Anatomy and Morphology of Glandular and Non-glandular trichomes of *Pteronia incana* in relation to their essential oil production.

by

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submitted in partial satisfaction of the requirements

for the degree of

MASTER OF SCIENCE

in the

Faculty of Science

of the University of Fort Hare

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Date submitted : January 2000

Declaration

I Buyisile Mayekiso do hereby declare that the work contained in this thesis is entirely my own work.

Signed at **University of Fort Hare** this **24th** day of month of **January** year **2000**

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Abstract

The morphology and anatomy of the non-glandular and glandular trichomes present on both abaxial and adaxial surfacea as well as on the stem surface of *Pteronia incana* were examined using light, scanning electron microscopy and transmission electron microscopy. The non-glandular trichomes were thread-like structures with elongated fibrous ends having a tubular basal cells. They occurred in greater abundance in both young and mature leaves and in stems. The fibrous ends of non-glandular trichomes seemed to be shielding the epidermal layer, the glandular trichome and the stoma. The electron micrograph of non-glandular trichomes showed that both trichomes originated from a single epidermal cell by anticlinal division. As the trichome matured, the cells at the end of the trichome became dehydrated and formed a thread-like fibre. Structurally, these fibrous ends were similar in composition to the components of the suberized cell walls.

The glandular trichomes which were club to oval shape and occurred in greater abundance in young leaves and stems. However, they decreased drastically, particularly in the old stems which was characterized by the periderm. The electron micrographs of these gland cells showed that they started as outgrowth of the epidermal cells, and subsequent periclinal division followed by anticlinal division, giving rise to the trichome with a basal epidermal cells, a stalk cell and six to eight celled secretory head. At the early stages of the glandular trichome development, the stalk cells adjacent to the gland contained significant number of plasmodesmata which showed a transport system between the basal cell and stalk cell. These gland cells contained various cell organelles such as modified mitochondria, a large nucleus with a dense cytoplasm containing osmophilic droplets.

A fully mature glandular trichome was characterized by the distinct numerous endoplasmic reticulum, mitochondria, osmophilic droplets, a relatively large vacuole and a distended cuticular sac due to the accumulation of the essential oil.

The chemical analysis indicated that essential oil of *P. incana* contained the following predominant compounds : Alpha-pine, Beta-pinene + Beta myrcene, Sabinene, 1.8 Cineole, Beta-thujene, dl-Limonene, Alpha terpinene and Delta-4 carene. It appear that the high distribution of glandular trichomes and high oil yield occurred prior to vegetative growth and the flowering season, that is, June and October. It appear that this would be ideal for commercial distillation and for industrial purposes.

1. Introduction

Eastern Cape Province in South Africa, is richly endowed with aromatic plants which are unpalatable to livestock, because of their high content of secondary metabolites, which are primarily located in the secretory tissues of the leaves, stems and flowers (*Webber* et al. 1999; *Bruns* and *Meiertorens* 1987; *Werker* and *Fahn*, 1981). These secretory tissues may occur either intracellular or extracellular. The extracellular secrectory tissues found in most angiosperms species as early as the nineteen century are covered with the unicellular or multicellular non-glandular trichomes which originated from the epidermal cells and developed on the surface of various organs (*Weiss*, 1867; and *De Bary*, 1877). With the development of the organized taxonomic classification, it has been shown that the most extracellular secretory tissues like the oil gland are the common characteristics of aromatic species (*Fahn*, 1988).

These aromatic species are very common in diverse families such as Asteraceae, Geraniaceae, Solanaceae, Cannabinaceae and Lamiaceae where many studies have been conducted on trichome anatomy and morphology in relation to their mode of essential oil release (*Massimo* et al. 1986; *Uphof* and *Hummel*, 1962; *Vermeer* and *Peterson*, 1979; *Werker* and *Fahn*, 1981; *Bruni* and *Modenesi*, 1983; *Bosabalidis* and *Tsekos*, 1982; 1984, *Peterson* and *Vermeer*, 1984; *Werker* et al. 1985a and 1985b; *Dudai* et al. 1988; *Antunes* and *Sevinate - Pinto*, 1991; *Ascensão* et al., 1985; *Fahn* and *Shimony*, 1998; *Serrato-Valenti* et al., 1997). It has also been shown that the seasonal variation in the composition of essential oils of a number of species of these families has also been extensively discussed (*Werker* and *Fahn*, 1981; *Bruni* and *Modenesi*, 1983; *Bosabalidis* and *Tsekos*, 1982, and 1984). These families are well known for their essential oil, herb and medicinal plant species by the international and local indigenous people. In many third world countries, these species are therefore, facing extinction. However, in highly industrialized countries, most natural flavoring and fragrances are derived from plant substances - either from the aromatic volatile plant oils called resins or from the extracellular secretory structures (Werker, 1993).

The aromatic substances derived from flowers, herbs and spices, are usually costly and limited in supply. Over the past century, success in reproducing some of these aromatic substances synthetically has contributed to industry such that hundreds of flavors and fragrances for use in food, perfumes and other products were synthesized. Many flavors and fragrances, have scored, if not hundreds, of components that contributed to their aroma. However, towards the end of the last century, there has been a tremendous increase of interest in genetic manipulation of these essential oil plants using plant biotechnology. Such recent developments have opened up new opportunities for breeding new cultivars of essential oil plants, and to point to some of the obstacles that must be overcome before their promise can be converted into commercial reality.

In addition, many of the natural products like essential oil, fatty oils, latex and resins, which have been used by man through the ages, are produced by the secretory tissues of vascular plants. Such secretory tissues are usually classified according to the substance they produce (*Fahn*, 1979; *Thomson* and *Healey*, 1984; *Peterson* and *Vemeer*, 1984). These various kinds of tissues are : oil secreting trichomes; oil cells; oil cavities; osmophores; flavonoid secreting tissues; laticifers; gum ducts; resin ducts; salt glands; nectaries; mucilage secreting glands; stinging trichomes; enzyme secreting glands of carnivorous plants; myrosin cells and hydathodes which produce guttation fluid (*Magwa* et al., 1993; *Fahn*, 1979). However, different compounds may be secreted by the same tissue, but a large spectrum of secretory substances and their production by a range of structures, differ greatly in their morphology and anatomy (*Fahn*, 1988).

Plants trichomes appeared to be unicellular or multicellular structures which originated from epidermal cells and develop on the surface of various organs (*Bosabalidis* and *Tsekos*. 1984), a definition which was initially given by *Weiss* (1867) and *De Bary* (1877) . The morphology of these structures can vary greatly with tissue and species (*Bosabalidis* and *Tsekos*. 1984). These characteristics have often been used in plant classification.

Some authors have also included the functions of the trichomes (absorption, secretion, storing) in their definitions, which means that, trichomes may be simple hairs which deter herbivores, guide the path of pollinators (nectary) or affect photosynthesis, leaf temperature, or water loss through increased light reflectance as in dry areas species (*Wagner*, 1991).

In some aquatic species, found in the salty marshlands, secretory glands are regarded as an adaptive mechanism to regulate the level of salt in the leaves to cope with high substrate salinity (*Naidoo* and *Naidoo*, 1998). The glandular trichomes, of these species adapted to aquatic environments, are assumed to regulate the salt concentration and are termed salt secreting trichomes (*Bosabalidis* and *Tsekos*.. 1984). In contrasts, the glandular trichome of the species adapted to arid environment, contained the secondary metabolites. Such metabolites are considered to be involved in major defensive strategies against the pests and predators. They are also regarded to be responsible for the vital role in pollination as these metabolites are able to attract small animals for the pollination process due to the presence of the attractive odour and nectar section (*Wagner*, 1991).

In other species, these trichomes may be more specialized tissues (glandular secreting trichomes) whose principal function may be to produce pest -or- pollinator inter-active chemicals which are stored or volatilized at the plant surface. It has been suggested that in species which are subjected to arid environments the principal role of the glandular secreting trichomes is to produce high levels of exudate that forms a continuous layer on the plant surface. This layer may increase light reflectance and thereby reduce leaf temperature (*Fahn*, 1982). It has also been suggested that these non-glandular trichomes appeared to cover the glandular trichomes of the aromatic plants (*Werker* et al., 1985).

The main common compounds in these aromatic plants appeared to be isoprenoids (*Kleinig*, 1989). Isoprenoids, also known as terpernoids are well known to represent a large family of natural compounds found mainly in higher plants, but also in lower plants, fungi, animals and prokaryotes (*Kleinig*, 1989).

A large number of isoprenoid structures have been cited in plants, and this clusters of these compounds continued to increase as research capacity advances. Many biological activities have been attributed to some members of these compounds, including functions as attractants and repellants, hormones, growth inhibitors, pigments, phytoalexins, constituents of the electron transport chain, and translocators of sugar residues through members (*Kleinig*, 1989). However, the principal or specific function of each isoprenoid and its origin found in nature, is not well-documented although, in most publications dealing with isoprenoid biosynthesis, it has been documented that there were three true plasmic phases; the cytoplasm, the mitoplasm (the mitochondrial matrix), and the plastoplasm (the plastids stroma), which were not directly connected, but separated from each other by the non-plasmic phases between the inner and outer envelop membranes of the mitochondria and plastids within the plant cell (*Fahn*, 1988).

All three plasmic compartments of the plant cell were regarded as the sites of isoprenoid biosynthesis. However, the question still arises about the mechanism of biosynthesis of isoprenoids constituency such as hydrophobic isoprenoids and amphiphic substances and their sites of origin. Plastids were regarded as the site of mon- and diterpenes synthesis and these were metabolized further in the cytoplasm/endoplasmic reticulum. The concept of metabolic channelling is still unclear (*Fahn*, 1988; *Saccheti* et al., 1999). This may partially be the case during the release of volatiles into the surrounding. Most of the volatile compounds are released by plants through a specialised epidermal appendages called trichomes. The most characteristic feature of a secretory tissue is the presence of small vacuoles, a relatively dense cytoplasm containing numerous mitochondria. The frequency of other cell compartments and organelles varies according to the particular compound secreted. Therefore cell compartments and organization of cell organelles frequency varies according to the particular compound secrected.

Generally, all secretory trichome have a complete cutinization of the side of the stalk cells (*Fahn*, 1988), similar to that occurring in the walls of the cells of the root endodermis.

This cutinization indicates that the flow of the secretory material or their precursors into the trichome take place exclusively through the symplast and that flow of the secreted substance back to the plant though the apoplast is prevented.

To our knowledge, most of the aspects mentioned above, in Asteraceae family particularly in the genus *Pteronia* have never been studied.

The genus *Pteronia* is characterized by several species which are predominantly common from Eastern Cape to Western Cape areas. These species are *Pteronia incana, Pteronia glauca and Pteronia adenocarpa*, and they are mostly found in the Eastern Cape. However, *P. incana* is more dominant than other species around Peddie and Alice district (*Bruns* and *Meiertorens*, 1987). *P. incana* is a small bush like perennial shrub, having small greyish leaves and often ramified branches reaches a height of approximately 100 cm and has invaded an estimated land of 60 000 hectares of semi arid areas of the Eastern Cape (*Webber* et al., 1999; *Bruns* and *Meiertorens*, 1987). It is also considered to be a weed in the Eastern Cape and thus render plant cultivation unnecessary and is reported to be unpalatable to livestock.

The plant appears whitish due to the hairs that cover the epidermal surface and thus render the plant visibility from afar as grey patches in grassland. The flowering period is May - July and October - December in the same area. The flowers are yellow and is characteristic of the Asteraceae family. The essential oil produced by this plant is pale-yellow and is extensively studied by *Bruns* and *Meiertoberens*, (1987) and also *Webber* et al., (1999), however the worldwide production value of this oil is still unknown.

P. incana is believed to contain essential oil which is competitive in industry (*Webber* et al., 1999). However there is little information regarding to the mode of growth of the essential oil glands and essential oil production.

This scientific data is very important for essential oil industry. Although *P. incana* has the ability to produce precious essential oil, there is no well documented scientific data (*Lawrence*, 1984). The purpose of this study is an attempt to address the following :

To establish the anatomical and morphological relationship of *P. incana* trichomes and their essential oil production.
Is the essential oil synthesized from the glands or transported from the intracellular palisade or intracellular cortical tissues ?
What is the appropriate period for essential oil sampling for large commercial scale and what is the composition of the essential oil ?
Is there any prediction with regard to the biosynthesis of essential oil ?
Is the production of oil suitable for commercial purposes ?

Materials and Methods

The plant material was collected from a site at Double Drift Nature Game Reserve between Alice and Peddie area in the Eastern Cape.

2.1 Scanning electron microscopy

Sections of leaves and stem (0.1 x 0.5 mm) thick, were collected randomly and immediately fixed in 6% glutaraldehyde in 0.05 mM Sodium cacodylate buffer (pH 7.3), washed in 0.05 mM Sodium cacodylate for 12 h. Sections were then dehydrated in an ethanol series. The leaves were dried in a Hitachi HCP-2 critical point dryer, coated with gold using sputter coater and viewed at 15 kV with a Hitachi S-450 Scanning electron microscope.

2.2 Anatomical investigation by light microscope

Leaf and stem sections (0.1 - 0.5 mm) thin were cut and fixed in FAA - Formaldehyde, ethanol / acetic acid. Sections were then dehydrated in a series of alcohols. This involved a graded series of ethyl alcohol, tertiary butyl alcohol (TBA) and substituted TBA with liquid paraffin. Infiltration with wax was achieved after the material was embedded in the paraplast wax. Infiltration involved the dissolving of wax in a solvent, which contained the sections and decreasing the concentration of the solvent. The process was performed in an oven at 70 ^{O}C. The top and bottom faces of the specimen were trimmed in order to form a ribbon during sectioning by Leitz rotary microtome. The sections were stained with safranin, fast green, and aniline blue (*Johansen*, 1940) and were permanently mounted on a slide under the cover slip in a mountant and were examined with a Zeiss Photo-microscope III.

2.3 Transmission electron microscopy

Mature and young leaves and stems of *P. incana* were randomly selected from the natural environment at double drift game reserve in Alice. The leaf portions were cut into small segments approximately 2-3 x 5 mm in cold 50 mM Sodium cacodylate buffer, (pH 7.3). The plant segments were fixed in a buffered 6 % glutaradehyde (50mM Na-cacodylate, pH 7) and stored overnight in a refrigerator. After rinsing in a 50 mM Na-cacodylate buffer, the sample were then postfixed in 2 % Osmium tetroxide (OsO_4) in 50 mM Na-cacodylate buffer, pH 7.3, overnight at 4 ^{o}C, infiltrate in a graded series resin (*Spurr* 1969). Thin sections (0.5 - 2.0 mm) were cut with glass knives on an LKB Ultramicrotome, stained with Uranyl acetate followed by lead citrate and observed in a Hitachi at 75 - 100 kV. Some of the sections were stained with 0.05 % toludine blue and examined with a Zeiss photo-microscope III.

2.4 Essential oil extraction (water distillation)

The plant sample (branches, flowers) was collected at a site at Double drift Nature Reserve on a monthly bases from January to December 1998 and 1999. The leaves were removed from stem and branches, and were weighed. The weighed mass of sample of leaves about 550g was placed in a cleavenge flack to which a 1000 ml distilled water was added and subjected to high temperatures for a period of 2 hrs. This process repeated three times using consistent mass in order to obtain statistical accepted results. Essential oil collected from cleavenger flack from the distilled sample was stored at -10 ^{0}C and was ready for oil analysis.

2.5 Gas chromatography analysis

The essential oil, which was cooled, was analysed on a Hewlet Packard, Series II Gas Ghromatograph. with a flame ionization mass selective detector and a Hewlett Packard 2971 Series. The column consisted of a cross-linked 5 % pH ME Siloxane on 30m x 0.25mm x 0.25μm film thick and the column head pressure was 55 Kpa. The carrier gas used was Helium and the flow was 35 cm/s-split flow 30 - 40:1.

The temperature programmes, initial temperature was 50 ^{0}C and accelerated to a temperature of 240 ^{0}C in the temperature range of 50 ^{0}C - 240 ^{0}C at an acceleration of 3 ^{0}C/min.

Results :

3.1 Morphological investigation using SEM

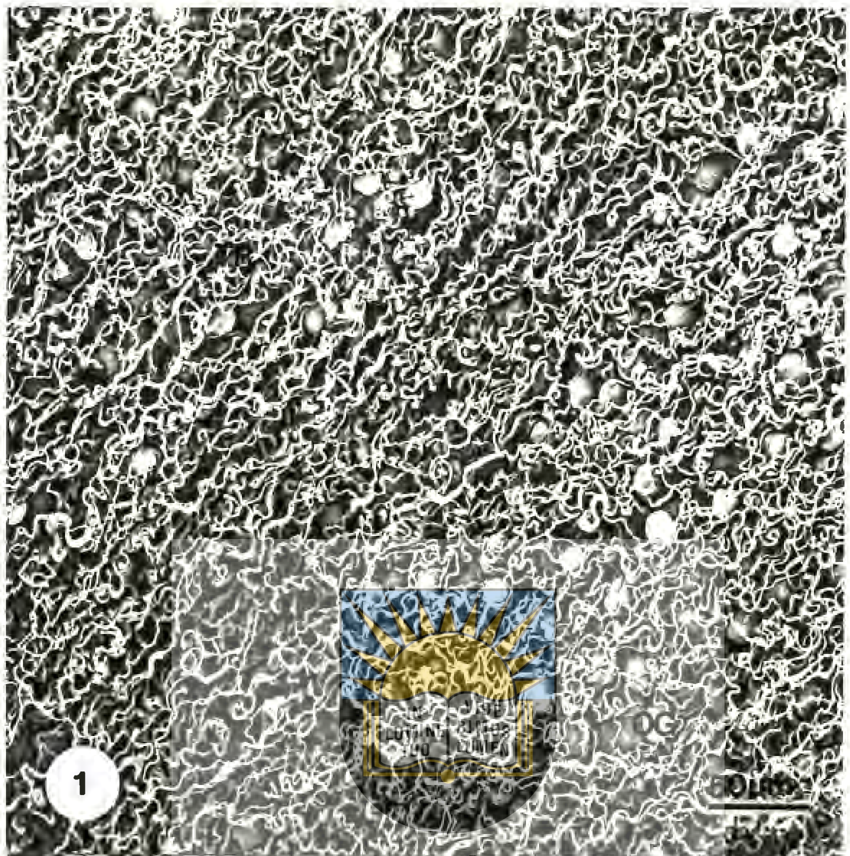
Scanning Electron Microscopy (SEM) results have shown that the leaves and stems of *P. incana* are characterized by uniseriate non-glandular and multicellular glandular trichomes. The dead non-glandular trichomes appeared to be threadlike fibrous in nature and appeared to be shielding the oil glands and epidermis, and they were covering almost the large portion of the epidermal surface (figs.1, 2, 4 and 6). These trichomes were densely populated on the leaf and stem surface, and their characteristic whitish epidermal layer rendered the plant visibility from afar as grey patches in grassland (figs. 1 - 6). They appeared to be tubular and fleshy in nature at the early stages of development when they are viewed by scanning electron microscopy. They seemed to be formed by the crystalline aggregates of cellulose molecules which constituted the well-organized macrofibrils (figs. 1-5). These fibrous threadlike non-glandular trichomes which were dead at maturity, appeared to be shielding and protecting the glandular trichomes, epidermal layer and the stoma (figs. 4, 5, 6 and 7).

The mode of development of non-glandular trichomes appeared to be a basipetal development (fig. 8) As this basipetal development proceeded from the terminal end to the base of the non-glandular trichome, the mature portion of the trichome became dehydrated and fibrous (fig. 5, 8 and 9). However, the basal portion of the non-glandular trichome remained alive without being dehydrated (fig. 8). The other common characteristics which is associated with the maturation of the non-glandular trichome, was the development of the spongy-like structures (fig. 5, 10 and 12). These structures appeared to be more dominant where the epidermal layer of leaves or stems are characterized by the number of matured non-glandular trichomes and are also associated with the oil glands (fig. 5 and 12).

In some instances, a complete growth of these non-glandular trichomes often resulted in their removal from the stem epidermis, as epidermis was substituted by the periderm during the development of the secondary growth, while the basal cell remained alive when the progressive growth continued in a basipetal direction (fig. 8).

Fig. 1. This is an electron micrograph of *P. incana* leaf at low magnification showing the surface with high distribution of glandular and non-glandular trichomes. TR = Trichome, OG = Oil gland.

Fig. 2. This is an electron micrograph of *P. incana* leaf at high magnification showing distribution pattern of glandular and non-glandular trichomes in relation to stomata. TR = Trichome, OG = Oil gland, E = Epidermis, S = Stomata



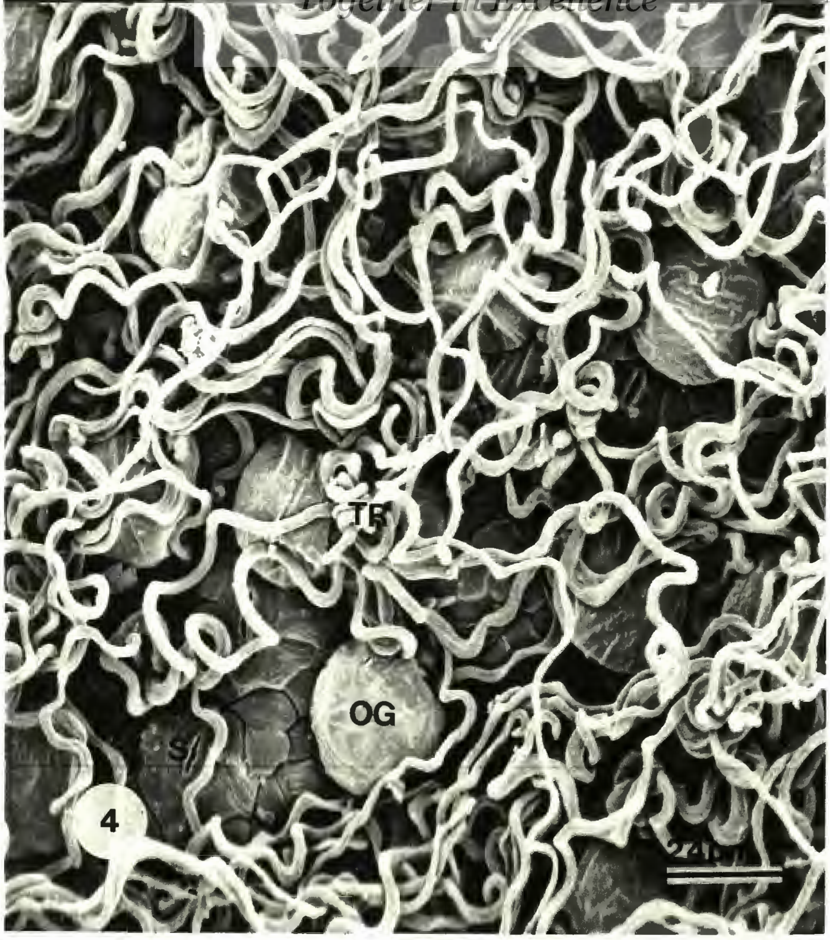
1

2

Fig. 3. This is an electron micrograph of young *P. incana* stem displaying the characteristic glandular trichome with a shrivelled epidermis and fibrous-like non-glandular trichomes. TR = Trichome, OG = Oil gland, E = Epidermis.

Fig. 4. This is an enlarged electron micrograph of young *P. incana* leaf displaying the characteristic pattern of high distribution of glandular and non-glandular trichomes on the leaf surface. TR = Trichome, OG = Oil gland, S = Stomata.

S
TR
OG
3
15um

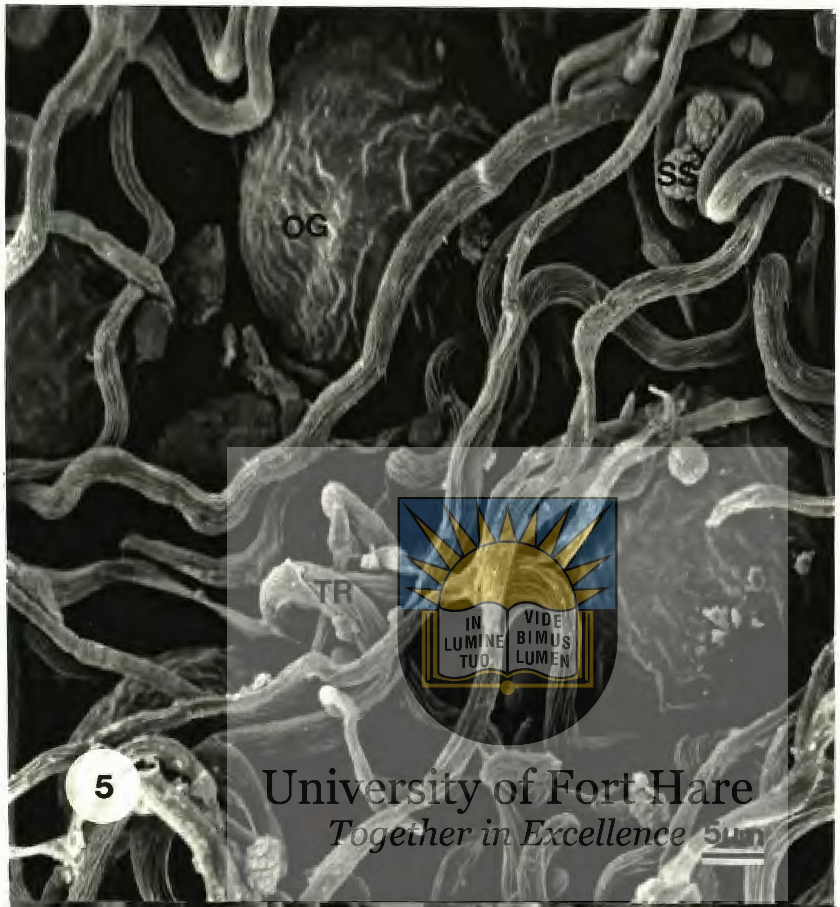


TR
OG
S
4
24µm

Fig. 5. This is an electron micrograph of *P. incana* leaf showing the interwoven fibrous non-glandular trichomes which proctect the glandular trichomes. Note the spongy structures which start to develop on the non-glandular trichome.

TR = Trichome, OG = Oil gland.

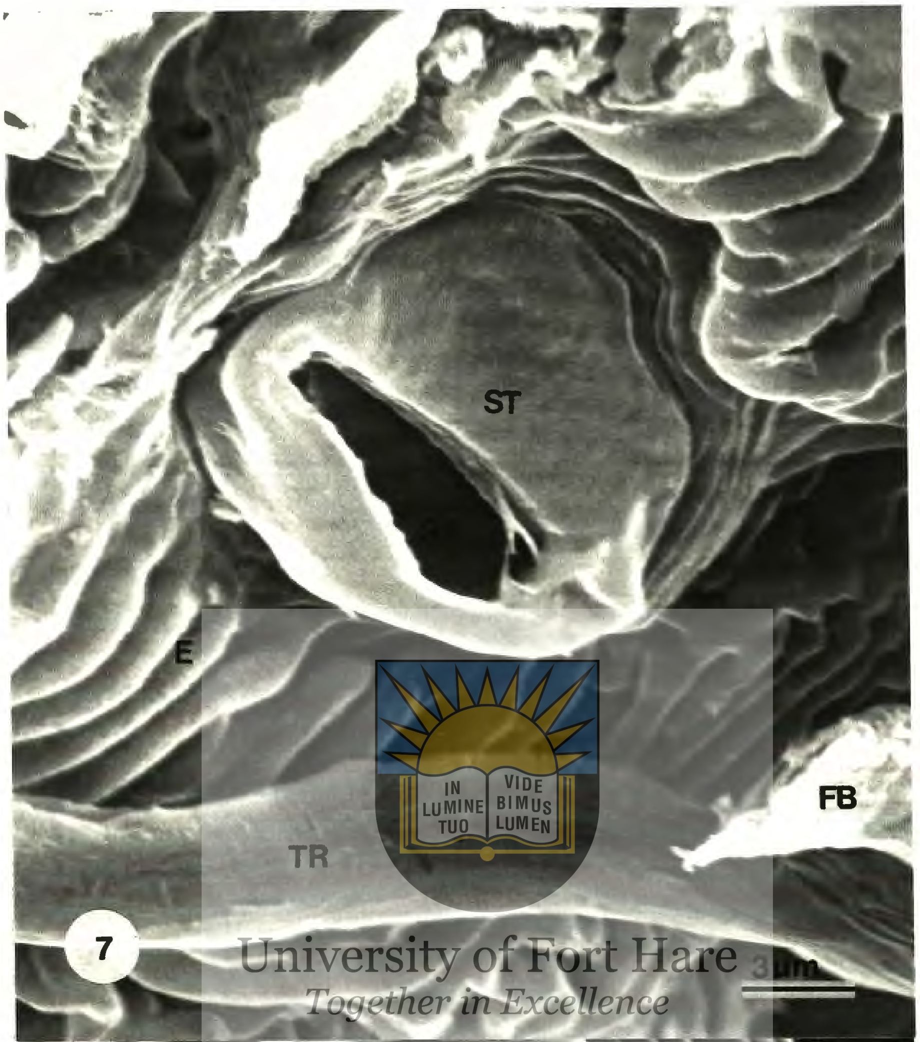
Fig. 6. This is an electron micrograph of an old *P. incana* stem showing the surface of the stem with a dense network of the fibrous non-glandular trichomes. Note the low distribution of the glandular trichome. TR = Trichome, FB = Fibrous end, OG = Oil gland, E = Epidermis


SS
OG
TR
5
5µm


E
FB
OG
T
6

Fig. 7. This is an electron micrograph of *P. incana* leaf at high magnification showing the characteristic shape of stomata, the protective nature of non-glandular over the grooved epidermal surface which is assumed to adsorb the essential oils. FB = Fibrous end, E = Epidermis, SP = Stomatal pore, ST = Stomata.

Fig. 8 This is an electron micrograph of *P. incana* stem showing a similar pattern of grooved epidermis. Note the fibrous thread-like structure of non-glandular trichomes due to progressive basipetal development. TR= Trichome, FB = Fibrous end, BC = Basal cells, E = Epidermis.


ST
E
FB
TR
7
3um


BC
E
TR
8
FB
5um

In contrast, the glandular trichomes resembled a sac like structures that form a protrusion outside the epidermal layer and they are relatively club shape (figs. 9, 11 and 12). During the early stage of leaf and stem development, glandular trichomes appeared to be scattered in a densely random pattern (figs.1, 2, 4 and 5). As the stems become mature, these glandular trichomes appeared to decrease, their distribution were far apart and the number of glandular trichomes decreased progressively particularly in the stem (figs. 6, 9 and 11). In contrast, these glandular trichomes were more concentrated in the leaf vein (fig. 12). The glandular trichome cuticular sacs appeared to be intact, shrivelled and hard during the early stage of the oil gland development (figs. 2, 3, 5 and 9). As the oil gland cells approached maturity the surface seemed to be smooth, as these oil glands cell walls were transformed to cuticular sacs (figs. 11 and 12). A further development resulted to the accumulation of the essential oil in the oil glands, and consequently to the rupturing of the cuticular sacs (figs. 13 and 14)

4.2 Light microscopy and ultrastructure investigation

4.2.1 Structure of the leaf.

The leaf of *P. incana* was found to be dorsi-ventrally flattened and contained a continuous layer of palisade parenchyma cells both the abaxial and adaxial surface (figs. 15 and 16). These parenchymatous cells occupied almost the entire space of the leaf and therefore appeared to be the main photosynthetic tissue which formed a palisade mesophyll . However, there were inner tissue of parenchymatous cells that are associated with the vascular bundles (figs. 15 and 16). Since the anatomy of *P. incana* had a characteristics of a C_3 anatomical syndrome, there was no specialization as far as chloroplast dimorphism was concerned. A small number of these chloroplasts is visible in the ordinary parenchymatous cells associated with the vascular tissue compared to a high chloroplasts density in palisade tissue (figs. 16 - 18). These chloroplasts of palisade tissue appeared to contain some starch grains than the ordinary parenchyma cells associated with the vascular tissue.

Fig. 9 An electron micrograph of *P. incana* leaf showing the arrangement of glandular trichomes and consistent occurrance of the non-glandular trichomes throughout the year.TR = Trichome, OG = Oil gland, E = Epidermis

Fig. 10 This is an electron micrograph of *P. incana* stem showing a cluster of advanced cauliflower-like spongy structures attached to fibrous non-glandular trichome. TR = Trichome


E
OG
TR
9

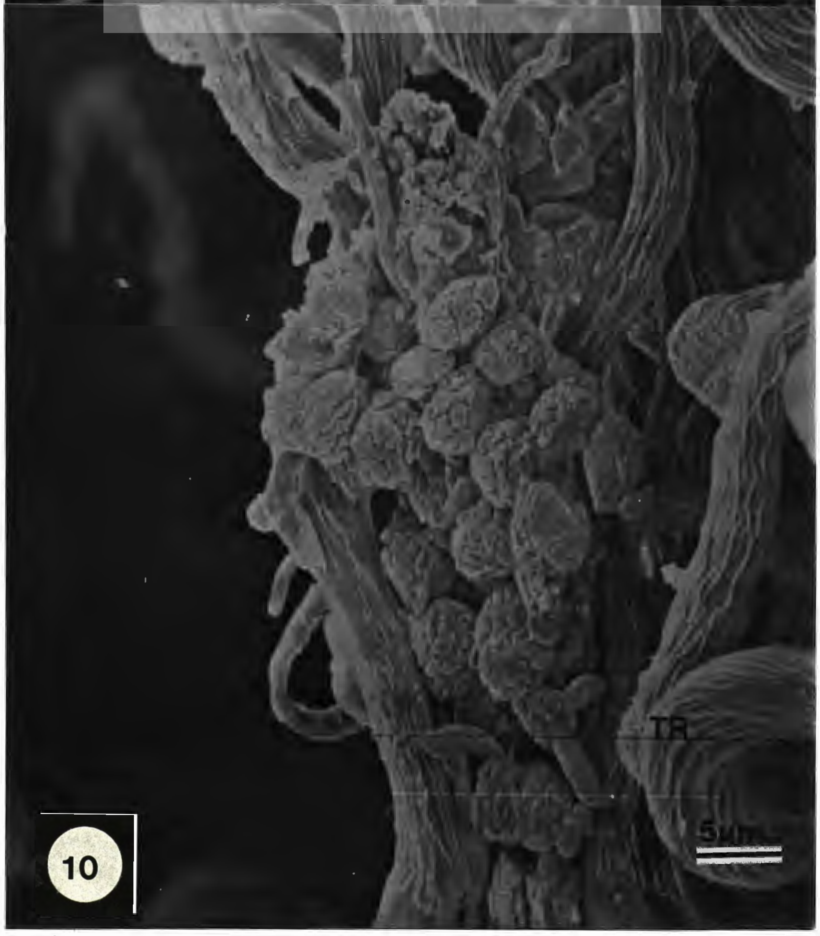

TR
5µm
10

Fig. 11. This is an electron micrograph of *P. incana* stem showing the characteristic glandular cell which was about to release essential oil. Note the distended cuticular sac. OG = Oil gland.

Fig. 12. This is an electron micrograph of *P. incana* leaf showing the arragement of glandular trichomes some of which have released the essential oil after the rupture of the cuticular sac. Note the developing oil gland cell and the distribution pattern of the non-glandular trichome. TR = Trichome, OG = Oil gland, ST = Stomata, UE = Upper epidermis


OG
11
5um


ST
TR
UE
OG
12

Fig.13. This is an electron micrograph of *P. incana* leaf after releasing the volatile compounds during distillation process. Note the collapsed cuticular sacs of the distilled leaves. CS = Cuticular sac, ST = Stomata, E = Epidermis.

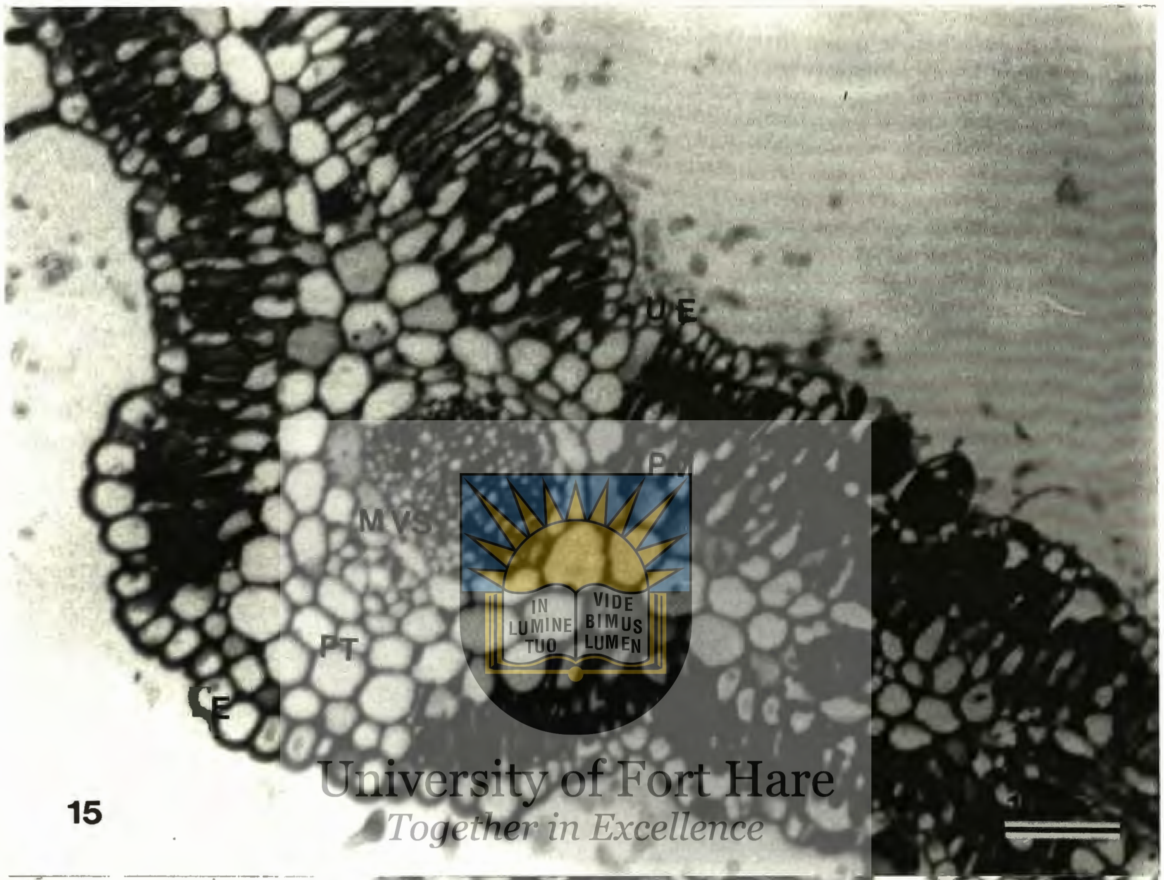
Fig. 14. This is an electron micrograph of *P. incana* stem after releasing the volatile compounds during distillation. TR = trichome, BC = Basal cell, E = Epidermis

13

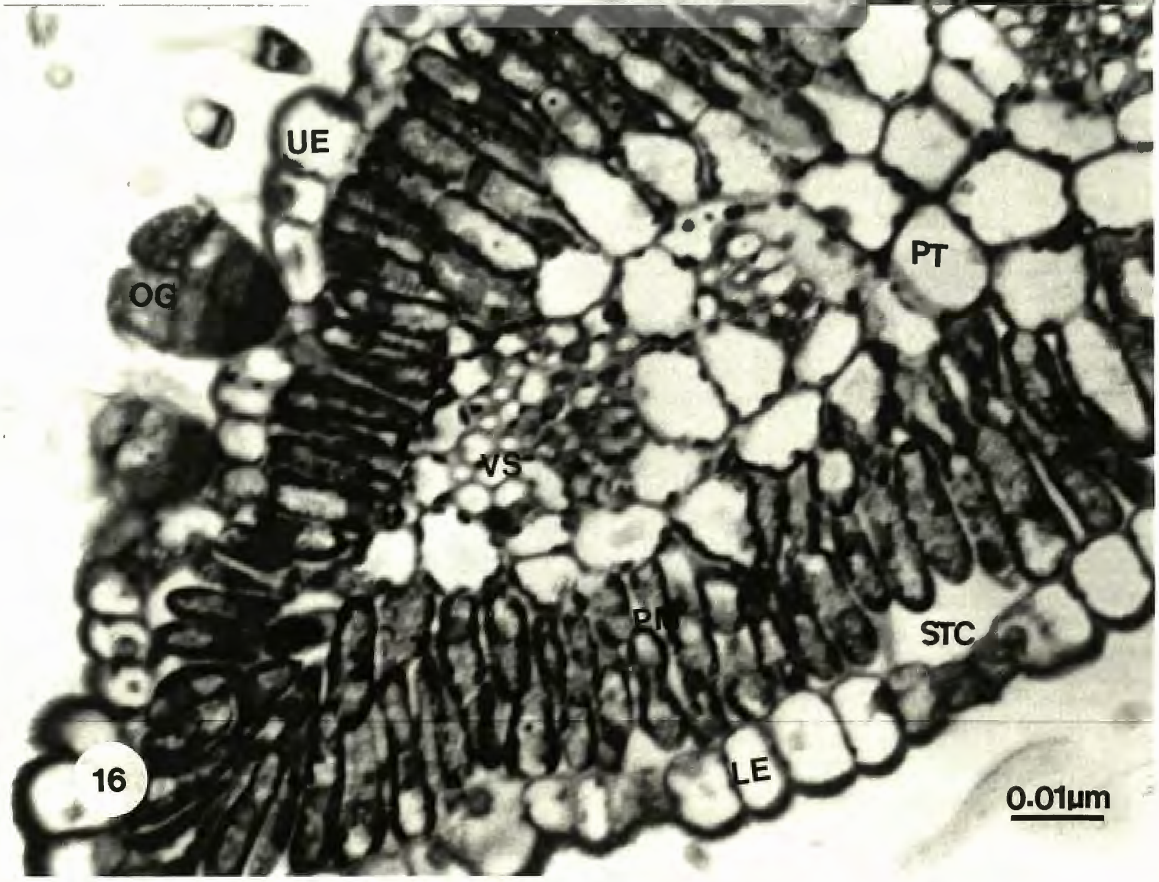
14

Fig. 15. The transverse section of *P. incana* leaf showing the abaxial and adaxial epidermis with specialized epidermal cells which contained glandular and non-glandular trichomes. UE = Upper (adaxial) epidermis, LE = Lower (abaxial) epidermis, MVS = Main Vascular tissue, PM = Palisade mesophyll cells,
PT = Parenchymatous tissues.

Fig. 16. This is the transverse section of *P. incana* leaf showing the vascular tissue of the minor veins, phloem, palisade mesophyll tissues, upper and lower epidermis. VS = Vascular tissue, PM = Palisade mesophyll cells, UE = Upper epidermis, LE = Lower epidermis, PT = Parenchymatous tissues,
STC = Stomatal cavity, OG = Oil gland.



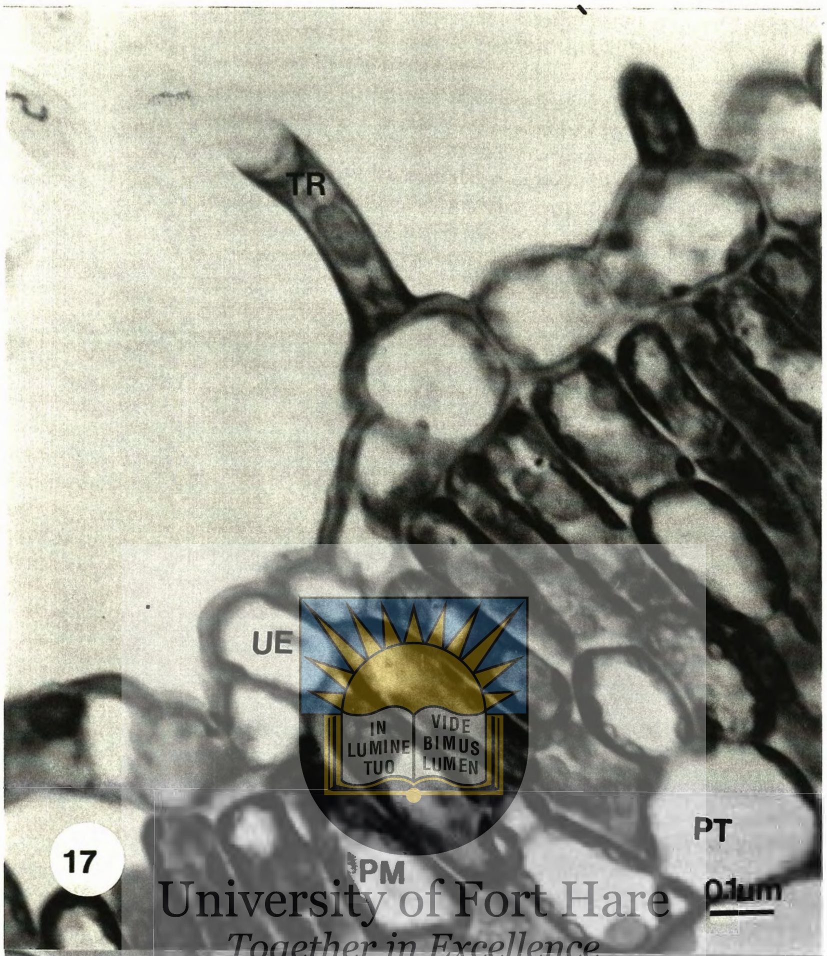
15

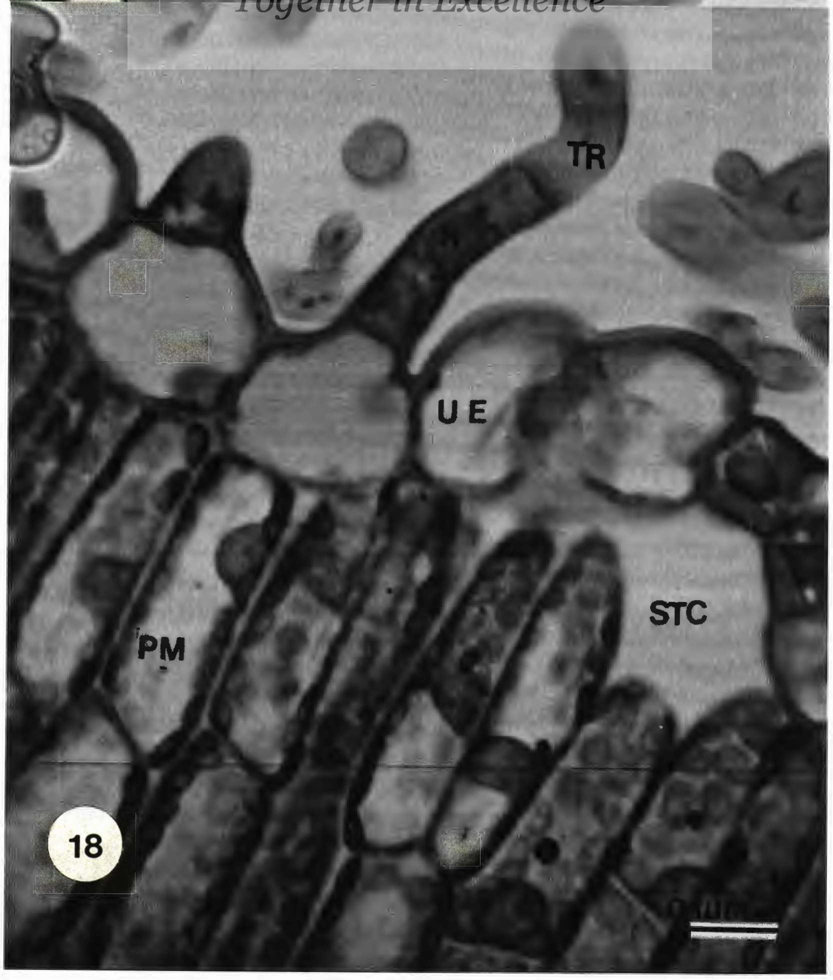


16

Fig. 17. This is the transerve section of *P. incana* leaf showing mature non-glandular trichomes and photosynthetic palisade mesophyll tissues below.
TR = Trichome, UE = Upper epidermis, PT = Parenchymatous tissues,
PM = Palisade mesophyll cells.

Fig. 18. An transerve section of *P. incana* leaf showing the developing trichomes and compact palisade mesophyll cells. TR = Trichome, N = Nucleus,
UE = Upper epidermis, STC = Stomatal cavity, PM = Palisade mesophyll cells.


TR
UE
PT
PM
17
0.1um


TR
U E
STC
PM
18

The leaves were also charecterized by the main vascular bundle which is associated with intermediate small vascular bundles (fig. 15). The large veins were located in the median part of the leaf and formed the central vein from which the small veins branched (figs. 15 and 16). The arrangement of the xylem vessels at the minor veins at the edges of the leaf lamina were obliquely compared to the xylem vessels of the vascular bundles found towards the middle which were facing the upper surface (fig. 16). It appeared that there were no connection between the vascular tissues and the glandular cells, however, it was noticed that a communication between the palisade, the basal glandular cells and stalk cells occurred through the plasmodesmata present on both sides of the epidermal cells connecting these palisade and stalk cells.

The epidermal layer was characterized by a thick cutinized cell (figs. 15 - 20). These thick cutinized epidermal cells were associated with the highly specialized cells non-glandular trichomes, glandular trichomes and various stomatal pores which are bordered by thick-walled subsidary cells (figs. 15, 16 and 18). The glandular trichomes were always bordered by the thick serrated edge cell walls of the adjacent epidermal cells (figs. 16).

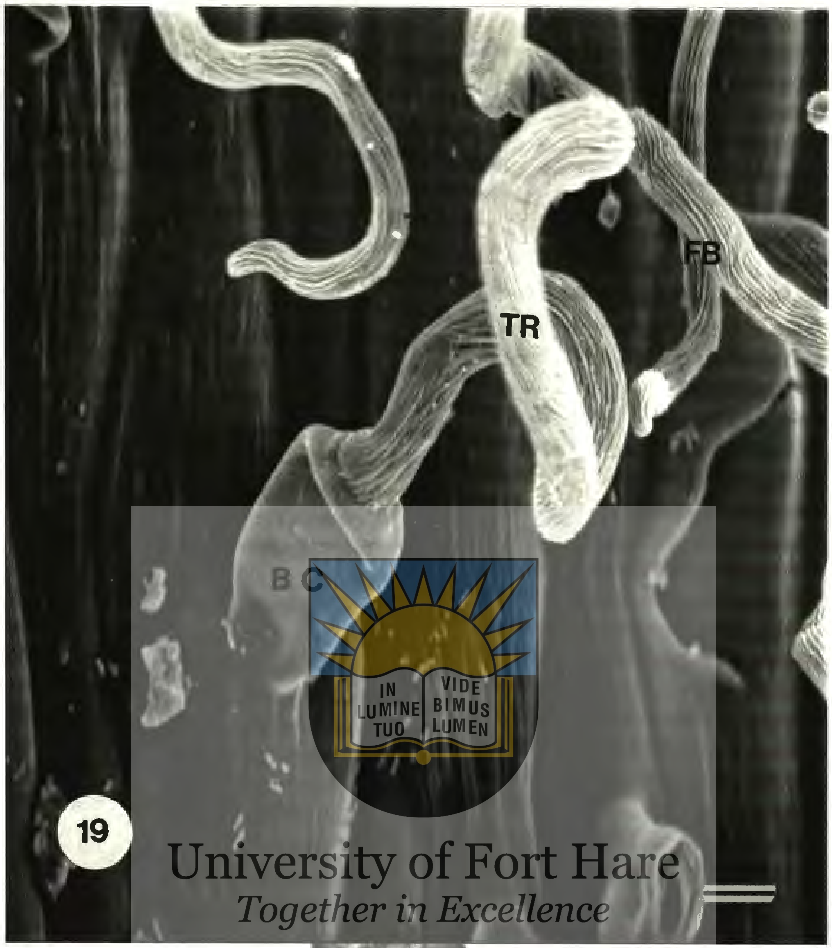
4.2.2 Non-glandular trichomes

The non-glandular trichomes of *P. incana* were abundant, forming a long dense covering that completely obscured the epidermal surface (figs. 1, 15 and 19). The ultrastructure of the non-glandular trichomes have showed that at an early stages of the trichome development all cells of the trichome are living (figs. 17, 18 and 20). At this young stage of development, the basal cell was characterized by a large nucleus surrounded by a dense cytoplasm (figs. 17 and 20). As these non-glandular trichome cells mature, a progressive growth occurred through anticlinal division (figs. 17, 18 and 20). At maturity the first non-glandular trichome cell which were produced, collapsed and died thereby giving the non-glandular trichome a more or less fibrous appearance (figs. 28 and 20).

Such ontogeny of the non-glandular trichome continued with repeated anticlinal division to produce the progressive cells of the non-glandular trichome (figs. 19). The development of the non-glandular trichome seemed to be more rapid than glandular trichomes.

Fig. 19. An electron micrograph of *P. incana* leaf showing a non-glandular trichome with a dehydrated fibrous end. Note the basipetal development of the non-glandular trichomes. E = Epidermis, TR = Trichome, BC = Basal cells, FB = Fibrous end.

Fig. 20. This is an ultrastructure of *P. incana* leaf non-glandular trichomes. Note the dense cytosol with a well-defined nucleus at the apical cell. C = Cytoplasm, N = Nucleus, EC = Epidermal cell, TR = Trichome.


TR
FB
BC
19

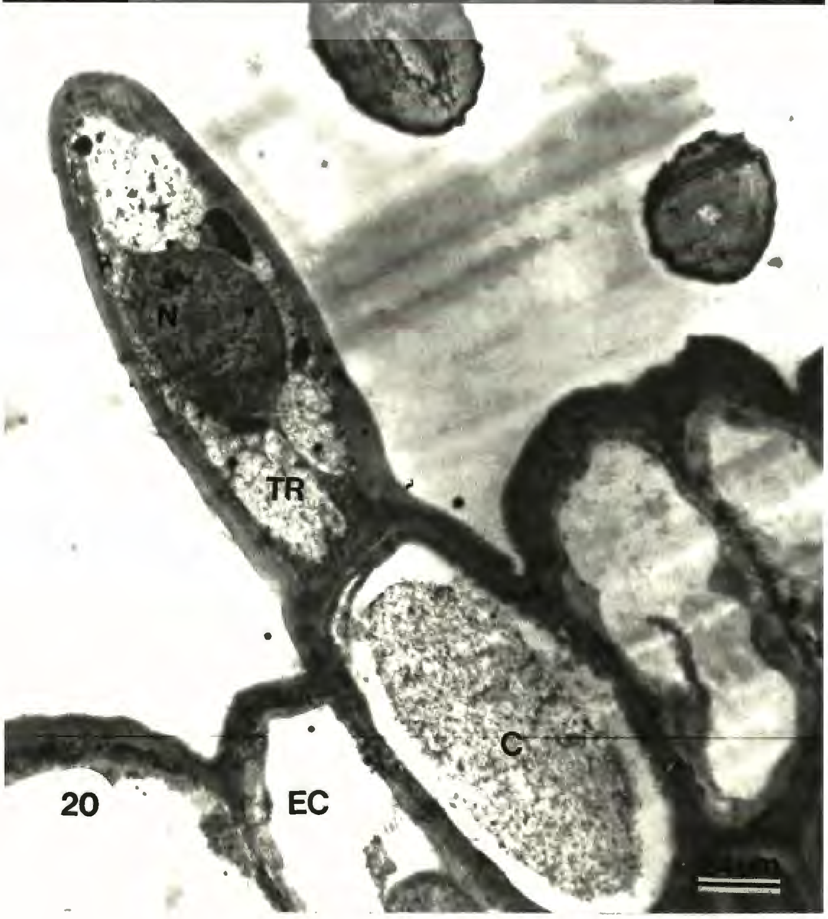
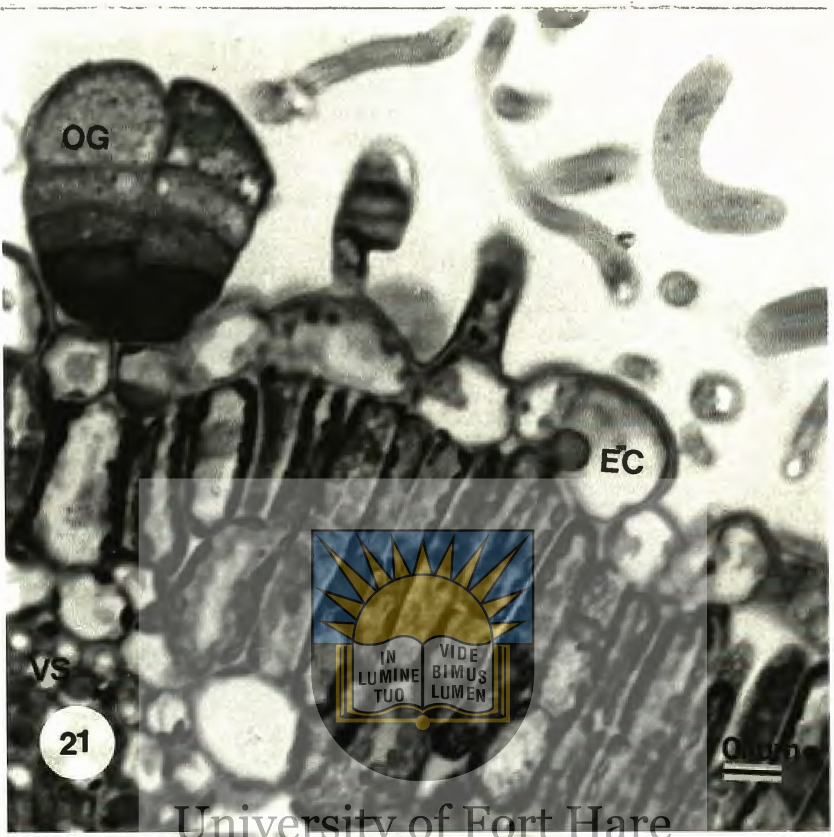

N
TR
C
EC
20

Fig. 21. This is an electron microgrph of *P. incana* leaf showing the epidermal cells, a portion of the palisade tissue and glandular trichome with cuticular sac. EC = Epidermal cell, OG = Oil gland, PM = Palisade mesophyll cells,
VS = Vascular tissue.

Fig. 22. An electron micrograph of *P. incana* leaf showing an early stage of non glandular trichome development, the palisade mesophyll cell below the upper epidermis containing chloroplasts. PM = Palisade mesophyll, N = Nucleus,
TR = Trichome.


OG
EC
VS
21
0.1µm


TR
N
PM
2 2
0.1µm

Fig. 23. This is an electron microgrph of *P. incana* leaf showing the glandular trichome with an eight celled head, and a collapsed cuticular sac. V = Vacoule, H = Head cells, S = Stalk cells.

Fig. 24. This is an electron microgrph of *P. incana* leaf at high magnification showing the glandular trichome head cells. V = Vacuole, H = Head cells.

23

24

Fig. 25. An electron micrograph of *P. incana* leaf exhibited a bulgy epidermal cell at an early glandular or non-glandular trichome development. EC = Epidermal cell, V = Vacuole.

Fig. 26. An electron micrograph of *P. incana* leaf displaying the stalk cells of a developing glandular trichome. Note the presence of plasmodesmata on the cell wall between the epidermal and stalk cells (arrows). H = Head cells, S = Stalk cell, N = Nucleus, Nn = Nucleolus, B = Basal cell, P = Plastids.


25

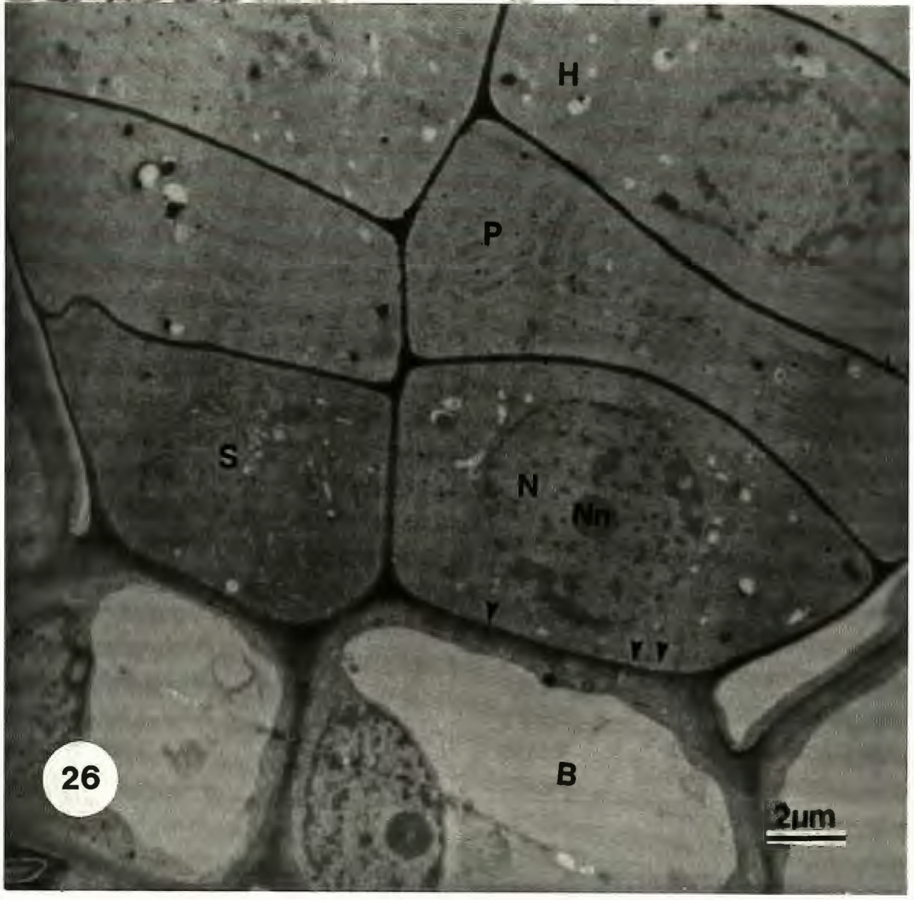
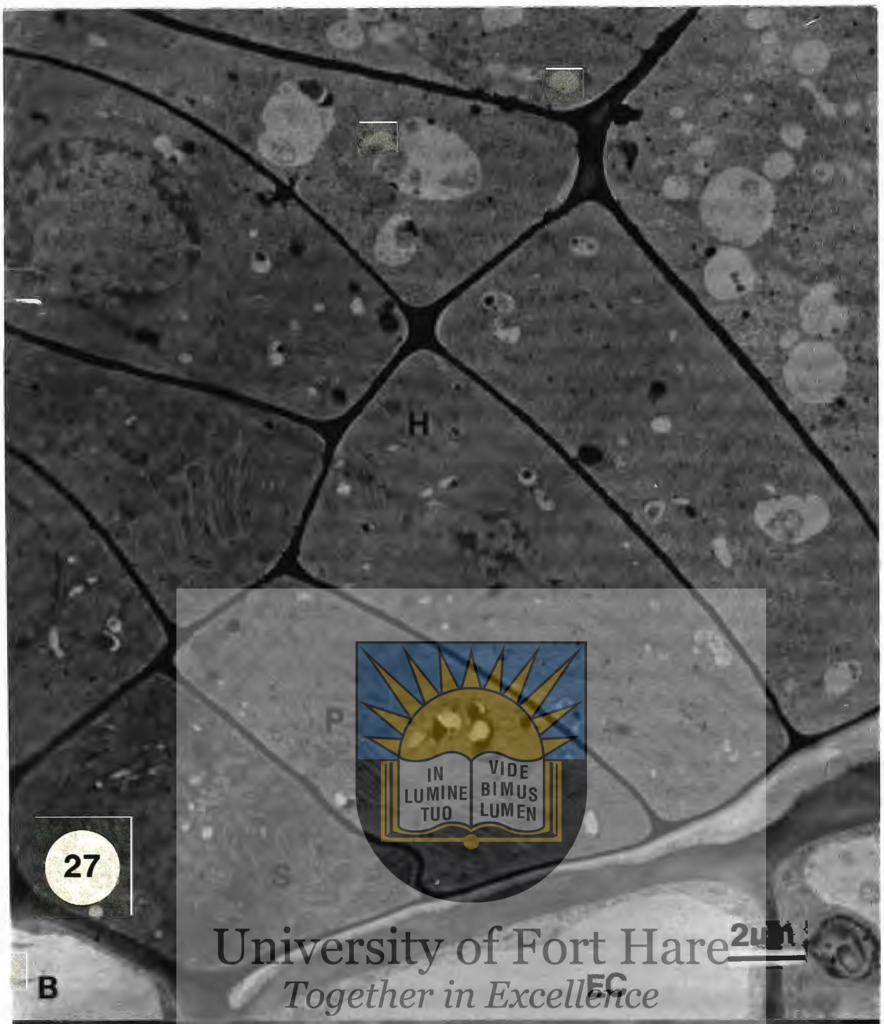
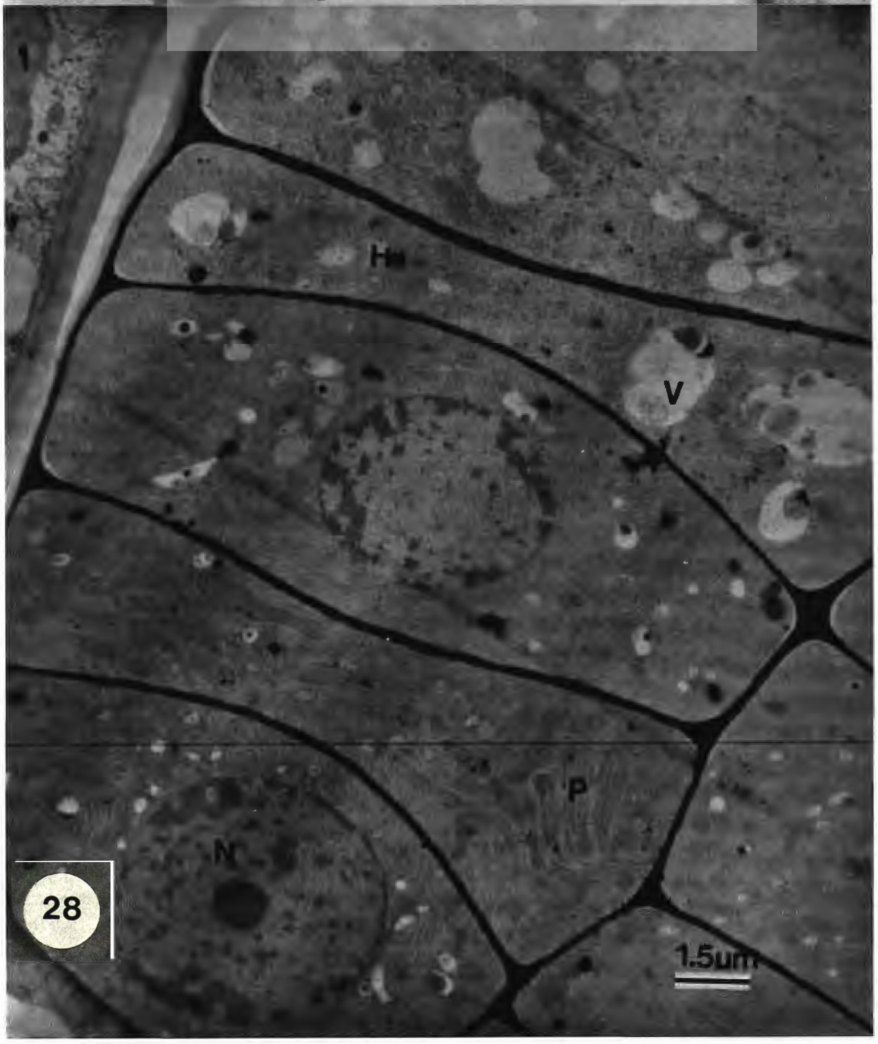

26

Fig. 27. This an electron micrograph of *P. incana* leaf at an early stage of development. A longitudinal section showed both side of gland head. H = Head cells, S = Stalk cells, P = Plastids.

Fig. 28. This is an electron micrograph of *P. incana* leaf at an early stage of development showing a longitudinal section of one side of gland head.
H = Head cells, N = Nucleus, P = Plastids, V = Vacuole.


H
P
S
27
B
EC
2um


H
V
P
N
28
1.5um

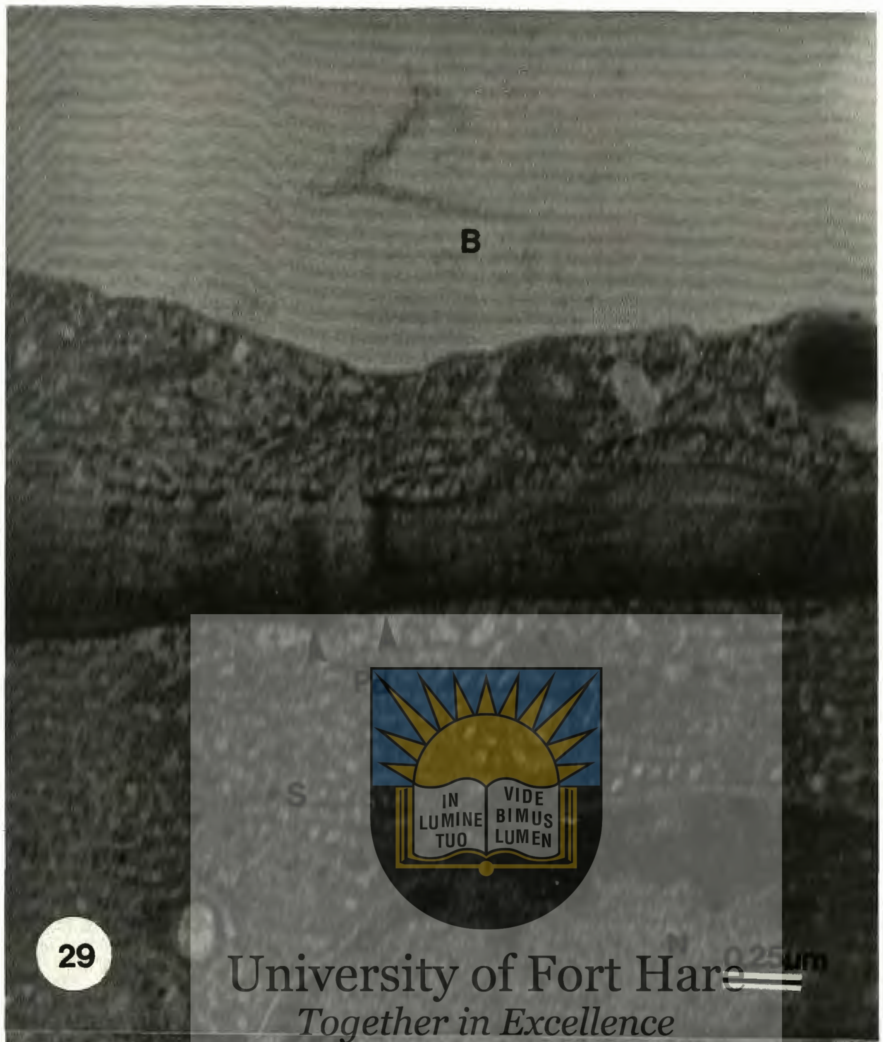
4.2.3 Glandular trichome

The glandular trichomes of *P. incana* were multicellular and biserate, globular to oval in shape (figs. 3, 11, 12, 21 and 23). Transmission electron microscopy have shown that, at the initial stages of trichome development, the expanding epidermal cell had an electron dense cytosol, with a clearly evident large basal vacuole (figs. 21 and 25). At this stage, it was difficult to distinguish whether the cell might be a non-glandular or a glandular trichome, because there was no clear indication of periclinal or anticlinal division with the exception of bulgeness of the epidermal cell which would formed the trichome initials (figs. 21, 22 and 25). The glandular trichome appeared to be originating from two epidermal cell as a result of a periclinal division which was subsequently followed by a anticlinal division to give rise to six or ten glandular head (figs. 21, 23 and 24). Interesting features were the presences of plasmodesmata between the stalk cells and basal epidermal cell of the glandular trichome at the early stages of development (figs. 26 - 30). However, these plasmodesmata were absent in the suberized cell walls of the matured glandular trichome cells (fig. 35). It was also noticed that there were numerous highly elongated granular structures which were considered to be modified plastids. These plastids seemed to occupy most of the volume of the cytoplasm of the cells above stalk cells, but below the apical cells of the developing glandular trichome (figs. 26 - 28). When the glandular trichome was fully mature, the upper surface of the oil gland cells were covered with a cuticular sac (figs. 35 and 38). This cuticular sac appeared to be modified cell wall of glandular cell(s) which occurred on the terminal position (figs. 35). The subcuticular sac became distended due to the accumulation of the essential oils which were produced by the oil gland cells. As the progressive production of the essential oil continued, it resulted to the slow disintegration of the subcuticular sac. The essential oil were subsequently released through pores of the cuticle or more likely after the rupturing of the cuticular sacs (figs. 13, 14, 23, 24, 36 and 37).

The apical cells of the glandular trichome structurally, had a dense cytosol, poorly developed vacuoles with small osmiophilic droplets (figs. 23, 35 - 38). Endoplasmic reticulum system appeared to wrap around the plastids in the oil glands cells (figs. 39, 41, 43 and 44). These plastids displayed a complex tubular structures (figs. 39 - 44).

Fig. 29. This is an electron micrograph of *P. incana* leaf at magnification showing the adjacent cell walls of the epidermis and stalk cell where these two cells are joined together. Note the plasmodesmata. Pd (arrows) = Plasmodesmata

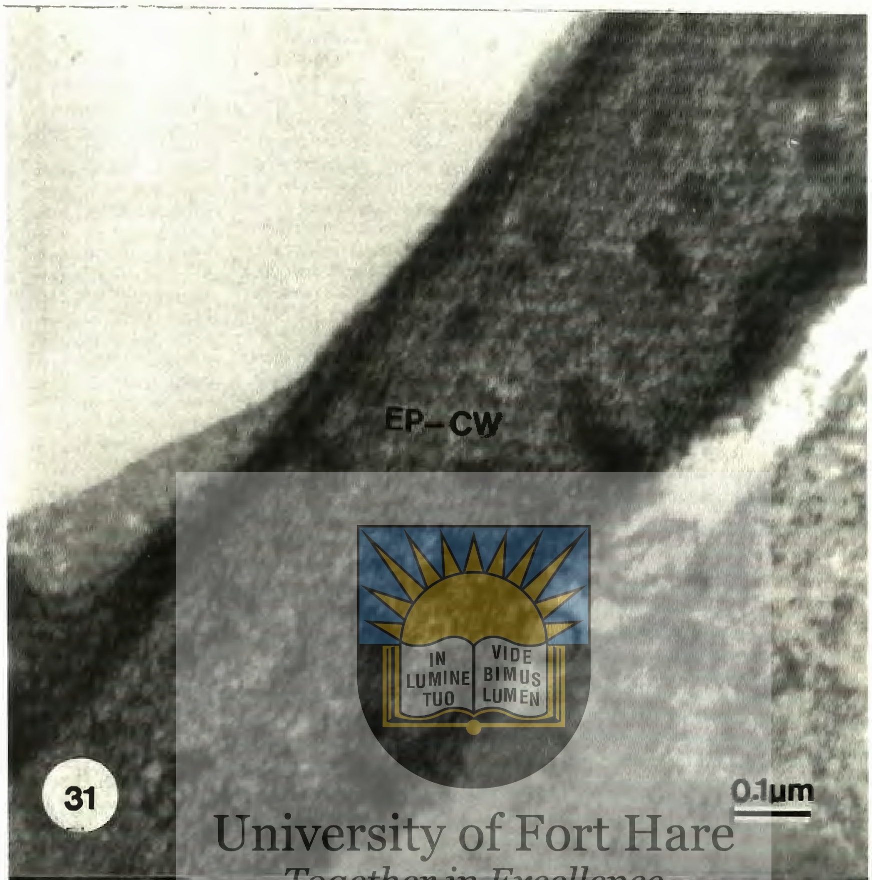
Fig. 30. This is an electron micrograph of *P. incana* leaf at high magnification showing cell walls between the epidermis and stalk cell. Note the presence of plasmodesmata which are assumed to be involved in transport and communication between these two cells. Pd (arrows) = Plasmodesmata


B
P
S
29
0.25 um

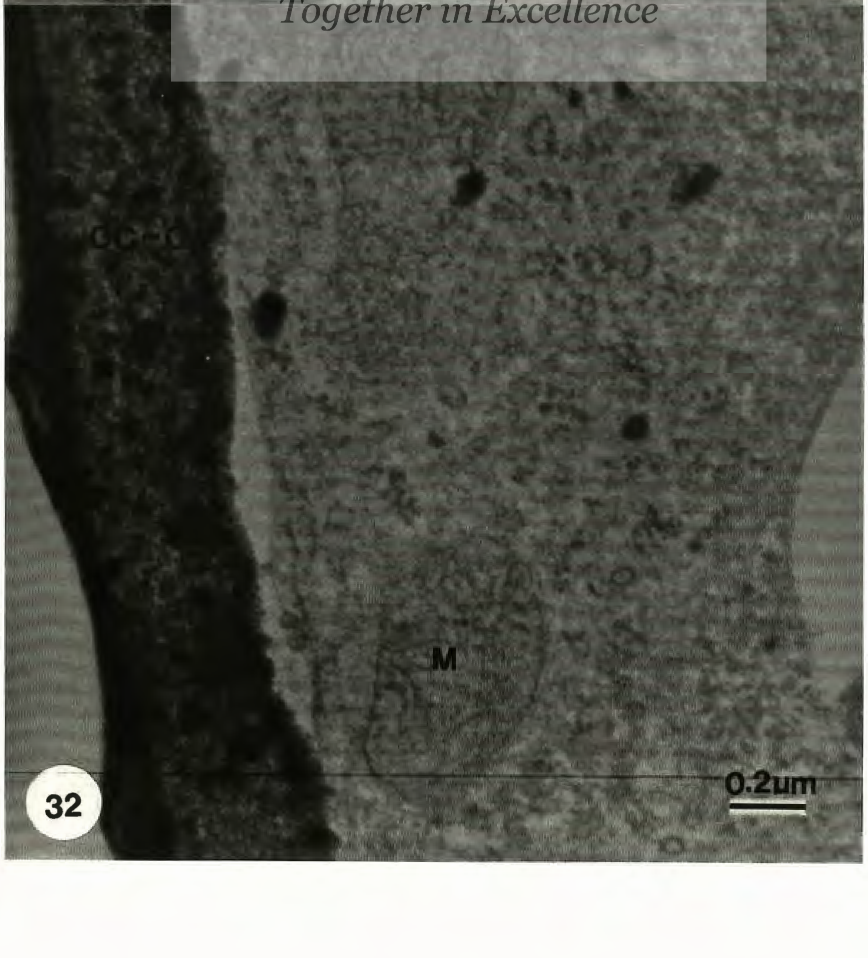

B
Pd
S
30
0.1um

Fig. 31. This is an electron micrograph of *P. incana* leaf showing the epidermal cell wall at high magnifications. EP-CW = Epidermal cell wall.

Fig. 32. This is an electron micrograph of *P. incana* leaf exhibiting the thick suberized cell wall of stalk (basal) cell, which is the lateral position, far from the joining junction between the epidermal cell wall and the stalk cell wall. Note that there is no plasmodesmata. OG-WC = Oil gland cell wall, M = Mitochondria



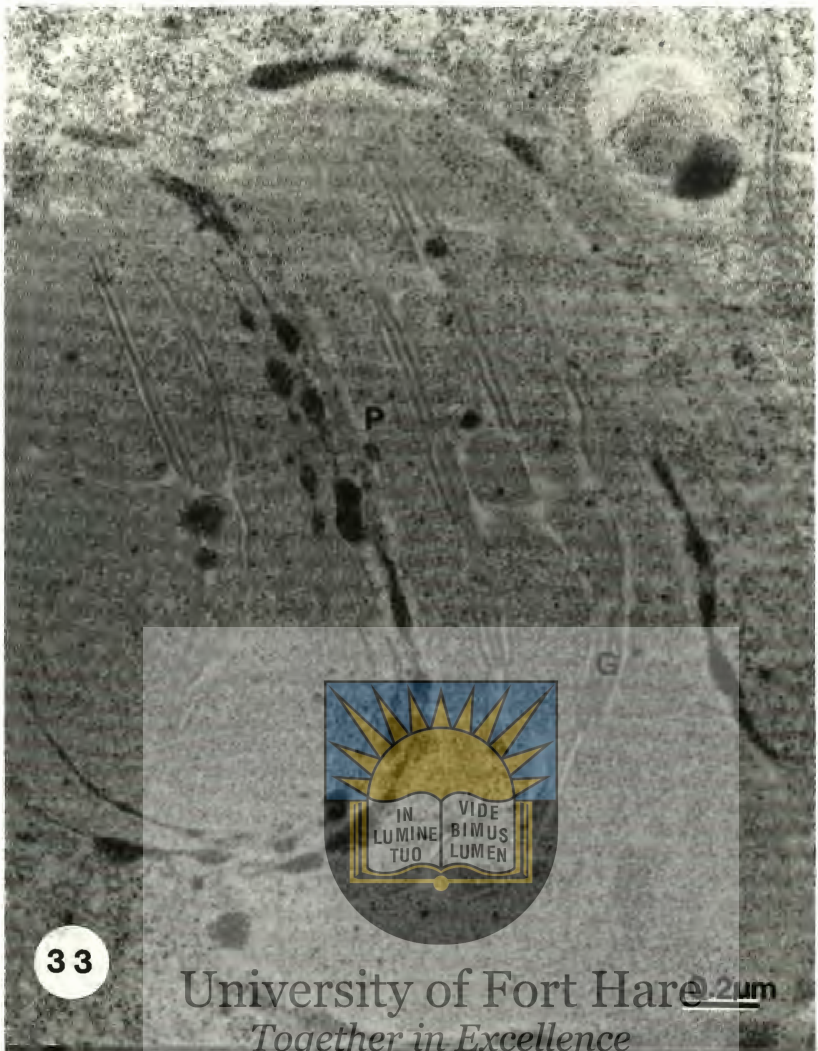
31



32

Fig. 33. This ia an electron micrograph section of *P. incana* leaf showing plastids in the second pair of the oil gland cells. P = Plastids, G = Grana.

Fig. 34. An electron micrograph of *P. incana* leaf showed the first pair of the oil gland cells which contained plastids. P = Plastids, G = Grana.

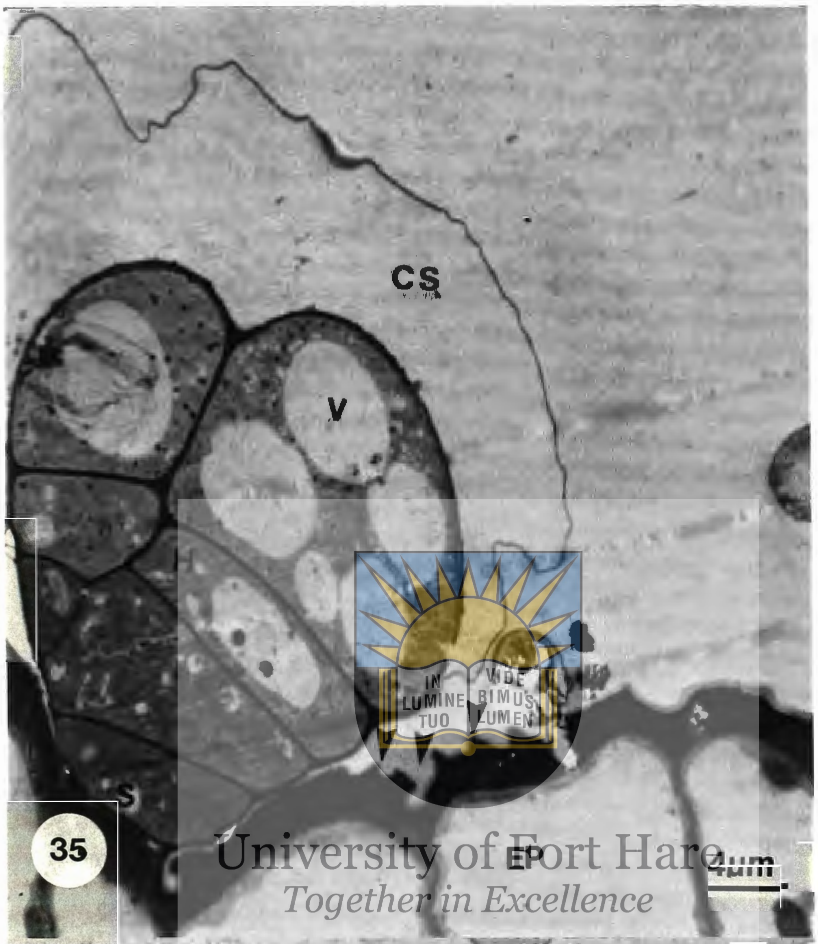


33

34

Fig. 35. This is an electron micrograph of *P. incana* leaf showing a mature oil gland cell before releasing an essential oil. CS = Cuticular sac, V = Vacuole, H = Head cells, S = Stalk cells, Arrows - showing serrated cell wall edges.

Fig. 36. This is an electron micrograph of *P. incana* leaf showing one oil gland cell with a raptured cuticular sac whilst the other oil gland cell still contained essential oil within the distended cuticular sac. CS = Cuticular sac, H = Head cells.


35

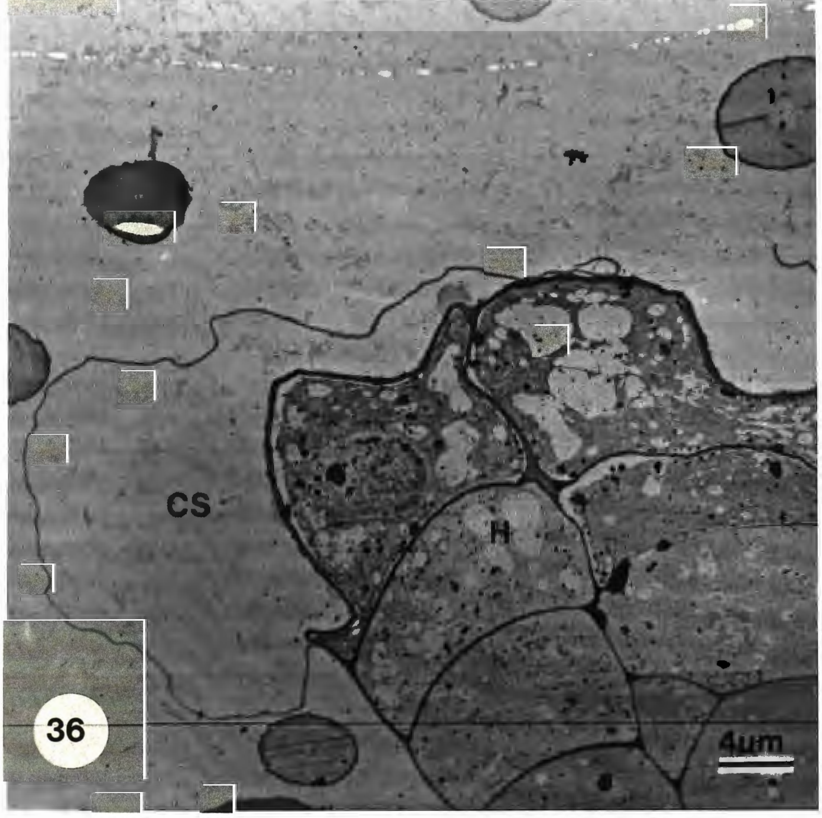
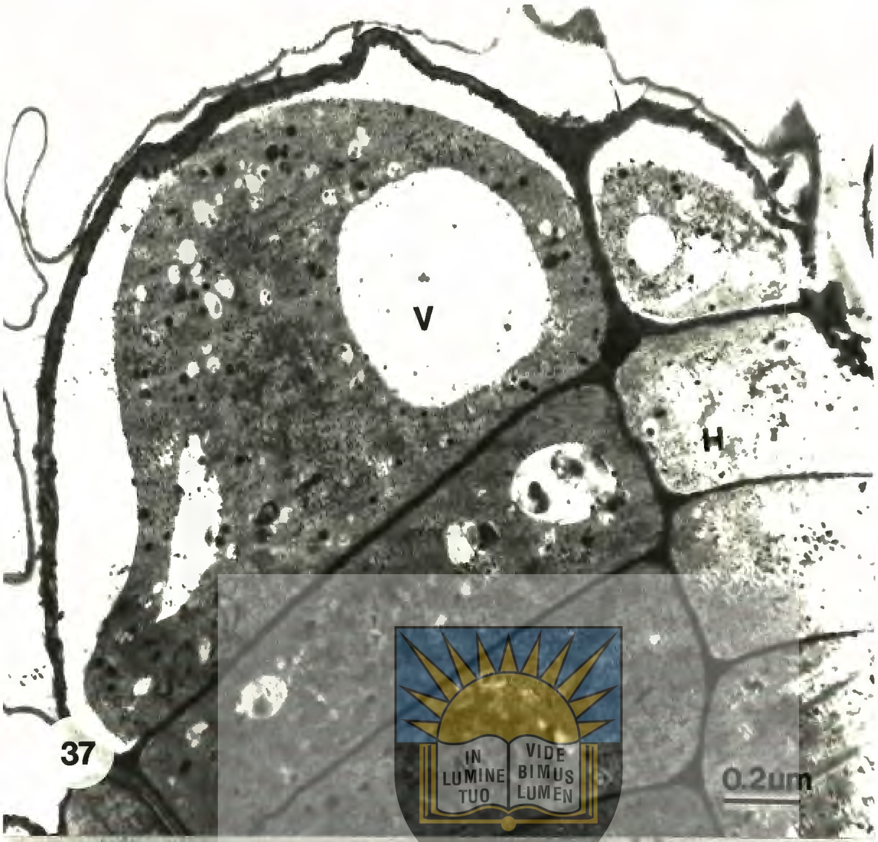
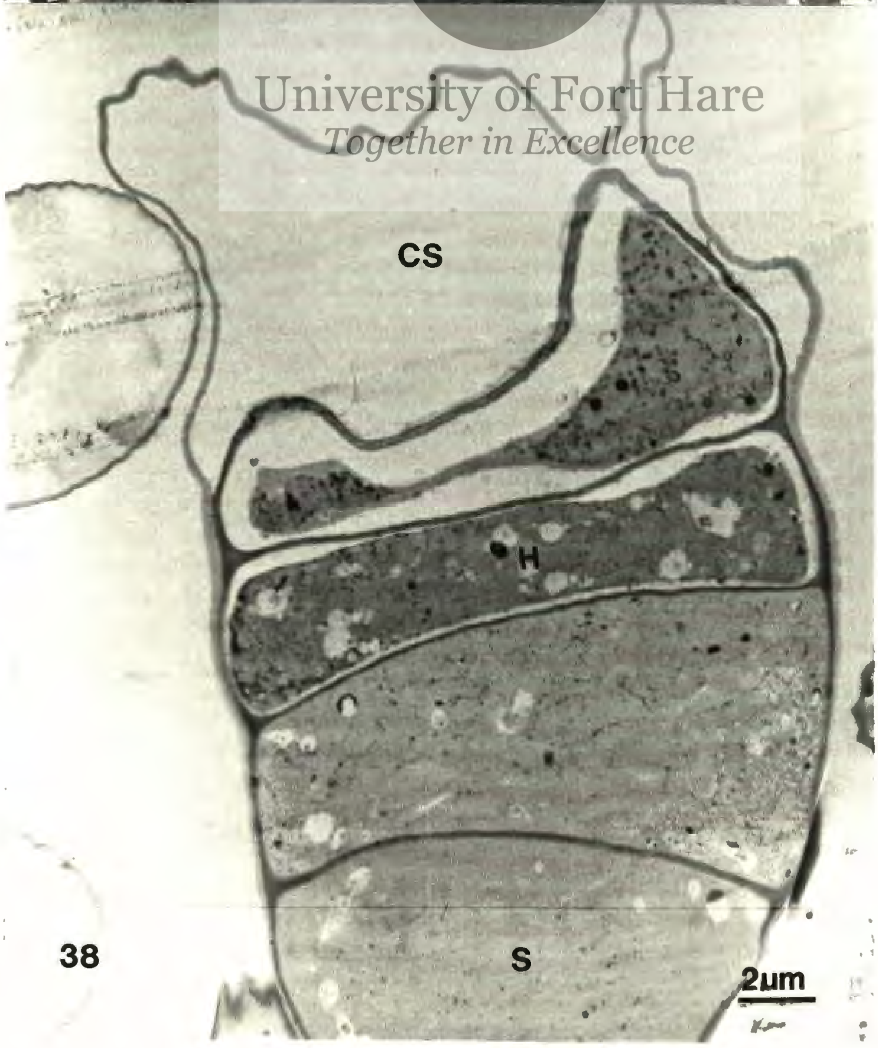

36

Fig. 37. This is an electron micrograph of *P. incana* leaf showing a raptured cuticular sac of oil gland cell at maturity after a release of the essential oil.
V = Vacuole, H = Head cells, N = Nucleus.

Fig. 38. This is an electron micrograph of *P. incana* leaf exhibiting another orientation an oil glands cell with cuticular sac containing essential oil.
CS = Cuticular sac. H = Head cell, S = Stalk cell.


V
H
37
0.2µm


CS
H
38
S
2µm

The significance of the high density of palisade mesophyll cell of the leaf is not yet known to the mature glandular trichomes although their presence at the early stages of glandular trichome development suggested an energy input mechanism to stalk-epidermal cells transport system (figs. 45 and 46). This hypothesis is supported by the presence of plasmodesmata between the stalk cells and basal epidermal cells at the early stages of glandular trichome developmental, however, the cell wall between the stalk cells, glandular trichome gland cell and also the cuticular sac showed no visibility of the transport system or presence of plasmodemata (figs. 31, 32, 47 and 48). .

Characteristic of *P. incana* essential oil

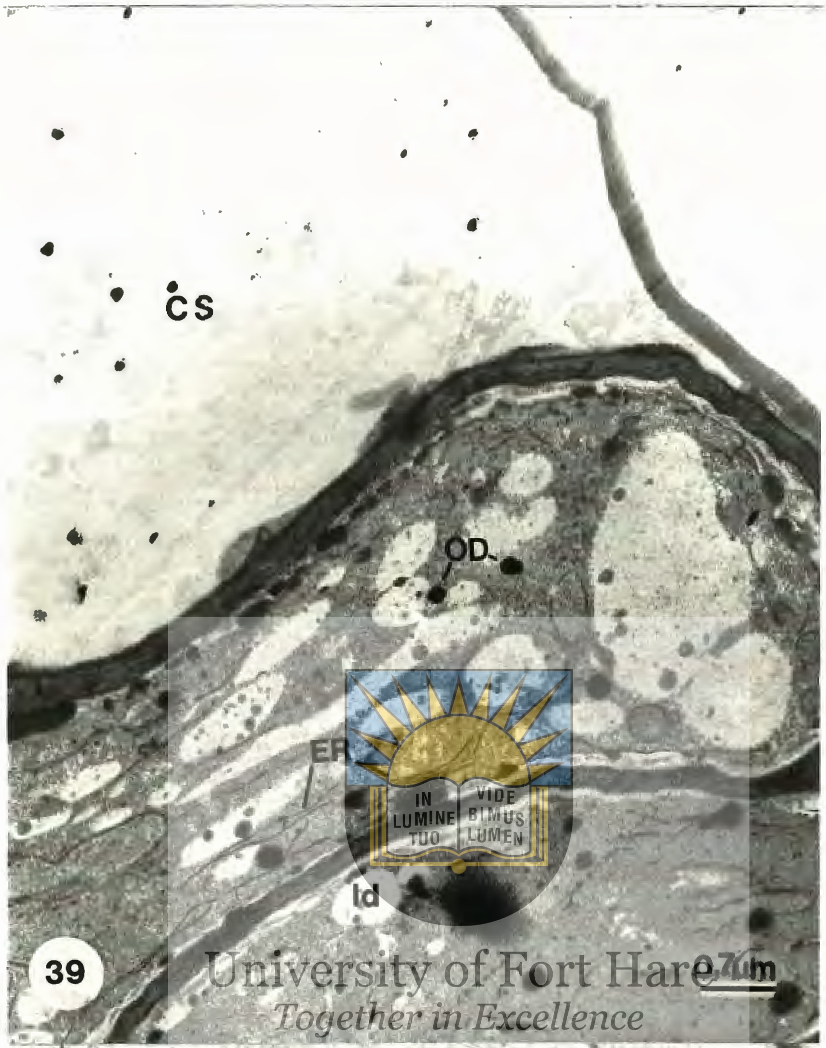
The essential oil obtained from *P. incana* was pale yellow in colour and had a fragrance somewhat reminiscent dwarf pine, siber and fir leaf oil. The oil yield appeared to be high during the flowering season, however it is reduced during the drought periods although high volume was obtained in the May to June months (fig. 66).

The results of the chemical analysis of the essential oil are presented in figs.68 - 76. GC-MS analysis have shown that the spectrum of the essential oil of *P. incana* sample varies. The GC spectrum of the oil sample in June had the characteristic speaks which appeared throughout the year these speaks seemed to represent the compounds : Alpha-pine, Beta-pinene + beta myrcene, sabinene and 1.8 Cineole, However the spectrum of the samples obtained between March and August respectivelly appeared to be characterized by the additional terpenoids like, Beta-thujene, dl-Limonene, Alpha terpinene and Delta-4 carene.

The oil glands are presumed to be the site of monoterpene as microscopic examination has indicated that, their extracellular secretory space beneath the cuticular sacs are filled with terpene at a very early stage, before the leaves have fully expanded (the results are not included).

Fig. 39. The enlarged electron micrograph of *P. incana* leaf displaying the first of gland cells of the glandular trichome with endoplasmic reticulum, osmiophilic droplets and cuticular sac. ER = Endoplasmic Reticulum. OD = Osmiophilic droplets, CS = Cuticular sac, ld = lipid droplets.

Fig. 40. The electron micrograph of *P. incana* leaf showing the second pair of oil gland cells with organized plastids and osmiophilic droplets. P = Plastids, OD = Osmiophilic droplets, PL = Plasmalemma.

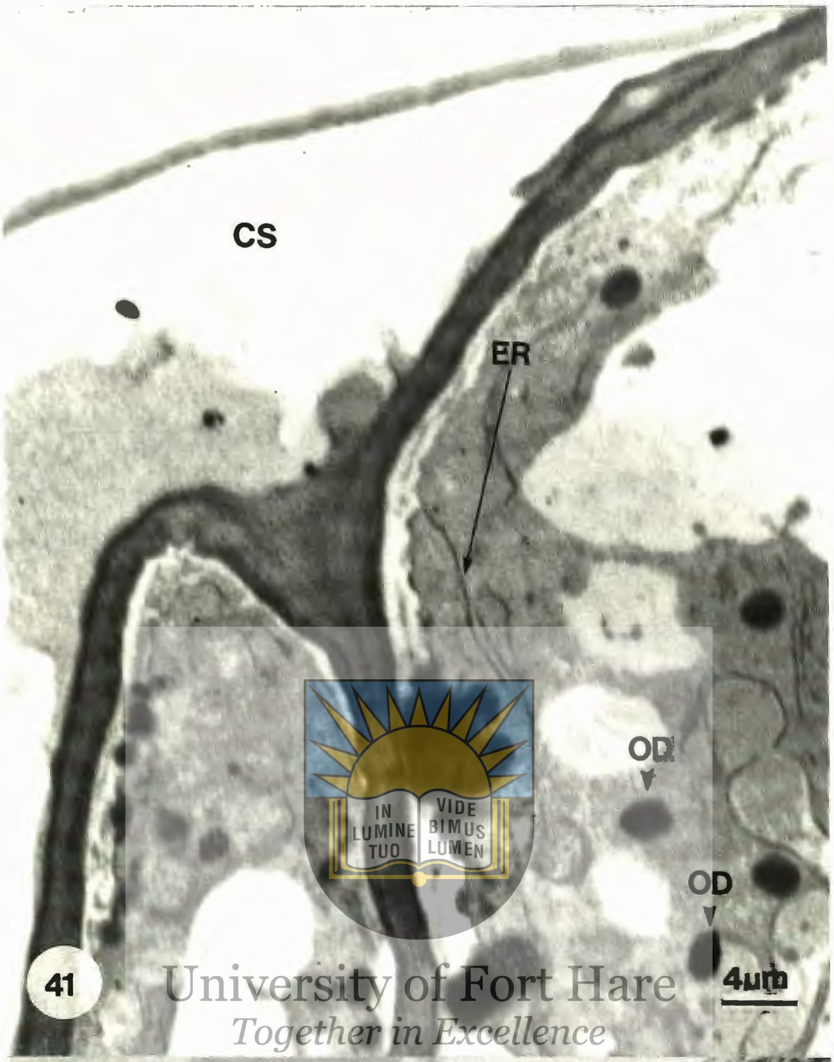


39

40

Fig. 41. The enlarged electron micrograph of *P. incana* leaf showed the apical gland cells (first pair of oil gland cells) with endoplasmic reticulum (ER) and Osmiophilic droplets occupying predominanlty the peripheral position.
ER = Endoplasmic Reticulum. OD = Osmiophilic droplets, CS = Cuticular sac.

Fig.42. The electron micrograph of *P. incana* leaf exhibited the thick the glandular trichome cell wall a with well defined plasmalemma.
PL = Plasmalemma.



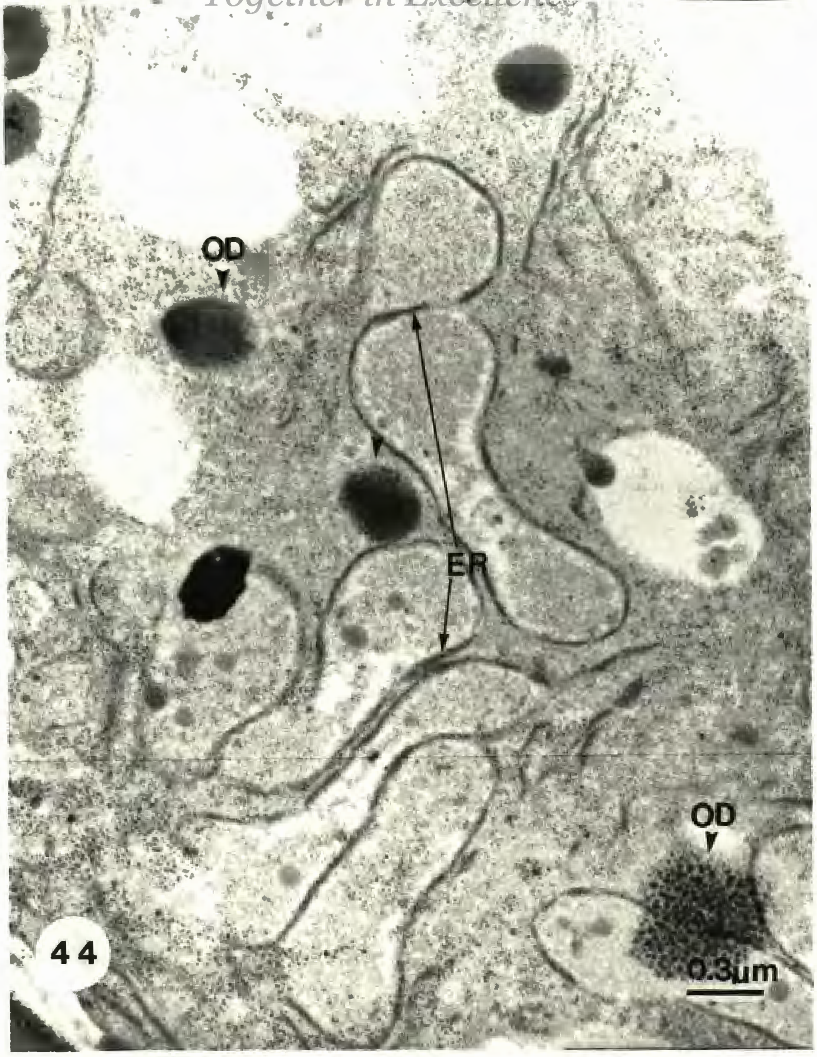
41

42

Fig. 43. The micrograph of *P. incana* leaf showing the lossed arranged endoplasmic reticulum with the osmiophilic droplets. ER = Endoplasmic reticulum, OD = Osmiophilic droplet.

Fig. 44. The micrograph of *P. incana* leaf at high mignification exhibited the loosely arranged endoplasmic reticulum and the osmiophilic droplets.
ER = Endoplasmic reticulum, OD = Osmiophilic droplet.

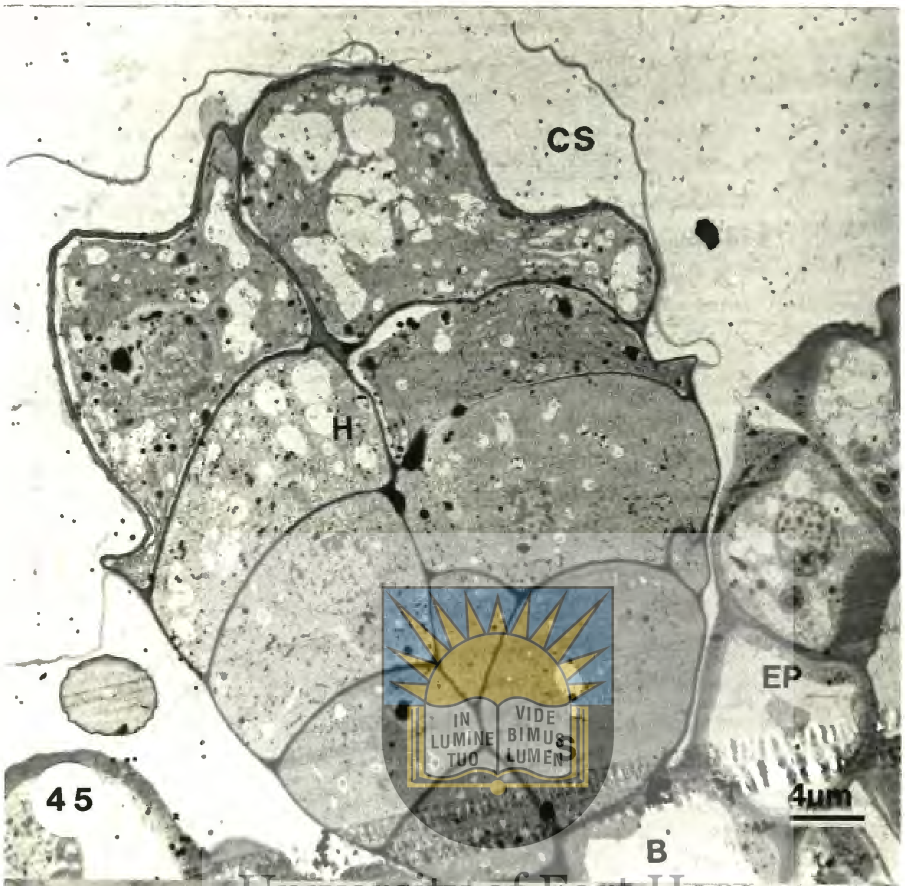
43



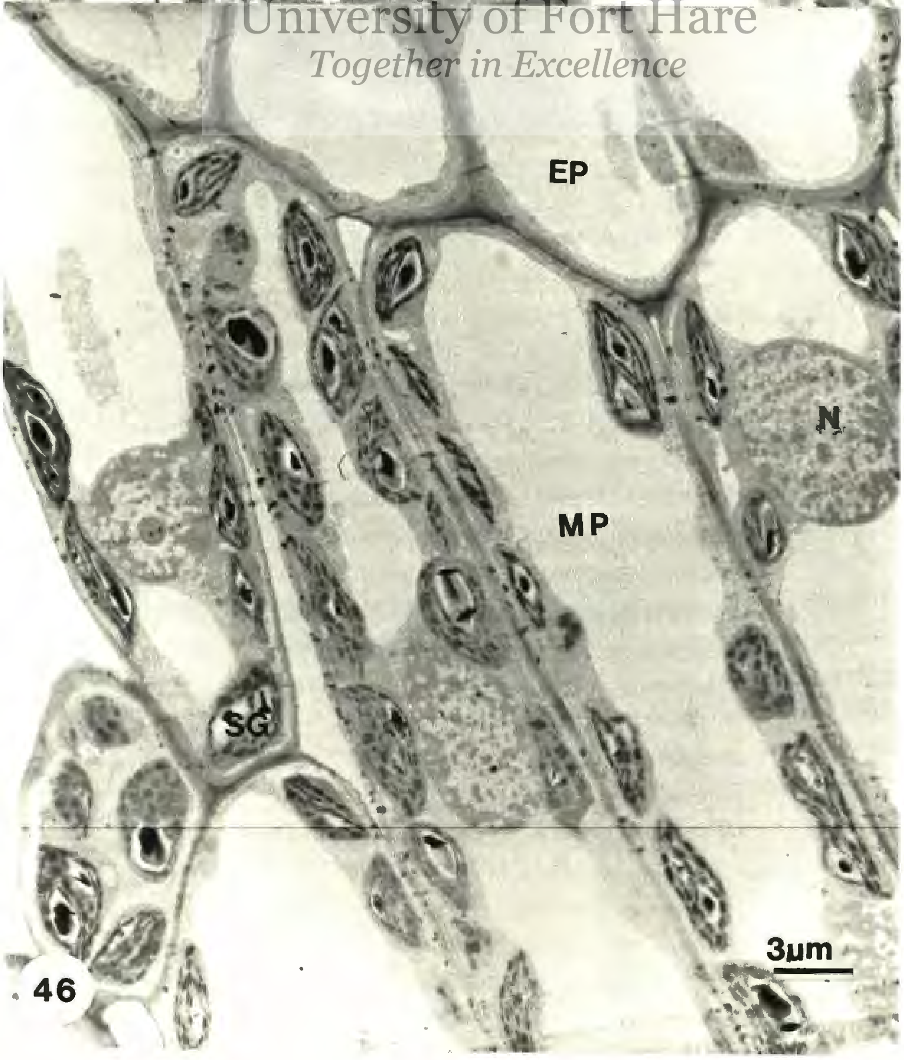
44

Fig. 45. The electron micrograph of *P. incana* leaf showing glandular trichome at it maturity with cuticular sac. OG = Oil gland, H = Head cell, CS = Cuticular sac, S = Stalk cell, EP = Epidermal layer, B = Basal cell,

Fig. 46. Electron micrograph of *P. incana* leaf showing the mesophyll cell containg chloroplasts below the epidermal layer to which the glandular trichome is attached. EP = Epidermal cell, MP = Mesophyll cell, SG = Starch grain.



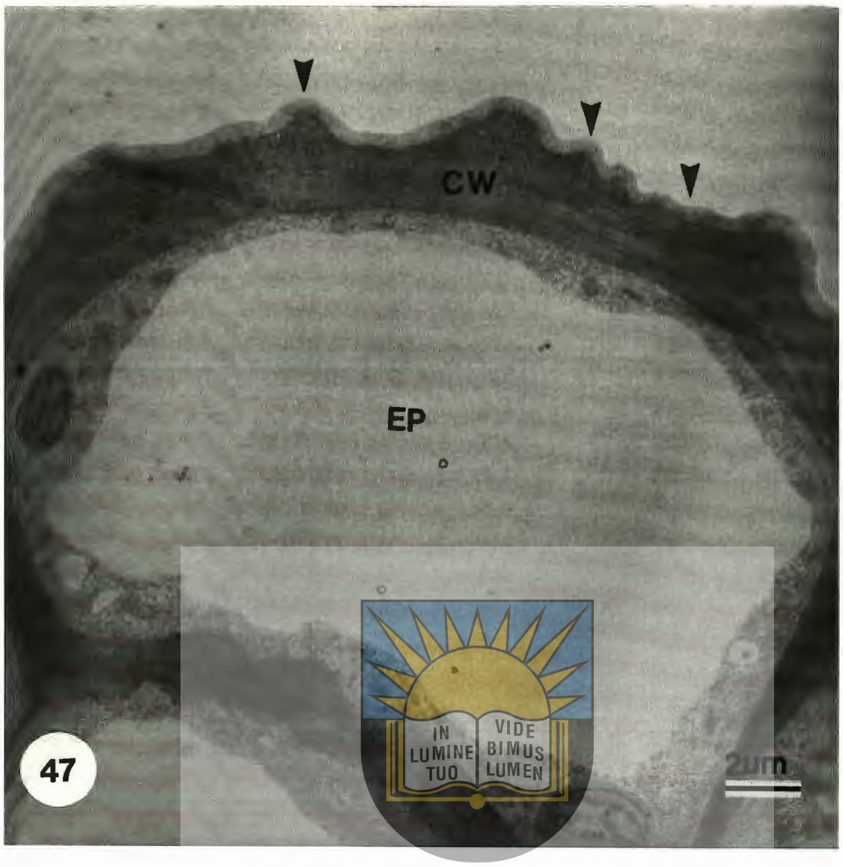
45



46

Fig. 47. The enlarged electron micrograph of *P. incana* leaf displaying a very thick serrated epidermal cell wall (arrows) which is subtended the glandular trichome. CW = Cell wall, EP = Epidermal cell.

Fig. 48. The electron micrograph of *P. incana* leaf showing the portion of the cell wall of the oil gland cell in comparison to the cell wall of the epidermis.
OG-CW = Oil gland cell wall.


CW
EP
47
2um

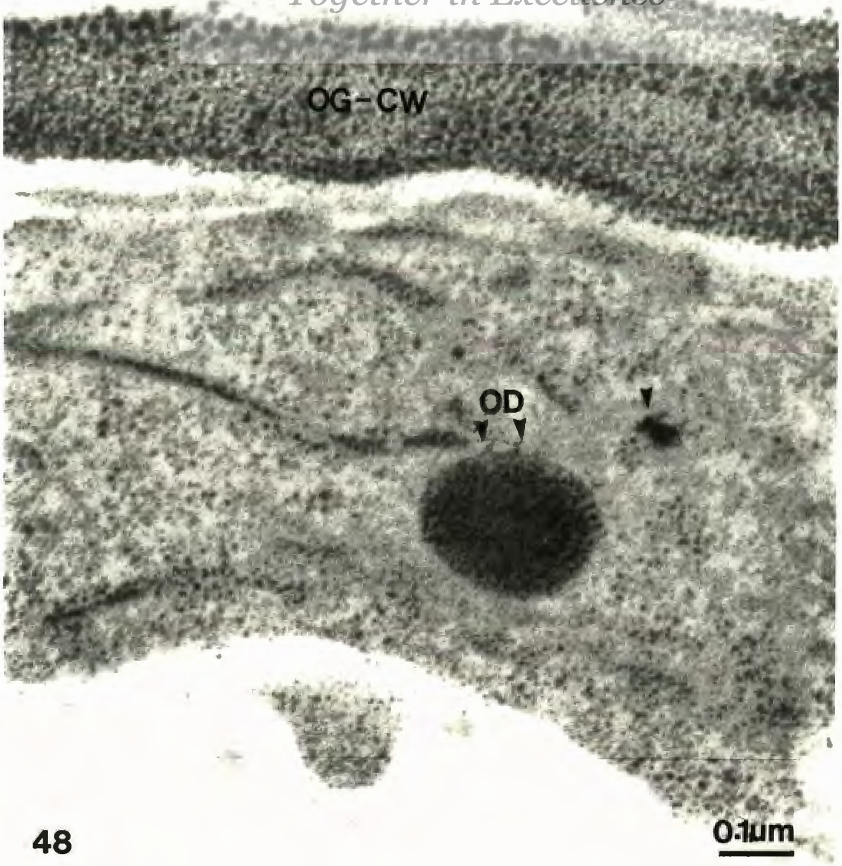

OG-CW
OD
48
0·1um

Fig. 49. A histogram of *P. incana* representing the quantitave variation of essential oil measured in a two year period.

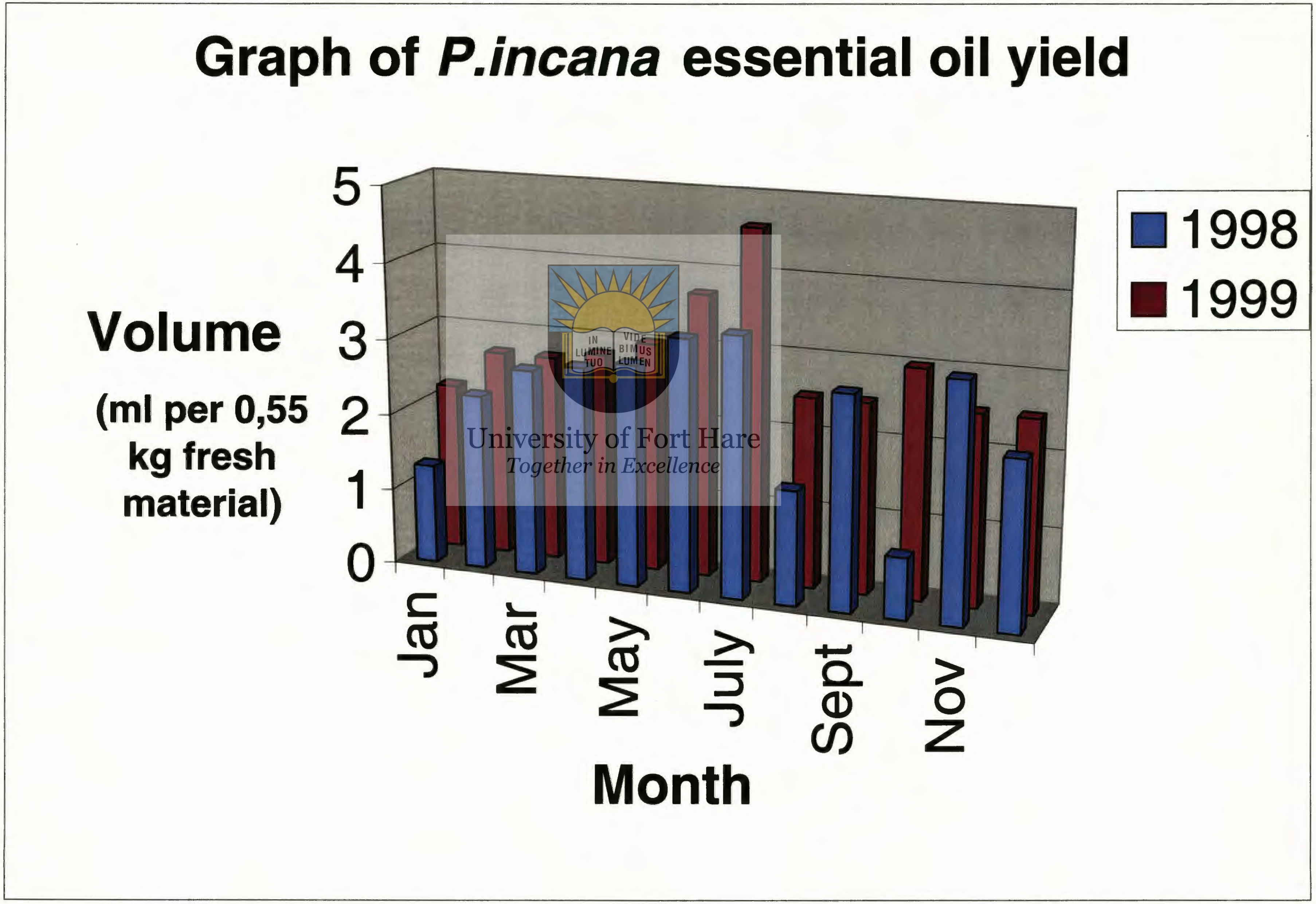

Graph of *P.incana* essential oil yield
Volume
(ml per 0,55 kg fresh material)
0
1
2
3
4
5
Jan
Mar
May
July
Sept
Nov
Month
1998
1999

Fig. 50. Gas chromatogram of essential oil of *P. incana* representing the analysis of the oil in March showing the following volatile compounds: alpha and beta-pinene, beta-thujene, dl-limonene, alpha-terpinene and 1.8 cineole

File: C:\CHEMPC\DATA\P01073I.D
Operator: FVN
Date Acquired: 15 Sep 98 12:42 pm
Method File: ESS_OIL2.M
Sample Name: 2874
Misc Info:
ALS vial: 1

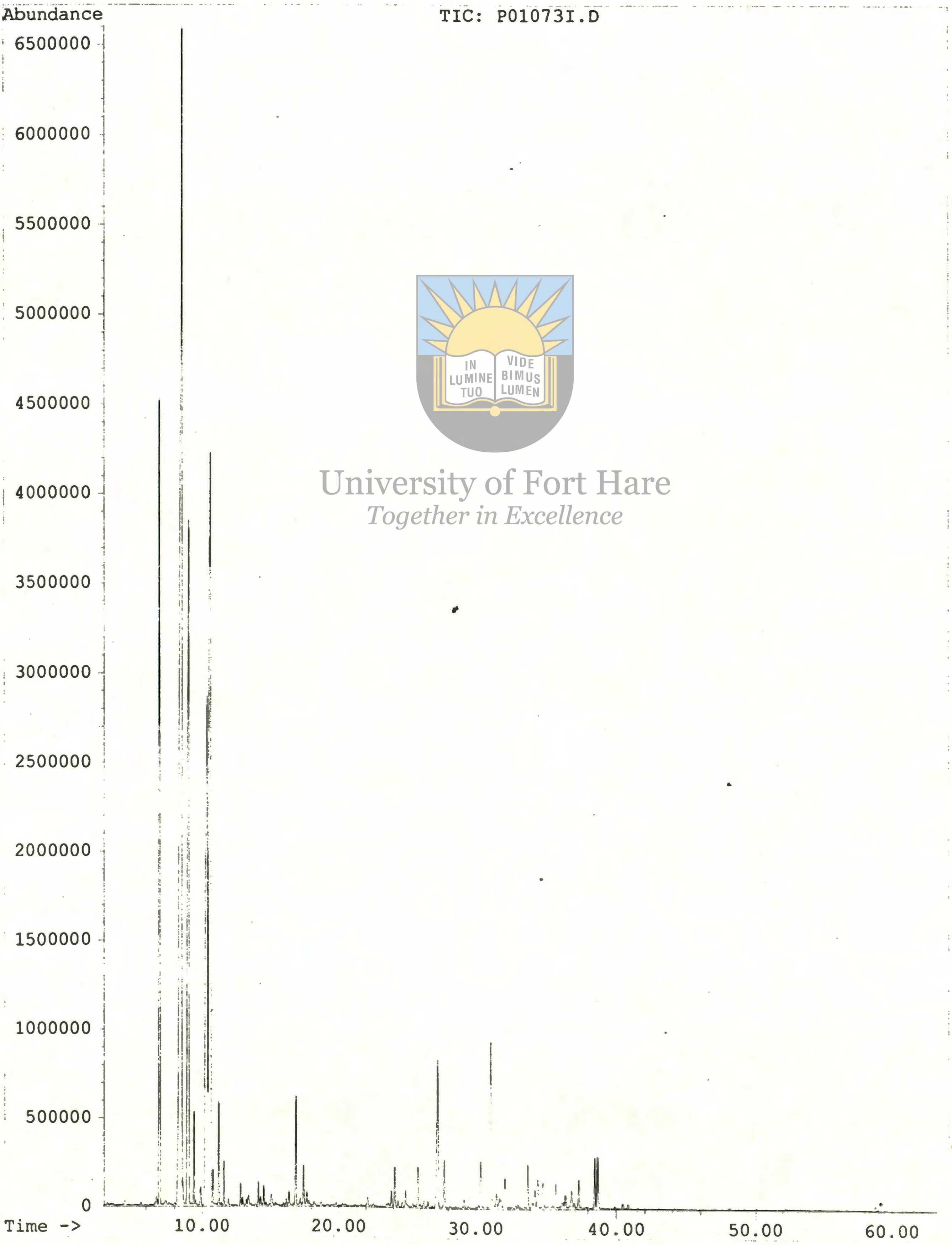


Fig. 51. Gas chromatogram of essential oil of *P. incana* representing the analysis of the oil in April showing the following volatile compounds: alpha and beta-pinene, dl-limonene, gamma-terpinene, delta carenene, alpha-terpinene and 1.8 cineole

File: C:\CHEMPC\DATA\P01073M.D
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Date Acquired: 16 Sep 98 9:42 am
Method File: ESS_OIL2.M
Sample Name: 2878
Misc Info:
ALS vial: 1

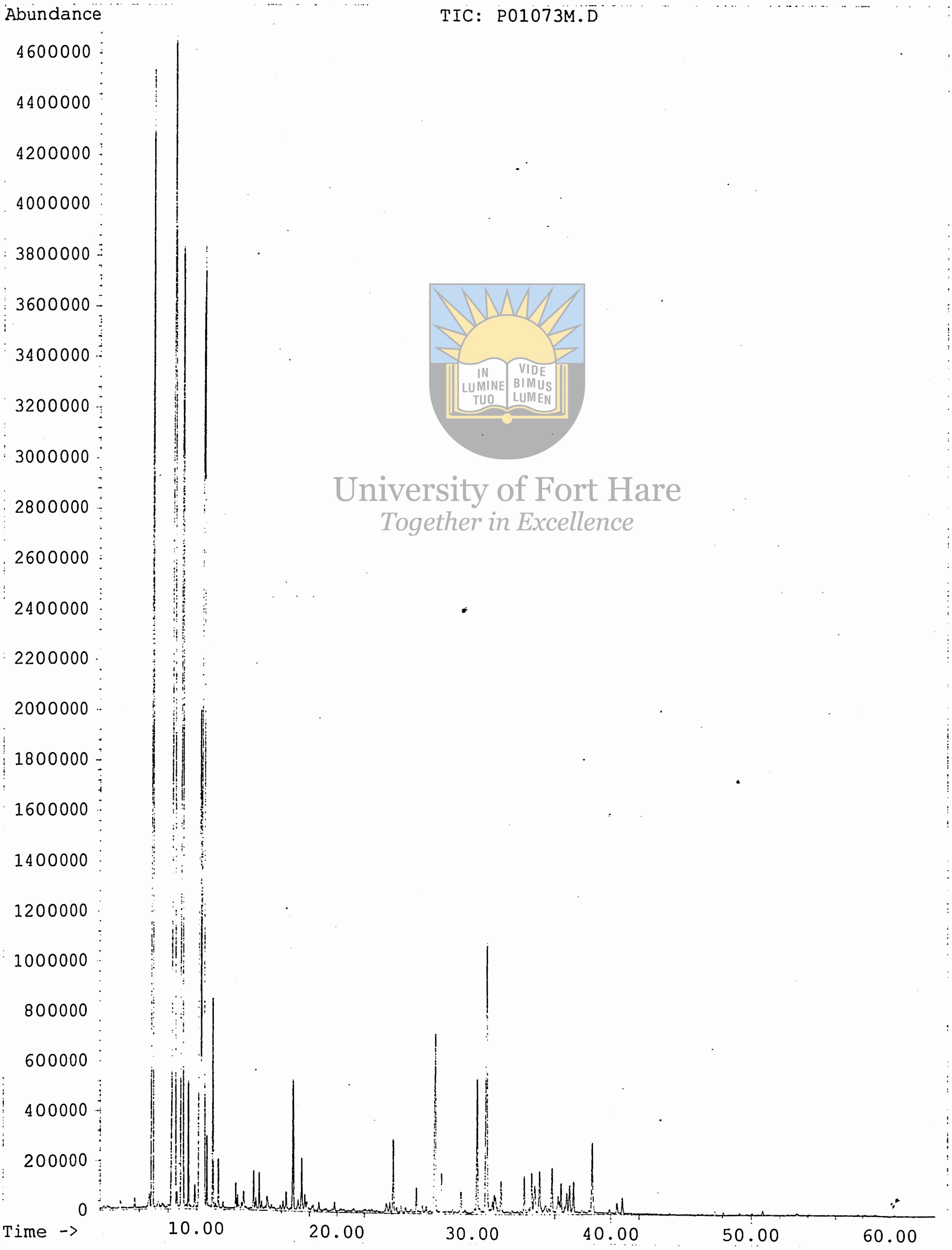

Fig. 52. Gas chromatogram of essential oil of *P. incana* representing the analysis of the oil in June showing the following volatile compounds: alpha pinene, beta-myrcene, 2-beta-pinene, alpha-thujene and 1.8 cineole

File: C:\CHEMPC\DATA\P01073C.D
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Date Acquired: 14 Sep 98 12:38 pm
Method File: ESS_OIL2.M
Sample Name: 2868
Misc Info:
ALS vial: 1

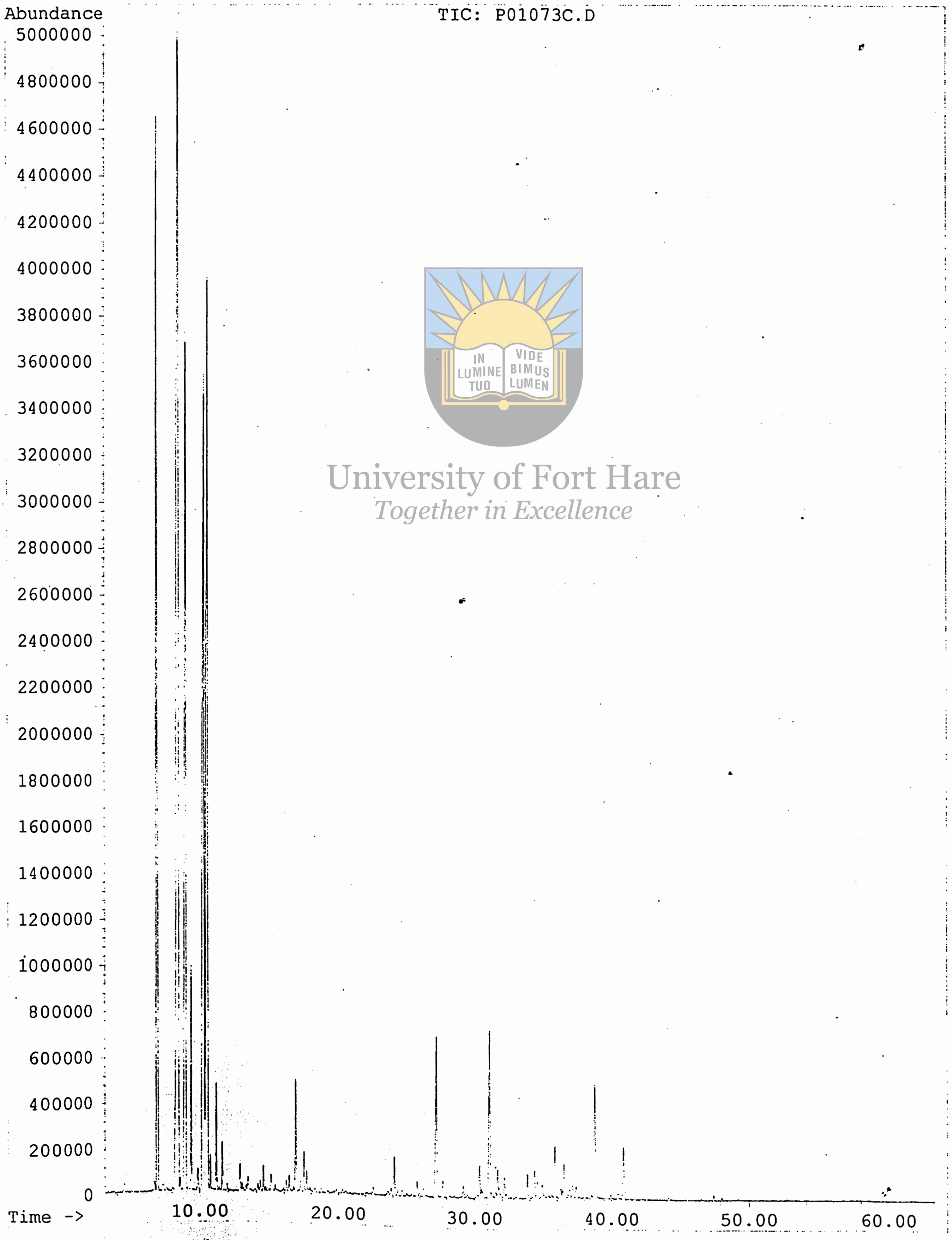

Fig. 53. Gas chromatogram of essential oil of *P. incana* representing the analysis of the oil in July showing the following volatile compounds: alpha pinene, beta-myrcene, sabine, delta-3-carene and 1:8 cineole

```
File:          C:\CHEMPC\DATA\P01073B.D
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Date Acquired: 14 Sep 98  11:22 am
Method File:   ESS_OIL2.M
Sample Name:                    2867
Misc Info:
ALS vial:      1
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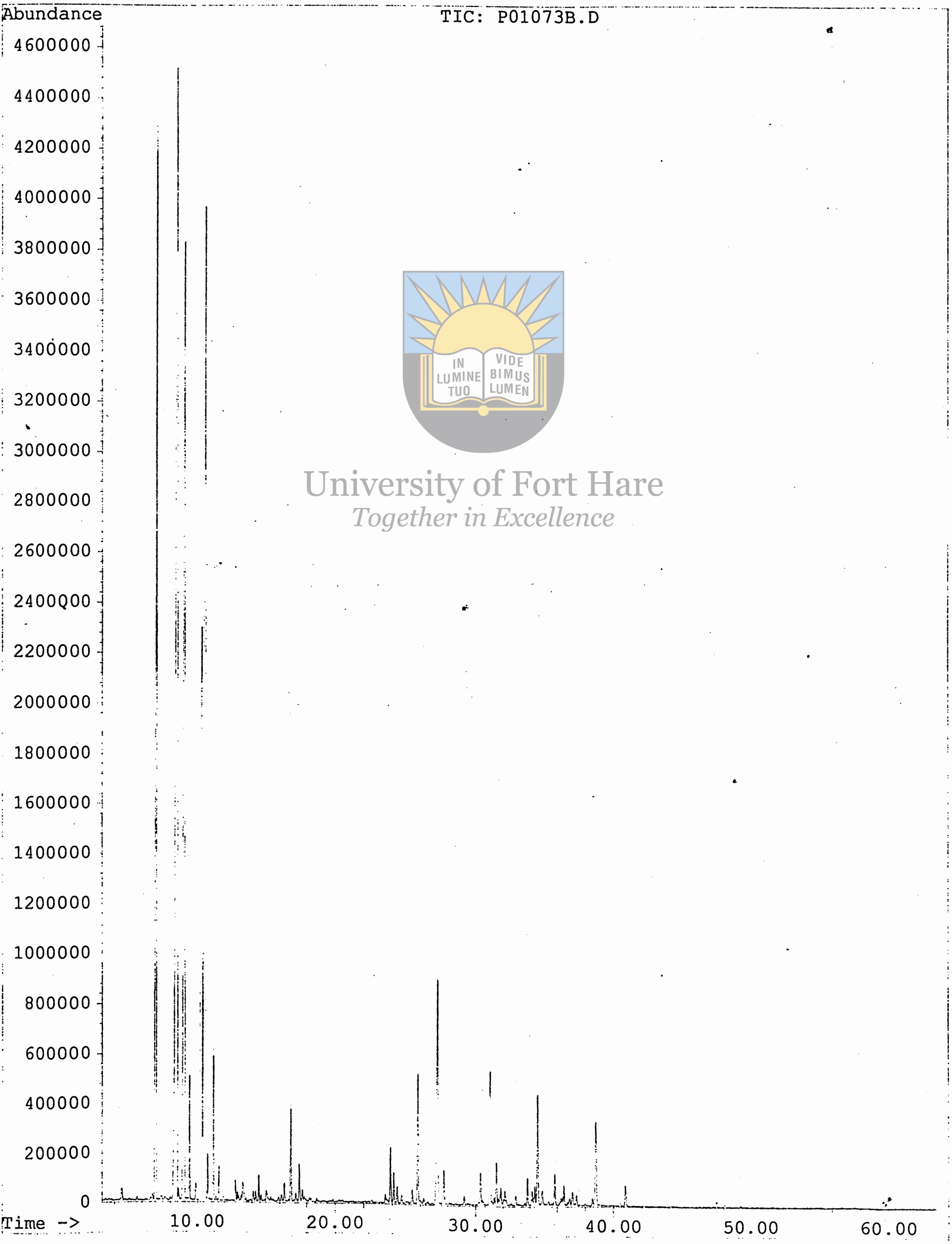
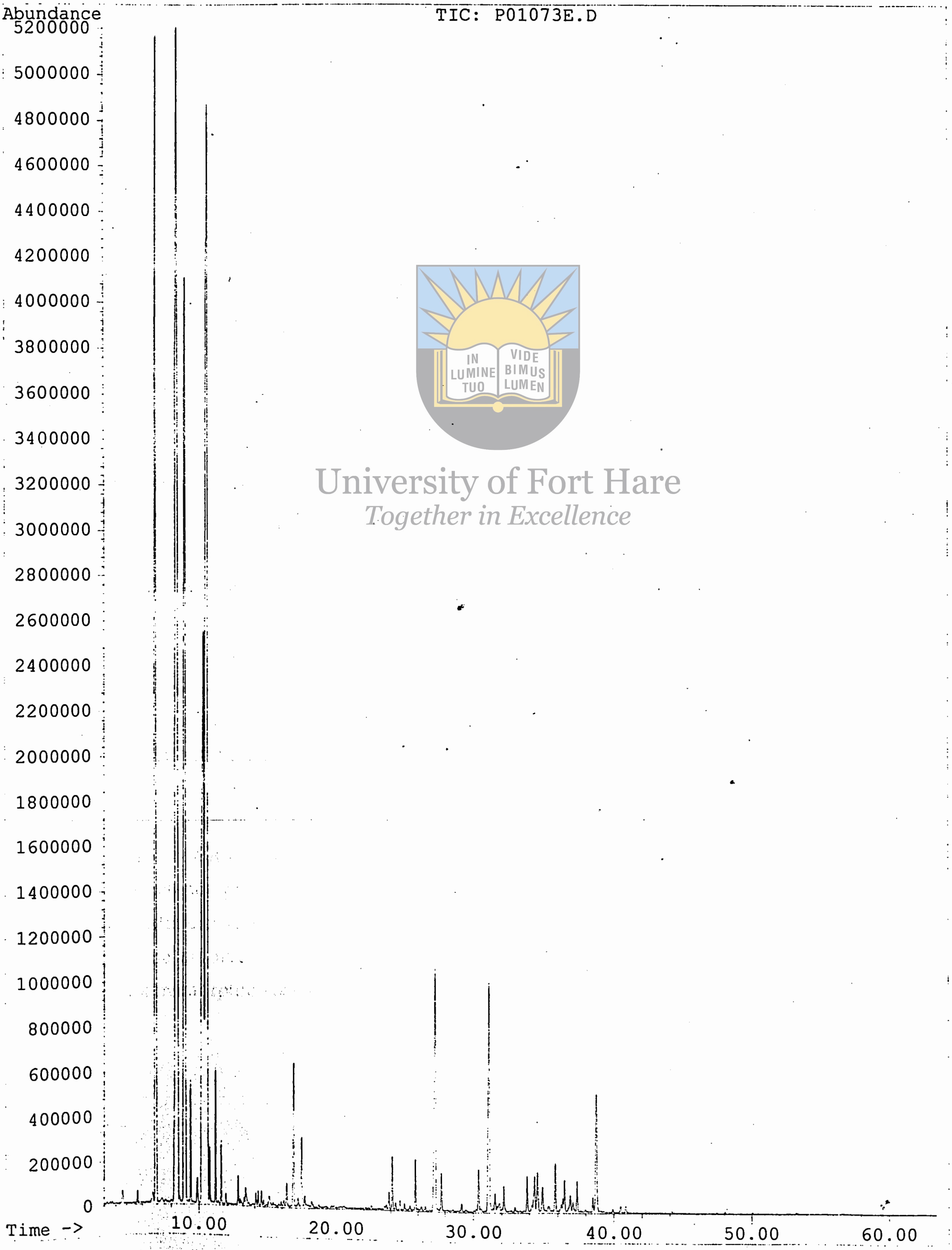


Fig. 54. Gas chromatogram of essential oil of *P. incana* representing the analysis of the oil in August showing the following volatile compounds: alpha-pinene, bete-pinene, beta-thujene, alpha terpinene and delta-4-carene

File: C:\CHEMPC\DATA\P01073E.D
Operator: FVN
Date Acquired: 14 Sep 98 3:14 pm
Method File: ESS_OIL2.M
Sample Name: 2870
Misc Info:
ALS vial: 1



Discussion.

The non-glandular trichomes were composed of uniseriate cells. The terminal cells of the non-glandular trichomes were the first cells to mature but thereafter was no further growth. A further progressive maturity resulted in continuous death of the uniseriate cells until on many occasions there were approximately one or two cells which remained alive at the basal ends of the trichome, and the dead cells resembled fibrous like clothing threads which were covering the epidermis. The observation indicated that, the basal or the terminal cells of the non-glandular trichomes were characterized by suberin similar to the root endodermal cells. It was assumed that this suberin barrier on the dead cells of the non-glandular trichomes were able to prevent apoplastic water flow into the trichomes. When such uniseriate cells retained living protoplasts, it was assumed that the transpiration stream of the trichomes might continue through the symplast. The same contention was supported by *Fahn* and *Shimony* (1998). However a possibility existed that when a water deficit balance developed, incipient plasmolysis might have occurred in the trichome cells. The detachment of the protoplasts from the cell wall or a complete death of the terminal cells might minimize the effect of the symplastic water transport to the uncutinized walls of the upper or the adjacent cells of the trichomes. This stage of non-glandular trichome development occurred in great abundance in mature leaves and stems

It appeared that, the high distribution of non-glandular trichomes on the leaves and young stems of *P. incana* might be regarded as an adaptation associated with arid conditions. The trichomes were assumed to affect transpiration by influencing the water diffusion boundary layer of the transpiring leaf surface. In addition, they might also indirectly influence the water economy of the leaves or young stems through temperature. This might also occur either through a reduction energy dissipation by trichomes, which have high reflectance properties, or the non-glandular trichome were able to shield the stoma and oil glands from the extensive heat during the dry and hot seasons. Such basic phenomena involved in characterization of the boundary layer resistance have also shown been in xeromorphic species (*Gates*, 1968; *Chafe* and *Wardrop*, 1972; *Johnson*, 1975).

Such relationship between the stomatal and boundary layer resistance was also of great significance in attempting to assign an adaptive role to the indumentum layer shown by *P. incana*. The greyish-white cast which occurred in the dead non-glandular trichomes of the leaves and young stems supports the contention that high reflectance of visible radiation was frequently associated with the pubescence (*Johnson*, 1975). The dead non-glandular trichome cells and the epidermis appeared to have wavy anticlinal walls and the undulation which covered the entire depth of the walls of the leaf and stem epidermis, and the dead portion of the non-glandular trichome. This differentiation has resulted in a thick fibrous epidermal layer and thick fibrous thread-like and rod-like non-glandular trichome. It has been suggested that the undulation and waviness of the epidermal and dead non-glandular trichomes might be related to the development of stresses during the leaf non-glandular trichome and cuticle differentiation in the xeromorphic species (*Chafe* and *Wardrop*, 1972; *Esau*, 1953). Since these characters were also present in *P. incana*, it appeared that this species might also be adapted to a xeromorphic environment.

The non-glandular trichomes appeared to originate from the epidermal layer by periclinal division. This process continued by periclinal division until several cells, which formed uniseriate trichomes, are produced. A similar uniseriate trichome ontogeny was observed in several species (*Bosabalidis* and *Tsekos*, 1984). An ultrastructure of the non-glandular trichome cells was characterized by a large nucleus surrounded by the cytoplasm which contained oil globules and phenolic compounds that were associated with the cell wall. It appeared that these phenolic compounds and oil globules found in living cells of non-glandular trichomes might be ascribed for defence and wound healing process (*Webber* et al., 1999). This physiological aspect had been observed in the trichomes of may species by *Beckman et al.*, (1972). This proposition on physiological specialization of non-glandular trichomes was also confirmed in some general of Asteraceae family (*Duke and Paul*, 1993; *Afolayan* and *Meyer*, 1995) Furthermore, it appeared that, such phenomenon has a been demonstrated in young leaves of *Olea europaea* (*Fahn*, 1986).

The essential oils and oil globules found in the non-glandular trichome of *P. incana* might be related in defence function to alkaloids which were present in the same compartments in other species (*Robinson*, 1974).

The glandular trichomes were also the characteristic feature of *P. incana* leaf and stem. These trichomes were multicellular and uniseriate. The glandular trichomes ranged from club to oval in shape and they were composed of a basal cell, a short stalk cell with large six to eight celled head. The orientation of the glandular trichome is not uniform, however, a different orientation have been observed both in the leaf and stem. Morphological studies have shown that the distribution of these glandular trichomes was high during the vegetative growth prior to the flowering period. At this stage of development high glandular trichome distribution were present on both the abaxial and adaxial surface of the leaf. These glandular trichomes were also found in the old stems, however, their distribution frequency is lower in comparison to the mature leaves. It appeared that, the glandular trichomes occurred in abundance during the early stages of leaf and stem differentiation, however, these glandular trichomes seemed to decrease in the old stems. What is not yet known in the literature, is whether the new glandular trichomes which exist throughout the lifetime of the leaf or stem are formed in the same position of the previous glandular trichomes by regeneration of new cuticular sac, and to our knowledge there is no literature available, which dealt with this aspect. However, it appeared that there is strong evidence that, after the essential oil have been released due to the rupture of cuticular sac, there was a regeneration of the new oil glands which would replace the old cells. It was proposed that the process occurred in the following sequence.

The initial event was the apical oil gland cell lysis. Thereafter the first layer of the oil gland cells, beneath the apical oil gland cells undergo a process of differentiation and replace the former apical oil gland cells. It is also, proposed that, the new stalk cells are regenerated from the basal epidermal cell by periclinal and anticlinal divisions. However, the possibility of glandular trichome ontogeny in other parts of the epidermis due to ordinary expansion of leaf or stem as the result of growth and differentiation is not excluded.

Some authors have also suggested that there was a high density occurrence of glandular trichomes during early leaf differentiation (*Ascensão* and *Pais*, 1987; *Fahn*. 1988). However, other authors have observed that there is a high glandular trichome distribution throughout all stages of leaf or stem development (*Bosabalidis*, 1990; *Bosabalidis* and *Tsekos*, 1982a and 1984; *Vermeer* and *Peterson*, 1984).

These glandular trichomes of *P. incana* are regarded as long term glandular trichomes in which the secretory material appeared to accumulate gradually and consistently under elevated cuticular sacs during the development and growth of the aerial parts of *P.incana*. The glandular trichomes of similar nature, which occurred in some species of most genera are believed to have played vital role in defensive mechanisms against the pathogens and herbivores (*Werker*, 1993). These metabolites which were present in the glandular trichomes of the stems and leaves, were also found in the various floral parts. The function of such glandular trichomes is attributed to the attraction of pollinators (*Werker* et al., 1985 b).

In contrast to the varieties of uniseriate, biseriate and capitate, short term glandular trichomes commonly found in some genera of the Asteraceae, and Laminiaceae, *P. incana* is only characterized by the long term peltate glandular trichomes. These short term glandular trichomes are assumed to function for a very short period during the early development of young organs (*Fahn*, 1988; *Duke* and *Paul*,1993).

The other common characteristic of *P. incana* glandular trichomes was that when the glandular trichome was mature, the stalk cell showed clear cutinization of the lateral wall. This cuticular sac is believed to enclose the secondary metabolites such as essential oil, and when this sac raptured due to the external pressure exerted to the wall, it releases the content. It was suspected that these glandular trichomes did not only accumulate and store essential oil, but also the phytotoxic compounds which rendered these species unpalatable to herbivores. Consequently *P. incana* has invaded successfully the large hectares in Alice and Peddie districts in the Eastern Cape.

A similar notion was supported by *Wagner* (1991) when he had demonstrated that the toxic compounds were able to defend the plants as these phytotoxin were excreted to the surface of the plants. The secreted metabolites appeared to be adsorbed by the fibrous non-glandular trichomes which shielded these glandular trichomes and stoma.

Ultrastructural studies showed that *P. incana* is characterized by three to four pairs of glandular cells. It appeared that these pairs did not reach maturity at the same time. The first pair which is produced at the early stages of the glandular trichome, the first glandular cells to be development were responsible for the production of the essential oil. After the release of the essential oil, it is suspected that this first pair of the oil gland cells undergo cell lysis. It is assumed that the second pair of the oil gland cells took the function of the first pair. This proposition was supported by the fact that these oil gland cells undergo extensive differentiation before they became highly specialized to deal with the biosynthesis of the essential oil. It was observed that, at the initial stages of glandular trichome development, there was an expansion of the epidermal cell through anticlinal and periclinal divisions. The two cells produced, were characterized by electron-dense cytoplasm, a prominent nucleus with well-defined nucleolus and a large vacuole. At this stage, the cells which were the precursor of the first oil glands were in direct contact with the epidermis and palisade mesophyll cell through the prominent plasmodesmata. It is believed that the precursors which are required for the biosynthesis of essential oils, were transported at this period, if their source of origin is mesophyll tissue. The reason for this recommendation is that a further development resulted to the production of heavily suberized cells walls around all the three or four pairs of oil gland cells and there were no plasmodesmata at all. Therefore, the possibility of symplastic or apoplastic translocation of essential oil precursors to either the stalk or gland cells of the glandular trichome is possible (*Sacchetti et al.*, 1999)

The other possibility is that the essential oil precursor were *de novo* synthesized within the individual oil gland cell. Such proposition could be supported by cell organelles differentiation which occurred in these cells.

These cells were initially characterized by the well-developed vacuoles, with osmiophilic material, mitochondria and plastids. As the development progressed, the anatomical characteristics of the cells inclusions changed dramatically. These cells showed dense cytosol and poorly developed vacuoles with the scattered small osmiophilic droplets. There were numerous mitochondia, and endoplasmic reticulum system appeared to engulf the plastids which exhibit complex tubular structures. The arrangement of this nature, where there is a proximity between plastids and endoplasmic reticulum, was assumed to be typical of terpene-secreting system (*Figueireds* and *Pais*, 1994). The complex relationship between endoplasmic reticulum and platsids in *P. incana* is believed to exhibite the connection between membranes of the plastidal envelop and the periplastidal endoplasmic reticulum as it was demostrated in the oil gland cells of the essential oil producing species (*Benayoun* and *Fahn*, 1979). It was also noted that there was a high distribution of motochondria in addition to this complex endoplasmic reticulum and plastids relationship in these oil gland cells. These three organelles became modified as the essential oil gradually accumulated the cells. The presence of a combination of this tight network of endoplasmic reticulum, numerous plastids and modified mitochondria in oil gland cells appeared to be an indication that these cells had the typically active metabolism, presumably involved in biosythesis, transport and secretion of the essential oil through the cuticular sac. This proposition was confirmed by *Fahn* (1988) that the endoplasmic reticulum might also be involved in the synthesis of transport materials in addition to the biosynthesis of lipophilic substances.

This process of oil synthesis in glandular trichomes development was accompanied by a formation of the cuticular sac as the essential oil gradually accumulated the cell. The portion of thick cell wall which would be the future cuticular sac, became gradually thinner and thinner until the cuticular sac was formed. This process was believed to occurred through the inner cell wall lysis, although the mechanism is not yet known at this moment. The essential oil were finally released where the cuticular sac ruptured. This resulted to a complete distruction of the cell rather than dedifferentiation of the modified organelles in the same cells. It was believed that the continuous differentiation process engaged the next pair of oil gland cells which became also involved in essential oil biosynthesis.

It was assumed that this continuous repeated process could result to a continuous production of the stalk cells particularly in the leaves as the stalk cells consistently replaced the shifted oil gland cells and became oil gland cells themselves. This was in contrast to some of the compounds, found in the guttation fluid, which were shown to be synthesized in the roots (*Magwa et al*., 1993).

The occurrence of osmiophilic compounds in several different cell compartment in tissues which secreted essential oil raises several questions regarding the possible site of synthesis of this materials. There are three possible interpretations that can be suggested, each organelle in which osmiophilic droplets occurred, was capable of synthesizing all the oil components independently; or several steps in essential oil synthesis, each taking place in a particular organelle and finally, different components were synthesized by different organelles (*Fahn*, 1988).

Chemical analysis have shown a pronounced quantitative difference in essential oil components in a number of species, and between the chemotype, also between the different organs of each chemotype (*Dudai* et al., 1988; *Werker* et al., 1993; *Fahn* and *Shimony* 1996). *P. incana* is characterized by pale yellow essential oil which had a fragrance associated with dwarf pine, siber and fir leaf oil. However the volatile material produced by *P. incana* could be of potential economic importance if could be properly and scientifically examined, because most of flavoring and fragrance are derived from plant substances either from the aromatic volatile plant oils called resins (*Theimer*, 1989).

The composition of the essential oil of *P. incana* appeared to contain following important constitutes, alpha-pinene at 14,2%, beta-pinene and sabinene at 29,8% myrcene at 17,7%, 1.8 cineole and lemonene at 14%, beta-cymene 2,3% and terpinolene 9,4% (*Webber* et al., 1999). However there was an indication of some of the appearance of other constituance such as delta 4 cerene and 1 phellandene. However, a thoroughly investigation is needed to establish the relationship between glandular trichomes of *P. incana* and the essential oil produced, because essential oil users are reluctant to develop new products based upon unreliable supplies of raw materials. A solution to this problem is to produce reserve stocks of the oil during favourable periods when there is an abundance of plant material.

Conclusion

In this investigation, the uniseriate non-glandular trichomes were shown to be originating from epidermal cells by periclinal division. After the completion of this process, further growth occurred by means of basipetal development. This resulted in the suberisation of fibrous non-glandular trichome cells and finally to the death of the same cells. It is believed that when the non-glandular trichome with suberized cells died, they greatly extended the diffusion boundary layer of the transpiration leaf surface. It is suspected that the detachment of the protoplasts from the wall or a complete death of the non-glandular trichome cells also minimize the effect of the symplastic and apoplastic water transport. The greyish-white cast which also occurred in dead non-glandular trichome appeared to support the contention that high reflectance of visible radiation is frequently associated with the heat dissipation process. It is also assumed that this process is able to facilitate the plant cooling effect.

The glandular trichomes appeared to originate from the epidermal cells, initially by anticlinal division followed by periclinal divisions. It is also proposed that the precursors for the biosynthesis of the essential oils, originated from the mesophyll cells. They are thus assumed to be transported to the oil gland cells at the early stages of the development. During this transportation period, the plasmodesmatal connections still occurred between the mesophyll and oil gland cells. Once the heavy suberin developed around each glandular trichome cell, the symplastic and apoplastic transport systems appeared to be blocked and these cells are assumed to function metabolically independent of one another.

It is also proposed that the continuous release of the essential oils, which is shed to the grooved epidermis and fibrous non-glandular trichomes, might render the plant unpalatable to the herbivores and might also inhibit the effect pathogens. These morphological behavioural characteristics of glandular and non-glandular trichomes are ascribed to plants which are commonly adapted to arid conditions.

However, despite the unpalatability to herbivores, *P incana* is regarded to be economically important due to the characteristic aromatic fragrance of terpenoids present in its essential oil.

Acknowledgement

I wish to express my sincere gratitude, first and foremost to Dr M.L Magwa, my promoter, and to Mr E. Kelly for their skillful assistance in preparing this project. The research and ultimately the thesis was completed under Dr M.L. Magwa's guidance and his advice on numerous aspects of this work is appreciated. His competence, willingness to help and instruct, enthusiasm and genuine interest are highly esteemed and appreciated. Furthermore I wish to acknowledge my friend and colleague Mr. R. M. Coopoosamy for driving me to the field for sampling, and my colleages for their skillful assistance in preparation this of project.

I am deeply indebted to the University for allowing me the opportunity to further my studies.

It is difficult to express adequately my gratitude to Miss N. Magazi, Nkcube my son, for their encouragement, sustained interest and affection and, and my mother Mrs E.N. Mayekiso, brothers and sisters for making my career possible.

Sincere thanks to National Research Foundation for their financial assistance for this project, also to CSIR for GC-MS analysis.

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