Abstract. Flowers of cvs. Winter Nelis and Bartlett contained an equivalent amount of an acidic, auxin-like substance, which decreased in the following 30 days. Thereafter, until 70 days after full bloom (AFB), the substance increased in parthenocarpic ‘Bartlett’, remained relatively constant in seeded ‘Bartlett’, and declined in ‘Winter Nelis’. A neutral auxin-like promoter was detectable in flowers of the 2 cultivars, reaching a maximum between 40 and 65 days AFB. Also, flowers and fruitlets of both cultivars contained extractable gibberellin-like substances which were in greatest abundance about 25 days AFB. The concn of these GA-like promoters at this time was greater in parthenocarpic ‘Bartlett’ fruits than in seeded fruits of either cultivar. Relatively large amounts of an abscisic acid-like (ABA-like) inhibitor were present in ‘Bartlett’ and ‘Winter Nelis’ fruits for a short time AFB. The concn subsequently decreased in both cultivars, but in ‘Winter Nelis’ it increased again toward maturity. Peer extracts contained a bound inhibitor which was active after hydrolysis with β-glucosidase. Seeds of both cultivars and unfertilized ovules of parthenocarpic ‘Bartlett’ fruits had similar levels of an ABA-like inhibitor which increased concurrently with fruit growth. Inhibitors are present throughout fruit development, in contrast to promoters, which occur in sequential order.

The relation between fruit-set or development and endogenous growth regulators in several species has been studied, without being understood (4, 6, 11, 13, 14, 18, 20, 25). Little is known, however, about endogenous hormones in pears, although abscisic acid (ABA) has been reported to increase in ripening pears (21). In the present work, the levels of endogenous growth promoters and inhibitors were studied in relation to pear fruit growth. Additionally, comparisons of growth regulators on parthenocarpic and seeded pears were made, in order to examine their relationship to fruit retention and growth.

Materials and Methods

Extractable auxin-like, GA-like, and inhibitory substances of developing ‘Winter Nelis’ and ‘Bartlett’ pear fruits were studied in 1970 and 1971. Both parthenocarpic and seeded fruits of ‘Bartlett’ were included. All fruits were from the Leary and Fay-Sturtz Orchards in the Sacramento River district of California. In the Fay-Sturtz Orchard, ‘Winter Nelis’ pollinizers are interplanted with ‘Bartlett’ at an approximate rate of every fifth tree in every fifth row. ‘Bartlett’ fruits assumed to contain developing seeds were collected from trees adjacent to a ‘Winter Nelis’ pollinizer. Presumed parthenocarpic ‘Bartlett’ fruits were obtained from the Leary Orchard, which consists of a solid block of ‘Bartletts’ which in the past have produced 95-100% seedless fruit, a result of vegetative parthenocarpy (8). Late in the season of this study it was found that most of the ‘Bartlett’ fruits from the trees adjacent to the pollinizers contained seeds, and virtually all fruits from the Leary Orchard were seedless. At that time the fruit were easily separated into seeds and seedless groupings.

Extraction of growth substances. Flower samples were collected just prior to pollination. Thereafter, fruit samples were collected 7 times during the 1970 season and 6 times in 1971 at intervals varying from 1 week during the early period of fruit growth to a maximum of 3 months when ‘Winter Nelis’ fruits were approaching maturity. Fresh wt of the fruits were determined in the laboratory. In 1970, the samples of fruit tissue and seeds were extracted separately; in 1971, the intact pears were freeze-dried, and no attempt was made to remove the seeds. Though seeds contain large amounts of growth regulators, their size and wt relative to the fruit was so small that they did not affect the bioassay when left in the fruit. Samples were freeze-dried in 1971 for convenience as only IAA-like compounds appear unstable in that procedure.

Fresh (1970) and freeze-dried (1971) samples were homogenized in absolute methanol, shaken for 24 hr at 0°C and the methanol was decanted. Two subsequent extractions followed with 80% methanol for 24 hr each time. The methanolic extracts were then combined, filtered, and evaporated to the aqueous phase under reduced pressure at 30°C.

Growth substances were separated essentially as described by Badr et al. (2). Three fractions were obtained for bioassay in 1970: 1) neutral ethyl acetate, 2) acidic ether, and 3) acidic butanol. As the acidic butanol fraction in 1971 contained a gummy substance that interfered with chromatographic procedures, the fraction was evaporated to dryness, and the residue was dissolved with phosphate buffer, pH 2.8, and passed through a 3-cm charcoal:celite (1:9 v/v) column. The adsorbed material was then eluted with acetone (acidic acetone fraction). Also in 1971, the first water phase remaining after partitioning with ethyl acetate was hydrolyzed at pH 6.0 with β-glucosidase for 15 hr. After centrifugation and acidification, the hydrolysate was partitioned with ethyl acetate as before. Thus, 4 fractions were bioassayed in 1971: 1) neutral ethyl acetate, 2) acidic ether, 3) acidic acetone, and 4) hydrolysate.

Determination of auxin-like growth promoters. Portions of the neutral ethyl acetate fraction equivalent to 1 g of freeze-dried flowers or fruits, and of the acidic ether fraction equivalent to 80 mg of extracted dry material, were streaked on 3 MM Whatmann paper strips. Duplicate strips were developed by ascending chromatography to a distance of 20 cm using isopropanol:ammonia:water (IAW, 8:1:1 v/v/v). The chromatograms were dried and divided into 10 equal sections which were placed individually in 1.5 x 15-cm test tubes and bioassayed by the wheat coleoptile straight growth test (19). As controls, sections of unstreaked but developed chromatograms were bioassayed. The results were expressed as percentage growth in relation to growth of the controls.

Determination of GA-like growth promoters. Only the acidic n-butanol (1970) and acidic acetone (1971) fractions were assayed for GA content as they were the only fractions with activity in preliminary trials. Equivalents of 200 mg of extracted...
Preliminary work indicated that the inhibitory activity of the seed or entire fruit of most of the inhibitory activity was in the acidic ether fraction and in the hydrolysate; therefore, analyses were done on these fractions. Dry material equivalent to 5 mg (1970) or 10 mg of freeze-dried material (1971) from the acidic fraction was used for assay by the wheat coleoptile test following ITLC. The inhibitor content of the hydrolysate was assayed with an equivalent of 80 mg of freeze-dried fruit.

Duplicate chromatograms were developed twice to a distance of 10 cm with benzene: acetic acid: water (BAW, 8:3:5 v/v/v, upper phase). After drying, they were steamed in a closed jar to eliminate acetic acid, and sections were bioassayed by the wheat coleoptile straight growth test (19). Results were expressed as percentage growth in relation to that of unstreaked controls.

### Results

**Auxin-like growth promoters**

**A. Acidic ether fraction**

The peak of growth activity in the acidic ether fraction of fruit in 1970 was generally between Rf 0.4 and Rf 0.5, the Rf of pure IAA. In both cultivars, IAA-like activity was highest in flower samples (Fig. 1). The level in the fruitlets had decreased after 10 days, and differences between cultivars then became evident. The parthenocarpic 'Bartlett' pears increased markedly in acidic promoter 20 to 30 days AF, reaching a peak 70 days AF. At this later time, 'Winter Nelis' fruits contained the least acid promoter, and seeded 'Bartlett' fruits contained intermediate amounts. In all instances the content of the promoter decreased as fruits approached maturity. No activity was detected in the 1971 samples though previous work indicated their presence (10). Their absence in 1971 could be due to oxidation of the promoter during freeze-drying of the initial sample, however, during both years freeze-drying was used to concentrate the acidic ether fraction following solvent partitioning.

**B. Neutral ethyl acetate fraction**

The neutral promoters in fruit which were measurable in 1970 and 1971 were active in the wheat coleoptile test, and migrated to a zone on paper chromatograms between Rf 0.6 and Rf 1.0. The peak in activity occurred at Rf 0.8 to 0.9. This neutral promoter co-chromatographed with indoleacetonitrile (IAN). At the beginning of the 1971 season, the level of promoter was relatively low (Fig. 2); it started to increase 10 days ('Bartlett') and 22 days ('Winter Nelis') AF to the season's maximum at 40 and 65 days AF, respectively, then decreased. The parthenocarpic fruits of 'Bartlett' contained a slightly higher level than the seeded ones, while the seeded fruits of 'Winter Nelis' showed the highest maximum levels. The results of the 3 samples analyzed in 1970 were similar to those of a comparable date in 1971.

No growth promoter was detectable by the wheat coleoptile bioassay in the hydrolysate fraction.

**Gibberellin-like growth promoters.** The GA-like substances from the acidic acetone fraction were between Rf 0.6 and Rf 0.8 when chromatographed on ITLC and assayed with dwarf rice. Only 1971 results are reported because the 1970 samples for GA-like analysis was not sufficiently purified for bioassy. GA-like substances were detectable at bloom time (below 30% promotion), increased with some deviations until 23 to 26 days AF, then dropped sharply to nondetectable levels by 40 days AF (Fig. 3). The flowers of the 2 cultivars had similar levels of GA-like substances, but at the time of maximum concn, parthenocarpic 'Bartlett' fruits had a higher level (55% growth promotion) than did seeded 'Bartlett' fruits (32%) or 'Winter Nelis' fruits (41%). The Rf of the promoter in IAW was similar to that of gibberellins A3, A4, and A7. In BAW, the stimulatory activity was located at the origin where standards of gibberellins A1 and A3 were found.

**Inhibitors.** The inhibitory activity of the seed or entire fruit samples was found at Rf 0.1 to 0.2, and had chromatographic characteristics similar to those of ABA. Unfertilized flowers contained a high level of the inhibitor (Fig. 4). The level had increased 10 days later at fruit set; thereafter it decreased. During both years the concn of inhibitor tended to be lower in seedless than the seeded pears. Shortly before harvest, there was an increase of inhibitor in 'Winter Nelis'. The concn of inhibitor in seeds and ovules steadily increased throughout the season (Fig. 5). Although the levels were initially lower in 'Winter Nelis' seeds, no differences were found when fruits were mature. An inhibitor similar in chromatographic behavior to the
DAYS AFTER FULL BLOOM

Fig. 3. Changes in the levels of extractable GA-like substances in the acidic acetone fraction of flowers and parthenocarpic 'Bartlett', seeded 'Bartlett', and seeded 'Winter Nelis' fruits in 1971 estimated by the dwarf rice test. Each point represents activity in 1.0 g freeze-dried sample. The GA-like compound migrated to Rf 0.6-0.8 on ITLC with isopropanol:ammonia:water (8:1:1, v/v/v).

acidic one just described was the only growth substance obtained from the water fraction after hydrolysis with β-glucosidase. An amount equivalent to 80 mg of freeze-dried material was necessary to induce a level of inhibition similar to that caused by a 10-mg equivalent in the acid ether fraction. The concn of hydrolyzable inhibitor in seeded and seedless 'Bartlett' fruits paralleled those of the free form (compare Fig. 4 with Fig. 6). In the 'Winter Nelis' fruits the concn of the glucosidase-sensitive substance through the season was opposite to that of the free inhibitor with the exception of the harvest sample, in which both increased to levels as high as those found at bloom time.

Discussion

The same level of auxin-like compounds existed in 'Bartlett' flowers destined to develop parthenocarpically into fruits, as in the flowers of 'Winter Nelis' which set fruit only after pollination and fertilization (Fig. 1).

The relatively high concn of auxin-like compounds, acidic and neutral, from about "June drop" until 1 month (seedless 'Bartlett') or 2 months ('Winter Nelis') before harvest maturity may indicate their involvement in growth and retention of pear fruits (Fig. 1 and 2). Though seeds are required for apple fruit retention up to "June drop" they are not required later (1). Auxins perhaps synthesized in the fruit exclusive of the seed may play a regulatory role in fruit growth of pear for a time after "June drop." Whether the neutral growth promoter which co-chromatographed with IAN actually exerts a direct hormonal influence on the growth of pear fruits, or whether it is metabolically related to the acidic promoter, is not known. Indoleacetonitrile is said to promote wheat coleoptile elongation because the compound is converted to IAA by the enzyme nitrilase (22). The neutral promoter may be in dynamic equilibrium with the acidic promoter, and may be detected more easily because of its stability during extraction, purification, and storage.

Application of GA to pear flowers will induce fruit retention and growth (15, 17). Our results establish the presence of endogenous GA-like substances in pear flowers and fruits during the early stages of growth. In fact, the peak of gibberellin activity occurred 24 to 26 days AFB, about 10 days prior to "June drop" and at a time when cell division and rapid morphological changes take place (3). Gil et al. (7) found that gibberellin treatment at bloom will induce parthenocarpic fruit-set in 'Winter Nelis', but that additional sprays are

Fig. 4. Seasonal changes in ABA-like inhibitor level in pear flowers and parthenocarpic 'Bartlett', seeded 'Bartlett', and seeded 'Winter Nelis' fruits assayed with the wheat coleoptile straight growth test. Each point represents the inhibitor in an equivalent of 5 mg extracted dry material (1970) or 10 mg freeze-dried material (1971).

Fig. 5. Seasonal trends of the ABA-like inhibitor level in 'Bartlett' and 'Winter Nelis' pear seeds and in the unfertilized ovules of parthenocarpic 'Bartlett' pears in 1970, estimated by the wheat coleoptile straight growth test. Each point represents the inhibitor level in 40 mg of extracted material.

The fact that the level of bound, water-soluble inhibitor released by hydrolysis with β-glucosidase followed the same trend as that of the free acidic form in 'Bartlett' but not in 'Winter Nelis' pears, may mean that the metabolism of the inhibitor is different in the 2 cultivars. The identity of the bound inhibitor, however, is not known, and the hydrolyzed moiety may not be the same as the free acidic inhibitor previously described.

The level of the inhibitor extracted from pear seeds, and which had the same Rf in 2 chromatographic systems as did the inhibitor from pear fruits, increased until fruit maturity, at which time there was no difference in concn among 'Winter Nelis' seeds, 'Bartlett' seeds, and unfilled ovules from parthenocarpic 'Bartlett'. This increase in concn of inhibitor (Fig. 5) may be associated with pear seed dormancy at harvest. Waring and Saunders (24), cited evidence indicating participation of inhibitors, particularly ABA, in the dormancy phenomenon. As the inhibitor levels were high in pear fruit tissue, and the compound was mobile, the possibility exists that it is translocated from the fruit flesh to the seeds, where it accumulates.

That the hormonal requirements of plant tissues vary with their physiological age was demonstrated by Wright (26) with wheat leaves. He found that the basal meristematic sections responded to GA and kinetin, but the midsection, consisting predominantly of expanding cells, responded to GA, kinetin, and IAA. Terminal leaf sections containing the more mature tissues responded only to IAA. Similarly, pear fruit-set and development may be associated with a sequential role of different endogenous growth substances. Soon after anthesis, gibberellins are the main growth-promoting substances detectable. When gibberellin levels decrease, auxins, either acidic, neutral, or both, appear and remain conspicuous for most of the fruit growth period. During the time when promoters were detectable, the fruits developed at a fast rate. The growth-promoting hormones from full bloom until prior to harvest may overcome any effect on abscission or growth exerted by growth inhibitors. As the fruits approach maturity, the auxins (3) disappear, the inhibitor is the only growth regulator detectable, and the rate of fruit growth slows. This scheme is not much different from the one visualized by Crane (4) for fruits that have a double sigmoid growth curve.

**Literature Cited**


Variation in Essential Leaf Oil Components of Citrus Due to Stubborn Disease and Leaf Size

R. W. Scora, C. N. Roistacher, and C. K. Labanauskas²,³

University of California, Riverside

Abstract. The comparative analysis of leaf-oil components separated by vapor phase chromatography was tested on stubborn infected and non-infected sweet orange leaves as a possible new technique for detection of stubborn disease of citrus. Three leaf-oil components: citronellol, nerol, and geraniol were found reduced in the stubborn infected leaves, but the reduction was shown to be related to the smaller leaf size and not due to the stubborn pathogen. However, one component, linalool, showed an increase in stubborn or stunt infected leaves relative to respective controls. This increase was not related to leaf size, but probably due to the presence of the pathogen.

The stubborn and greening diseases of citrus caused by mycoplasma-like bodies are now considered to be the most serious and potentially destructive maladies affecting citrus throughout the world (2, 5). Any diagnostic technique that could enhance rapid detection would be materially beneficial, since current indexing techniques for stubborn disease are time consuming and apparently erratic (2, 3, 4). A specific fluorescent marker developed for the rapid detection of greening disease by Schwarz (9), has not proved applicable for the detection of stubborn disease of citrus. Three leaf-oil components: citronellol, nerol, and geraniol were found reduced in the stubborn infected leaves, but the reduction was shown to be related to the smaller leaf size and not due to the stubborn pathogen. However, one component, linalool, showed an increase in stubborn or stunt infected leaves relative to respective controls. This increase was not related to leaf size, but probably due to the presence of the pathogen.

Materials and Methods

Early in 1969, 1-year-old ‘Madam Vinous’ sweet orange (Citrus sinensis [L.] Osb.) and ‘Sexton’ tangelo (C. paradisi x C. reticulata Blanco) seedlings were inoculated by tissue grafts with a severe isolate of the stubborn disease pathogen (C-276) and grown in a greenhouse under 28°C to 36°C daytime and 22°C to 24°C nighttime temp. Leaves of these plants, exhibiting various degrees of stunting and mottling were then tested over a 2-year period against control seedlings grown under the same storage conditions. Bul. Acad. Polon. Sci. Ser. Sci. Biol. 16:509-512.

The essential oil profiles from leaf samples from all stubborn disease-infected plants tested at intervals over a 2-year period resembled those of zinc deficiency, duplicate leaf samples of the field trees were collected for macro- and micronutrient analyses (6). The leaves were analyzed for N, P, K, Ca, Mg, Na, Zn, Cu, B, and Fe.

Results and Discussion

No association was found between the nutrient concn in the leaves and the monoterpene reductions. Leaves from stubborn-infected trees which appeared to exhibit typical zinc deficiency patterns were found to be free of zinc deficiency upon analysis.

The essential oil profiles from leaf samples from all stubborn disease-infected plants tested at intervals over a 2-year period showed substantial reductions in leaf oils for 3 acyclic terpenes namely, citronellol, nerol, and geraniol, as compared with leaves from control plants. This reduction appeared proportionate to the severity of leaf symptoms, which in the case of stubborn disease is characterized by a decreased leaf size. See Calavan (3) for typical leaf stunting caused by stubborn.

However, when the small, second flush leaves of greenhouse