	34.3 a	28.4 a	24.8 a	23.7 a
	2,4-D		45.7 b	41.6 b	35.1 a	29.0 a	26.4 bc	24.6 a
C_{33}	control	5.8	8.8 b	7.6 b	6.4 b	4.8 b	3.4 bc	1.8 a
	GA_3		7.8 a	6.6 a	5.7 a	4.0 a	2.5 a	2.1 a
	GA_3 + 2,4-D		7.7 a	6.9 a	5.8 a	3.9 a	3.0 b	1.7 a
	2,4-D		8.6 b	7.6 b	6.2 b	4.5 b	3.6 c	2.3 a
subtotal (odd)	control	82.0	87.7 b	88.0 b	89.0 b	90.2 b	91.6 a	91.9 a
	GA_3		85.0 a	85.2 a	86.8 a	88.5 a	90.6 a	90.4 a
	GA_3 + 2,4-D		85.0 a	85.0 a	86.3 a	87.6 a	89.6 a	90.7 a
	2,4-D		87.5 b	88.1 b	88.5 b	90.0 b	90.5 a	92.0 a
C_{24}	control	0.8	0.4 a	0.3 a	1.0 a	1.1 a	0.8 a	1.1 a
	GA_3		0.6 b	0.5 a	1.2 a	1.3 a	0.9 a	1.4 a
	GA_3 + 2,4-D		0.7 b	0.8 b	1.3 a	1.2 a	1.5 b	1.3 a
	2,4-D		0.3 a	0.3 a	1.1 a	1.2 a	1.4 b	1.0 a
C_{26}	control	4.1	2.1 a	2.2 a	2.1 a	2.1 a	2.2 ab	2.4 a
	GA_3		2.8 b	3.0 b	2.6 b	2.5 b	1.9 a	2.4 a
	GA_3 + 2,4-D		2.8 b	2.9 b	2.8 b	2.4 b	2.5 b	2.5 a
	2,4-D		2.2 a	2.2 a	2.2 a	2.2 a	2.2 ab	2.4 a
C_{28}	control	3.4	1.7 a	2.0 a	1.6 a	1.6 a	1.3 a	1.1 a
	GA_3		2.4 b	2.5 b	2.2 b	1.9 b	1.7 b	1.7 b
	GA_3 + 2,4-D		2.4 b	2.4 b	2.2 b	1.9 b	1.8 b	1.6 b
	2,4-D		1.9 a	1.9 a	1.8 a	1.6 a	1.4 a	1.2 a
C_{30}	control	5.5	4.3 a	4.0 a	3.6 a	2.8 a	2.5 a	2.5 a
	GA_3		5.0 b	5.0 b	4.2 b	3.5 b	3.0 a	3.0 b
	GA_3 + 2,4-D		4.9 b	5.1 b	4.4 b	3.5 b	2.8 a	2.9 b
	2,4-D		4.3 a	4.1 a	3.6 a	2.9 a	2.8 a	2.3 a
C_{32}	control	4.2	3.8 a	3.5 a	2.7 a	2.2 a	1.6 a	0.9 a
	GA_3		4.2 b	3.8 b	3.0 b	2.3 a	1.9 a	1.1 a
	GA_3 + 2,4-D		4.2 b	3.8 b	3.0 b	2.4 a	1.8 a	1.0 a
	2,4-D		3.8 a	3.4 a	2.8 a	2.1 a	1.7 a	1.1 a
subtotal (even)	control	18.0	12.3 a	12.0 a	11.0 a	9.8 a	8.4 a	8.1 a
	GA_3		15.0 b	14.8 b	13.2 b	11.5 b	9.4 a	9.6 a
	GA_3 + 2,4-D		15.0 b	15.0 b	13.7 b	11.4 b	10.4 a	9.3 a
	2,4-D		12.5 a	11.9 a	11.5 a	10.0 a	9.5 a	8.0 a

[a] All data are means of six replications. [b] Date of treatment application. [c] Mean separation within dates, for each compound, by Duncan's multiple range test at 5% level.

fruit from control and 2,4-D-treated trees (Table II). This difference in odd/even composition was not apparent during the two subsequent sampling dates. For most dates, 2,4-D did not differ from the control, and GA_3 alone did not differ from the GA_3 plus 2,4-D combination. This says that GA_3 has a stronger influence on the odd/even composition than 2,4-D. However, whether odd/even differences have significant horticultural or physiological implications is unknown.

The epicuticular wax from GA_3-treated trees had higher percentages of C_{26}, C_{23}, and C_{30} chains than the wax from the two non-GA_3 treatments. The influence of GA_3 on C_{25}, C_{27}, and C_{29} was time dependent. During the maturation phase, fruit from GA_3-treated trees had relatively high percentages of C_{25} and C_{27} and relatively low percentages of C_{29}. During senescence, GA_3-treated fruit had relatively low percentages of C_{25} and relatively high percentages of C_{29}. 2,4-D produced minor fluctuations in alkane composition.

Table III shows that for most individual *n*-alkane components, the quantity per unit of fruit surface area increased regardless of treatment, but the rate of increase differed among hydrocarbons and among treatments. The slowest rate of increase was observed for the even-numbered hydrocarbon chains, and C_{27}, C_{29}, and C_{31} had the highest rate of accumulation. For all components, GA_3-treated and GA_3 plus 2,4-D-treated fruits accumulated *n*-alkanes at a slower rate than did the control fruit. However, GA_3 treatment exhibited slower rates than the 2,4-D treatment for odd-numbered chains, but not for the

Table III. Effect of GA_3 (10 ppm), 2,4-D (16 ppm), and Fruit Age on the Quantity ($\mu g/cm^2$) and Rate of Accumulation ($\mu g\ cm^{-2}\ month^{-1}$) of Individual *n*-Alkanes in the Epicuticular Wax of the "Washington" Navel Orange Fruit[a]

hydrocarbon chain length	treatment	date of sampling: 1983 Oct 6[b]	1983 Nov 3	1983 Dec 1	1983 Dec 29	1984 Jan 27	1984 Feb 23	1984 Mar 22	overall rate of accumulation[d]
C_{23}	control	0.02	0.02 a[c]	0.10 bc	0.35 b	0.85 b	1.50 d	1.92 c	0.34
	GA_3		0.01 a	0.08 a	0.24 a	0.50 a	0.69 a	0.98 ab	0.17
	GA_3 + 2,4-D		0.02 a	0.09 ab	0.20 a	0.49 a	0.94 b	0.93 a	0.16
	2,4-D		0.02 a	0.11 c	0.34 b	0.78 b	1.15 c	1.18 b	0.21
C_{25}	control	0.24	0.21 a	0.44 bc	0.94 c	1.85 b	2.98 c	3.95 c	0.66
	GA_3		0.21 a	0.37 a	0.68 b	1.12 a	1.70 a	2.06 a	0.33
	GA_3 + 2,4-D		0.23 b	0.38 ab	0.54 a	1.13 a	1.81 a	1.76 a	0.27
	2,4-D		0.26 c	0.47 c	0.91 c	1.75 b	2.28 b	2.46 b	0.40
C_{27}	control	0.65	0.53 ab	0.89 b	1.46 c	2.69 b	4.41 c	5.73 c	0.91
	GA_3		0.50 a	0.72 a	1.16 b	1.87 a	2.80 a	3.31 ab	0.48
	GA_3 + 2,4-D		0.55 b	0.77 a	0.89 a	1.81 a	2.85 a	2.80 a	0.38
	2,4-D		0.67 c	0.99 b	1.47 c	2.56 b	3.31 b	3.66 b	0.54
C_{29}	control	0.70	1.01 b	1.88 b	2.23 c	3.18 d	4.70 c	6.16 c	0.98
	GA_3		0.71 a	1.10 a	1.63 b	2.57 b	3.74 b	4.35 b	0.65
	GA_3 + 2,4-D		0.77 a	1.20 a	1.27 a	2.31 a	3.20 a	3.29 a	0.45
	2,4-D		1.17 c	1.91 b	2.18 c	2.94 c	3.47 ab	3.78 ab	0.55
C_{31}	control	1.34	2.53 b	3.83 b	3.77 b	4.59 c	5.99 c	6.73 c	0.96
	GA_3		1.83 a	2.29 a	2.69 a	3.15 a	3.79 a	4.07 b	0.49
	GA_3 + 2,4-D		2.00 a	2.55 a	2.16 a	2.95 a	3.53 a	3.18 a	0.33
	2,4-D		2.92 c	3.73 b	3.64 b	4.12 b	4.46 b	4.20 b	0.51
C_{33}	control	0.22	0.48 b	0.67 b	0.67 b	0.73 c	0.75 c	0.45 b	0.04
	GA_3		0.33 a	0.38 a	0.45 a	0.43 a	0.37 a	0.34 ab	0.02
	GA_3 + 2,4-D		0.36 a	0.44 a	0.35 a	0.41 a	0.44 a	0.23 a	0.00
	2,4-D		0.55 c	0.68 b	0.64 b	0.64 b	0.62 b	0.38 ab	0.03
subtotal (odd)	control	3.17	4.78 b	7.81 b	9.42 c	13.89 c	20.33 c	24.94 c	3.89
	GA_3		3.59 a	4.94 a	6.85 b	9.64 a	13.09 a	15.11 b	2.14
	GA_3 + 2,4-D		3.93 a	5.43 a	5.41 a	9.10 a	12.77 a	12.19 a	1.59
	2,4-D		5.59 c	7.89 b	9.18 c	12.79 b	15.29 b	15.66 b	2.24
C_{24}	control	0.03	0.02 a	0.01 a	0.11 b	0.17 b	0.19 b	0.29 b	0.05
	GA_3		0.02 a	0.03 a	0.10 ab	0.13 a	0.12 a	0.25 ab	0.04
	GA_3 + 2,4-D		0.03 a	0.05 b	0.08 a	0.12 a	0.21 b	0.17 a	0.03
	2,4-D		0.02 a	0.02 a	0.11 b	0.17 b	0.23 b	0.15 a	0.02
C_{26}	control	0.16	0.12 a	0.17 a	0.22 b	0.33 b	0.49 c	0.65 c	0.09
	GA_3		0.12 a	0.18 a	0.21 b	0.26 a	0.27 a	0.40 b	0.04
	GA_3 + 2,4-D		0.13 ab	0.19 a	0.17 a	0.25 a	0.35 ab	0.34 a	0.03
	2,4-D		0.14 b	0.20 a	0.23 b	0.31 b	0.38 b	0.40 b	0.04
C_{28}	control	0.13	0.09 a	0.16 a	0.17 b	0.24 c	0.28 a	0.30 a	0.03
	GA_3		0.10 ab	0.15 a	0.17 b	0.21 ab	0.23 a	0.28 a	0.03
	GA_3 + 2,4-D		0.11 ab	0.16 a	0.14 a	0.19 a	0.24 a	0.22 a	0.02
	2,4-D		0.12 b	0.17 a	0.18 b	0.23 bc	0.24 a	0.21 a	0.01
C_{30}	control	0.21	0.23 a	0.34 ab	0.38 b	0.44 c	0.55 b	0.66 b	0.08
	GA_3		0.21 a	0.29 a	0.34 b	0.37 ab	0.44 a	0.49 a	0.05
	GA_3 + 2,4-D		0.23 a	0.32 ab	0.28 a	0.36 a	0.40 a	0.39 a	0.03
	2,4-D		0.28 b	0.37 b	0.37 b	0.41 bc	0.47 ab	0.39 a	0.03
C_{32}	control	0.16	0.20 b	0.29 b	0.28 b	0.33 b	0.36 a	0.26 b	0.02
	GA_3		0.17 a	0.22 a	0.23 a	0.25 a	0.28 a	0.19 ab	0.01
	GA_3 + 2,4-D		0.19 ab	0.24 a	0.20 a	0.24 a	0.26 a	0.13 a	0.00
	2,4-D		0.24 c	0.30 b	0.29 b	0.30 b	0.29 a	0.19 ab	0.01
subtotal (even)	control	0.69	0.66 a	0.97 a	1.16 b	1.51 b	1.87 b	2.16 c	0.27
	GA_3		0.62 a	0.87 a	1.05 b	1.22 a	1.34 a	1.61 b	0.17
	GA_3 + 2,4-D		0.69 a	0.96 a	0.87 a	1.16 a	1.46 a	1.25 a	0.11
	2,4-D		0.80 b	1.06 a	1.18 b	1.42 b	1.61 ab	1.34 ab	0.11
total (odd + even)	control	3.86	5.44 b	8.78 b	10.58 c	15.40 c	22.20 c	27.10 c	4.16
	GA_3		4.21 a	5.81 a	7.90 b	10.86 a	14.43 a	16.72 b	2.31
	GA_3 + 2,4-D		4.62 a	6.39 a	6.28 a	10.26 a	14.23 a	13.44 a	1.70
	2,4-D		6.39 c	8.95 b	10.36 c	14.21 b	16.90 b	17.00 b	2.35

[a] All data are means of six replications. [b] Date of treatment application. [c] Mean separation within dates, for each compound, by Duncan's multiple range test at 5% level. [d] Overall rates for the period of Oct 6 through Mar 22.

even-numbered ones. Moreover, GA_3 plus 2,4-D-treated surfaces had slower rates of accumulation than did the 2,4-D-treated fruits for odd-numbered *n*-alkanes but not for the even-numbered chains.

Total *n*-alkane accumulation was decreased substantially by GA_3, but not by 2,4-D, during the month following treatment. This was especially obvious for C_{29} through C_{33} odd-numbered chains. This is consistent with previously reported rapid and long lasting effects produced by GA_3, but not 2,4-D, on rind firmness, rind color, and other changes associated with maturation and senescence (Coggins, 1969).

It appears safe to conclude that epicuticular *n*-alkanes accumulate to rather high levels during maturation and senescence, that there is a shift toward a higher ratio of shorter chains during maturation and senescence, and that GA_3 moderates both changes. Based on this, and on published evidence that GA_3 delays rind senescence, we conclude that these quantitative and qualitative changes are associated with senescence and that senescence is subject to considerable moderation by plant growth regulators. We speculate that GA_3 is acting at the level of wax component synthesis and encourage studies along these lines.

Registry No. 2,4-D, 94-75-7; GA_3, 77-06-5.

LITERATURE CITED

Atkin, D. S. J.; Hamilton, R. J. *J. Nat. Prod.* **1982**, *45*, 697.
Baker, E. A. In "The Plant Cuticle"; Cutler, D. F., Alvin, K. L., Price, C. E. Eds.; Academic Press: Los Angeles, 1982; p 139.
Baker, E. A.; Bukovac, M. J; Flore, J. A. *Phytochemistry* **1979**, *18*, 781.
Baker, E. A.; Procopiou, J.; Hunt, G. M. *J. Sci. Food Agric.* **1975**, *26*, 1093.
Coggins, C. W., Jr. *Proc. Int. Citrus Symp., 1st* **1969**, *3*, 1177.
Coggins, C. W., Jr.; Eaks, I. L.; Hield, H. Z.; Jones, W. W. *J. Am. Soc. Hortic. Sci.* **1963**, *82*, 154.
Coggins, C. W., Jr.; Lewis, L. N. *J. Am. Soc. Hortic. Sci.* **1965**, *86*, 272.
El-Otmani, M.; Coggins, C. W., Jr. *J. Am. Soc. Hortic. Sci.* **1985**, *110*, 371.
El-Zeftawi, B. M. *J. Hortic. Sci.* **1980**, *55*, 211.
Freeman, B.; Albrigo, L. G.; Biggs, R. H. *J. Am. Soc. Hortic. Sci.* **1979**, *104*, 801.
Gangopadhyay, S.; Chattopadhyay, S. B. *Indian Phytopathol.* **1973**, *26*, 169.
Kuraoka, T.; Iwasaki, K; Ishii, T. *J. Am. Soc. Hortic. Sci.* **1977**, *102*, 651.
Leece, D. R. *Aust. J. Plant Physiol.* **1976**, *3*, 833.
Nagy, S.; Nordby, H. E. *Phytochemistry* **1971**, *10*, 2763.
Nagy, S.; Nordby, H. E. *Phytochemistry* **1972**, *11*, 2789.
Nagy, S.; Nordby, H. E. *Phytochemistry* **1973**, *12*, 801.
Nagy, S.; Nordby, H. E.; Lastinger, J. S. *Phytochemistry* **1975**, *14*, 2443.
Nordby, H. E.; Hearn, C. J.; Nagy, S. *Proc. Fla. State Hortic. Soc.* **1975**, *88*, 32.
Nordby, H. E.; Nagy, S. *Proc. Fla. State Hortic. Soc.* **1974**, *87*, 70.
Nordby, H. E.; Nagy, S. *Phytochemistry* **1975**, *14*, 1777.
Nordby, H. E.; Nagy, S. *J. Am. Soc. Hortic. Sci.* **1976**, *101*, 262.
Nordby, H. E.; Nagy, S. *J. Agric. Food Chem.* **1977a**, *25*, 224.
Nordby, H. E.; Nagy, S. *Phytochemistry* **1977b**, *16*, 1393.
Nordby, H. E.; Nagy, S.; Smoot, J. M. *J. Am. Soc. Hortic. Sci.* **1979a**, *104*, 3.
Nordby, H. E.; Nagy, S.; Smoot, J. M. *J. Am. Soc. Hortic. Sci.* **1979b**, *104*, 280.
O'Toole, J. C.; Cruz, R. T.; Seiber, J. N. *Physiol. Plant.* **1979**, *47*, 239.
Riehl, L. A.; Coggins, C. W., Jr.; Carman, G. E. *J. Econ. Entomol.* **1966**, *59*, 615.
Schulman, Y.; Monselise, S. P. *J. Hortic. Sci.* **1970**, *45*, 471.
Scora, R. W.; Kumamoto, J.; Clerx, W. A. *J. Agric. Food Chem.* **1982**, *30*, 692.
Trimble, J. L.; Skelly, J. M.; Tolin, S. A.; Orcutt, D. M. *Phytopathology* **1982**, *72*, 652.
Turrell, F. M. "Tables of Surfaces and Volumes of Spheres and Prolate and Oblate Spheroids and Spheroidal Coefficients", 1st ed.; University of California Press: Berkeley and Los Angeles, 1946.

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Volatile Constituents of Mountain Papaya (*Carica candamarcensis*, syn. *C. pubescens* Lenne et Koch) Fruit

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The volatiles of fresh mountain papaya (*Carica candamarcensis*, syn. *C. pubescens* Lenne et Koch) were separated from the fruit pulp by high-vacuum distillation and subsequent solvent extraction (pentane–dichloromethane, 2:1). In three fractions obtained by preseparation of the concentrated extract with adsorption chromatography on silica gel (pentane–diethyl ether gradient) the volatiles were analyzed by capillary gas chromatography and combined capillary gas chromatography–mass spectrometry. From 199 volatiles identified by these methods 103 compounds showed structures of esters, among them some uncommon substances such as, e.g., ethyl 3-mercaptopropanoate, ethyl 4-hydroxy- and 4-acetoxybutanoate, methyl (*E*)-2- and (*E*)-3-octenoate, butyl and hexyl (*E*)-2-butenoate, and butyl 2-furoate and butyl nicotinoate were found.

The mountain papaya (*Carica candamarcensis*, syn. *C. pubescens* Lenne et Koch) originates in Colombia and Ecuador where it grows at elevations about 2400–2800 m (Brücher, 1977). As the fruit ripens its skin color changes from dark green to lemon yellow. The ripe fruit has an outer layer of firm pale orange to white translucent flesh, and a cavity filled with seeds and soft pulp. Its strong aromatic flavor and attractive color are both stable to heating or prolonged storage. Mountain papayas are grown commercially as a processing crop in Chile where the fruits are mostly canned as peeled pieces in syrup. In New Zealand, the fruits are used for homemade preserves, jams, and chutneys; recently, they have been recommended for commercial production of nectars, canned fruit slices, and fruit leather (MacKenzie and Strachan, 1980). Nonvolatile fruit constituents such as sugars and acids have been already investigated (Heatherbell, 1974), but the volatile components have not been studied as yet.

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EXPERIMENTAL SECTION

Sample Preparation. Fresh ripe mountain papayas (*C. candamarcensis*, syn. *C. pubescens* Lenne et Koch) were obtained from the fruit market in Santiago, Chile (Dec, 1983; Jan, 1984), transported by air freight, and analyzed the day after arrival. After removal of the kernels, crushing by a Waring blender, and separation by a hydraulic press (Hafico) 2.6 kg of fresh fruit pulp was

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