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9. ABSTRACT

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SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY ANALYSIS OF CHALK SECRETING LEAF GLANDS OF PLUMBAGO CAPENSIS[1]

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ABSTRACT

Chalk secreting leaf glands of *Plumbago capensis* Thunb. were examined by polarized reflected light microscopy, scanning electron microscopy, x-ray diffraction, and energy dispersive x-ray analysis. There are about 23 glands per mm² on the lower leaf surface and none on the upper surface. Each gland measures 20–30 μm in diam and consists of four small secretory cells surrounded by four subsidiary cells. Secretion of chalk is apparently through a pore in the surface of each secretory cell. The secreted material was crystalline and x-ray diffraction confirmed the presence of two minerals: calcite ($CaCO_3$) and nesquehonite ($MgCO_3 \cdot 3H_2O$). Energy dispersive x-ray analysis revealed the presence of magnesium, silicon, phosphorus, sulfur, chlorine, potassium, and calcium in the epidermal cells. However, only calcium and magnesium and traces of silicon were detected in the secreted material. A distribution analysis showed calcium and magnesium to be uniformly distributed through the secreted material.

THE FIRST DESCRIPTION of chalk secretion by epidermal glands was by Mettenius (1856) in the flowering plant family Plumbaginaceae. He described variation in the chalk secretion by these glands. Some species secreted large amounts and others little. Later workers described the structure of these glands and noted differences in amount of secretion dependent upon environmental as well as specific differences (Licopoli, 1879, in Wilson, 1890; de Bary, 1884; Volkens, 1884, in Wilson, 1890; Maury, 1886; Vuillemin, 1887; Wilson, 1890; de Fraine, 1916). Glands which secreted chalk, termed chalk, Licopoli, or Mettenian Glands, consisted of eight cells, while those without secreted chalk, termed mucilaginous glands, consisted of up to 20 cells. Glands displaying all stages of gradation in structure and chalk secretion between the two types of glands were described. However, all glands secreted mucilage (Wilson, 1890).

Schtscherback (1910), Ruhland (1915), and Faber (1923) reported inorganic salt (sodium chloride) secretion by these glands. Thus, the term salt gland was used by these and subsequent investigators (see review in Luttge, 1971). Apparently, most of the later workers thought that all of these secretory glands were functional salt glands and no descriptions of chalk glands occur in literature since the early 1900's. However, in the family Tamaricaceae, *Tamarix aphylla* is reported to have glands which secrete potassium, calcium, and magnesium, in addition to sodium chloride (Waisel, 1961; Berry and Thomson, 1967). The root environment greatly influenced the composition of the secretions and in some situations the divalent cations magnesium and calcium accounted for most of the cations present (Berry, 1970).

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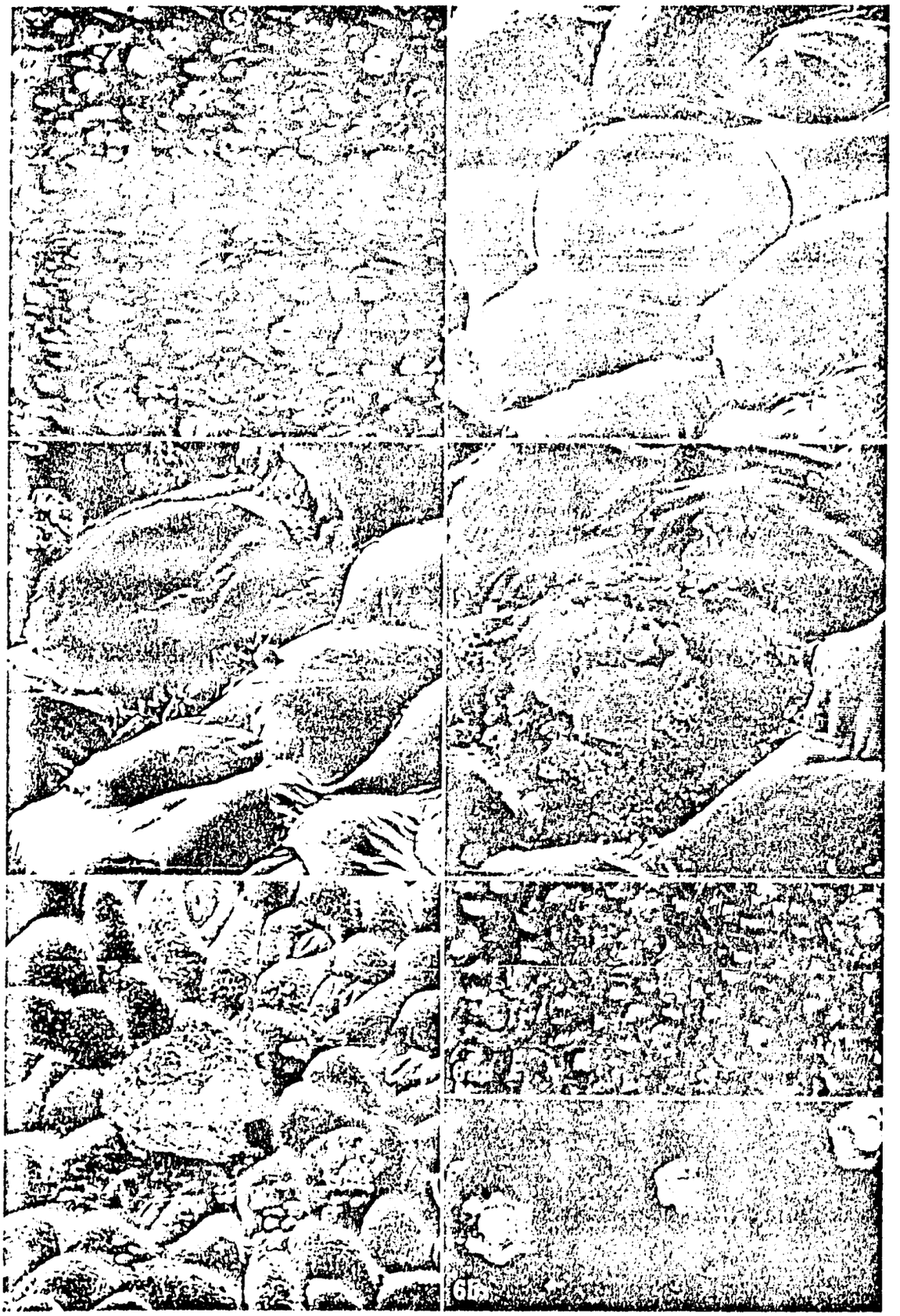
Fig. 1–6. Light and scanning electron micrographs of chalk-secreting leaf glands of *Plumbago capensis*. **1.** Reflected light micrograph of lower leaf surface of young leaf. Glands (g) are without chalk secretion. × 175. **2.** SEM of young leaf gland with no secretion. Note the pore in each of the four inner secretory cells. × 2000. **3.** SEM of leaf gland with some secretion. Note the rice grain-like appearance of some of the secretion. × 2000. **4.** SEM of leaf gland with secretion apparently exuding directly from the pores. × 2000. **5.** Large secretion covering gland and some adjacent cells. Note the rough appearance of the cuticle on the epidermal cells of this mature leaf. ×500. **6.** Reflected light (a) and polarized, with crossed polars, reflected light (b) micrographs of lower leaf surface of mature leaf. The secretions of two of the three glands appear to refract more light in areas over the glands and at the edges of the secretions. × 175.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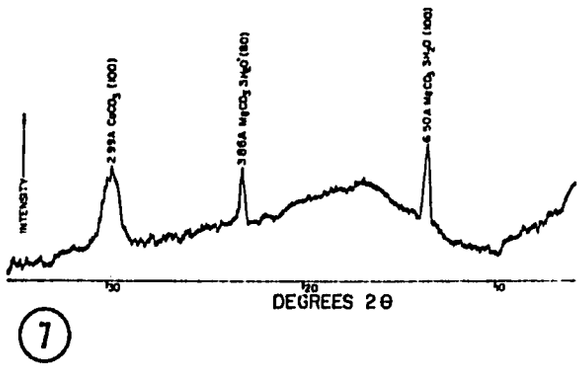

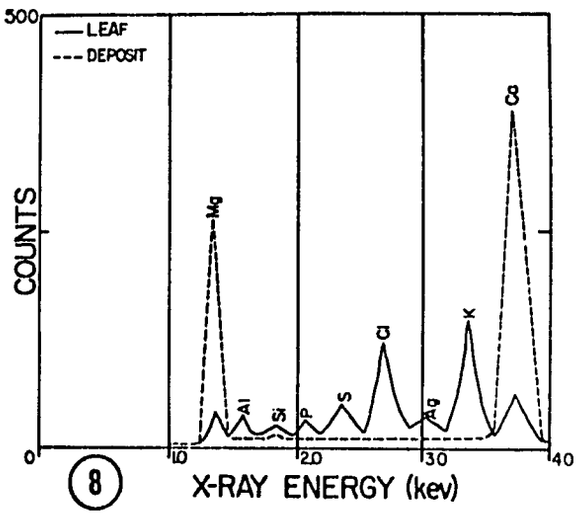

Fig. 7, 8. 7. X-ray diffractogram of leaf gland secretions of *Plumbago capensis*. The 100 and 80 intensity peaks for nesquehonite occur at 6.50 A and 3.86 A, respectively. The 100 intensity peak for magnesium-substituted calcite is at 2.99 A. 8. Energy dispersive x-ray spectrograph of leaf gland secretions of *Plumbago capensis*. The solid line shows the number of counts for elements (K_α) in epidermal cells shown in Fig. 9. The dotted line shows the number of counts for elements in the deposit shown in Fig. 9. Values are qualitative and are not corrected for background, fluorescence, or adsorption. Aluminum and silver are from specimen stub and silver conducting adhesive.

According to Wilson (1890), two genera with very active chalk glands were *Plumbago* and *Limoniastrum*. Neither of these genera have since been studied. This paper describes studies of *Plumbago capensis* which illustrate secretions of crystalline calcium carbonate (calcite) and hydrated magnesium carbonate (nesquehonite) by leaf glands.

MATERIALS AND METHODS—Leaf samples of *Plumbago capensis* Thunb. were collected from plants grown as ornamentals on the University of Hawaii Manoa campus. The soil, of the Makiki series, a fine, mixed, isohyperthermic, Andic Ustic Humitropept of the Low-Humic Latosols was supplemented with a yearly application of balanced fertilizer. For light microscopy, fresh material was viewed and photographed with a Zeiss Petrographic microscope equipped with a Photomicrographic Camera I. For scanning electron microscopy, fresh material was viewed and photographed with a Cambridge Steroscan S-4 operated at 5 or 10 kv, using Polaroid 55 P/N film. For energy dispersive x-ray analysis the same scanning electron microscope, operated at 10 kv, was equipped with a Nuclear Diodes EDAX 707 energy dispersive x-ray analyzer. Material was examined fresh or freeze dried by immersion in freon-22 in a liquid nitrogen bath followed by vacuum sublimation in a Virtis 10-010 Automatic Freeze-Dryer. The freeze-dried material was carbon coated by vacuum evaporation in a Denton DV-502 Vacuum Evaporator. For x-ray diffraction analysis, secreted material was collected from leaves, air dried, and analyzed with a Norelco X-ray diffractometer equipped with a fine-focusing, high intensity copper x-ray tube, focusing graphite monochrometer, scintillation counter, pulse height analyzer, and curved, autofocusing specimen holder.

OBSERVATIONS—*Light and scanning electron microscopy*—The leaf glands of *Plumbago capensis* occur abundantly on the undersides of leaves (Fig. 1). The average number of glands is 23 per mm^2 (50 counts). Each gland in surface view usually consists of four small secretory cells surrounded by four subsidiary cells (Fig. 2). Glands with three secretory and three subsidiary cells also occur, although infrequently. The glands are level with the surface of the leaf and measure 20–30 μm in diam. They are probably equivalent to the chalk, Mettenian, or Licopoli glands described by early workers (Mettenius, 1856; Licopoli, 1879, in Wilson, 1890; de Bary, 1884; Volkens, 1884, in

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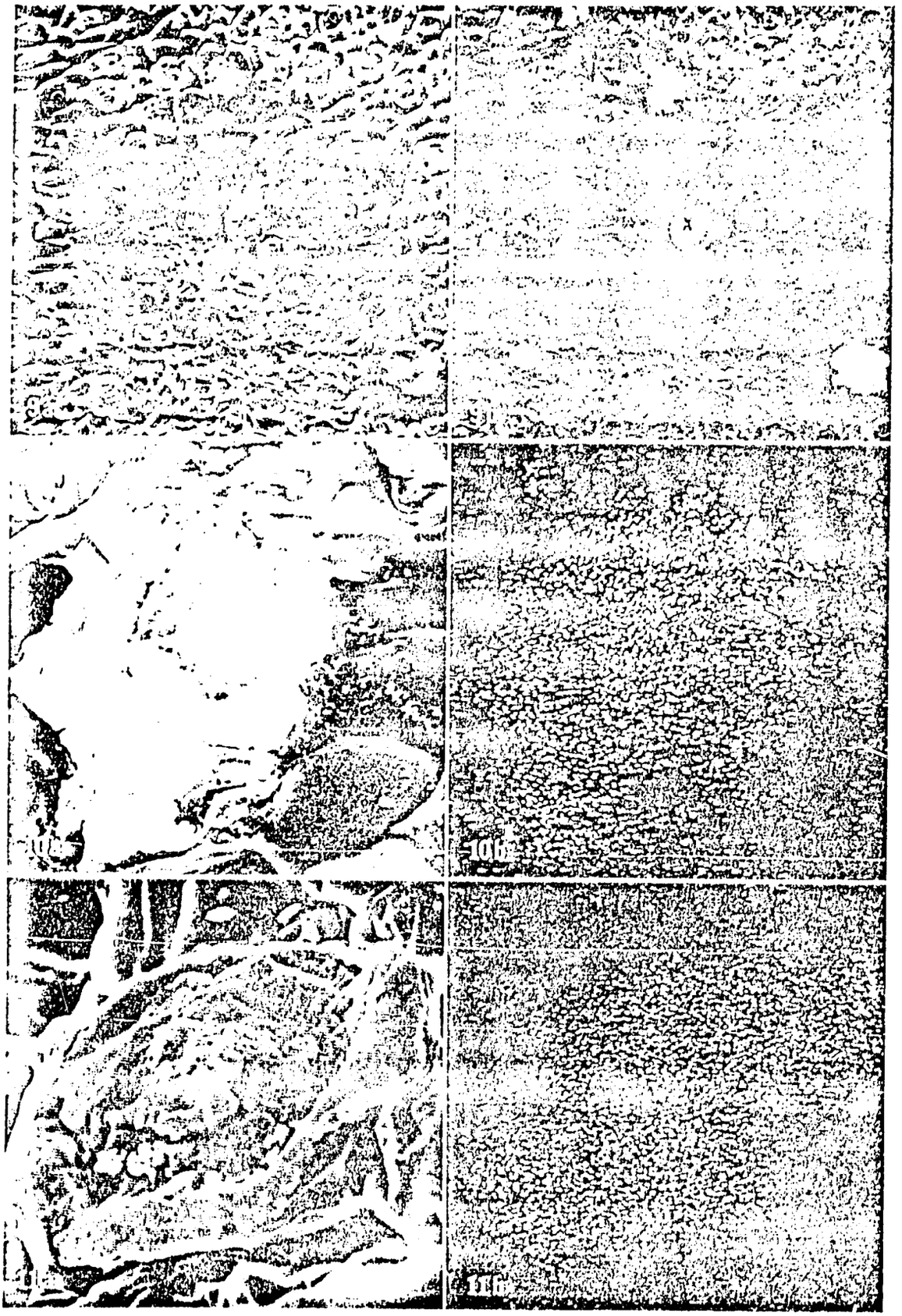
Fig. 9–11. SEM and energy dispersive x-ray elemental distribution pattern of leaf glands and secretions of *Plumbago capensis*. 9. Normal secondary electron image (a) and cathode luminescence mode image (b) of the lower surface of mature, fresh leaf. The secretions apparently absorb electrons and emit light in the visible spectrum; therefore, they appear white in the cathode luminescence mode (b). Area and deposit scanned for elements shown in Fig. 8 are indicated by the brackets and x, respectively. × 200. 10. Secondary electron image (a) and distribution pattern (b) of calcium (K_{α_1}, 3.64–3.74 keV) for mature chalk deposit from freeze dried, carbon coated leaf material. × 1000. 11. Secondary electron image (a) and distribution pattern (b) of magnesium (K_α, 1.20–1.30 keV) for immature chalk deposit from fresh uncoated leaf material. × 2000.



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Each of the secretory cells has a depression on its surface (Fig. 2). These apparently correspond to the pores described in salt glands (Arisz et al., 1955) as the site of secretion. In *Statice* the method of discharge is by simple exudation without rupture of the cell membrane (Wilson, 1890). In *P. capensis* initial deposits which are probably exuded from the pores occur near the periphery of the gland and subsequently solidify on the surface of the subsidiary cells (Fig. 3). The initial secretions have a rice grain-like appearance, measuring about 2.5 by 1.0 μm. Secreted material may also occur over adjacent epidermal cells (Fig. 3), although, it is not likely that these cells secrete any material resting on them (in agreement with Wilson, 1890).

Subsequent secretions remain primarily over the pores of the secretory cells. The build-up of secreted material is apparently rapid and few glands were observed with only moderate secretion, as in Fig. 4. The bulk of the secretion accumulates directly over the pores, with some spreading to the subsidiary cells. Secretion of material continues, and the entire gland and adjacent epidermal cells are covered by the chalk deposit (Fig. 5, 9). These large deposits are easily visible with the unaided eye. With reflected light microscopy the deposits appear dense over the glands (Fig. 6a). In polarized reflected light with crossed polars the deposits appear to refract more light directly over the glands and in areas near the edges of the deposits (Fig. 6b), indicating that these areas are more crystalline.

X-ray diffraction—Because of the apparent crystalline nature of the deposits, samples were analyzed by x-ray diffraction (Fig. 7). Values of 2θ and corresponding d-spacings confirmed the presence of two crystalline minerals: calcium carbonate ($CaCO_3$, calcite) and magnesium carbonate ($MgCO_3 \cdot 3H_2O$, nesquehonite) (Chave, 1952; ASTM File 1-0130, 1960). The d-spacing of 2.99 A for the 100 intensity peak of calcite, instead of the normal 3.04 A d-spacing (ASTM File 5-0586, 1960) is caused by a large amount of Mg substitution for Ca in the crystal lattice of calcite. According to Chave (1952), approximately 15% by weight of the calcite must be $MgCO_3$ for a d-spacing of 2.99 A. He suggested that a solid solution existed between calcite, $CaCO_3$, and dolomite, $CaMg(CO_3)_2$. However, no evidence of dolomite (2.89 A, 100 intensity peak) could be found in the *Plumbago* deposits; instead nesquehonite ($MgCO_3 \cdot 3H_2O$) was found (Fig. 7). This is the first report of the biological secretion of nesquehonite. Its discovery in association with Mg-substituted calcite suggests that there is only a partial solid solution between calcite and dolomite.

Scanning electron microscopy and energy-dispersive x-ray analysis—Analysis of epidermal cells and deposits shown in Fig. 9a with energy dispersive x-ray analysis revealed the presence of significant amounts of magnesium, silicon, phosphorus, sulfur, chlorine, potassium, and calcium (Fig. 8). Hydrogen, carbon, oxygen, and nitrogen cannot be routinely detected by energy-dispersive x-ray spectroscopy because only elements of the atomic number of sodium or greater release sufficient x-rays (Elad et al., unpublished paper; Frankel and Aitken, 1970). Analysis of the single deposit in Fig. 9b (arrow) revealed the presence of large amounts of calcium and magnesium and only a trace of silicon (Fig. 8).

Since calcium and magnesium were present in the deposit, a distribution analysis was performed on these elements. Calcium occurred uniformly throughout the deposit (Fig. 10a, b). The distribution of magnesium was similar (Fig. 11a, b). There appeared to be no variation in the distribution of these elements in fresh young (Fig. 11) or freeze-dried old (Fig. 10) deposits. Careful matching of the distribution pattern for calcium and magnesium with the secondary electron scanning electron micrographs showed the presence of these elements in the rice grain-like secretions.

DISCUSSION—Recently it was suggested that chalk glands do not exist and that all described chalk glands were functional salt glands. Helder (1956) felt that calcium bicarbonate in the salt gland solution was responsible for the early descriptions of chalk glands and Berry and Thomson (1967) thought that calcium and magnesium present in secretions of salt glands gave some support to the early descriptions of chalk glands. Apparently, most later workers ignored the report by Marloth (1887, in Arnott and Pautard, 1970) that deposits on leaves of *Tamarix* were 51% calcium carbonate. However, Berry (1970) reported that Mg and Ca accounted for most of the cations and that HCO_3 comprised 80% of the anions present in salts secreted by glands of *Tamarix aphylla*.

No observations were made of *Plumbago capensis* growing in its native habitat (South Africa). However, it is obvious that under the conditions in which the plant was observed in this study, the leaf glands function primarily in chalk secretion. In all probability chalk glands and salt glands are not separate secretory structures. Wilson (1890) has described a gradation in structure in the family Plumbaginaceae from typical eight-celled chalk glands to typical 12- to 20-celled mucilaginous

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