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Development and Essential Oil Content of Secretory Glands of Sage (*Salvia officinalis*)[1]

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ABSTRACT

Scanning electron microscopy of sage (*Salvia officinalis* L.) leaves confirmed the presence of two basic types of glandular trichomes consisting of a capitate stalked form containing a multicellular stalk and surmounted by a unicellular secretory head, and a capitate sessile form containing a unicellular stalk and unicellular, or multicellular, secretory head. In the latter type, secretory activity and filling of the subcuticular cavity may begin at virtually any stage of the division cycle affording fully developed glands containing from one to twelve cells in the secretory head. Gas liquid chromatographic analysis of the oil content of the most numerous gland species (capitate stalked, capitate sessile with one and with eight secretory cells) indicated only minor quantitative differences in essential oil composition. Thus, each gland type is capable of producing the four major monoterpene families (*p*-menthanes, pinanes, bornanes and thujanes) characteristic of sage.

The production of essential oils and resins in plants is generally associated with the presence of specialized secretory structures such as glandular trichomes and oil or resin ducts (10, 19). Such structures contain the monoterpenes, sesquiterpenes, and diterpenes characteristic of the oil or resin of the plant and there is little doubt that they are the primary sites of terpene accumulation (6, 9, 11, 14, 20). Although not conclusively demonstrated, considerable indirect evidence (6, 9, 17) suggests the various terpenoid types are synthesized within the secretory cells of such structures.

Among the Lamiaceae, the primary secretory organ is the glandular trichome, the detailed structure of which varies widely with species. *Mentha piperita*, for example, possesses two types of secretory trichomes, both types with unicellular base and stalk but bearing a head consisting of either one or eight secretory cells (1, 2). In both gland types, oil accumulates in a bulbous, subcuticular chamber. In *Monarda fistulosa* (12), *Origanum dictamnus* (4), and *Pogostemon cablin* (15), similar glandular trichomes are observed. However, in *O. dictamnus* the gland-head is comprised of 12 secretory cells and in *P. cablin*, glands with four-celled heads predominate. *P. cablin*, in addition, bears secretory hairs within the mesophyll (15). In various *Salvia* species, sessile glands are present as are long-stalked capitate glands (18, 21). At least four discrete gland types have been ascribed to *Salvia officinalis* (long-stalked glands and sessile glands bearing one-, two- to four, and eight-celled heads) and histochemical evidence has been presented to suggest that oil composition varies with gland type (21). Based on gas chromatographic analysis of the glandular contents of other Lamiaceae (3, 13, 15), both quantitative and qualitative variation in oil content between different gland types of the same tissue have been described.

We have previously noted the presence of several gland types on *S. officinalis* leaves, suggested an ontogenic sequence for the origin of the capitate sessile glands (containing from one to eight secretory cells), and showed the camphor content of the leaves to increase in proportion to the number of filled glands (peltate) which appear on the leaf surface during expansion (5). Since *S. officinalis* bears many apparent gland-types (or several developmental populations of but a few gland types) and produces an oil of considerable complexity (containing monoterpenes of the structurally distinct *p*-menthane, bornane, pinane and thujane families) (7, 16), it was of interest to investigate in greater detail the origin of the glandular trichomes and to examine directly the oil content of these structures.

MATERIALS AND METHODS

Sage (*S. officinalis* L.) plants were grown from seed under controlled conditions described previously (5, 8). Plant tissues (intact small leaves or leaf pieces) were fixed for 1 h at 28°C in 3% glutaraldehyde in 100 mM Na-phosphate buffer at pH 7.3. After rinsing in fixative buffer, the tissues were postfixed for 1 h at 28°C in 2% OsO_4 in distilled H_2O and subsequently rinsed with distilled H_2O (2–5 min). Tissues were dehydrated in a graded ethanol series, critical point dried, gold-coated by standard procedures and observed at 20 kv within a month of preparation. Preliminary field counts made while observing were verified using the resulting micrographs.

The different gland types were manually collected with the aid of a fine steel needle (under magnification × 50) and oil-extracted by immersion of the pooled glands in 0.2 ml of redistilled ether at 0°C. Analysis of the extracts was performed on a Perkin-Elmer Sigma 3B Chromatograph (FID) equipped with a 25- m fused silica capillary column coated with Carbowax 20 M and operated at 60°C for 15 min and programmed to 190°C at 5°C/min (injection temperature = 185°C, detection temperature = 220°C, 2 ml/min N_2 flow). Coupled GLC-MS was performed on a tandem Hewlett-Packard 5840A-5985B system using similar chromatographic conditions in the EI mode (ionization at 70 ev). Mass spectra were compared to those of authentic standards.

RESULTS AND DISCUSSION

Initial observations indicated a fairly uniform distribution of glandular trichomes on both abaxial and adaxial leaf surfaces,

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with higher densities on the abaxial surface (Fig. 1a). Two basic types of glandular trichomes are present: capitate stalked glands comprised of a multicellular (2–3 cells) stalk with a small bulbous head consisting of a single secretory cell with enlarged subcuticular chamber within which oil accumulates; and capitate sessile glands (peltate) consisting of a basal cell, unicellular stalk and heads presumed to comprise from one to many cells and with varying degrees of filling of the subcuticular space. Figure 1, a and b, shows fields with the two basic types of glandular trichomes as they appear on the abaxial leaf surface.

Many observations of leaves of differing maturity indicated that both juvenile and filled sessile glands are present on immature leaves, with the former outnumbering the latter. The proportion of juvenile glands decreases with leaf expansion, and few such glands are present on mature leaves. Juvenile glands are evidenced by the presence of a highly convoluted, unstretched cuticle with obvious division planes indicating an unfilled extracellular cavity (Fig. 1, c and d). sessile glands containing two-, four-, and eight-celled heads were also observed to be actively secreting oil as indicated by filling of the subcuticular chamber with separation of the cuticle from the uppermost walls of the secretory head cells (Fig. 1, c and e). The head cell numbers of fully enlarged peltate glands were difficult to determine since the cell outlines were seldom visible beneath the bulbous subcuticular chamber (Fig. 1f). However, the cuticular covering of a sufficient number of glands of this type did rupture during fixation and/or critical point drying, revealing the inner disc of the head and allowing ready determination of cell numbers (Fig. 1g). Most glands of this larger type (some 30–40% of the sessile gland population) contained eight cells in the head structure; however, head structures comprised of 10 or 12 cells were also noted (Fig. 1g) although they constituted a relatively small proportion of the sessile gland population. The larger the gland head, the more likely was the possibility of cuticular rupture, no doubt indicating a thinner, more fragile envelope surrounding these structures.

Few very immature stalked glands were obvious, yet careful observation did reveal heads of differing size which presumably reflects a difference in the time of onset of secretory activity of the head cell.

No obvious differences were observed in the nature of the stubby gland initials which may indicate that all types of sessile glands are derived from the same type of protodermal emergence. On the basis of these observations, it appears the development of the glands in sage proceeds, as in related species (2, 4, 12), by periclinal division of the initial to produce the stalk cell which in turn divides to provide the first secretory cell. This uppermost cell then divides anticlinally, providing glandular heads of two, four, eight and occasionally 10 or 12 cells. Of particular interest is the observation that significant numbers of glands initiate secretory activity at intermediate developmental stages (as evidenced by filling of the subcuticular space and the cessation of

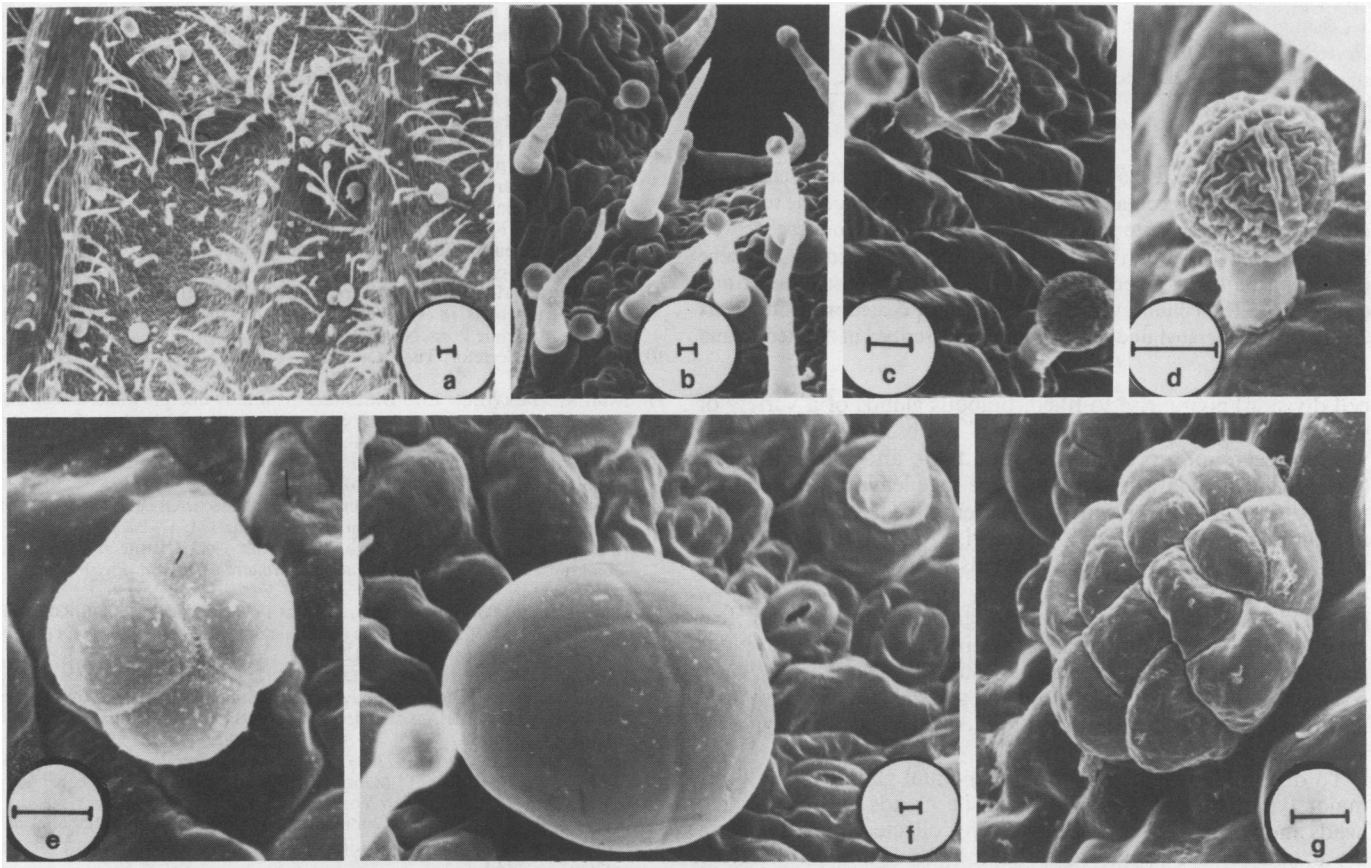

FIG. 1. Scanning electron micrographs of the glandular trichomes on the surface of sage (*S. officinalis*) leaves. a (calibration bar = 50 μm), abaxial surface illustrating the distribution of capitate stalked and sessile glandular trichomes; b (calibration bar = 10 μm), abaxial crevice containing capitate stalked glands and immature sessile glands; c (calibration bar = 10 μm), juvenile sessile gland with two-celled head (lower right) and similar gland after initiation of secretory activity (upper left); d (calibration bar = 10 μm), juvenile sessile gland with four-celled head; e (calibration bar = 7.5 μm), sessile gland with four-celled head after initiation of secretory activity and separation of the cuticle from the head cells; f (calibration bar = 5 μm), mature sessile gland with eight-celled head and secretory cavity near full expansion; g (calibration bar = 10 μm), mature sessile gland with ruptured cuticle and exposed twelve-celled secretory head.

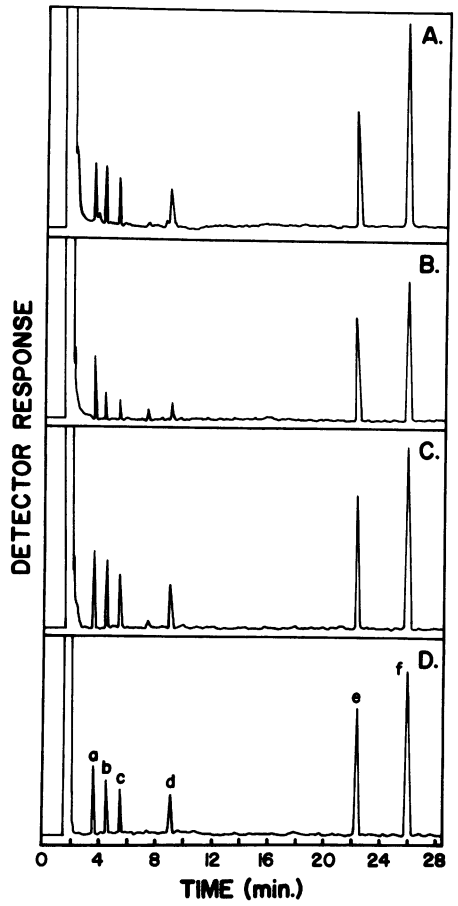

FIG. 2. Capillary gas-liquid chromatograms of the oil extracted from capitate sessile glands containing eight secretory cells (A) or one secretory cell (B) and from capitate stalked glands (C) isolated from sage leaves, and of the whole leaf oil (D). The components indicated are α-pinene (a), camphene (b), β-pinene (c), 1,8-cineole (d), 3-isothujone (e), and camphor (f). The column was 25 m fused silica coated with Carbowax 20 M and was operated under the conditions described in "Materials and Methods."

cell division) giving rise to filled, bulbous glands of a variety of sizes. Similar oil gland development is observed in *P. cablin* whereby secretion occurs at the one-, two-, and four-cell stages (15). On the abaxial surface of mature sage leaves, the predominant sessile gland is the type with an eight-celled head, with the remaining population (~65%) comprised largely of individuals with one-, two-, and four-celled heads. Capitate stalked glands outnumber capitate sessile glands by roughly two to one. As expected, the surface density of the glands decreases with leaf expansion, while the number of mature glands increases. Nonglandular hairs comprised of two to four cells above a large basal cell are numerous on both abaxial and adaxial surfaces of mature leaves, with a stouter modification of this trichome type on younger leaves.

To determine oil composition, individual glands of the three major types (stalked glands, and sessile glands with one-celled heads and with eight-celled heads) were collected manually from the abaxial surface of mature leaves, the gland contents extracted with ether and the extracts analyzed by capillary GLC. Analysis of the extract obtained from sessile glands with eight-celled heads produced a chromatogram (Fig. 2A) similar to that obtained on analysis of a total leaf extract (Fig. 2D), with α-pinene, camphene, β-pinene, 1,8-cineole, 3-isothujone, and camphor as major components. Extracts of sessile glands with unicellular heads (Fig. 2B) and of stalked glands (Fig. 2C) did not differ significantly in oil composition from that of the above, exhibiting but minor differences in the relative proportions of components present. The identity of the monoterpenes in the various extracts was confirmed by combined GLC-MS. Thus, contrary to earlier indications based on histochemical studies (21), direct analyses of glandular contents indicate the stalked glands and sessile glands (at both early and late developmental stages) produce a very similar oil and therefore each must be capable of synthesizing all the major monoterpene types associated with this species. The intracellular site(s) of monoterpene synthesis and the mechanism of oil transport to the extracellular chamber remain uncertain, and questions regarding the relationship of cell division to the onset of secretory activity have yet to be addressed. Further studies employing transmission microscopy are underway.

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LITERATURE CITED

1. AMELUNXEN F 1964 Electronenmikroskopische Untersuchungen an den Drüsenhaaren von *Mentha piperita* L. Planta Med 2:121–139
2. AMELUNXEN F 1965 Elektronenmikroskopische Untersuchungen an den Drüsenschuppen von *Mentha piperita* L. Planta Med 13: 457–473
3. AMELUNXEN F, T WAHLIG, H ARBEITER 1969 Über den Nachweis des ätherischen Öls in isolierten Drüsenhaaren and Drüsenschuppen von *Mentha piperita* L. Z Pflanzenphysiol 61: 68–72
4. BOSABALIDIS A, I TSEKOS 1982 Glandular scale development and essential oil secretion in *Origanum dictamnus* L. Planta 156: 496–504
5. CROTEAU R, M FELTON, F KARP, R KJONAAS 1981 Relationship of camphor biosynthesis to leaf development in sage (*Salvia officinalis*). Plant Physiol 67: 820–824
6. CROTEAU R, MA JOHNSON 1984 Biosynthesis of terpenoids in glandular trichomes. *In* E Rodriguez, PL Healy, I Mehta, eds, Biology and Chemistry of Plant Trichomes. Plenum, New York, pp 133–185
7. CROTEAU R, F KARP 1976 Biosynthesis of monoterpenes: enzymatic conversion of neryl pyrophosphate to 1,8-cineole, α-terpineol, and cyclic monoterpene hydrocarbons by a cell-free preparation from sage (*Salvia officinalis*). Arch Biochem Biophys 176: 734–746
8. CROTEAU R, F KARP 1977 Demonstration of a cyclic pyrophosphate intermediate in the enzymatic conversion of neryl pyrophosphate to borneol. Arch Biochem Biophys 184: 77–86
9. DELL B, AJ MCCOMB 1978 Plant resins—their formation, secretion and possible functions. *In* HW Woolhouse, ed, Advances in Botanical Research, Vol 6. Academic Press, New York, pp 278–316
10. FAHN A 1979 Secretory Tissues in Plants. Academic Press, New York, pp 158–222
11. FAHN A, J BENAYOUN 1976 Ultrastructure of resin ducts in *Pinus halepensis*. Development, possible sites of resin synthesis, and mode of its elimination from the protoplast. Ann Bot 40: 857–863
12. HEINRICH G 1973 Entwicklung, Feinbau und Ölgehalt der Drüsenschuppen von *Monarda fistulosa*. Planta Med 23; 154–166
13. HEINRICH G 1977 Die Feinstruktur und das ätherische Öl eines Drüsenhaares von *Monarda fistulosa*. Biochem Physiol Pflanz 171: 17–24
14. HEINRICH G, W SCHULTZE, I PFAB, M BÖTTGER 1983 The site of essential oil biosynthesis in *Poncirus trifoliata* and *Monarda fistulosa*. Physiol Vég 21: 257–268.
15. HENDERSON W, JW HART, P HOW, J JUDGE 1970 Chemical and morphological studies on sites of sesquiterpene accumulation in *Pogostemon cablin (patchouli)*. Phytochemistry 9: 1219–1228
16. LAWRENCE BM, JW HOGG, SJ TERHUNE 1971 Les huiles essentielles et leurs constituants. IV. Quelques nouveaux constituants á l' état de traces dans l'huile essentielle de *Salvia officinalis* L. Parfums Cosmetiques Savons de France 1: 256–259
17. LOOMIS WD, R CROTEAU 1973 Biochemistry and physiology of lower terpenoids. *In* VC Runeckles, TJ Mabry, eds, Recent Advances in Phytochemistry Vol 6. Academic Press, New York, pp 147–185
18. SCHNEPF E 1972 Tubuläres endoplasmatisches Reticulum in Drüsen mit lipophilen Ausscheidungen von *Ficus*, *Ledum* und *Salvia*. Biochem Physiol Pflanz 163: 113–125
19. SCHNEPF E 1974 Gland cells. *In* AW Robards, ed, Dynamic Aspects of Plant Ultrastructure. McGraw-Hill, London, pp 331–357
20. VASSILIEV AE 1970 On the localization of the synthesis of terpenoids in the plant cell (in Russian). Rastit Resur 6:29–45
21. VERZÁR-PETRI G, M THEN 1975 The study of the localization of volatile oil in the different parts of *Salvia sclarea* L. and *Salvia officinalis* L. by applying 2-^{14}C-sodium acetate. Acta Bot Acad Sci Hung 21: 189–205