solubility, turbidity, and titration studies. *J. Food Sci.* **1982**, *47*, 1976.
Puski, G. Modification of functional properties of soy proteins by proteolytic enzyme treatment. *Cereal Chem.* **1975**, *52*, 655.
Richardson, T. Functionality changes in proteins following action of enzymes. In *Food Proteins: Improvement through Chemical and Enzymatic Modification*; Feeney, R. E., Whitaker, J. R., Eds.; American Chemical Society: Washington, DC, 1977; p 185.
Shukla, T. P. Chemical modification of food proteins. In *Food Protein Deterioration: Mechanisms and Functionality*; Cherry, J. P., Ed.; American Chemical Society: Washington, DC, 1982; p 275.
Swift, C.; Sulzbacher, W. Comminuted meat emulsions: factors affecting meat proteins as emulsion stabilizer. *Food Technol.* **1963**, *17*, 244.
Thanh, V. H.; Shibasaki, K. Beta-conglycinin from soybean protein. Isolation and immunological and physicochemical properties of the monomeric forms. *Biochim. Biophys. Acta* **1977**, *490*, 370.
Weber, K.; Osborn, M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **1969**, *244*, 4406.
Wolf, W. J. Purification and properties of the proteins. In *Soybeans: Chemistry and Technology, Vol. 1, Proteins*; Smith, A. K., Circle, S. J., Eds.; AVI Publishing Co.: Westport, CT, 1972; p 43.
Wolf, W. J.; Tamura, T. Heat denaturation of soybean 11S protein. *Cereal Chem.* **1969**, *46*, 331.
Yamashita, M.; Arai, S.; Matsuyama, J.; Gonda, M.; Kato, H.; Fujimaki, M. Phenomenal survey on alpha-chymotrypsin plastein synthesis from peptic hydrolyzates of soy protein. *Agric. Biol. Chem.* **1970**, *34*, 1484.

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Effect of Gibberellic Acid and 2-(3,4-Dichlorophenoxy)triethylamine on Nootkatone in Grapefruit Peel Oil and Total Peel Oil Content

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The nootkatone content in grapefruit peel oil extracted from flavedo and the peel oil content of fruit receiving preharvest treatment with 20 or 50 ppm gibberellic acid (GA) and/or 50, 125, or 250 ppm 2-(3,4-dichlorophenoxy)triethylamine (DCPTA) were determined. Treatment by GA reduced the rate of increase in nootkatone concentration observed in control fruit with maturation, and the effect was dose-dependent. When DCPTA was used alone as the growth regulator, nootkatone content increased significantly. When 50 ppm GA followed DCPTA treatment at the three levels used above, the effect of GA predominated and nootkatone content was significantly lower than that found in untreated fruit. Treatment by GA generally increased peel oil concentration.

Traditionally, to export grapefruit from Florida to locations such as Japan with a quarantine against fruit flies, it has been necessary to subject fruit to fumigation or to adverse physical conditions to kill any Caribbean fruit fly eggs or larvae present. Treatment of grapefruit with the growth regulator gibberellic acid (GA) to sustain resistance to Caribbean fruit flies until late in the season might make it possible to ship GA-treated fruit early in the season (when fruit demand and price are high) without postharvest disinfection treatments. This is the period when the fruit is most susceptible to peel damage from the cold storage treatment currently used to kill any fruit fly eggs or larvae present (Ismail et al., 1986). Use of GA or other growth regulators could affect the content or composition of grapefruit peel oil, e.g., the nootkatone content, and thereby influence the infestation of the fruit by Caribbean fruit fly larvae (Greany et al., 1983).

Coggins and co-workers (1969) attempted to establish a maturity or senescence index for California navel oranges and to determine the effect of GA treatment on fruit senescence (Lewis et al., 1967). They steam-distilled the essential oil of GA-treated vs untreated fruit and analyzed volatile flavor components by gas chromatography (GC). The concentration of many of the oil components was not affected by GA treatment, but octanol, linalool, and geranial levels increased, while valencene content decreased after GA treatment. Since changes in valencene concentration were concomitant with biochemical and physiological changes associated with senescence, it was thought possible to use valencene content as an indicator of the

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maturity of oranges (Coggins et al., 1969).

Those studies were of interest to us as we are attempting to develop indicators of grapefruit senescence for studies on susceptibility of grapefruit to attack by the Caribbean fruit fly, *Anastrepha suspensa*. Susceptibility of citrus fruit to attack by fruit flies and plant pathogens increases with senescence (Greany et al., 1983, 1985, 1987; Coggins and Hield, 1962; Coggins, 1973; McDonald et al., 1987), and we would like to quantify susceptibility in relation to senescence indicators. Such indicators also would be helpful in our studies on use of bioregulators to delay senescence of citrus fruit and thereby extend the period of innate resistance of the fruit to fruit fly attack (McDonald et al., 1988). We have already found that some of the senescence-related changes that normally occur in the peel can be delayed by preharvest application of GA prior to color break (McDonald et al., 1987) but have not yet related GA treatment to the composition of grapefruit peel oil.

Some changes that occur in senescent citrus fruit include rind softening, decreases in chlorophyll, accumulation of carotenoid pigments, and changes in essential oil composition (Coggins et al., 1969; McDonald et al., 1987). Resistance of citrus fruit to fruit fly eggs and larvae is due in part to the toxicity of peel oil components and the duration of exposure of larvae to the peel oil (Greany et al., 1983; Styer and Greany, 1983; Calkins and Webb, 1988).

Because changes in peel oil composition might be responsible for, or could be useful indicators of, the effectiveness of GA treatment in retarding attack by the Caribbean fruit fly, we analyzed the volatile flavor components of grapefruit oil from fruit treated with GA and the bioregulator 2-(3,4-dichlorophenoxy)triethylamine (DCPTA). Analogues of triethylamine are known to affect the biosynthesis of terpenoid compounds in plant tissues and microorganisms (Yokoyama et al., 1986). These bioregulators have been shown to increase carotenoid accumulation in several citrus cultivars (Yokoyama et al., 1977). Lemons treated with DCPTA showed an increase in total aldehydes with major increases in geranial and neral. In addition, the terpene alcohols geraniol and nerol also increased (Yokoyama et al., 1986). Lemon oil quality is associated with total aldehydes, and citral, a mixture of geranial and neral, is considered important to the characteristic lemon aroma.

In addition to analyzing the volatile flavor components of grapefruit oil as possible senescence indicators and in relation to GA and DCPTA treatment, we also determined the effect of these bioregulators on changes in total peel oil content because of concern that total oil content might be important in determining fruit susceptibility to fruit flies.

MATERIALS AND METHODS

Sixteen Marsh grapefruit trees on rough lemon rootstock received eight treatments replicated two times each. Application of GA to grapefruit trees has been described previously (Greany et al., 1987; McDonald et al., 1987). For the 1986–1987 season, trees were treated with DCPTA, DCPTA + GA, and GA. The DCPTA, at levels of 50, 125, and 250 ppm, was applied in April 1986, right after fruit set when the green fruit were about 0.5–0.6 cm in diameter. The GA, at 50 ppm, was applied to both DCPTA-pretreated and untreated trees in Sept 1986, just before the fruit reached color break. For the 1987–1988 season, trees were treated as above with 20 and 50 ppm GA only. The bioregulators, along with 0.1% of the surfactant Triton X-100, were applied to the trees until runoff (ca. 25 L/tree).

Sample Preparation. Peel oil determinations were made on duplicate, weighed, eight-fruit composite samples for each of the four replicates for each treatment (McDonald et al., 1987). Flavedo from duplicate eight-fruit samples was removed with a peel shaver. The combined flavedo from eight fruit (about 200 g of flavedo/2000 g of fruit) was blended for 30–60 s in an explosion-proof blender with 350 mL of HPLC grade hexane. The mixture was allowed to stand for 20 min and filtered through a coarse sintered-glass funnel. The flavedo was stirred with 2 × 250 mL of hexane and refiltered, and the combined extracts were dried over sodium sulfate. The dried extract was concentrated under reduced pressure at 45 °C, transferred to a small-volume flask, and left on the rotary evaporator at 55 °C under reduced pressure until the residual oil reached a constant weight (10–20 min). Samples were weighed to determine peel oil content in the fruit and stored in screw-cap vials (ca. 10 mL) under nitrogen at 25 °C until use.

Chromatographic Methods. Oils were analyzed in duplicate on a Hewlett-Packard Model 5840 or Model 5890 GC equipped with a flame ionization detector and a glass-lined capillary inlet. Volatile flavor components were separated on a bonded-phase DB-5 fused silica capillary column, 60 m × 0.32 mm, with a 1-μm film thickness (J&W Scientific, Folsom, CA). The He flow (U) was 35 cm/s at 40 °C. The capillary inlet and flame detector temperatures were 250 and 350 °C, respectively. The capillary inlet was operated in the split mode, and the 0.2-μL sample injected was split 50/1. The oven temperature was held at 40 °C for 0.5 min, raised to 60 °C at 20 °C/min, then programmed at 4 °C/min to 225 °C, and held at 225 °C for 15 min.

Statistical Analyses. A regression line statistical program for comparing two or more regression lines (Lee and Lee, 1982) was used to determine differences in nootkatone content during the mature growing season in samples reported in Tables I–III.

Table I. Nootkatone Content[a] in Peel Oil from Grapefruit Treated with GA and DCPTA (1986–1987 Season)

harvest date	GC area, %							
	GA, ppm		GA[b] + DCPTA, ppm			DCPTA, ppm		
	0	50[c]	50[d]	125[d]	250[c]	50[c]	125[e]	250[c]
01-28-87	0.20	0.16	0.09	0.07	0.07	0.24	0.29	0.24
02-24-87	0.47	0.14	0.25	0.34	0.16	0.44	0.63	0.57
03-23-87	0.66	0.15	0.65	0.61	0.27	0.76	1.44	0.80
04-30-87	0.86	0.52	0.63	0.45	0.54	1.39	1.07	1.12

[a] Determined as GC area percent. [b] Trees treated with 50 ppm GA subsequent to application of DCPTA. [c] Seasonal trends are significantly different from control sample at a confidence level of 99.9%. [d] Seasonal trends are significantly different from control sample at a confidence level of 99%. [e] Seasonal trends are significantly different from control sample at a confidence level of 95%.

Table II. Changes in Nootkatone Content[a] in Peel Oil from Grapefruit Treated with GA (1987–1988 Season)

harvest date	GC area (%) at GA, ppm		
	0	20[b,c]	50[b]
12-10-87	0.10	0.01	0.00
01-11-88	0.24	0.06	0.06
01-20-88	0.35	0.15	0.15
02-04-88	0.52	0.17	0.16
02-18-88	0.70	0.32	0.29
03-03-88	0.88	0.35	0.37
03-17-88	0.82	0.48	0.32
04-08-88	1.10	0.92	0.74
04-21-88	1.23	0.70	0.48

[a] Determined as GC area percent. [b] Seasonal trends are significantly different from control sample at a confidence level of 99.9%. [c] Seasonal trends are significantly different from 50 ppm GA sample (1987–1988 season) at a confidence level of 95%.

RESULTS AND DISCUSSION

Grapefruit peel oil from fruit treated with GA and DCPTA was analyzed by GC for quantitative or qualitative changes in volatile flavor constituents. The only per-

Table III. Peel Oil Content in Grapefruit Treated with GA (1987–1988 Season)

	% oil in whole fruit at GA, ppm		
harvest date	0	20[a]	50[a]
12-10-87	0.158	0.158	0.164
01-11-88	0.129	0.170	0.187
01-20-88	0.130	0.149	0.172
02-04-88	0.134	0.157	0.155
02-18-88	0.121	0.150	0.149
03-03-88	0.123	0.175	0.134
03-17-88	0.112	0.164	0.162
04-08-88	0.150	0.129	0.111
04-21-88	0.117	0.140	0.177

[a] Significantly different from control at the 99.9% confidence level.

ceptible change among the volatile flavor constituents was in the nootkatone content. Nootkatone is a bicyclic sesquiterpene ketone present in grapefruit peel oil and juice; it has a typical grapefruit aroma and exhibits a low odor threshold (MacLeod and Buigues, 1964; MacLeod, 1965; Berry et al., 1967). It is an important contributor in synthetic grapefruit flavors, and its importance to grapefruit flavor has been assessed (Furia and Bellanca, 1975; Shaw and Wilson, 1981). Nootkatone is probably formed in vivo from valencene through 2-hydroxyvalencene (nootkatol), since several cell suspension cultures from citrus were able to convert valencene to nootkatone (Drawert et al., 1984).

Nootkatone variations in GA- and DCPTA-treated fruit for the 1986–1987 growing season are shown in Table I and in the GA-treated fruit for the 1987–1988 season are shown in Table II. Treatments with DCPTA were not used for the 1987–1988 growing season because fruit treated with this agent showed no difference in total peel oil content, as compared to control fruit. Early-season fruit is relatively low in nootkatone with values of 0.02–0.065% reported for oils from grapefruit harvested in November (Kesterson et al., 1971; Wilson and Shaw, 1980). During the 1986–1987 growing season no measurable amounts of nootkatone were present through December. However, for the 1987–1988 season 0.10% nootkatone was detected by mid-December.

Nootkatone values for control fruit (0 ppm, Tables I and II) increased from 0.20 to 0.86% from January to April during the 1986–1987 season and from 0.24 to 1.23% during the 1987–1988 season. Late-season oils usually contain the highest nootkatone levels (0.75–0.81%) (Wilson and Shaw, 1980). Nootkatone levels were higher in oils from control fruit for the 1987–1988 season, compared to the control fruit from the same trees for the 1986–1987 season, probably because of seasonal variation. Grapefruit treated with GA had significantly less nootkatone than control fruit (Tables I and II), and there was a significant difference (95% confidence level) in nootkatone values between 20 and 50 ppm GA treatments (Table II) when regression lines for data from Dec 10 through April 21 were compared.

Nootkatone values from fruit treated first with 50, 125, or 250 ppm DCPTA and then 50 ppm GA showed results similar to those shown for GA treated fruit, in that all samples were significantly lower in nootkatone content than were control fruit (Table I). There was no significant difference in nootkatone content among treatments involving GA and DCPTA combinations.

Changes in nootkatone levels in fruit treated only with DCPTA were different from those of fruit treated with GA or GA–DCPTA combinations (Table I). Nootkatone levels increased at a faster rate during the season with all DCPTA treatments than did control samples. There was no significant difference in the nootkatone content among fruit treated at the different levels of this compound (50, 125, 250 ppm).

Increases in nootkatone content in grapefruit oil with maturity parallel those reported in navel orange for valencene by Coggins et al. (1969). In addition, the decreased nootkatone content in grapefruit treated with GA was similar to decreases in octanol, linalool, and geranial reported for GA-treated California navel oranges by those workers. They reported increased valencene in GA-treated fruit as well. In our study, valencene did not seem to be affected by GA treatment, but the small amount present in grapefruit oil made quantitation of this component difficult. Quantitative changes in nootkatone are probably due to enzyme inhibition in the farnesyl pyrophosphate pathway for sesquiterpene biosynthesis, since growth regulators similar to DCPTA have been shown to affect this biosynthetic pathway for terpene synthesis (Sponsel, 1987).

Bioregulators such as DCPTA have a generalized effect on plants depending on their genetic constitution (Yokoyama et al., 1986). In the guayule plant, DCPTA increases activity of enzymes in the biosynthetic pathway for terpenoids. Enzymes such as isopentyl pyrophosphate synthetase and farnesyl pyrophosphate synthetase are affected (Benedict et al., 1983). The increase of nootkatone in grapefruit oil could be due to an increase in these and other enzymes.

Since polyisoprenoid hydrocarbon content in plants is sometimes affected by growth regulators (Yokoyama et al., 1984), we measured the effect of GA and DCPTA on total peel oil content in these samples. The peel oil contents for untreated grapefruit and fruit treated with GA for the 1987–1988 season are listed in Table III. Using statistical comparison of regression lines, we found significant differences between the control fruit and fruit treated with either 20 or 50 ppm GA (increased peel oil content with GA usage). However, for the 1986–1987 season, neither GA nor DCPTA nor combinations of the two caused significant changes in peel oil content in the grapefruit (data not shown).

In summary, decreased amounts of nootkatone were found in oils from grapefruit subjected to preharvest treatments with GA and with GA combined with DCPTA. In contrast, use of DCPTA alone at three different levels resulted in increased levels of nootkatone in the fruit. This latter finding could lead to improved grapefruit oil quality, since a high nootkatone level is desirable. The relationship of nootkatone content in grapefruit peel oil to susceptibility toward infestation by the Caribbean fruit fly has not been determined. However, naturally occurring oxygenated compounds, some of which are present in grapefruit peel oil, have been found to be toxic to Caribbean fruit fly larvae (Davis et al., 1976; Styer and Greany, 1983). Thus, any change in peel oil level or nootkatone content might affect survival of the larvae in grapefruit.

LITERATURE CITED

Benedict, C. R.; Riebach, P. H.; Madhavan, S.; Stipvanovic, R. V.; Keithly, J. H.; Yokoyama, H. Effect of 2-(3,4-Dichlorophenoxy)-triethylamine on the Synthesis of *cis*-Polyisoprene in Guayule Plants (*Parthenuium argentatum* Gray). *Plant Physiol.* **1983**, *72*, 879–899.

Berry, R. E.; Wagner, C. J., Jr.; Moshonas, M. G. Flavor Studies on Nootkatone in grapefruit juice. *J. Food Sci.* **1967**, *32*, 75–78.

Calkins, C. O.; Webb, J. C. Temporal and seasonal differences in movement of the Caribbean fruit fly larvae in grapefruit

and the relationship to detection by acoustics. *Fla. Entomol.* **1988**, *71*, 409–416.

Coggins, C. W., Jr. Use of Growth Regulators to Delay Maturity and Prolong Shelf Life of Citrus. *Acta Hortic.* **1973**, *34*, 469–472.

Coggins, C. W., Jr.; Hield, H. Z. Navel Orange Fruit Response to Potassium Gibberellate. *Proc. Am. Soc. Hortic. Sci.* **1962**, *81*, 227–230.

Coggins, C. W., Jr.; Scora, R. W.; Lewis, L. N.; Knapp, J. C. F. Gibberellin-Delayed Senescence and Essential Oil Changes in the Navel Orange Rind. *J. Agric. Food Chem.* **1969**, *17*, 807–809.

Davis, P. L.; Munroe, K. A.; Selhime, A. G. Laboratory bioassay of volatile naturally occurring compounds against the Caribbean fruit fly. *Proc. Fla. State Hortic. Soc.* **1976**, *89*, 174–175.

Drawert, F.; Berger, R. G.; Godelmann, R. Regioselective Biotransformation of Valencene in Cell Suspension Cultures of Citrus sp. *Plant Cell Rep.* **1984**, *3*, 37–40.

Furia, T. E.; Bellanca, N. *Fenarolis Handbook of Flavour Ingredients*, 3rd ed.; CRC Press: Cleveland, OH, 1975; Vol. 2, p 438.

Greany, P. D.; Styer, S. C.; Davis, P. L.; Shaw, P. E.; Chambers, D. L. Biochemical Resistance of Citrus to Fruit Flies. Demonstration and Elucidation of Resistance to the Caribbean Fruit Fly, *Anastrepha suspensa*. *Entomol. Exp. Appl.* **1983**, *34*, 40–50.

Greany, P. D.; Shaw, P. E.; Davis, P. L.; Hatton, T. T. Senescence-Related Susceptibility of Marsh Grapefruit to Laboratory Infestation by *Anastrepha Suspensa* (Diptera: Tephritidae). *Fla. Entomol.* **1985**, *68*, 144–150.

Greany, P. D.; McDonald, R. E.; Shaw, P. E.; Schroeder, W. J.; Howard, D. F.; Hatton, T. T.; Davis, P. L.; Rasmussen, G. K. Use of gibberellic acid to reduce grapefruit susceptibility to attack by the Caribbean fruit fly *Anastrepha suspensa* (Diptera: Tephritidae). *Trop. Sci.* **1987**, *27*, 261–270.

Ismail, M. A.; Hatton, T. T.; Dezman, D. J.; Miller, W. R. In Transit Cold Treatment of Florida Grapefruit Shipped to Japan in Refrigerated Van Containers: Problems and Recommendations. *Proc. Fla. State Hortic. Soc.* **1986**, *99*, 117–121.

Kesterson, J. W.; Hendrickson, R.; Braddock, R. J. Florida Citrus Oils. *Univ. Fla., Inst. Food Agric. Sci. Bull.* **1971**, *No. 769*, 72.

Lee, J. D.; Lee, T. D. *Statistics and Numerical Methods in BASIC for Biologists*; Van Nostrand Reinhold: New York, 1982; p 186.

Lewis, L. N.; Coggins, C. W., Jr.; Labanauskas, C. K.; Dugger, W. M., Jr. Biochemical Changes Associated with Natural and Gibberellin A3 Delayed Senescence in the Navel Orange Rind. *Plant Cell Physiol.* **1967**, *8*, 151.

MacLeod, W. D. The Constitution of Nootkatone, Nootkatene, and Valencene. *Tetrahedron Lett.* **1965**, *52*, 4779–4783.

MacLeod, W. D.; Buigues, N. Sesquiterpenes I. Nootkatone, A New Grapefruit Flavor Constituent. *J. Food Sci.* **1964**, *29*, 565–568.

McDonald, R. E. Unpublished data, 1988.

McDonald, R. E.; Shaw, P. E.; Greany, P. D.; Hatton, T. T.; Wilson, C. W. Effect of Gibberellic Acid on Certain Physical and Chemical Properties of Grapefruit. *Trop. Sci.* **1987**, *27*, 17–21.

McDonald, R. E.; Greany, P. D.; Shaw, P. E.; Schroeder, W. J.; Hatton, T. T.; Wilson, C. W. Use of gibberellic acid for Caribbean fruit fly (*Anastrepha suspensa*) control in grapefruit. In *Proceedings*, Sixth International Citriculture; Goron, R., Mendel, K., Eds.; Margraf Science Books: Weikersheim, 1988; pp 1147–1152.

Shaw, P. E.; Wilson, C. W., III. Importance of Nootkatone to the Aroma of Grapefruit Oil and the Flavor of Grapefruit Juice. *J. Agric. Food Chem.* **1981**, *29*, 677–679.

Sponsel, V. M. Gibberellin Biosynthesis and Metabolism. In *Plant Hormones and Their Role in Plant Growth and Development*; Davies, P. J., Ed.; Martinus Nijhoff: Boston, MA, 1987; pp 46–48.

Styer, S. C.; Greany, P. D. Increased Susceptibility of Laboratory-Reared vs. Wild Caribbean Fruit Fly, *Anastrepha suspensa* (Lowe) (Diptera:Tephritidae), Larvae to Toxic Citrus Allelochemicals. *Environ. Entomol.* **1983**, *12*, 1606–1608.

Yokoyama, H.; Hayman, E.; Hsu, W. J.; Poling, S.; Bauman, A. Bioinduction of Rubber in Guayule Plants. *Science* **1977**, *197*, 1076–1078.

Yokoyama, H.; Hsu, W. H.; Hayman, E.; Poling, S. Bioregulation of Plant Constituents. In *Recent Advances in Phytochemistry—Phytochemical Adaptation to Stress*; Plenum Press: New York, 1984; pp 231–250.

Yokoyama, H.; Gold, S.; DeBenedict, C.; Carter, B. Bioregulation of Essential Oils of Lemon. *Food Technol.* **1986**, *40*, 111–113.

Wilson, C. W.; Shaw, P. E. Glass Capillary Gas Chromatography for Quantitative Determination of Volatile Constituents in Cold-Pressed Grapefruit Oil. *J. Agric. Food Chem.* **1980**, *28*, 919–922.

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