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Observations on Leaf Characteristics of Afghanistan Pine[1]

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Abstract. **Observations on leaf morphology and on the fine-structure and quantity of epicuticular wax of a newly introduced (Plant Introduction (PI) 303638) Afghanistan pine (*Pinus brutia* Ten.) indicated that needles were borne 2 or 3 per fascicle with 1.7- to 4-fold more 3- than 2-needled fascicles depending on the flush of growth. Needles were curved and twisted, margins serrulate, and apices acute. An amorphous epicuticular wax, about 180±48 μg cm^{-2}, covered the needles. Crystalline platelet or fiber-like wax occurred in irregular patches, frequently around stomata. Stomata on both abaxial and adaxial surfaces were deeply depressed and in rows parallel to the long axis of the needle. In many stomata the antechamber was partially occluded with fiber-like wax. Thin-layer chromatograms indicated that the more polar constituents, namely fatty acids, *sec*-alcohols and esters were most prevalent, while only traces of alkanes, ketones and aldehydes were present.**

Seeds of an unidentified species of pine collected in the Herat Region of Afghanistan were introduced into the United States in 1960 (7). Seedlings of this accession (PI 271431) were widely distributed to cooperators for evaluation in 1961. The young tree grows rapidly and is tolerant to drought, high temperature and frost (7, 8, 16). Its attractive form and rapid growth in semi-arid regions make it potentially useful for Christmas trees and for shelterbelts in the arid southwestern United States (7, 8).

There is some question whether this pine belongs to *P. brutia* Ten. (W.B. Critchfield, E. L. Little, F. G. Meyer, personal communications) or to the closely related Aleppo pine, *P. halepensis* Mill. (7). Classification is complicated because 3 collections were made. The original one was labelled PI 271431. Subsequent collections also made near Herat were identified as PI 303638 and 362153. The 3 collections may or may not have been from the same source. Classification is further complicated by opinions as to whether *P. brutia* is an established species. Turrell (20) and others (1, 3, 6) list *P. brutia* as *P. halepensis* var. *brutia* (Tenore) Elwes and Henry, while Mirov (17) considers it an independent species. For this report, we use the name *P. brutia* as suggested by Meyer (personal communication).

Here we report on the morphological characteristics of the leaves and on the quantity, fine-structure and chemistry of the epicuticular wax. These data should be useful in further taxonomic and drought tolerance studies of this plant.

Materials and Methods

General. All leaf samples were collected from 5-year-old seedlings of PI 303638 at the University Farm at Las Cruces, New Mexico. Total moisture per annum averaged 50-60 cm, 20 to 30 cm from natural precipitation and 30 cm from 3 to 4 irrigations.

Gross morphology. Branches from current season's growth, consisting of 3 flushes, were removed from each of 5 representative trees on December 3, 1976. Fasicicles were removed in sequence from each flush of growth and the number of needles and length of the longest needle in each fascicle was determined. Scale needles at the base of each flush of growth frequently persisted but were not studied.

Length of needles in 100 2- and 3-needle fascicles collected at random from current season's growth and width of 20 randomly selected needles were measured. The width of the adaxial, or flat (4), surface of semi-circular needles and the length of a line representing a chord formed with the arc of the curved surface (abaxial), as well as the two flat (adaxial) surfaces of triangular needles were measured with a binocular dissecting scope using an eyepiece reticle.

Surface morphology and leaf structure. Leaves from 2- and 3-needle fascicles were selected from the median portion of the third flush of growth, frozen immediately on dry ice and then lyophilized and stored in a desiccator. Segments about 5

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mm in length were excised from the basal, median and apical portions of the needles and mounted on aluminum stubs with a suspension of carbon. Mounted specimens were first coated lightly with carbon and then with about 100 Å of gold-palladium (Au, 60%; Pd, 40%). Hand-cut transverse sections (about 2 mm) were mounted on end and prepared as described above. Specimens were examined with a scanning electron microscope (Mini-Scan, International Scientific Instruments) at 5 kV. Scanning electron micrographs were taken with Polaroid type 55 or 105 film.

Epicuticular wax. One hundred fascicles were collected from current season's growth of each of 5 representative trees. The epicuticular wax was removed from the needles by 4 successive 10 sec dips in redistilled chloroform. The 4 extracts were pooled, dried over anhydrous sodium sulfate and filtered. The solvent was removed with a rotary evaporator and the wax dried to constant weight at 40°C. Surface area of the extracted needles was estimated by viewing the fascicle as a cylindrical rod and adding appropriate values for the flat surfaces. The major constituents were separated by thin-layer chromatography (TLC) on precoated silica gel G plates (250 μm) using benzene or petroleum ether:diethyl ether:acetic acid (50:50:2 by volume) as the developing solvents. Constituents were localized by spraying with 40% H_2SO_4 (by volume) and charring at 180°C (9).

Results

Gross morphology. Seedlings of *P. brutia* grew vigorously, usually producing 3 flushes of growth annually in the Las Cruces area. About one-half of the total current season's shoot elongation and fascicles were produced in the first flush with progressively less in the second and third flushes (Table 1).

Each fascicle bore 2 or 3 acicular leaves with marked variation in frequency, but with no apparent pattern within a flush of growth. There were 1.7- to 4-fold more 3-needle than 2-needle bearing fascicles. The frequency of 2-needle fascicles was lowest (20%) on the second flush and similar (37 vs. 31%) on the first and third flushes (Table 1).

The slender, bright to dark green needles tended to curve and twist. The apex had a horn-like point (acute) and the margins were serrulate. The persistent basal sheath was about 5 mm in length (Fig. 1A). Needle length averaged 11 to 14 cm, being somewhat shorter in the second and third flushes (Table 1). There were no significant differences in the length of needles within fascicles, mean difference being 0.4 mm. Mean (followed by SD) width of the semi-circular needles was 1.15±0.08 mm, 1 of the 2 in each fascicle being slightly narrower. Mean dimensions of the triangular needles were 1.24±0.15, 0.73±0.08 and 0.76±0.09 mm for the curved and the 2 flat surfaces, respectively.

Surface morphology and internal structure. The surface of the needle portion beneath the basal sheath (Fig. 1A) had little epicuticular wax fine-structure and few, if any, stomata (Fig.

Table 1. Characterization of fascicles and needles produced on the various flushes of growth on current season's shoots of *P. brutia* seedlings.[z]

Flush of growth[y]	Fascicles No.	Fascicles 2-needle (%)	Fascicles 3-needle (%)	Needles No.	Needles Length (mm)
First	76 ± 23	37 ± 30	63 ± 30	198 ± 57	14.0 ± 1.1
Second	48 ± 18	20 ± 11	80 ± 11	133 ± 62	12.9 ± 1.9
Third	33 ± 5	31 ± 22	69 ± 26	89 ± 19	10.9 ± 1.1

[z]Mean of 5 replications ± SD.

[y]Data collected December 3, 1976.

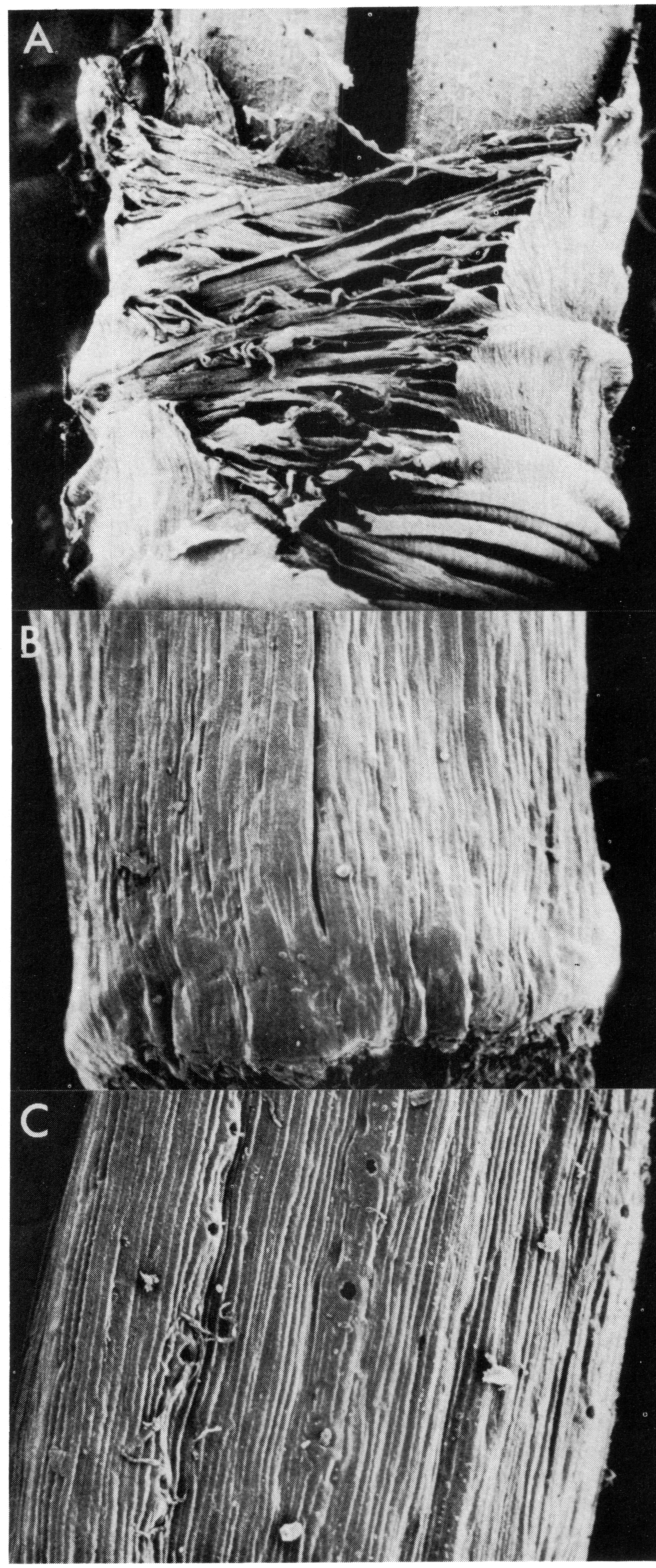

Fig. 1. Scanning electron micrographs illustrating the surface morphology of the basal sheath (A) and of the leaf beneath the basal (B) and apical (C) portions of the basal sheath of *P. brutia* needles. Magnifications are 25×, 45×, and 75× for A, B, and C, respectively.

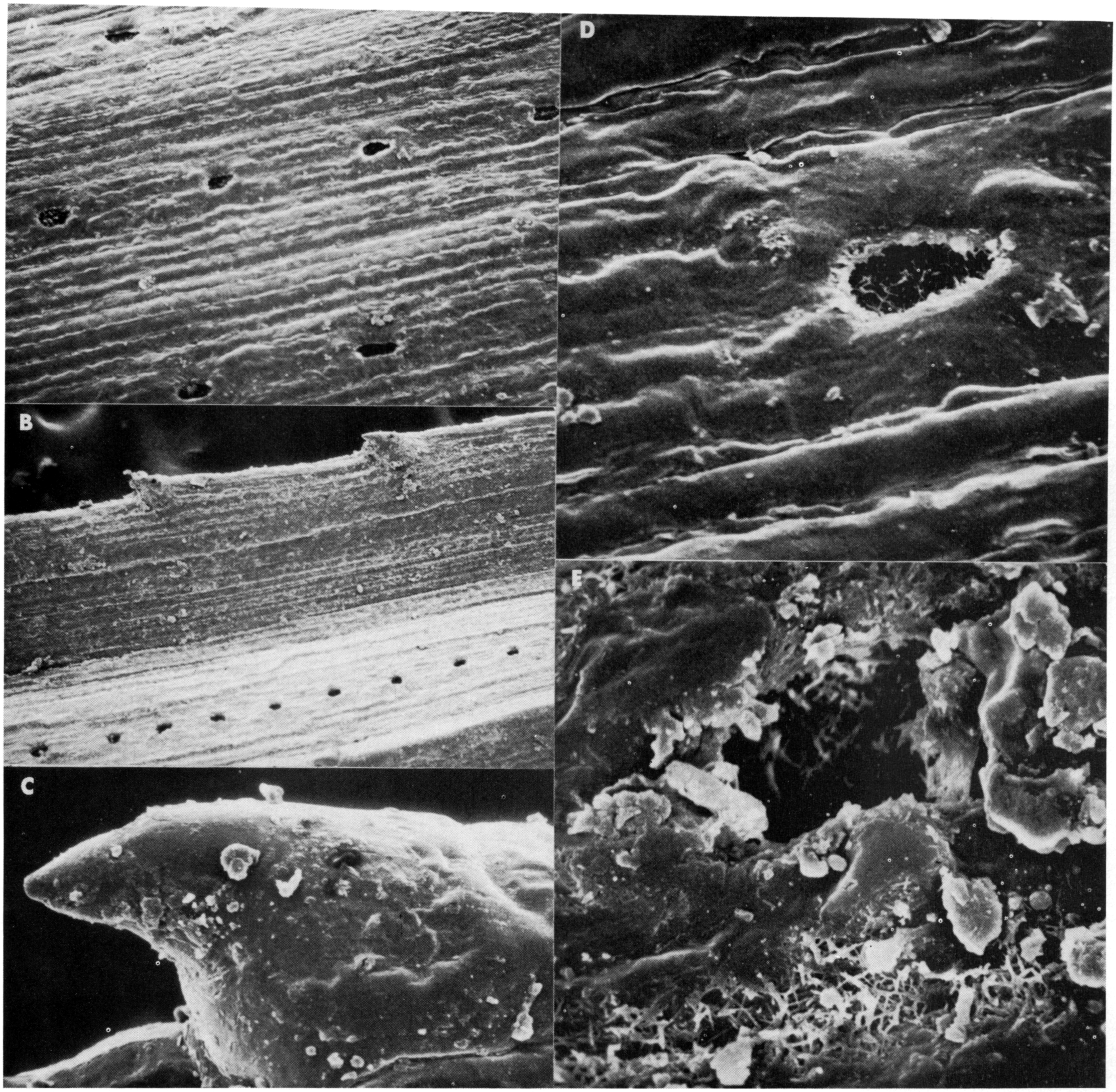

Fig. 2. Scanning electron micrographs illustrating the fine-structure of the surfaces of *P. brutia* needles. A) Abaxial surface of a semi-circular leaf, 207×. B) Adaxial surface of a triangular leaf, 103×. C) Tooth-like projection found on the leaf margins, 1255×. D) Stoma on the abaxial surface with wax in the antechamber, 1255×. E) Platelets and fibrous-like epicuticular wax on the adaxial surface near a stoma, 2509×. All sections taken from the median portion of the needles.

1B). From base to apex, stomata first became evident at the apical region of the basal sheath (Fig. 1A, C). Many stomata were plugged with an unidentified material (possibly wax), and fibrous strands were associated with the leaf surface (Fig. 1C). The frequency of stomata decreased toward the tip of the needle (data not shown).

The abaxial (Fig. 2A) and adaxial (Fig. 2B) surfaces of the median region of the leaves were characterized by parallel ridges and rows of stomata. Teeth-like projections, pointing to the base of the leaf, were present on the leaf margins (Fig. 2B). These projections, like the surfaces of the leaves, were covered with an amorphous epicuticular wax with isolated areas of wax platelets (Fig. 2C). Strands of wax were present in the antechamber of many stomata (Fig. 2D), and dense platelets and fiber-like strands of wax were prominent around the stomatal openings (Fig. 2E).

Transverse sections of semi-circular and triangular needles (Fig. 3A, B, respectively) showed that 2 vascular bundles were embedded in transfusion tissue and encircled by a clearly defined endodermis (Fig. 3A, B). Two resin ducts were present

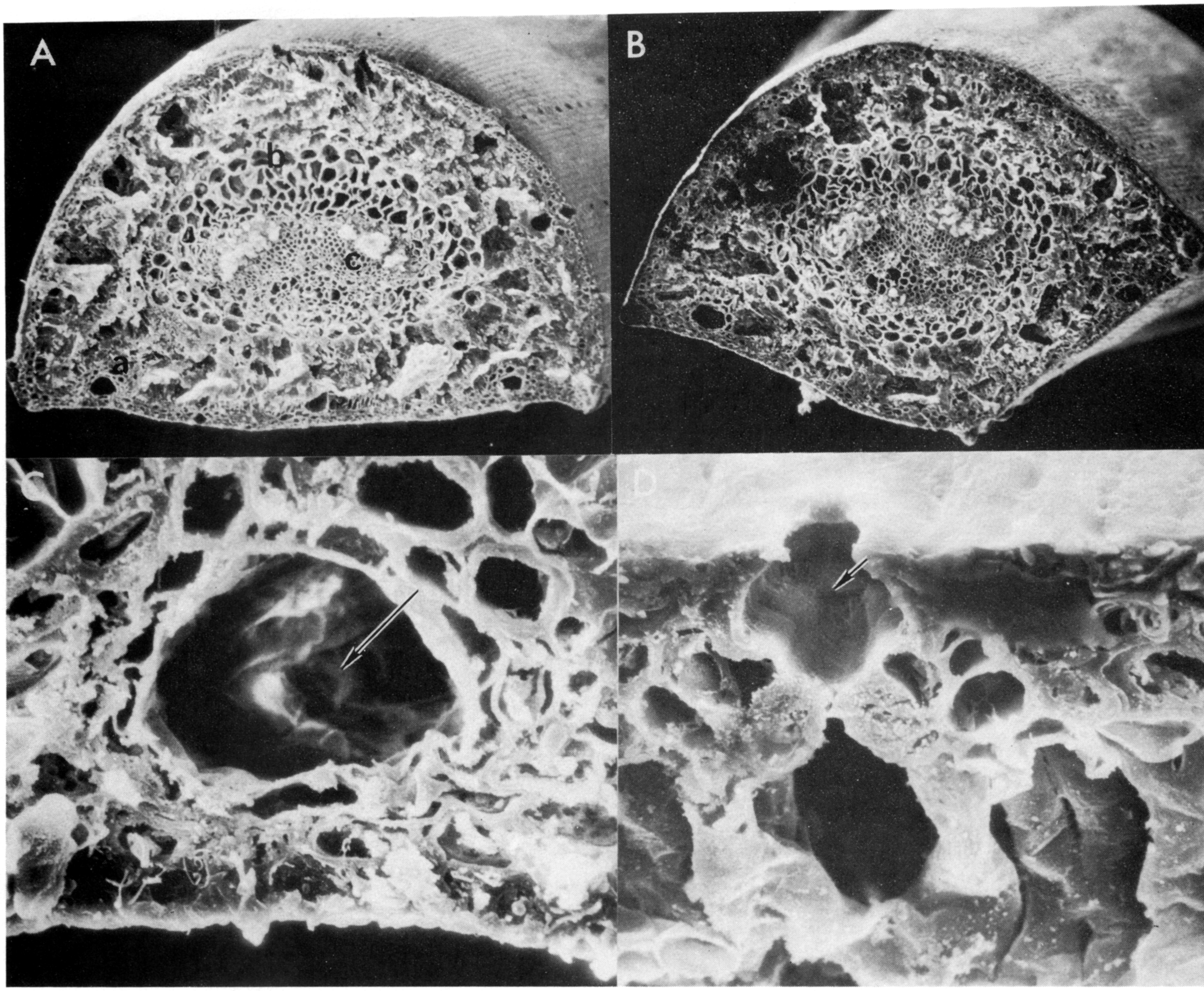

Fig. 3. Scanning electron micrographs of hand-cut transverse sections of *P. brutia* leaves. A) Semi-circular leaf (100×), Note resin duct (a), endodermis (b), and vascular bundle (c). B) Triangular leaf (90×), C) Resin duct (arrow) (1150×). D) Sunken stoma; note antechamber (arrow) (815×).

near the adaxial surface (Fig. 3A, C). Stomata were deeply sunken into the epidermis with a clearly delineated antechamber above the stomatal pore (Fig. 3D).

Epicuticular wax. Surfaces of the needles were covered with about 180±48 μg cm^{-2} of chloroform-soluble epicuticular wax, which was rich in polar constituents, primarily fatty acids, *sec*-alcohols and esters (Fig. 4). The alkane content was low and no appreciable amounts of ketones or aldehydes were noted even when 150 μg of wax was chromatographed. The fatty acids remained at the origin when benzene was used as the developing solvent (Fig. 4). When chromatographed with petroleum ether: diethyl ether:acetic acid (50:50:2 by volume), the fatty acids moved from the origin but were not adequately resolved into individual components (data not presented).

Discussion

Leaf growth pattern and surface morphology of PI 303638 are variable. Two or 3 acicular leaves were borne per fascicle and the ratio of one to the other differed depending on the flush of growth examined (Table 1). We found 1.7- to 4-fold more 3- than 2- needled fascicles. This observation is significant from a taxonomic point of view, since there is disagreement as to the classification of this accession (7, 8). Recently, Fisher (7) reported that a specimen from PI 271431 was classified as *P. halapensis*. However, other authorities (1, 3) have reported that leaves of *P. halapensis* are borne in pairs and seldom in threes. This suggests that our specimen is not *P. halapensis*.

The fine-structure and distribution of the epicuticular wax also varied markedly from needle to needle. Needles are covered with a layer of amorphous wax overlaid with crystalline platelet or fiber-like wax. The crystalline wax is frequently localized in irregular patches, as is true for *P. radiata* (14) and *P. sylvestris* (11). Although fiber-like crystalline wax was observed in stomatal antechambers, few stomata were completely occluded as in *P. nigra* and *P. sylvestris* (11), and the frequency of partial occlusion was relatively low. We did not attempt to establish whether the occlusion was wax; however, Reicosky and Hanover (18) showed that this material could be removed from antechambers with chloroform in *Picea pugens*.

The quantity of epicuticular wax was relatively high (180 μg cm^{-2}) in comparison with many plants (15). This, along with the deeply sunken and partially occluded stomata, may

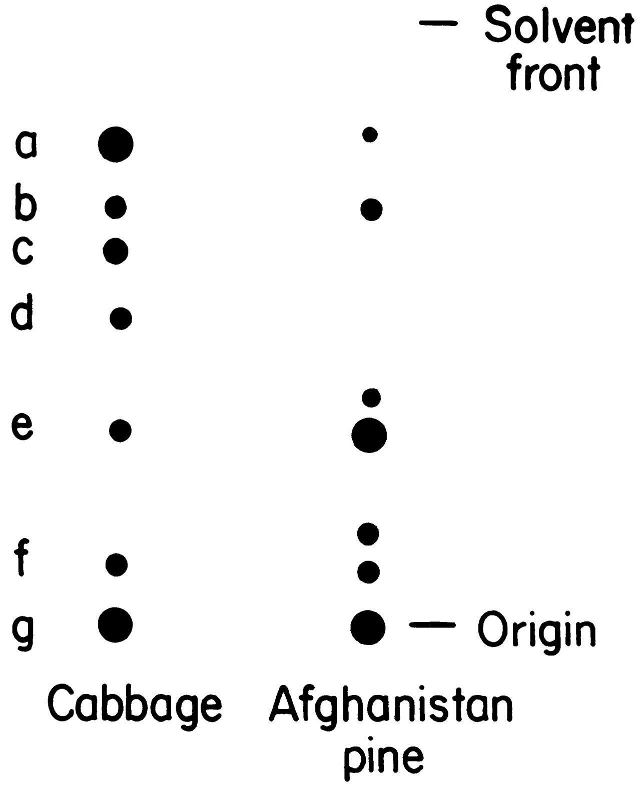

Fig. 4. Graphic representation of thin-layer chromatograms illustrating the separation of epicuticular waxes (50 μg) from cabbage (left) and Afghanistan pine (right) leaves. Developing solvent was benzene. Major constituent classes: a, alkanes; b, esters; c, ketones; d, aldehydes; e, *sec*-alcohols; f, *p*-alcohols; g, fatty acids (9).

contribute to the drought tolerance of this species (5, 12, 19). However, the epicuticular wax is rich in polar constituents and relatively poor in alkanes, and thus would be expected to be more permeable to water than one rich in nonpolar constituents (2, 10). Nevertheless, the chemistry of this wax, if it is generally representative of pines, coupled with the limited fine-structure present, may explain the low contact angles and penetration of water into pine needles (13).

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