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# Quantitative aspects of *Origanum dictamnus* L. glandular scales

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## Abstract

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Leaves of *Origanum dictamnus* are densely covered with numerous glandular scales which produce an essential oil. There are approximately 2680 glandular scales, which occur on both sides of the leaf, with an average horizontal diameter of about 90 μm per scale head. The subcuticular space of a differentiated glandular scale was found to contain $1.81 \times 10^5$ μm$^3$ of essential oil and the sum of the secretory cells (head cells) $0.37 \times 10^5$ μm$^3$. The theoretical essential oil yield of mature leaves was estimated to be 1.4% v/w (1.4 cm$^3$ of essential oil per 100 g dry leaf material).

The changes in the relative volume of the ground plasm, plastids, mitochondria, endoplasmic reticulum, dictyosomes, nucleus, vacuoles and essential oil accumulated in the cytoplasm of the secretory cells, were measured at six stages of glandular scale development. Values for the ground plasm varied from 25% to 53% of the total scretory cell volume during these stages. Corresponding values for the nucleus progressively decreased from 23% to 6%. The plastids, mitchondria, endoplasmic reticulum and dictyosomes did not exceed 10% during all stages. The average volume percentage of the cytoplasmic essential oil was estimated to be 38% at the stage of glandular scale maturity. Correlation between the quantitative estimations and the secretory function of the gland are discussed.

*Key words: Origanum dictamnus,* glandular scales, quantitative estimations.

## Introduction

*Origanum dictamnus* L. is an endemic shrub of the island of Grete, Greece. It grows wild in rocky regions at an altitude of 300–1500 m. The essential oil derived from the plant is of great commercial interest because of its use in vinification and cosmetics. Analytical studies made on the constitution of *O. dictamnus* essential oil have revealed that the major component (60–80%) is carvacrol (Schaden and Hesse 1976, Skrubis 1979, Katsiotis and Oikonomou 1986). Other components of minor importance are β-phellandrene, p-cymene, α-pinene, β-myrcene, γ-terpinene, linalool and β-caryophyllene.

In the present report an attempt was made to determine morphometrically the theoretical essential oil yield of mature leaves of *O. dictamnus*. The quantitative changes taking place in the ground plasm, plastids, mitochondria, endoplasmic reticulum, dictyosomes and the cytoplasmic essential oil accumulations of the secretory cells, were also studied at six stages of glandular scale development, to clarify their contribution to the secretory function of the glands.

## Material and methods

Developed leaves (2 cm-long) of *Origanum dictamnus* L. plants kept in a growth chamber, were used. For light and electron microscopy, small leaf segments were prefixed for 3 h with a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.5 M sodium cacodylate (pH 7.2) at room temperature. After several rinses in buffer, the specimens were postfixed with 1% $OsO_4$ in the same buffer for 3 h at 4 °C. Alternatively, the material was fixed with 1% $OsO_4$ in 0.025 M phosphate buffer (pH 7.2) for 12–14 h at 4 °C. After dehydration in an alcohol series, the samples were infiltrated and finally embedded in Spurr's (1969) resin. Semithin sections were cut on a Reichert Om $U_2$ ultramicrotome, and then stained with toluidine blue O and examined using a Zeiss Photomicroscope III. Ultrathin sections were obtained on a Reichert-Jung Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds 1963) and observed in a Jeol 100 B electron microscope.

To estimate the theoretical essential oil yield of mature leaves of *O. dictamnus,* the following parameters were determined: (a) leaf surface area (b) number of glandular scales on each leaf surface (c) horizontal diameter of the head of a glandular scale (fully formed subcuticular space) (d) height of the subcuticular space of a glandular scale (e) volume fraction of the essential oil accumulations in the cytoplasm of the secretory cells (f) volume ratio of sum of head cells/subcuticular space in a glandular scale and (g) weight of dry leaves.

The surface area of the leaf was measured by tracing it on paper with a 1-mm square lattice. The number of glandular scales on each leaf side was determined from magnified micrographs of entire leaves taken with a Zeiss Stereomicroscope SV8. The horizontal diameter of the glandular scale head and the height of the subcuticular space were measured from light micrographs (leaf paradermal and cross sections).

The subcuticular space which is formed at the apex of the gland was considered an ellipsoid. In this ellipsoid the two horizontal axes (D) represent the medially crossed equal diameters of the disc-like area exhibited by a glandular scale in surface view. The third vertical axis (D′) corresponds to the maximum height of the subcuticular space. The volume of the essential oil contained within the subcuticular space was obtained from the formula

$$V = \frac{4}{3}\pi \frac{D'}{2} \frac{D^2}{4}.$$

The volume of the essential oil in the cytoplasm of the secretory cells was estimated by superimposing a transparent sheet bearing a square lattice of points upon electron micrographs at a magnification of 12,000 (Steer 1981). By using the same method, the volume ratio between the sum of the secretory cells (head cells) and the subcuticular space, was determined.

Six stages of glandular scale development were chosen for studying the changes in volume percentage of the secretory cell components. The stages were: (1) glandular scale initiation (2) glandular scale early differentiation (3) just preceding secretion (4) early secretion (5) late secretion and (6) glandular scale maturity. The following criteria were used to identify each stage: the volume of the secretory cells, the size of the nucleus and the vacuoles, the amount of ground plasm, the time at which essential oil droplets first appeared in the ground plasm of the secretory cells, the number and volume of the essential oil droplets, the presence of extensive cytoplasmic accumulations of essential oil and, finally, the extent of detachment of the cuticle from the gland apical walls to form a subcuticular space.

To characterize the cytoplasmic lipophilic droplets as essential oil, their high osmiophilia, the developmental stage in which they first appeared, their subsequent increase in number and volume, their coalescence to form extensive accumulations and, finally, their ultrastructural resemblance to the content of the subcuticular space, have been considered.

The secretory cell components for which the changes in total volume during glandular scale development were studied, involved the ground plasm, the nucleus, the plastids, the mitochondria, the endoplasmic reticulum, the dictyosomes, the vacuoles, and the cytoplasmic essential oil accumulations. For each of these, and for each developmental stage, a series of volume fraction values were taken by applying a square lattice of point arrays 6 mm apart, to electron micrographs of entire cells ( $\times$ 25,000) (Steer 1981). The averages of the collected values were processed by a computer to obtain histograms (Macintosh Plus, Statview 512+).

## Results

Morphometric estimation of the essential oil yield of leaves (2 cm long) of *O. dictamnus* initially comprises the determination of several parameters listed in Table 1.

When viewed from above, a glandular scale exhibits a disc-like area the average surface of which was found to be 6285.23 $\mu m^2$ [parameter (d) of Table 1]. The latter value, together with the values of parameters (a) and (b) of Table 1, allow an estimation of the percentage of the surface area occupied by the glandular scales at the upper leaf side. This

Tab. 1. Morphometric characteristics of developed leaves of *Origanum dictamnus*.

| Parameters | n | Mean $\pm$ SD |
|---|---|---|
| (a) Surface area of each side of the leaf | 38 | $358 \pm 45$ $mm^2$ |
| (b) Number of glandular scales on the upper side of the leaf | 32 | $1213.23 \pm 92.37$ |
| (c) Number of glandular scales on the lower side of the leaf | 32 | $1471.38 \pm 96.12$ |
| (d) Horizontal diameter of the head of a glandular scale | 32 | $89.48 \pm 7.35$ µm |
| (e) Maximum height of the subcuticular space of a glandular scale | 27 | $43.11 \pm 9.25$ µm |
| (f) Volume ratio of sum of secretory cells/subcuticular space | 27 | $0.54 \pm 0.048$ |
| (g) Volume fraction of the essential oil accumulations in the cytoplasm of the secretory cells | 19 | $0.38 \pm 0.029$ |
| (h) Weight of a dry leaf (2 cm long) | 55 | $0.042 \pm 0.009$ g |

Tab. 2. Quantitative estimations associated with essential oil secretion in *Origanum dictamnus*.

| | Values |
|---|---|
| Essential oil content of the fully extended subcuticular space of a glandular scale | $1.81 \times 10^5$ $\mu m^3$ |
| Volume of essential oil contained within the secretory cells (secretory head) of a glandular scale | $0.37 \times 10^5$ $\mu m^3$ |
| Total essential oil content of a glandular scale | $2.18 \times 10^5$ $\mu m^3$ |
| Total essential oil content of a single leaf (essential oil content of all glandular scales at both leaf sides) | 0.59 $mm^3$ |
| Theoretical essential oil yield (ml/100 g dry leaf material) | 1.4% |

percentage was 2.1%. The corresponding percentage for the lower leaf side was 2.6%. Thus, glandular scales cover 4.7% of the total leaf surface area.

By using parameters presented in Table 1 and considering those described in material and methods, a series of quantitative characteristics associated with the essential oil-content of glandular scales and the essential oil-yield of mature leaves, can be additionally determined (Table 2).

The changes in volume of various secretory cell components were also determined with respect to total cell volume at six stages of glandular scale development (Fig. 1). During all stages, the relative volumes of the plastids, mitochondria, endoplasmic reticulum elements, and dictyosomes did not exceed 10% of the total secretory cell volume. At the stage of glandular scale initiation, the ground plasm represented 42%, the nucleus 23% and the vacuoles 19% of the entire secretory cell volume. At the stage of early differentiation, the volume of the ground plasm increased to 53%, while the corresponding volumes of the nucleus and the vacuoles decreased to 19% and 8%, respectively. At the stage preceding secretion, the volume percentages of the ground plasm and the nucleus decreased to 25% and 12%. Vacuoles appeared highly developed occupying a cell volume of 48%. The volume of the cytoplasmic droplets of essential oil at the stage of early secretion, was estimated to be 3% of the total secretory cell volume (Fig. 2). The vacuoles and the ground plasm occupied a considerable cytoplasmic portion (44% and 31%). At the stage of late secretion, the volume of the cytoplasmic essential oil droplets increased to 12%. The ground plasm and the vacuoles were still the major components of secretory cells (33% and 37% of the total secretory cell volume). Finally, at the stage of glandular scale maturity, the essential oil was found to occur in the form of irregular cytoplasmic accumulations (Fig. 3) occupying a relative volume of 38%. The ground plasm itself exhibited a high volume (41%), unlike the vacuoles (15%) and the nucleus (6%).

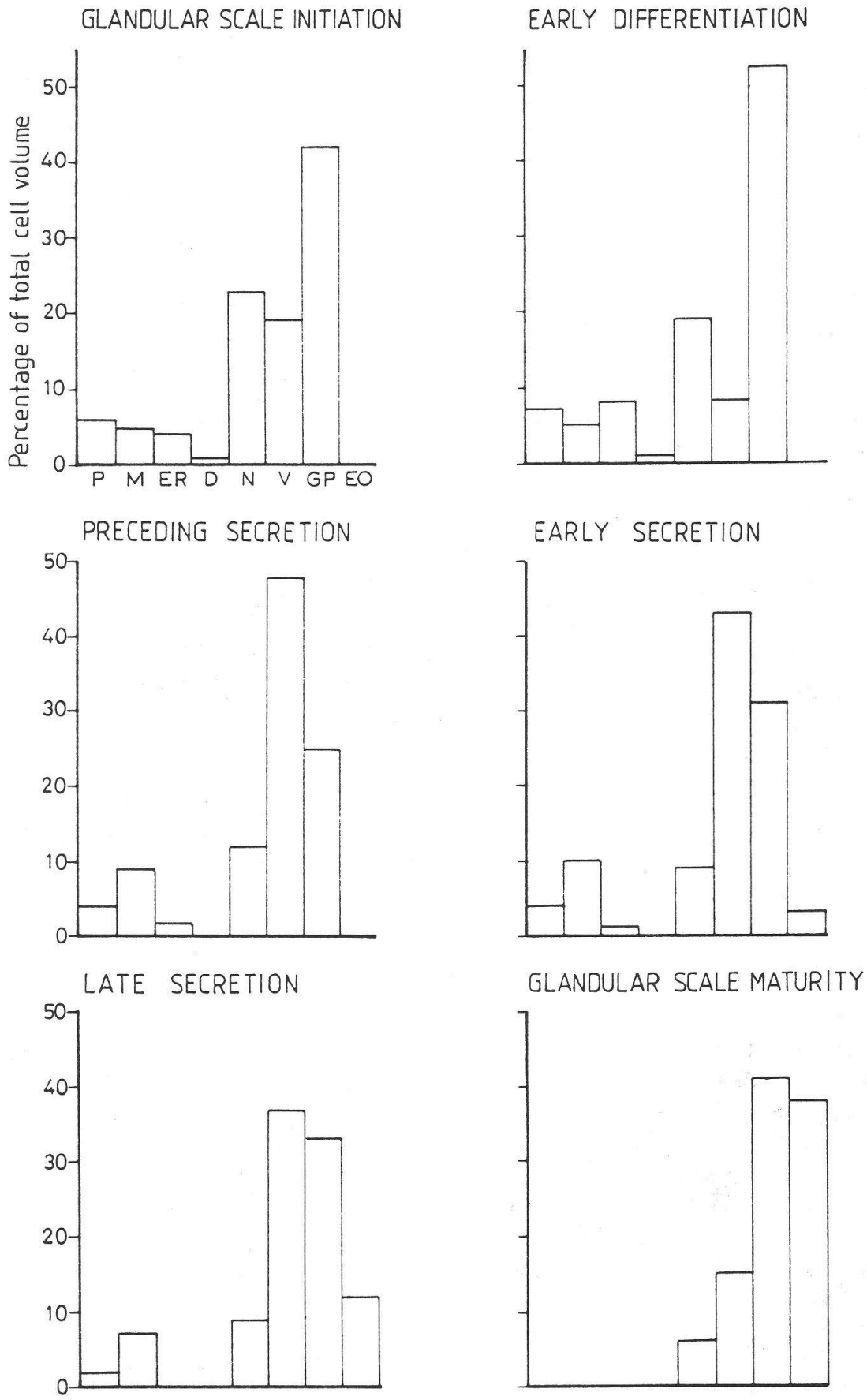
## Discussion

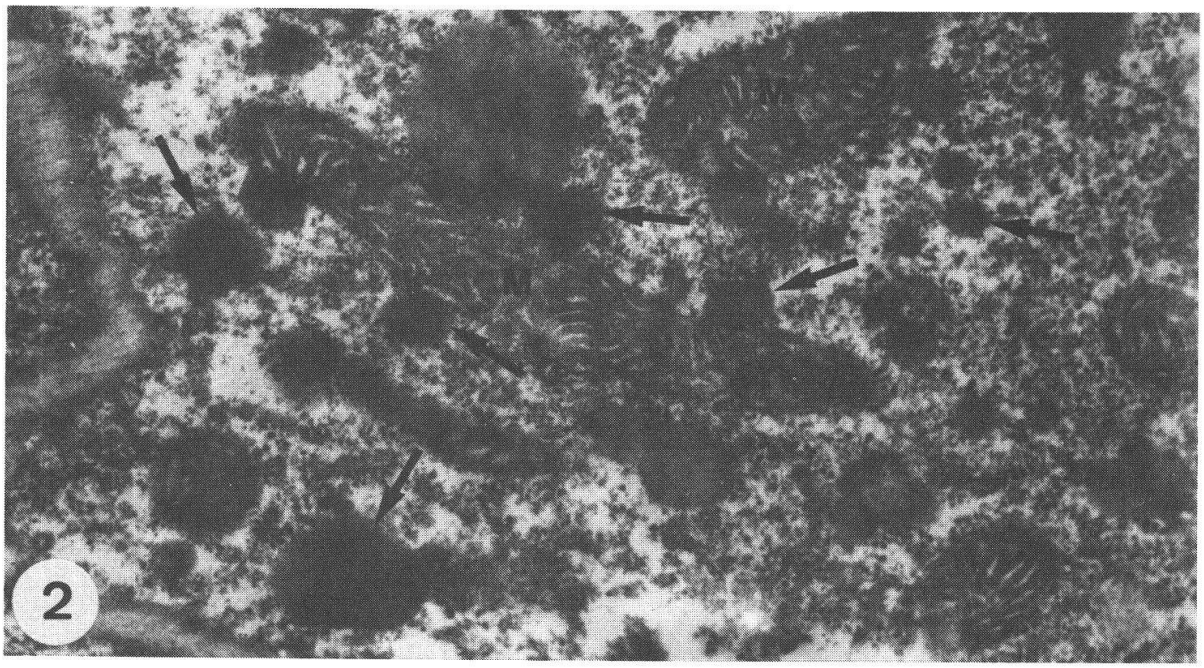
Leaves of *Origanum dictamnus* are covered with glandular trichomes of two distinct types, glandular scales and glandular hairs. Glandular scales are considered to be the principal source of essential oil production, since glandular hairs are manifold smaller. Furthermore, glandular hair abundance per leaf surface area is low in mature leaves. In the differentiated leaves studied, glandular scales appear numerous occupying about 4.7% of the entire leaf surface area.

Our results give a theoretical essential oil yield of 1.4% v/w. This value which corresponds to glandular scales only, appears reasonable since for the same plant Katsiotis and Oikonomou (1986) by distilling dry leaf samples found an essential oil yield varying from 1.33% to 1.60% v/w.

Plastids, endoplasmic reticulum elements, and mitochondria of the secretory cells which in some species are considered the principal organelles involved in essential oil secretion (Amelunxen and Arbeiter 1967, Schnepf 1969, Schnepf and Klasova 1972,

Fig. 1. Average volume percentages of secretory cell (head cell) components of *Origanum dictamnus* at six stages of glandular scale development. P plastids, M mitochondria, ER endoplasmic reticulum, D dictyosomes, N nucleus, V vacuoles, GP ground plasm and EO cytoplasmic essential oil.


GLANDULAR SCALE INITIATION
EARLY DIFFERENTIATION
Percentage of total cell volume
50
40
30
20
10
0
P M ER D N V GP EO
PRECEDING SECRETION
EARLY SECRETION
LATE SECRETION
GLANDULAR SCALE MATURITY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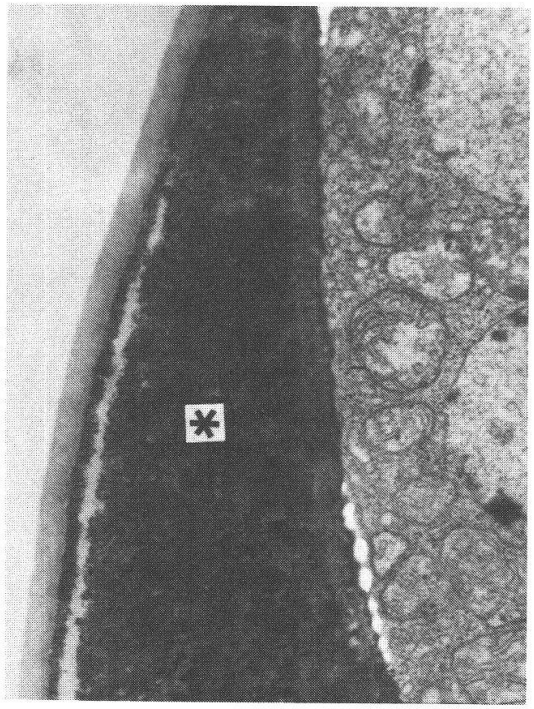
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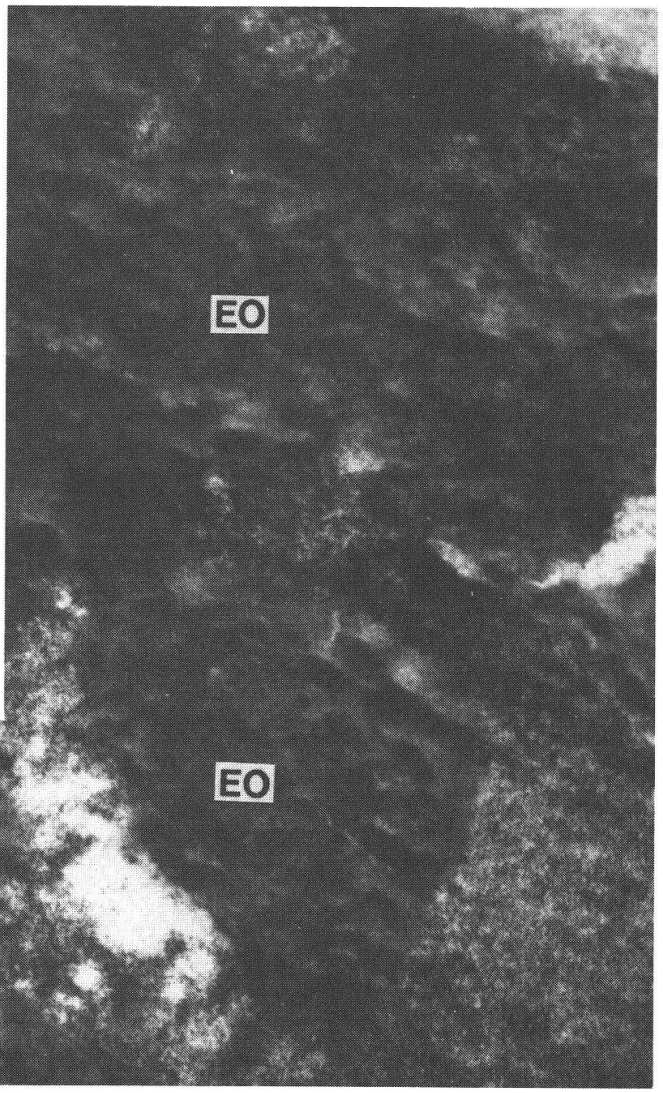
EO
EO


3

Bosabalidis and Tsekos 1982a), were found not to be particularly developed in *O. dictamnus*. The unexpected disappearance of the elements of the endoplasmic reticulum, particularly during the stages of secretion, may be due to membrane disintegration associated with the appearance of the essential oil in the cytoplasm. This is most evident at the stage of glandular scale maturity where the membranes of plastids and mitochondria of the secretory cells also disintegrated.

Dictyosomes are rarely found in the secretory cells during the early stages of *O. dictamnus* glandular scale development. The limited presence of dictyosomes is in accordance with analogous observations made with other glands secreting essential oils (Heinrich 1969, Wollenweber and Schnepf 1970).

Of particular interest is the high volume percentage of the ground plasm. The values estimated for the secretory stages disclose the important role the ground plasm may play in the process of essential oil secretion. Actually, ultrastructural observations on oil glands in members of the Lamiaceae have indicated that the ground plasm of the secretory cells is the possible site of essential oil formation (Amelunxen 1965, Heinrich 1973, Bosabalidis and Tsekos 1982b).

The appearance of the first droplets of essential oil not within an organelle but in the ground plasm of the secretory cells, and the subsequent formation of extensive essential oil accumulations also in the ground plasm, favour the suggestion that the enzymes of monoterpene metabolism are probably localized there. However, the presence of at least some enzymes (particularly those associated with sesquiterpene biosynthesis, i.e. of β-caryophyllene, see introduction) in cell compartments like the plastids, endoplasmic reticulum and vacuoles, cannot be excluded (Amelunxen 1965, Heinrich 1970, Tsekos and Schnepf 1974, Curry 1987). The view that monoterpenes and sesquiterpenes are synthesized at different intracellular sites (within membrane-bound compartments and in the ground plasm) has been expressed earlier by Loomis and Croteau (1973).

Although vacuoles are well developed during the stages of secretion (vacuoles occupy 37%–43% of the secretory cell volume) they are not used by the secretory cells to store essential oil. The latter is temporarily deposited in the ground plasm and is later moved into the subcuticular space. The presence of large accumulations of essential oil in the ground plasm of mature secretory cells (38% of the cell volume) indicates that the rate of essential oil exudation is rather low compared to its rate of formation.

Fig. 2. Osmiophilic droplets of essential oil (arrows) in the ground plasm of a secretory cell at the stage of early secretion, × 33,000.

Fig. 3. Irregular accumulations of essential oil (EO) formed in the ground plasm of a secretory cell during the stage of glandular scale maturity, × 33,000. Inset: Portion of essential oil deposited within the subcuticular space (asterisk). Note its apparent resemblance to the cytoplasmic droplets and irregular accumulations of essential oil, × 21,000.

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