creased 5%. This gain compares favorably with the 46% maximum gain in field hill wt from family selection (Table 2).

The above mass-selection sequence fits field and greenhouse procedures well. By evaluating root yield before completion of seed harvest, seed harvest can be concentrated on those plants with good root yield. After the seeds are cleaned, seed samples from each potential parent can be weighed and only those weighing at least 2g/100 seed planted. Before transplant, subjective vigor evaluations can be used to cull 50% of the seedlings from each family. Since this sequence requires no greenhouse notes and a culling level of only 50%, it represents a practical method for improved yield selection. Our data suggest that seed set is conditioned by factors unrelated to root yield, but improved seed set is important in sweet potato for increased breeding ease and efficiency.

The increase in progeny yields through inclusion of maternal seed wt in the selection sequence is not due to simple associations (Table 4). Obviously yield is conditioned much more by other genetic factors than by seed size. However, seed wt may be a good index of relative physiological balance within those plants that have high yield factors. Such improved physiological balance may represent lower genetic loads and could lead to improved long term gains. This conjecture is based on the theory that given yield genes would condition higher yields on a genetic background with a low genetic load than on one with a high load. On the other hand, seed size may be conditioned more by genetic factors than by physiological balance. In that case, the inclusion of seed size in the selection index would rapidly approach a point of diminishing return.

Our study indicates the possible usefulness of seed wt as a selection criterion for root yield. On an individual plant basis, greenhouse root wt can be very misleading as an indicator of subsequent field yield. Accordingly, selection based on other characters, such as flesh color, vigor, or perhaps disease or nematode resistances, seems more promising.

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# Gibberellic and Abscisic Acid-like Substances and the Regulation of Apple Shoot Extension[1]


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*Additional index words. Malus domestica,* dwarfing rootstocks and interstocks.



*Abstract.* **Shoots on interstem-dwarfed apple (*Malus domestica* Borkh.) trees ceased growing earlier and showed a stronger tendency towards becoming summer dormant than did corresponding shoots on vigorous trees. Substances similar to gibberellic acid ($GA_3$) and abscisic acid (ABA) were extracted from 1-year-old stems at 7 stages during the growing season using the centrifugation technique. ABA-like activity was higher in stems from dwarf than vigorous trees at full bloom, and remained higher until after the onset of summer dormancy. GA-like activity also was significantly higher in dwarf tree stems at the tight flower cluster stage. Injection experiments demonstrated that increasing ABA concn decreased terminal elongation in a linear manner. ABA-induced inhibition was reduced in direct proportion to the concn of $GA_3$ in the injected solution.**


Despite numerous studies (22) the mechanism of rootstock and interstock dwarfing is still not understood. Recently differences in growth substances have been measured in attempts to explain dwarfing. Both direct (5, 14) and indirect (21, 26, 28) evidence has accumulated indicating an auxin effect in dwarfing of apple trees. However, callus cultures derived from both vigorous and dwarfing apple rootstocks achieved maximum growth at the same concn of naphthaleneacetic acid (NAA) and kinetin (13). Higher NAA levels neither stimulated nor inhibited the growth of the dwarf callus. There is less growth promoting activity and more growth inhibiting activity in the bark of a dwarf than vigorous rootstock (10). Progressively lower levels of an ABA-like inhibitor occurred in extracts of scions on malling (M) 9, M 7, M 1, and M 16 (29). Single stem trees on dwarfing, intermediate, and vigorous rootstocks all stopped elongating and set terminal buds characteristic of summer dormancy when injected with 100 mg/liter of ABA (23). Trees on the more dwarfing rootstocks responded more quickly. Injection of similar trees with 10 mg/liter of $GA_3$ stimulated shoot elongation, the stimulation increasing as rootstock vigor decreased. The dwarfing response may be due to a lower production or more rapid metabolism of GAs, since there is less GA-like activity in the root xylem exudate from a dwarfing than from more vigorous rootstocks (7). In the present study we examined further the role of ABA and $GA_3$ in the


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[5]Donated by Merck Chemical Division.

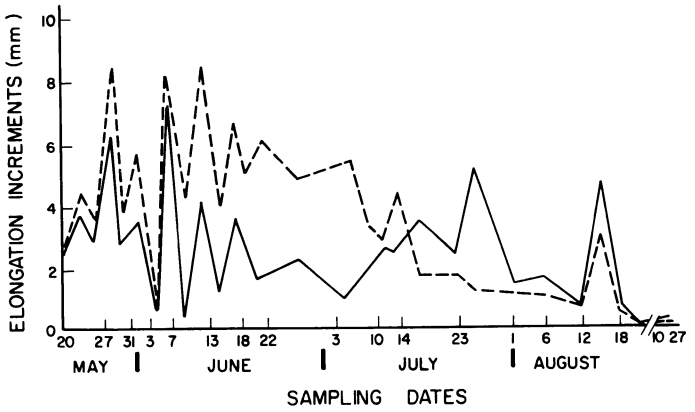


Fig. 1. Elongation increments of 40 dwarf (solid line) and 40 vigorous terminal shoots randomly selected on 4 trees of each type and measured every 2 to 4 days during the growing season.

dwarfing phenomena of apple trees.

## Materials and Methods

*Growth pattern studies.* Ten uniform branches on 4 trees each of 'Jonathan'/20 cm M 8 interstock/Alnarp-2 rootstock (dwarfing) and 'Jonathan'/seedling rootstock (vigorous) were selected and tagged before bud break. Length and girth of 80 terminal shoots originating from these tagged branches were measured 1 week following full bloom and every 2–3 days thereafter for the remainder of the growing season. Shoot diam was measured 3 cm above the previous season's growth.

*Injection experiments.* The technique has been previously described (23). Solutions were injected using "Plastipac" disposable 20 ml syringes (Becton and Dickinson, Rutherford, NJ) with a section of tygon tubing between the syringe and the needle (Yale No. 18 GL). The tapered needle was inserted at a higher point in the trunk with each injection. The effect of ABA on the growth of apple trees was studied using MM 111 (vigorous) non-grafted rootstocks. Single stem, 1-yr-old trees were growing vigorously in the greenhouse in 20 cm pots at the start of treatments. Forty-five uniform trees, blocked according to terminal shoot length, were given 5 injections each of 100, 80, 60, 40, 20, 10, 1 and 0 mg/liter of ABA[3] (50% cis, trans-50% trans, trans) or 10 mg/liter of succinic acid-2,2-dimethyl hydrazide (SADH)[4] at 4 day intervals. The mg of ABA taken up by each tree was determined. Shoot length was determined initially, and then measured 13× during the experiment. Regression analysis was used to examine the relationship between elongation and mg of ABA taken up.

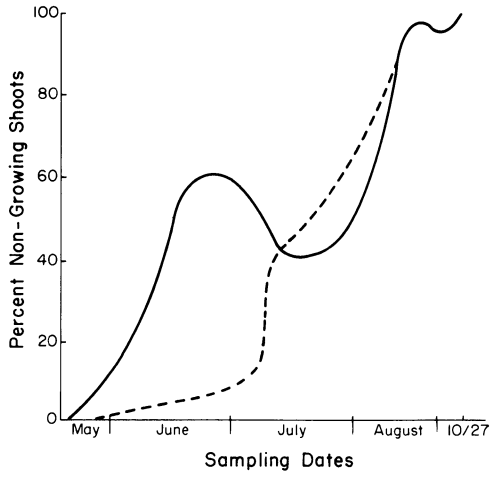


Fig. 2. Variation in percentage of 40 dwarf (solid line) and 40 vigorous shoots that were summer dormant during the growing season.

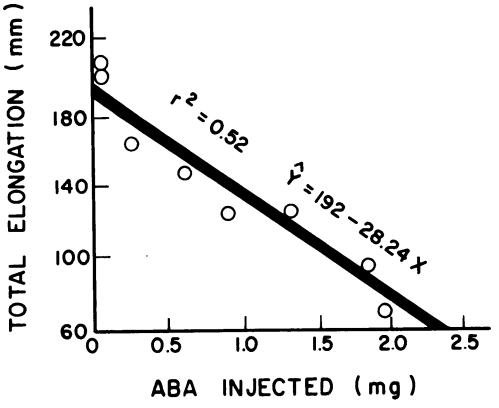


Fig. 3. Response in terminal shoot elongation of 1-yr-old apple trees to injections of increasing amounts of ABA. ABA injected (mg) equals the total of 5 injections. Elongation was measured 25 days after the initial injection.

The effect of $GA_3$ on ABA induced inhibition of elongation was studied using 1-yr-old 'Red Queen Delicious'/MM 111 (vigorous) trees. Trees, growing vigorously in 7.5 liter containers at the start of treatments, were given 5 injections each of 15, 10, 5, 1 and 0 mg/liter of $GA_3$[5] each plus and minus 50 mg/liter of ABA. Shoot length was determined initially, and again 1 week following the last injection. Treatments were replicated 4× in a randomized block design.

*Engogenous growth substances.* The centrifugal method for extracting growth substances (4) was adopted, because preliminary experiments with extracts emphasized the importance of contamination in masking differences between vigorous and dwarf stems. We collected 60 g of previous season's terminal growth of uniform diam from 5-yr-old 'Red Prince Delicious'/30 cm M 8/Alnarp-2 (dwarf) and 'Red Prince Delicious'/seedling (vigorous) trees at the following developmental stages: silver-tip, tight flower cluster, full bloom, rapid shoot growth, summer dormancy, and onset of winter dormancy. Stem samples were collected between 8 and 9 AM on clear days and brought immediately to the laboratory. Stems were stored intact and refrigerated until centrifuged, but all centrifugation

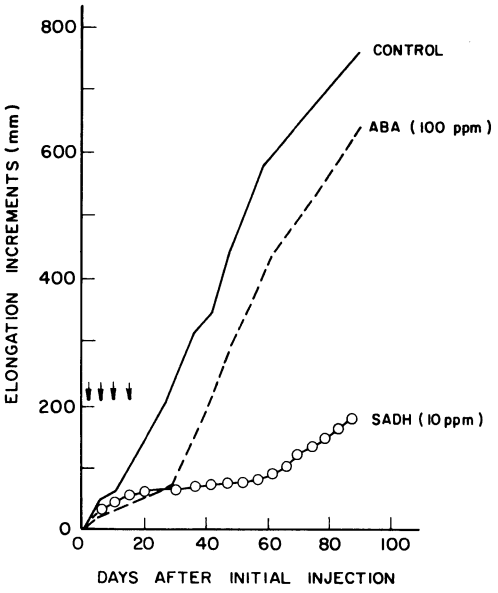


Fig. 4. Elongation increments for 1-yr-old apple trees injected with 4 mg/liter of ethanol (controls), 100 mg/liter of ABA, and 10 mg/liter of SADH. Arrows indicate dates when fresh injections were made. Note that at 25 days elongation increments equal those shown in Fig. 3.

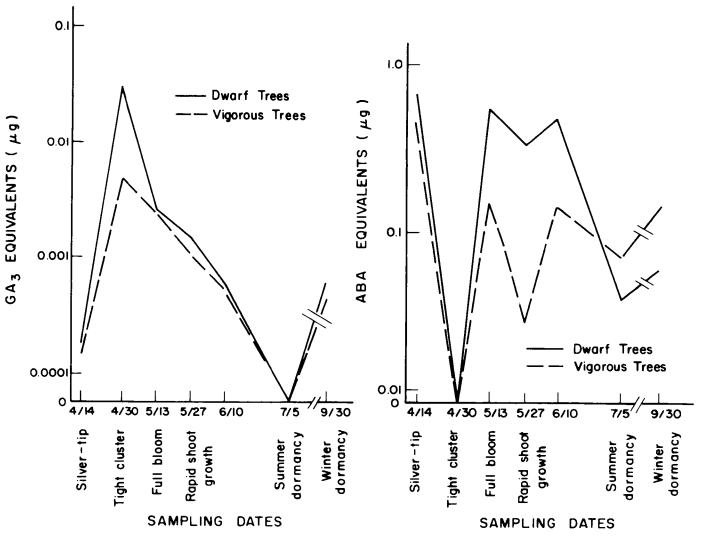


Fig. 5. Left, the stage of shoot development and level of a GA3-like substance in centrifugates from 1.5 g of dwarf and vigorous stems on 7 sampling dates during the growing season. The difference between the 2 stem types at the tight cluster stage of development was significant at the 0.05 level. Right, the stage of shoot development and level of an ABA-like inhibitor in centrifugates from 3 g of dwarf and vigorous stems on 7 sampling dates.

was completed within 2 hr after sample collection. Five cm stem segments were fully immersed apical-end up in 20% methanol in centrifuge tubes, and spun for 45 min at 1,500 × g. The methanol bathing solution was immediately evaporated to dryness *in vacuo* and resuspended in 0.33 M phosphate buffer at pH 8. This buffered phase was washed 2×, with both petroleum ether and ethyl acetate, adjusted to pH 2.5 with concd HCl, and partitioned 4× with ethyl acetate. The acidic ethyl acetate phase was evaporated to dryness *in vacuo* and the residue was redissolved in 2 ml of 100% methanol and stored in paraffin sealed vials at 5°C. All solvents used for extraction and chromatography were glass distilled.

GAs were purified by TLC on Eastman Chromatogram Sheets (no. 6060, silica gel) in isopropyl ether:acetic acid (95:5 v/v). Plates were divided into 11 equal sections including a control section below the origin, eluted with methanol, dried, and bioassayed for GA activity using the dwarf pea epicotyl bioassay (17) modified by placing 2 ml of distilled water in each vial and by measuring after 4 days at 22°C. All assay runs were replicated 3× and included GA3 standards.

Because 80% of the TLC plate strongly inhibited wheat coleoptile growth even after polyvinylpyrrolidone treatment, silica gel column chromatography was utilized for purification of inhibitors (19). Samples were dried on glass wool, introduced onto the column, and eluted with an increasing gradient of ethyl acetate in hexane. Five ml fractions were collected and halved to allow for duplicate bioassays of each column. A modified wheat coleoptile straight-growth assay was adopted from different sources and used to test for inhibitor activity. 'York Star' wheat seeds were germinated in the dark for 72 hr. Coleoptiles 2.5 cm long and of uniform diam were selected, and 4 mm sections were cut 3 mm below the apex. Five subapical sections were incubated in 0.3 ml of phosphate-citrate buffer plus 2% sucrose in tubes containing the dried column eluates (16). All operations were conducted under green light. Tubes were rotated at 1 rpm in the dark (6) for 22 hr before the sections were measured. All assays included ABA standards.

GLC was on a Packard 7300 series chromatograph with a 1.8 m glass column packed with 2% QF-1 Chromasorb W at 180 C, using flame ionization detection. Samples were prepared for chromatography by methylation with diazomethane (24).

Table 1. Elongation of 1-yr-old 'Red Queen Delicious' apple trees in response to injections of 50 mg/liter of ABA in the presence of increasing concn of GA3.

| Treatment | | Total elongation (% of control) |
|---|---|---|
| ABA (mg/liter) | GA3 (mg/liter) | |
| 0 | 0 | 100 |
| 0 | 15 | 115 |
| 50 | 0 | 41 |
| 50 | 1 | 43 |
| 50 | 5 | 57 |
| 50 | 10 | 70 |
| 50 | 15 | 96 |

Table 2. A comparison of the Rfs of GA3, GA4+7, and the centrifugal sap GA-like substance in 3 solvent systems.

| GA | Solvent systems[z] | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Centrifugal sap | 0.07-0.13 | 0.33-0.40 | 0.07-0.13 |
| GA3 | 0.08 | 0.37 | 0.14 |
| GA4+7 | 0.26 | 0.73 | 0.42 |

[z]Solvent systems: 1, di-isopropyl ether/acetic acid (95:5); 2, benzene/n-butanol/acetic acid (75:20:5); 3, chloroform/ethyl acetate/acetic acid (60:40:5).

## Results

*Growth pattern studies.* Shoots on dwarfed trees elongated at a slightly slower rate than did shoots on vigorous trees (Fig. 1) and a larger percentage of them stopped growing early in the season (Fig. 2). Over 60% of the dwarf shoots were summer dormant by mid-June vs. less than 10% of the vigorous shoots. One third of these dormant dwarf shoots resumed growth again later in the season. This early reduction in the elongation of dwarf tree shoots has previously been noted (27). The dwarf trees as expected had more fruit per unit length of branch, a factor which may have influenced shoot growth.

*Injection experiments.* The injection of less than 0.5 mg of ABA per tree decreased shoot elongation, and injections of increasing amounts reduced elongation in a linear manner (Fig. 3). Trees injected with the highest ABA concn showed adverse reaction in the form of chlorotic, twisted and brittle leaves, but leaves formed after the resumption of elongation were normal. Inhibition of elongation occurred rapidly after injection; likewise trees recovered quickly and again grew normally demonstrating the reversibility of ABA inhibition (Fig. 4). By comparison, the synthetic plant growth regulator SADH (Fig. 4) induced prolonged inhibition when injected at 10-fold lower concn than the natural hormone ABA.

Although ABA has been reported to induce terminal bud formation in apple (20, 23), in this experiment elongation was either depressed, or in the case of the largest quantity injected (2.0 mg), temporarily suspended without formation of buds. When injected alone, GA3 did not significantly affect shoot elongation even at 15 mg/liter. However, GA3 effectively overcame ABA-induced inhibition of shoot elongation, suggesting that these 2 growth substances may interact *in vivo* to control apple shoot elongation (Table 1).

*Endogenous growth substances.* A substance present in centrifugates was tentatively identified as GA3-like on the basis of mobility in 3 solvent systems (Table 2). Activity was first detected at the tight cluster stage, and decreased gradually over the next 2 months (Fig. 5 left). At this stage of development there was significantly higher activity in the dwarf than in the vigorous stems, while at all other stages activity was similar in both stem types. After having dropped to undetectable levels in early July, activity was once again present in samples taken

in late September.

A zone of strong inhibitory activity, eluted from columns in the same fraction as standard ABA, was found in centrifugate samples. Further confirmation that the inhibitor in this zone was ABA was obtained by gas chromatography of 2 μg of standard ABA together with 2 μg of the centrifugate inhibitor. One peak only, equivalent to 4 μg of ABA, was obtained at the retention time for ABA.

ABA activity was high at silver-tip, dropped to an undetectable level at tight cluster, and then rose again to a high level at full bloom (Fig. 5 right). Activity remained high during the period of rapid shoot elongation, and was higher in dwarf than in vigorous shoots on the 3 sampling dates spanning that period. In early July the activity in dwarf stems fell to a level lower than in vigorous stems, while activity in the latter remained fairly constant.

## Discussion

Centrifugation in solution was found to concentrate rooting substances in the base of cuttings, and to cause their diffusion out into the surrounding medium (8). Dry centrifugation was ineffective. Four hr of centrifugation at 2,750× g extracted the same amount of radioactive auxin, which had been applied to the tops of cuttings, as did a 24 hr non-centrifuged soak (11). In both cases 40% ethanol extracted almost 3× the radioactivity as did distilled water. Other workers showed that centrifugates and methanol extracts obtained from identical tissues were qualitatively similar, but that centrifugates had higher auxin and lower inhibitor activity (4). Purification procedures are simplified and levels of interferring substances are lowered, but intracellular and tightly bound growth substances, which are more likely to be important in gorwth processes, may be left behind in centrifugal extraction.

The higher inhibitor levels in dwarf stems during the period of shoot elongation is consistant with the earlier cessation of growth exhibited by dwarf shoots. The rapid growth that occurred in May when inhibitor activity was equally high may have been due to the higher GA3-like activity at that time; an activity, however, that was steadily decreasing. A dominating promoter may have to be removed before the ABA influence becomes apparent (26). We showed GA3 to be very effective in overcoming ABA induced inhibition of elongation. Re-growth of summer dormant dwarf shoots corresponded to a period of decreased inhibitor activity.

While our centrifugal extraction data corroborates field observations and measurements, it does not completely agree with the masceration extraction data of others which show high ABA levels in mid-August which then declined until bud swell the following spring (3, 18, 26). The renewed presence of an inhibitor at bud break that had previously decreased to very low levels after mid-winter, however, was also reported (3). Results obtained with older stems could differ from those obtained with new shoots or buds. ABA distribution has been shown to be nonuniform in apple shoots (26).

The tight cluster stage of development appears ideal for shoot growth initiation, since there is no detectable inhibitor activity combined with high GA3-like activity in the dwarf stems. GA3 can initiate the release of sugars from starch in twigs (9) and can regulate starch and sugar levels in tobacco leaves (15). Although the more precocious dwarf trees do have more reserve carbohydrates available for fruit bud formation (1), the high level of GA3-like activity occurred long before the time of fruit bud initiation. Another GA-like substance tentatively identified as GA4+7-like on the basis of migration in several solvent systems was present in centrifugates, but was not detected early enough to monitor because of the low sensitivity of dwarf pea epicotyls to GA4+7. The picture on growth substance levels in relation to dwarfing is therefore very incomplete.

The vascular tissue of dwarfing apple clones is more parenchymatous with a higher ratio of bark to wood than of vigorous rootstocks (1, 2, 12). This characteristic, which is transmitted to the scion (2, 12), could be responsible for a greater synthesis or metabolism of growth substances in dwarf tree stems.

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# Characterization of Major Plastid Pigments in Skin of 'Mission' Fig Fruits[1]

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*Additional index words. Ficus carica,* chlorophyll, carotene, lutein, violaxanthin, neoxanthin

*Abstract.* **Plastid pigments in the fruit skin of fig (*Ficus carica* L. cv. Mission) were extracted in acetone:0.1 N $NH_4OH$ (9:1 v/v) and purified by thin layer chromatography. Spectral analysis indicated the pigments were chlorophylls a and b, β-carotene, lutein, violaxanthin, and neoxanthin. These constituents were similar to those found in the leaves. Carotenoids present in the skin of bluish-black mature fruits were the same as those present in green, immature fruits.**

Pigments commonly found in leaf chloroplasts of most higher plants are chlorophylls a and b, β-carotene, lutein, neoxanthin, and violaxanthin (2, 8, 9, 10). The fig fruit is stem-like in nature, i.e., derived, in part, from peduncular tissue, hence it was of interest to determine whether the plastids in the fruit skin contained any unusual or unknown pigments. A 'Mission' fig fruit ripens over a period of 2 to 3 weeks, during which time the skin turns from green to bluish-black. Pigments involved were identified, as described in this report, as part of an investigation into the nature of changes in the chloroplast pigments during ripening.

## Materials and Methods

*Plant material.* Fruit, leaf and bark samples from 'Mission' fig trees growing in the Wolfskill Experimental Orchards, Winters, CA, were used in this study. Fruits of different degrees of ripeness were harvested and analyzed while fresh, or after storage in the dark at −20°C. Any pulp clinging to the skins was removed.

*Pigment extraction.* Extraction and chromatographic separation were done under reduced light in a cold room at 1°C to reduce pigment degradation. Various fig organs were ground in a porcelain mortar with quartz sand and a 4 × fresh wt volume of chilled acetone:0.1 N $NH_4OH$ (9:1 v/v) for qualitative studies. The pigment-containing extract was decanted, and the residue re-extracted twice with 3 × fresh wt volume of acetone: 0.1 N $NH_4OH$ (9:1 v/v). Following the basic acetone extraction, the residue was extracted with a 5 × fresh wt volume of anhydrous diethyl ether and finally with a 5 × fresh wt volume of a 1:1 (v/v) mixture of acetone and 0.1 N $NH_4OH$ (9:1 v/v) and anhydrous diethyl ether. A sufficient volume of diethyl ether was added to the combined extracts, when no additional pigment was extracted, to yield a final 1:1 (v/v) proportion of diethyl ether and basic acetone.

*Liquid-liquid chromatography.* Pigment extract was centrifuged at 10,000 × g for 10 min. The supernatant was then decanted and washed in a separatory funnel with chilled $H_2O$ saturated with $MgCO_3$. Water-soluble substances and acetone were retained in the $MgCO_3$ solution, while the ether fraction containing the plastid pigments collected as a supernatant in the funnel. A brief centrifugation was given to remove any remaining $H_2O$, then the pigment-containing supernatant fluid was concentrated in the dark under a nitrogen stream.

*Thin layer chromatography (TLC).* The concentrated pigment extract was streaked 1.5 cm from the base of 5 × 20 cm silica gel plates. Plates were developed immediately in benzene: ethyl acetate:absolute ethanol 80:20:5 (v/v/v). Chlorophyll pigments and derivatives were resolved by TLC on 20 × 20 cm cellulosepulver plates. Plates were developed in ligroine (63–75°C):acetone:n-propanol 90:10:0.45 (v/v/v). All TLC plates were developed in glass tanks for a distance of 18 cm.

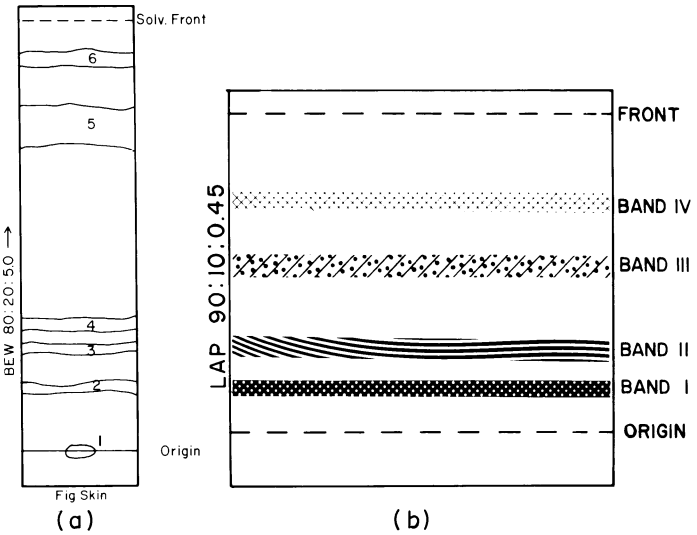


Fig. 1 (a) Chromatographic distribution of plastid pigments in fig skin. The numerals denote the following pigments: 1, chlorophyllide-like; 2, neoxanthin; 3, violaxanthin; 4, lutein; 5, chlorophyll; 6, β-carotene. (b) Rechromatography of the chlorophyll-containing band 5 following its elution from silica gel H plates. The numerals denote the following pigments: I, unknown; II, chlorophyll b; III, unknown; IV, chlorophyll a. BEW = benzene:ethyl acetate:absolute ethanol LAP = ligroine:acetone:n-propanol.

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